

POTYGUARD

Laboratory Notebook

iGEM 2026

Herstellung kompetenter Zellen

Project: iGEM Bonn 2026 Shared Project
Author: Franzi Müller
Status: In progress
Entry Created On: 15 Oct 2025 16:58:47 UTC
Entry Last Modified: 12 Nov 2025 15:51:41 UTC
Export Generated On: 05 Oct 2026 11:20:56 UTC

MITTWOCH, 15.10.2025

Preparation of TB (Transformation Buffer, Inoue et al., 1990) for the Preparation of Chemically Competent *E. coli* XL1-Blue Cells

Background:
The TB (Transformation Buffer) according to Inoue et al. (1990) is used for generating chemically competent *E. coli* cells. The ionic composition (Ca^{2+}, K^{+}, and Mn^{2+}) and a pH range of 6.7 are crucial for permeabilizing the cell membrane and thus for enabling the uptake of plasmid DNA during heat shock. Mn^{2+} acts as an essential cofactor that modifies membrane integrity and significantly enhances transformation efficiency.
The aim of this experiment was to prepare 100 ml of TB buffer for later use in competence induction of *E. coli* XL1-Blue.

Procedure:
For preparation, PIPES (M = 302.37 g/mol, salt form), KCl (M = 74.55 g/mol), $CaCl_2 \cdot 2H_2O$ (M = 147.02 g/mol), and KOH (5 N) were used. Calculations were made for a final volume of 100 ml with target concentrations of 10 mM PIPES, 15 mM $CaCl_2$, 250 mM KCl, and 55 mM $MnCl_2$.
In a 250 ml beaker, 90 ml of distilled, non-precooled water was added. Subsequently, 302 mg PIPES, 1.864 g KCl, and 220 mg $CaCl_2 \cdot 2H_2O$ were dissolved one after another. The pH value of the solution was adjusted to 6.7 at room temperature using 5 N KOH.
After pH adjustment, $MgCl_2 \cdot 6H_2O$ (1.09 g, equimolar to 55 mM) was mistakenly added instead of the intended $MnCl_2 \cdot 4H_2O$. The solution was then filled up to a final volume of 100 ml with distilled water, homogenized, and sterile-filtered through a 0.22 µm syringe filter. The filtrate was transferred into two sterile 50 ml Falcon tubes and stored at 4 °C protected from light.

Discussion:
The preparation was correct in terms of concentrations, pH adjustment, and filtration, but a critical error occurred in the choice of the metal ion. $MgCl_2$ was used instead of $MnCl_2$. This mistake initially went unnoticed but had a major impact on the functionality of the buffer.

Preparation of 250 ml SOC Medium (according to Hanahan, 1983) for the Regeneration of Transformed *E. coli* Cells

Background:
SOC (Super Optimal broth with Catabolite repression) is a nutrient-rich medium used after heat-shock transformation to allow *E. coli* cells a short recovery phase and promote expression of the antibiotic resistance gene. The medium is based on SOB (Super Optimal Broth) and is supplemented with magnesium ions and glucose after autoclaving to provide optimal growth conditions.
The aim of this experiment was to prepare 250 ml of sterile-filtered and supplemented SOC medium for the planned transformation of *E. coli* XL1-Blue.

Procedure:

The following components were used according to the standard SOC recipe:

2% (w/v) tryptone, 0.5% (w/v) yeast extract, 10 mM NaCl, 2.5 mM KCl. After autoclaving, the medium was aseptically supplemented to final concentrations of 12.5 mM $MgCl_2$ and 25 mM glucose.

Calculated amounts for 250 ml final volume:

Tryptone: 2% (w/v) → 5.00 g

Yeast extract: 0.5% (w/v) → 1.25 g

NaCl: 10 mM → 0.146 g

KCl: 2.5 mM → 0.047 g

The solids were dissolved in 250 ml distilled water, the pH was adjusted to 7.0 with 1 M NaOH, and the medium was autoclaved.

After cooling to room temperature, the medium was aseptically supplemented with 1 M $MgCl_2$ (final concentration 12.5 mM; 3.125 ml) and 1 M glucose stock solution (final concentration 25 mM; 6.25 ml). The finished SOC medium was stored at 4 °C. The medium appeared clear and light yellow and showed no precipitation or turbidity after addition of the supplements.

Plating of *E. coli* XL1-Blue for Obtaining Fresh Single Colonies

For the preparation of chemically competent cells, freshly grown and metabolically active *E. coli* colonies are required. Colonies from older plates or frozen stocks often show reduced transformation and growth efficiency.

To obtain a homogeneous starting population, a fresh LB agar plate was inoculated from a frozen aliquot of competent *E. coli* XL1-Blue cells. This served as the source material for the preculture (SOC or LB medium) to be used in the following days for competence induction.

Thursday, 16.10.2025

Inoculation of a Liquid Culture with *E. coli* XL1-Blue for Preparation of Competence Induction

Background:

For preparing chemically competent *E. coli* XL1-Blue cells, an actively growing log-phase culture is required. According to the protocol of Inoue et al. (1990), a fresh single colony from the LB plate prepared the previous day should be inoculated into liquid medium to obtain sufficient biomass for the subsequent TB treatment process. The aim of this step was to start a 250 ml preculture in LB medium, which would be processed once an OD_{600} of approximately 0.5–0.7 was reached.

Procedure:

A single morphologically typical colony from the XL1-Blue LB plate prepared on 15.10.2025 was picked under sterile conditions using a sterile toothpick and inoculated into 250 ml LB medium. The medium contained 1% tryptone, 0.5% yeast extract, and 1% NaCl (pH 7.0).

During the procedure, it was found that the 250 ml culture vessel did not fit into the available shaking incubator. Therefore, immediately after inoculation, the culture was divided into two 100 ml Erlenmeyer flasks, each containing 10 ml LB medium. Both cultures were incubated overnight at 21 °C in a shaking incubator at 180 rpm.

Discussion:

After overnight incubation, the culture showed no measurable growth. It was likely that the inoculation density was too low or the incubation temperature (21 °C) was too low to support significant cell growth. Contamination or technical malfunction could be excluded. Since no significant increase in cell density was observed even after increasing the temperature to 38 °C, the sample was deemed unusable and discarded.

Monday, 20.10.2025

Plating of *E. coli* XL1-Blue for Obtaining Fresh Single Colonies

Procedure as described on 15.10.2025.

Tuesday, 21.10.2025

Repetition of *E. coli* XL1-Blue Inoculation in LB Medium after Failed Initial Attempt

Procedure:

A single colony from the freshly prepared XL1-Blue LB plate (20.10.) was picked with a sterile toothpick and directly inoculated into 100 ml LB medium. The toothpick was left in the medium after inoculation to allow continuous release of cells during the initial phase. The culture was then incubated overnight at 21 °C in a shaking incubator at 180 rpm.
The next morning (22.10.2025, 9:30 a.m.), the cell density was measured photometrically at 600 nm and showed an OD_{600} < 0.1. The culture was therefore further incubated at 37 °C to promote growth.

Wednesday, 22.10.2025

Background:

After successful growth of the *E. coli* XL1-Blue culture to an optical density of about 0.6, chemical competence induction was carried out. The preparation of chemically competent cells follows the protocol of Inoue et al. (1990), involving sequential treatment of the cells with calcium-, potassium-, and manganese-containing TB buffer (Transformation Buffer). This treatment causes reversible destabilization of the cell membrane, increasing its permeability to DNA during the subsequent heat-shock transformation. After the final resuspension in TB with DMSO, the cells are aliquoted and cryopreserved by flash-freezing.

Procedure:

On 22.10.2025, the *E. coli* XL1-Blue culture (100 ml LB in a 500 ml Erlenmeyer flask) had reached an optical density of approximately 0.6 by 17:30 and was immediately processed to prepare chemically competent cells.
The entire culture was first placed on ice for ten minutes to halt cell growth and stabilize membranes. It was then transferred into 50 ml Falcon tubes and centrifuged at 4 °C for ten minutes at 2500 rpm. The supernatant was discarded, and the cell pellet was gently resuspended in ice-cold TB buffer. Instead of the 40 ml TB buffer specified in the protocol, only enough was used to completely cover the pellet. The suspension was incubated on ice for ten minutes and then centrifuged again at 4 °C and 2500 rpm for ten minutes. The supernatant was completely removed, and the pellet was resuspended in 10 ml ice-cold TB containing 7% (v/v) DMSO. This suspension was again incubated on ice for ten minutes to allow DMSO penetration into the cell membranes. After thorough mixing, the cell suspension was aliquoted into pre-chilled Eppendorf tubes (200 µl each), flash-frozen in liquid nitrogen, and stored at −80 °C. All steps were carried out on ice to minimize cellular stress.

Thursday, 23.10.2025

Testing the Transformation Efficiency of the Prepared Chemically Competent *E. coli* XL1-Blue Cells via Heat-Shock Transformation with pUC Plasmid (Amp^R)

Background:

To assess the quality of the chemically competent *E. coli* XL1-Blue cells prepared the previous day, a heat-shock transformation was performed using a pUC plasmid carrying an ampicillin resistance cassette (Amp^R). This test serves to evaluate cell competence and efficiency: the higher the number of colonies on the selection plates, the better the quality of the competent cells. Plating was done on LB agar plates containing ampicillin, allowing growth only of transformed cells.

Procedure:

An aliquot (200 µl) of the competent *E. coli* XL1-Blue cells prepared on 22.10.2025 was thawed on ice.

To each sample, 2 µl of pUC plasmid DNA (Amp^R) was added and the mixture was incubated on ice for 30 minutes. Heat shock was then performed at 42 °C for 90 seconds, followed by immediate cooling on ice. After an additional 5-minute incubation on ice, 700 µl SOC medium was added to each sample, and the suspensions were shaken for 1 hour at 37 °C at 850 rpm to allow expression of the resistance gene. Following recovery, 100 µl of each culture was plated onto LB agar plates containing ampicillin. Plates were incubated overnight at 37 °C.

Discussion:

The next day, the ampicillin plates showed no colonies, indicating a failed transformation. Since the procedure and plasmid were correct, the cause was most likely the insufficient competence of the cells due to the use of $MgCl_2$ instead of $MnCl_2$ during TB preparation. The batch was rated as non-transformable and discarded.

Single-temperature Double Digest

Introduction

This is the Double Digest Protocol with Standard Restriction Enzymes, using a common reaction and same incubation temperature for both enzymes.

More information from NEB can be found here.

Double Digests can be designed using NEB's Double Digest Finder.

See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.

NEBcloner will help guide your reaction buffer selection when setting up double digests.

Materials

- DNA **1 µg**
- NEBuffer
  - 1X
- NEB Restriction Enzymes
- Deionized Water

Procedure

Single Temperature DD Reaction

1. Set up the following reaction (total reaction volume 50 µl).

Table2

	A	B
1		**Reagent Volumes (µl)**
2	**Buffer (10x)**	5
3	**DNA ***	Input Volume for ng
4	**Restriction Enzyme #1 ****	1
5	**Restriction Enzyme #2 ****	1
6	**Deionized Water (µl)**	48
7	**Total Volume (µl)**	50

* Recommended maximum of 1 µg of substrate per 10 units of enzyme.

** Restriction Enzymes should be added to the mixture last.

2. Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube.

3. Quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.

4. Incubate for 1 hour at the enzyme-specific appropriate temperature.

01:00:00

Can be decreased to 5-15 minutes by using a Time-Saver™ Qualified Restriction Enzyme

See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.

Production of competent cells

Project: iGEM Bonn 2026 Shared Project
Author: Jula Ommerborn
Status: In progress
Entry Created On: 29 Oct 2025 22:53:36 UTC
Entry Last Modified: 03 Feb 2026 18:44:04 UTC
Export Generated On: 05 Oct 2026 11:21:05 UTC

MITTWOCH, 15.10.2025

Production of TB (Transformation Buffer, Inoue et al, 1990) for the preparation of chemically competent E.coli XL1 blue cells

Background

The TB (transformation buffer) according to Inoue et al. (1990) i used to generate chemically compenet E.coli cells. The ion composition (Ca^{2+} , K^{+} and Mn^{2+})and the pH of 6,7 are crucial for the permeabilization of the cell membrane and thus for the ability of the cells to takw up plasmid DNA during heat shock. Mn^{2+} is described as an essential cofactor that modifies membrane integrity and significantly increases uptake efficiency. The aim of today's approach was to produce 100 ml of TB buffer for later use in the compentence induction of E. col XL1-Blue

Procedure

PIPES (M=302,37 g/mol, as a salt), KCl (M=74,55 g/mol), $CaCl_2 \cdot 2\ H_2O$ (M=147,02 g/mol) and KOH (5) were used for the preparation. The calculations were made for a final volume of 100 ml with target concentrations of 10 mM PIPES, 15 mM $CaCl_2$, 250 mM KCl and 55 mM $MnCl_2$
First, 90 ml of distilled water was placed in a 250 ml beaker. Then 302 mg PIPES, 1.864 g KCl and 220 mg $CaCl_2 \cdot 2\ H_2O$ were completely dissolved. The pH was adjusted at room temperature using 5N KOH.
Lastly, $MgCl_2 \cdot 6H_2O$ (1,09 , equimolar to 55 mM) was accidentally added instead of the intended $MnCl_2 \cdot 4\ H_2O$. The solution was filled up to the final volume of 100 ml with distilled water, homogenized and sterilely filtered through a 0,22 µm syringe filter. The filtered buffer was transferred to two sterile 50 ml Falcon tubes and stored at 4°C protected from light.

Discussion

The preparation was correct in terms of concentrations, pH value and filtration but a critical error was made in the selection of the metal ion. $MgCl_2$ was used instead of $MnCl_2$. This error went unnoticed at first, but had a significant impact on the functionality of the buffer.

Preparation of 250 ml SOC Medium (according to Hanahan, 1983) for the regeneration of transformed E.coli

Background

SOC (Super Optimal Broth with Catabolite Repression) is a nutrient-rich medium used after heat shock transformation to allow E. coli cells a short recovery period and promote the expression of the antibiotic resistance gene. The medium is based on SOB (Super Optimal Broth) and is supplemented with magnesium ions and glucose after autoclaving to create optimal growth conditions.
The aim of this approach was to produce 250 ml of sterile filtered and supplemented SOC medium for the planned transformation of E. coli XL1-Blue.

Procedure:

The following components were used for preparation according to the standard recipe for SOC medium:
2% (w/v) tryptone, 0.5% (w/v) yeast extract, 10 mM NaCl, 2.5 mM KCl. After autoclaving, sterile supplementation was carried out to final concentrations of 12.5 mM MgCl2 and 25 mM glucose.
Calculated quantities for a final volume of 250 ml:

Tryptone: 2% (w/v) → 5.00 g
Yeast extract: 0.5% (w/v) → 1.25 g
NaCl: 10 mM → 0.146 g
KCl: 2.5 mM → 0.047 g

The solids were dissolved in 250 ml of distilled water, the pH was adjusted to 7.0 with 1 M NaOH, and the medium was then autoclaved.
After complete cooling to room temperature, the medium was supplemented under sterile conditions with 1 M MgCl2 (final concentration 12.5 mM; corresponding to 3.125 ml) and a 1 M glucose stock solution (final concentration 25 mM; corresponding to 6.25 ml). The finished SOC medium was stored at 4 °C.
The medium was clear, light yellow, and showed no precipitation or cloudiness after the additives were added.

Plating E. coli XL1-Blue to obtain fresh single colonies

Background

Freshly grown, viable E. coli colonies are always required for the production of chemically competent cells. Colonies from older plates or from frozen stocks often show reduced transformation and growth efficiency.

Procedure

In order to obtain a homogeneous starting population, an LB agar plate was freshly inoculated from a frozen aliquot of competent E. coli XL1-Blue cells. This serves as the starting material for the preculture (SOC or LB medium), which will be used for competence induction in the following days.

DONNERSTAG, 16.10.2025

Inoculation of a liquid culture with E. coli XL1 Blue to prepare for competence induction

Background:

An actively growing log-phase culture is necessary for the production of chemically competent E. coli XL1 Blue cells. According to the protocol by Inoue et al. (1990), a fresh single colony from the LB agar plate prepared the day before should be inoculated into liquid medium in order to obtain sufficient biomass for the subsequent TB treatment process. The aim of today's step was to prepare a 250 ml pre-culture in LB medium, which should be further processed after reaching an OD600 of approx. 0.5–0.7.

Procedure:

A single, morphologically typical colony was picked from the XL1 Blue LB plate prepared on October 15, 2025, under sterile conditions using a sterile toothpick. This was inoculated into 250 ml of LB medium. The medium contained 1% tryptone, 0.5% yeast extract, and 1% NaCl (pH 7.0).

During the work, it became apparent that the 250 ml culture vessel did not fit into the available shaking incubator. Therefore, immediately after inoculation, the culture was transferred to two 100 ml Erlenmeyer flasks, each containing 10 ml of LB medium. Both preparations were incubated overnight at 21 °C in a shaking incubator at 180 rpm.

Discussion:

The culture showed no measurable growth after overnight incubation. Presumably, the inoculation density was too low or the temperature of 21 °C was too low to achieve significant cell proliferation. Contamination or technical malfunction could be ruled out. Since no significant increase in cell density was observed even after raising the temperature to 38 °C, the sample was classified as unusable and discarded.

MONTAG, 20.10.2025

Plating of E. coli XL1-Blue to obtain fresh single colonies
Procedure see October 15, 2025

DIENSTAG, 21.10.2025

Repeating the inoculation of E. coli XL1-Blue in LB medium after the initial attempt failed Procedure:
A single colony from the XL1-Blue LB plate newly created on October 20 was picked up with a sterile toothpick and inoculated directly into 100 ml of LB medium. The toothpick was left in the medium after inoculation to allow continuous release of the cells during the start phase. The culture was then incubated overnight at 21 °C in a shaking incubator at 180 rpm. On the morning of the following day (October 22, 2025, 9:30 a.m.), the cell density was measured photometrically at 600 nm and yielded an OD600 < 0.1. The culture was then further incubated at 37 °C to promote growth.

MITTWOCH, 22.10.2025

Preparation of Chemical Competent Cells

Background:
After successful growth of the E. coli XL1 Blue culture to an optical density of approximately 0.6, chemical competence induction was to take place on this day. Chemically competent cells were produced according to the protocol of Inoue et al. (1990) by successive treatment of the cells with calcium-, potassium-, and manganese-containing TB buffer (Transformation Buffer). This treatment causes reversible destabilization of the cell membrane and increases its permeability to DNA during the subsequent heat shock transformation. After the final resuspension in TB with DMSO, the cells are aliquoted and preserved by shock freezing.
Procedure:

On October 22, 2025, the E. coli XL1 Blue culture (100 ml LB in a 500 ml Erlenmeyer flask) inoculated the previous day had reached an optical density of approximately 0.6 at around 5:30 p.m. and was then immediately processed further to produce chemically competent cells. The entire culture was first placed on ice for ten minutes to stop cell growth and stabilize the membranes. It was then transferred to 50 ml Falcon tubes and centrifuged at 4 °C for ten minutes at 2500 rpm. The supernatant was discarded and the cell pellet was carefully resuspended in ice-cold TB buffer. Instead of using the 40 ml of TB specified in the protocol, only enough buffer was used to completely cover the pellet. The suspension was then incubated on ice for ten minutes and centrifuged again at 4 °C and 2500 rpm for ten minutes. The supernatant was completely removed and the pellet was resuspended in 10 ml of ice-cold TB with 7% (v/v) DMSO. This suspension was again incubated on ice for ten minutes to allow the DMSO to penetrate the cell membrane. After thorough mixing, the cell solution was distributed in 200 µl aliquots into pre-cooled Eppendorf tubes, shock-frozen in liquid nitrogen, and then stored at −80 °C. All steps were performed on ice to avoid cell stress.

DONNERSTAG, 23.10.2025

Testing the transformability of the chemically competent E. coli XL1 Blue cells produced by heat shock transformation with pUC19 plasmid (Amp^R)

Background:
To check the quality of the chemically competent E. coli XL1 Blue cells produced the previous day, heat shock transformation was performed with a pUC19 plasmid carrying an ampicillin resistance cassette (Amp^R). This test serves to assess the functionality and efficiency of the competent cells: the higher the number of colonies formed on the selection plates, the better the quality of the cells. Plating was performed on LB agar plates with ampicillin, which should allow only transformed cells to grow.

Procedure:
An aliquot (200 µl) of the competent E. coli XL1 Blue cells produced on October 22, 2025, was thawed on ice.
To each sample, 2 µl of pUC19 plasmid DNA (Amp^R) was added and the mixture was incubated on ice for 30 min. This was followed by heat shock at 42 °C for 90 seconds, after which the samples were immediately placed back on ice. After a further 5 minutes of incubation on ice, 700 µl of SOC medium was added to each sample and the suspensions were shaken for 1 hour at 37 °C at 850 rpm to enable expression of the resistance gene. After the regeneration phase, 100 µl of each culture was plated on LB agar plates containing ampicillin. The plates were incubated overnight at 37 °C.

Discussion:
The following day, the ampicillin plates showed no colonies, indicating a lack of transformation. Since the procedure and plasmid were correct, the cause was most likely the insufficient competence of the cells due to the use of MgCl2 instead of MnCl2 in TB production. The batch was assessed as non-transformable and discarded.

DIENSTAG, 28.10.2025

Production of TB (Transformation Buffer, Inoue et al, 1990) for the preparation of chemically competent E.coli XL1 blue cells

Procedure

PIPES (M=302,37 g/mol, as a salt), KCl (M=74,55 g/mol), $CaCl_2 \cdot 2\ H_2O$ (M=147,02 g/mol) and KOH (5) were used for the preparation. The calculations were made for a final volume of 50 ml with target concentrations of 10 mM PIPES, 15 mM $CaCl_2$, 250 mM KCl and 55 mM $MnCl_2$
First, 40 ml of distilled water was placed in a 250 ml beaker. Then 151 mg PIPES, 932 g KCl and 110 mg $CaCl_2 \cdot 2\ H_2O$ were completely dissolved. The pH was adjusted at room temperature using 5N KOH.
Lastly, 544mg $MnCl_2 \cdot 4\ H_2O$ was added. The solution was filled up to the final volume of 50 ml with distilled water, homogenized and sterilely filtered through a 0,22 µm syringe filter. The filtered buffer was transferred to a sterile 50 ml Falcon tube and stored at 4°C protected from light.

Plating E. coli XL1-Blue to obtain fresh single colonies

see 15.10.2025

MITTWOCH, 29.10.2025

Plating E. coli XL1-Blue to obtain fresh single colonies

see 15.10.2025

DONNERSTAG, 30.10.2025

Inoculation of LB medium with *E. coli* XL1-Blue

see 21.10.25. The colony was picked from the plate plated on 29.10.25. The culture was grown overnight at 24 °C.

FREITAG, 31.10.2025

Preparation of chemical competent cells

After measuring the OD at 600 nm until the desired value of 0.55-0.65 was achieved the cells were incubated on ice for 10 minutes.
Then the liquid was transferred to centrifuge tubes and centrifuged at 2500g for 10 minutes at 4 °C..
The Supernatant was removed and the pellet dried by draining the excess medium. 20 ml of ice cold TB buffer were added to each tube (total Volume of TB buffer used: 40ml) and the cell pellet resuspended.
Once again, the liquid was centrifuged for 10 minutes at 4°C.
Then, the supernatant was removed again and the pellet dried by draining the excess medium.
3 ml of ice cold TB buffer were added to the cell pellet and then resuspended. Then the liquid of one was transferred into the other and 1,5 ml of TB buffer added.
After that 0,56 ml of DMSO were added and the suspension was gently shaken.
Then, the cells were incubated on ice for 10 minutes.
100 microliters of the suspension were aliquoted into tubes and immediately plunged into liquid nitrogen.

MONTAG, 3.11.2025

Testing the transformability of the chemically competent E. coli XL1 Blue cells produced by heat shock transformation with pUC19 plasmid (Amp^R)

see 23.10.2025. The transformation was performed using 1 µl of pUC19 plasmid concentrated 50 pg/µl and an aliquot of the competent cells prepared on 31.10.2025. After expression of the antibiotics resistance the cells were pelleted by centrifugation at 3000x g for 5 minutes. The supernatant, except the last 100 µl, was discarded, and the cells were resuspended in the remaining medium. The full 100 µl were plated on a LB(Amp30) media plate.

MITTWOCH, 5.11.2025

Calculating the transforamtion efficiency of the competent cells

The colonies on the plate plated on 3.11.2025 were counted and using the number of colony forming units (cfu) and the used amount of plasmid the transformation efficiency was calculated by dividing the cfu with the amount of used plasmid.

cfu / m(plasmid) = transformation efficiency

207 cfu / 50 pg = 4.14 cfu/pg = $4.14*10^6$ cfu/µg

MONTAG, 10.11.2025

Preparing buffers for future plasmid isolation

Background

Plasmid isolation is one of the irreplaceable methods when working in molecular biology. In our case alkaline lysis will be performed utilising alkaline lysis. The protocol is modified from Birnboim & Doly 1979. The used buffers are Bibdo 1, Bibdo 2 and Bibdo 3. Bibdo 1 deactivates the nucleases that could degrade the plasmid, Bibdo 2 lyses the cells and Bibdo 3 neutralises the alkaline pH of Bibdo 3 and allows the Plasmid to resuspend.

Procedure

100ml of each buffer was mixed to the following specifications:
Bibdo 1: 25mM tris, 10mM EDTA and 50mM glucose. pH was adjusted to 8.0 using NaOH and HCl. 0.5% of RNAseA is added freshly before use.
Bibdo 2: 0.2M NaOH and 1% SDS
Bibdo 3: 3M potassium acetate and 11.5% acetic acid

Due to an issue with the volume of destilled water when solving the potassium acetate 200ml of Bibdo 3 buffer was produced.

MONTAG, 8.12.2025

Purification of Q5 polymerase from Q5 polymerase expressing *E. coli*

Preparing buffers for the polymerase purification

The following buffers and media were prepared to the following specifications:
800ml of dYT medium was prepared to culture the bacteria.
dYT medium: trypton 16g/l, yeast extract 10g/l and NaCl 5g/l
50ml of lysis buffer was prepared to lyse the bacteria and extract the polymerase.
lysis buffer: 20mM TAPS, 50mM KCl, 1mM EDTA, 1mg/l lysozyme and 5% glycerol. The pH is adjusted to 8.5 using KOH. Because the buffer contained lysozyme the buffer was not autoclaved, but rather sterile filtered using a 0.22µm filter. Before use 1mM PMSF is freshly added.
10ml of 5xQ5 reaction buffer was prepared for later use with the purified polymerase.
5x Q5 polymerase reaction buffer: 125mM TAPS, 250mM KCl_2, 10mM $MgCl_2$ and 5mM β-mercapthoethanol. The pH was adjusted to 9.3 using KOH. Because the buffer contained β-mercapthoethanol the buffer was not autoclaved, but rather sterile filtered using a 0.22µm filter.

DIENSTAG, 9.12.2025

Inoculation of the pre-culture of Q5 polymerase expressing *E.coli*

5ml of dYT medium (50µg/ml kanamycin) was inoculated with Q5 polymerase expressing *E. coli*, by pricking a colony from a plate of bacteria with a toothpick and submerging that toothpick in the liquid medium. The liquid culture was grown overnight at 37°C and 180rpm.

MITTWOCH, 10.12.2025

Upscaling of the liquid culture and inducing transcription of Q5 polymerase with IPTG

250ml of dYT medium (50µg/ml kanamycin) was inuculated with the 5ml liquid culture prepared on 9.12.25. The culture was grown at 37°C and 180rpm until an OD_{600} of 0.6 was reached. Once the OD_{600} of 0.6 was reached the transcription of Q5 polymerase was induced by adding IPTG to a final concentration of 0.5mM. Afterwards the culture was grown overnight at 16°C, to stop the bacterial growth and to promote proper folding of the polymerase.

DONNERSTAG, 11.12.2025

Purification of the Q5 polymerase

Q5 polymerase was purified from the liquid culture grown on 9.12.25 & 10.12.25.
First the bacteria were pelleted by centrifugation at 4000xg and 4°C for 10min. Afterwards the supernatant was discarded and the pellet was weighed and resuspended in 5ml lysis buffer per gramm of pellet. Then the cells were sonifyed in 8 intervals of 30sec with 30sec rest in between. After sonification the cell debris was pelleted by centrifugation at 15000xg and 4°C for 15min. The cleared lysate was transferred into a new reaction tube and incubated at 95°C for 10min. After the incubation the lysate was immediately placed on ice and centrifuged at 16639xg (the used protocol recommends 20000xg, but this was the maximum speed for the centrifuge available)and 4°C for 30min. Afterwards the supernatant was collected and glycerol was added to a final concentration of 50% for storage at -20°C.

DIENSTAG, 3.2.2026

Test of the purified Q5 polymerase

Q5 polymerase was tested in a PCR with an control plasmid (known concentration)
A common mastermix for all 10 25 µL PCR-reactions was set up. The template is the puc19 plasmid (conc. 50 pg/µL) and the amplicon is 2.4 kb. long. The amount of Q5 polymerase and of the DNA template was varied to test for the best PCR success.

PCR Mastermix

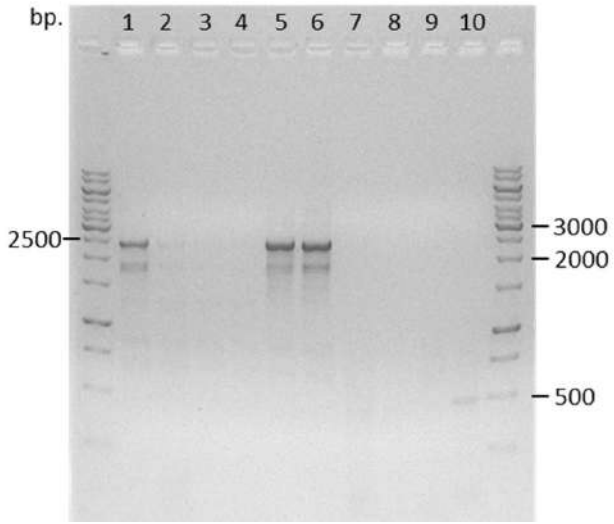
		1x	11 x
1	5x Q5 Buffer	5 µL	55 µL
2	5 mM dNTPs	1 µL	11 µL
3	10 µM PUC19_bbfwd	1.25 µL	13.75 µL
4	10 µM PUC19_bbrev	1.25 µL	13.75 µL

PCR reactions

	Number	1	2	3	4	5	6	7	8	9	10
1	**Q5 volume [µL]**	**0.5**	**1**	**1.5**	**2**	**1**	**1**	**1**	**3**	**5**	**1**
2	**template volume [µL] template final amount [pg]**	4 µL (200 pg)	4 µL (200 pg)	4 µL (200 pg)	4 µL (200 pg)	1 µL (50 pg)	4 µL (200 pg)	10 µL (500 pg)	1 µL (50 pg)	1 µL (50 pg)	0
3	**Volume dH20**	12	11.5	11	10.5	14.5	11.5	5.5	12.5	10.5	15.5

The standard PCR conditions (insert) were used adapted with a 57 °C annealing temperature and 1.15 min elongation time. 10 µL were afterwards mixed with 2 µL 6x TriTracks (DNA Loading dye) and 10 µL were run in 1% Agarose gel (with 3 µL Ethidumbromde / 100 mL) at 130 V and were imaged afterwards. 3 µL of 1 kp. GenRuler were added as a DNA ladder.

image.png



Discussion:

When varing the added Q5 polymerase volume (samples 1 to4), we observed the amplicon in the reaction with the lowest and slightly in the second lowest Q5 volume. This could potentially hint towards copurified inhibiting factors. Addtionally, an increase the template amount did also not positivly influence the outcome. We observed with 50 and 200 pg the amplicon, but not with 500 pg (samples 5 to 7). The negative control has a small band at 500 bp.

Production of electrocompetent GV3101 A. tumefaciens

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 05 Feb 2026 18:15:03 UTC
Entry Last Modified: 05 Feb 2026 20:46:53 UTC
Export Generated On: 05 Oct 2026 11:21:15 UTC

MONTAG, 2.2.2026

Inoculation of the pre culture

Background:

A. tumefaciens is one of the most used used systems for stable and transient transformation of plants. In order to transform the plant *A. tumefaciens* needs to first be transformed with the desired plasmid, containing the to be inserted T-DNA. In order to facilitate *A. tumefaciens* transformation competent cells, capable of picking up plasmids need to be generated first.

Procedure:

A 5 ml pre culture of Yep (rifampicin 100 ng/ml; gentamicin 30 ng/ml) medium was inoculated with GV3101 *A. tumefaciens* from a glycerol stock. The culture was grown for 2 days at 28 °C, 180 rpm.

Formulation Yep medium:
Tryptone 1%
Yeast extract 1%
Sodium chloride 0.5%

MITTWOCH, 4.2.2026

Scaling of the culture

Background:

In order to improve bacterial growth the bacteria are first grown in a small volume and later transferred to a bigger volume.

Procedure:

A 200 ml culture of Yep (rifampicin 100 ng/ml; gentamicin 30 ng/ml) medium was inoculated with the 5 ml pre culture generated on monday 2.2.2026. This new culture was grown over night at 28 °C, 180 rpm.

DONNERSTAG, 5.2.2026

Harvesting of *A. tumefaciens* and preparation for electroporation

Background:

In order to facilitate even electroporation of the cells all salts need to be removed from the solution. The final cells are stored in 10% glycerol for better storage at -70 °C.

Procedure:

The 200 ml culture generated on 4.2.2026 was spun down at 5000x g, 4 °C for 5 minutes. Afterwards the supernatant medium was discarded and the cell pellet was resuspended in 200 ml of ice cold deionized water. The cells were once again pelleted by centrifugation at 5000x g, 4°C for 5 minutes. The supernatant water was discarded and the cells were for a second time resuspended in 200 ml of ice cold deionized water. The cells were spun down at 5000x g, 4 °C for 5 minutes. The supernatant water was discarded, the pellet was resuspended in 30 ml of ice cold 10% glycerol and then the sample was transferred to a 50 ml reaction tube. The cells were spun down one last time at 5000x g, 4 °C for 5 minutes and the supernatant 10% glyceol was discarded. Finally the cells were resuspended in 1 ml ice cold 10% glycerol. 50 µl of the cell suspension was loaded into a cold electropartion cuvette and pulsed at 2.2 kV. The resulting time constant was 5 ms, which is to be expected for properly washed competent cells. Finally the competent cells were aliquoted into 25 µl aliquots and stored at -70 °C.

PCR 35S_Ruby_Bar Backbone

Project: iGEM Bonn 2026 Shared Project
Author: Joana Heckmann
Status: In progress
Entry Created On: 11 Feb 2026 16:59:57 UTC
Entry Last Modified: 13 Feb 2026 19:02:18 UTC
Export Generated On: 05 Oct 2026 11:21:24 UTC

MITTWOCH, 11.2.2026

Q5 PCR Mastermix

	A	1x [µl]	7x [µl]
1	dd H20	14.5	101.5
2	Q5 Buffer	5	35
3	5mM dNTPs	1	7
4	PP 35S_Ruby_Bar_2	1	7
5	Q5	1	7
6	Primer for	1.25	8.75
7	Primer rev	1.25	8.75

Used Primers

	A	B	C
1	JK20	oSNM38	Nr 1
2	oSNM39	oSNM40	Nr 2
3	oSNM41	AP04	Nr 3
4	I2605	oSNM38	Nr 4
5	oSNM41	I2606	Nr 5

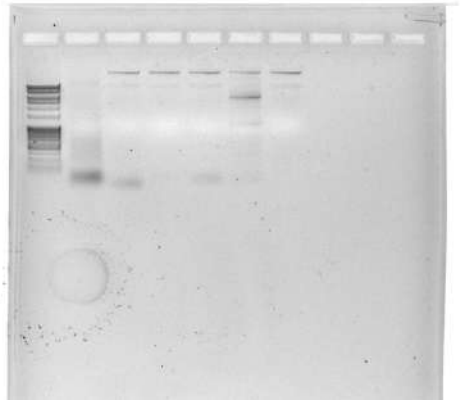
PCR programm

	A	B	C	D	E	F
1	1 cycle	45 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	95°C	95°C	58°C	72°C	72°C	4°C
4	05:00	00:15	00:30	02:00	02:00	endless

Gel loading scheme

	A	B	C	D	E	F	G
1	100 bp ext. ladder	1	2	3	4	5	- control

IGEM_35S_BAR_backbone_PCR_11.02.26.png



Loading scheme from left to right: 100 bp ladder, F1, F2, F3, F1 opt, F2 opt, control
Succesful amplifikation for F2 opt

DONNERSTAG, 12.2.2026

Q5 PCR PCR reaction setup

	A	B	C
1		x1[µl]	x11[µl]
2	dH20	14,5	159,5
3	Q5 buffer	5	55
4	5 mM dNTPs	1	11
5	Q5 Pol	1	11
6	primer for	1,25	
7	Primer rev	1,25	
8	Template	1	

Templaten concentration was diluted 1:10.
PCR Programm and Primer combination are the same as 11.02.2026

IGEM_35S_backbone_PCR_12.02.26.png



Loading scheme from left to right: F1, F1-, F2, F2-, F3, F3-, F1 opt, F1 opt-, F2 opt, 100 bp ruler
Succesful amplification for F2 and F1 opt

FREITAG, 13.2.2026

The PCR with 35S_Ruby_Bar Backbone for the fragment 1 and 2 were repeated. The annealing temperature was decreased by two degrees to improve primer hybridization.

Q5 PCR Reaction Mix2 (ddH20, Q5 Buffer & dNTPs via Mastermix for 5 reactions)

	A	1x [µl]	5x [µl]
1	dd H20	14.5	72.5
2	Q5 Buffer	5	25
3	5mM dNTPs	1	5
4	template	1	
5	Q5	1	
6	Primer for	1.25	
7	Primer rev	1.25	

Reactions2

	fwd_primer	rev_primer	template	D
1	JK20	oSNM38	PP 35S_Ruby_Bar_2	Nr 1
2	JK20	oSNM38	neg. ctrl	Nr 2
3	oSNM41	AP04	PP 35S_Ruby_Bar_2	Nr 3
4	oSNM41	AP04	neg. ctrl	Nr 4

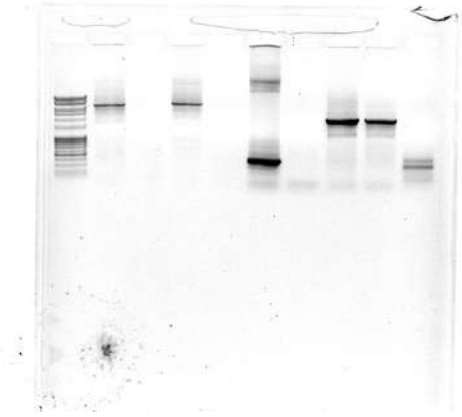
PCR programm2

	A	B	C	D	E	F
1	1 cycle	45 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	95°C	95°C	56 °C	72°C	72°C	4°C
4	05:00	00:15	00:45	02:00	02:00	endless

Gel loading scheme1

	A	B	C	D	E	F	G	H	I	J
1	100 bp ext. ladder	35S_F1	35S_F1 -	35S_F3	35S_F3 -	lexA	lexA -	TEV	TEV 1:5	TEV -

IGEM_PCRs_13.02.26.png



NLSsplitGFP PCR amplification

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 12 Feb 2026 22:47:42 UTC
Entry Last Modified: 18 Feb 2026 20:44:56 UTC
Export Generated On: 05 Oct 2026 11:21:26 UTC

DONNERSTAG, 12.2.2026

PCR amplification was performed for both proteins of the NLSsplitGFP system.
As templates the gene synthesis for both proteins was used. Both templates were used in the original 10 ng/µl concentration and a 1:5 dilution.
For protein A I2601 was used as fwd primer and I2602 was used as reverse primer, while for protein B I2603 and I2604 were used.

Q5 PCR Mastermix

	A	1x [µl]	3.5x [µl]
1	dd H20	15	52.5
2	Q5 Buffer	5	17.5
3	5mM dNTPs	1	3.5
4	Q5	1	3.5
5	Primer fwd	1	3.5
6	Primer rev	1	3.5

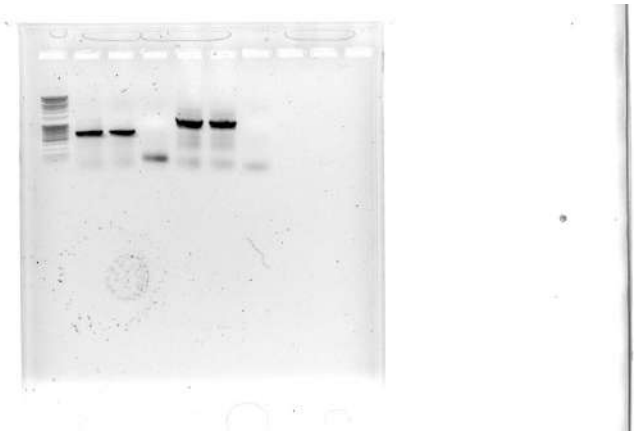
PCR programm

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	95°C	95°C	56°C	72°C	72°C	4°C
4	03:00	00:10	00:15	00:45	02:00	endless

Gel loading scheme

	A	B	C	D	E	F	G
1	100 bp ext. ladder	A	A 1:5	A -	B	B 1:5	B -

IGEM_NLSsplitGFP_PCR_12.02.26.png



PCR_35S_Ruby_Bar Backbone

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 12 Feb 2026 22:53:43 UTC
Entry Last Modified: 12 Feb 2026 23:22:21 UTC
Export Generated On: 05 Oct 2026 11:21:36 UTC

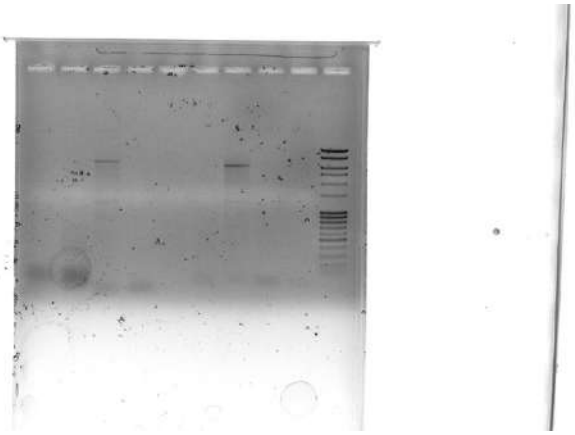
DONNERSTAG, 12.2.2026

Q5 PCR PCR reaction setup

	A	B	C
1		x1[µl]	x11[µl]
2	dH20	14,5	159,5
3	Q5 buffer	5	55
4	5 mM dNTPs	1	11
5	Q5 Pol	1	11
6	primer for	1,25	
7	Primer rev	1,25	
8	Template	1	

Templaten concentration was diluted 1:10.
PCR Programm and Primer combination are the same as 11.02.2026

IGEM_35S_backbone_PCR_12.02.26.png



Loading scheme from left to right: F1, F1-, F2, F2-, F3, F3-, F1 opt, F1 opt-, F2 opt, 100 bp ruler
Succesful amplification for F2 and F1 opt

PCR lexA

Project: iGEM Bonn 2026 Shared Project
Author: Friedrich Nitschke
Status: In progress
Entry Created On: 13 Feb 2026 18:27:27 UTC
Entry Last Modified: 13 Feb 2026 19:02:32 UTC
Export Generated On: 05 Oct 2026 11:21:45 UTC

FREITAG, 13.2.2026

Q5 PCR Reaction Mix2 (ddH20, Q5 Buffer, dNTPs, primer, Q5 pol via Mastermix for 2 reactions)

	A	1x [µl]	2x [µl]
1	dd H20	14.5	29
2	Q5 Buffer	5	10
3	5mM dNTPs	1	2
4	template	1	
5	Q5	1	2
6	Primer for	1.25	2.5
7	Primer rev	1.25	2.5

Reactions2

	fwd_primer	rev_primer	template	D
1	I2607	I2608	758_SP-PPR56_nad4_46_in_pPGX8	
2	I2607	I2608		neg. ctrl.

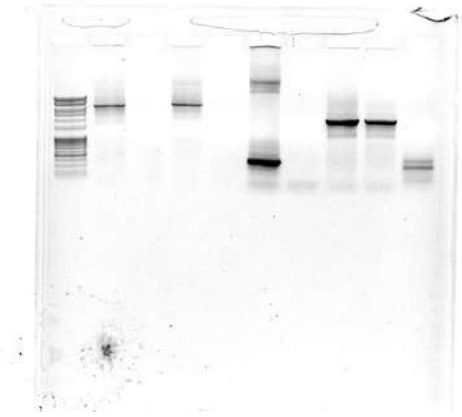
PCR programm2

	A	B	C	D	E	F
1	1 cycle	45 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	95°C	95°C	64 °C	72°C	72°C	4°C
4	05:00	20	20	00:20	02:00	endless

Gel loading scheme

	A	B	C	D	E	F	G	H	I	J
1	100 bp ext. ladder	35S_F1	35S_F1 -	35S_F3	35S_F3 -	lexA	lexA -	TEV	TEV 1:5	TEV -

IGEM_PCRs_13.02.26.png



PCR XVE

Project: iGEM Bonn 2026 Shared Project
Author: Friedrich Nitschke
Status: In progress
Entry Created On: 13 Feb 2026 18:31:46 UTC
Entry Last Modified: 23 Feb 2026 16:17:35 UTC
Export Generated On: 05 Oct 2026 11:21:54 UTC

FREITAG, 13.2.2026

Q5 PCR Reaction Mix2 (ddH20, Q5 Buffer, dNTPs, primer, Q5 pol via Mastermix for 2 reactions)

	A	1x [µl]	2x [µl]
1	dd H20	14.5	29
2	Q5 Buffer	5	10
3	5mM dNTPs	1	2
4	template	1	
5	Q5	1	2
6	Primer for	1.25	2.5
7	Primer rev	1.25	2.5

Reactions2

	fwd_primer	rev_primer	template	D
1	I2609	I2610	758_SP-PPR56_nad4_46_in_pPGX8	
2	I2609	I2610		neg. ctrl.

PCR programm2

	A	B	C	D	E	F
1	1 cycle	45 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	95°C	95°C	58 °C	72°C	72°C	4°C
4	05:00	20	20	02:30	02:00	endless

image.png



Lane 1 100bp marker, lane 2 pcr xve, lane 3 pcr xve neg. ctrl.

PCR TEV protease

Project: iGEM Bonn 2026 Shared Project

Author: Julius Krupke

Status: In progress

Entry Created On: 13 Feb 2026 18:32:27 UTC

Entry Last Modified: 13 Feb 2026 19:02:35 UTC

Export Generated On: 05 Oct 2026 11:22:03 UTC

FREITAG, 13.2.2026

As templates the 63.4 ng/µl concentrated pRK793 plasmid, as well as a 1:5 dilution of the plasmid were used.

Q5 PCR Mastermix

	A	1x [µl]	3.5x [µl]
1	dd H20	15	52.5
2	Q5 Buffer	5	17.5
3	5mM dNTPs	1	3.5
4	Q5	1	3.5
5	Primer I2009	1	3.5
6	Primer I2010	1	3.5

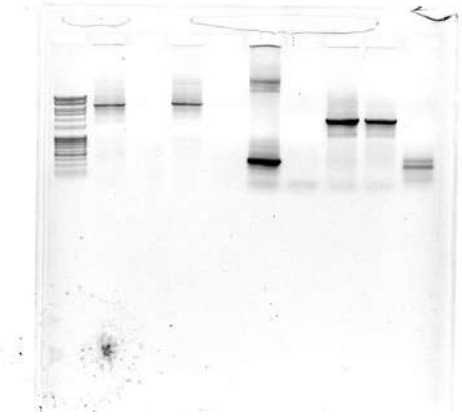
PCR programm

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	95°C	95°C	58 °C	72°C	72°C	4°C
4	05:00	0:10	0:20	1:15	5:00	endless

Gel loading scheme

	A	B	C	D	E	F	G	H	I	J
1	100 bp ext. ladder	35S_F1	35S_F1 -	35S_F3	35S_F3 -	lexA	lexA -	TEV	TEV 1:5	TEV -

IGEM_PCRs_13.02.26.png



PCR clean up, assembly and E. coli transformation

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 17 Feb 2026 16:27:49 UTC
Entry Last Modified: 08 Mar 2026 09:58:52 UTC
Export Generated On: 05 Oct 2026 11:20:59 UTC

DIENSTAG, 17.2.2026

PCR clean up

PCR clean up was performed using the Macherey-Nagel NucleoSpin PCR clean-up kit. The kits manual was followed for all steps of the clean up and all reccomended optional steps were performed.

Nanodrop measurement of the clean up

	A	B	C	D
1	Sample	Concentration (ng/µl)	A260/280	A260/230
2	Backbone fragment 1	7,4	3,02	0,30
3	Backbone fragment 2	15,7	1,70	0,37
4	Backbone fragment 3	14,7	1,57	1,05
5	Backbone fragment 1 without terminator	19,0	1,75	0,69
6	Backbone fragment 3 without promotor	16,6	1,78	0,34
7	lexA	65,8	1,92	1,63
8	XVE	50,4	1,86	0,84
9	TEV	64,0	1,96	2,01
10	NLSsplitGFP protein A	64,9	1,90	2,11
11	NLSsplitGFP protein B	128,8	1,80	1,75

Assembly

From the fragments the plasmids 35S_NLSsplitGFP_A_BAR, 35S_NLSsplitGFP_B_BAR and 35S_lexA_TEV_XVE_BAR were assembled. In a 1.5 ml reaction tube 5 µl of 2x NEBuilder mix was prepared. To the mix an equal molecular amount of all fragments of the final plasmid were added. For the 35S_NLSsplitGFP_A_BAR plasmid 1.5 µl each of backbone fragments 1 - 3 and 0.5 µl of the NLSsplitGFP protein A gel elution were used. For the 35S_NLSsplitGFP_B_BAR plasmid 1.5 µl each of backbone fragments 1 - 3 and 0.5 µl of the NLSsplitGFP protein B gel elution were used. For the 35S_lexA_TEV_XVE_BAR plasmid 1.2 µl each of backbone fragment 1 without terminator, backbone fragment 2 and backbone fragment 3 without promor, 0.6 µl of XVE, 0.5 µl of TEV and 0.3 µl of the lexA gel elution were used.

The samples were incubated at 50°C for 1 hour and immediately after the incubation transformed into E. coli.

E. coli transformation

Three aliquots of chemically competent XL1-blue *E. coli* were thawed on ice. Once thawed the assembled plasmids were added and the bacteria were incubated on ice for 15 minutes. After incubation the bacteria were heat shocked at 42°C for 90 seconds. Following the heat shock the bacteria were put back on ice and incubated for another 5 minutes. Afterwards 850 µl of SOC medium preheated to 37°C were added and the bacteria were icubated at 37°C, 850 rpm for 1 hour to express the antibiotics resistance. After incubation the cells were pelleted by centrifugation at 3000x g for 5 minutes and 850 µl of the supernatant was discarded. The pellet was dissolved in the remaining supernatant and plated on a LB (spectinomicin 100 ng/µl) plate. The bacteria were grown overnight at 37°C.

Sequential Double Digest

Introduction

This is the Sequential Double Digest Protocol with Standard Restriction Enzymes. If there is no buffer in which the two enzymes exhibit > 50% activity, this sequential digest can be performed.

More information from NEB can be found here.

Double Digests can be designed using NEB's Double Digest Finder.

NEBcloner will help guide your reaction buffer selection when setting up double digests.

See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.

Materials

- DNA **1 µg**
- NEBuffer
  - 1X
- NEB Restriction Enzymes
- Deionized Water

Procedure

Sequential Double Digest

1. Set up the following reaction using the restriction endonuclease that has the lowest salt concentration in its recommended buffer (total reaction volume **50 µl**).
2. Set up the following digest reaction on ice

Table1

	A	B	C
1		Volume (µl)	
2	**Buffer (10x)**	5	
3	**DNA ***	Input Volume for ng	
4	**Restriction Enzyme #1 ****	1	
5	**Deionized Water (µl)**	1	
6	**Total Volume (µl)**	#VALUE!	

*A 50 µl reaction volume is recommended for digestion of 1 µg of substrate.

** Restriction Enzyme, 10 units is sufficient, generally **1 µl** is used

***The enzyme should be the last component added to reaction

3. Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube.

4. Quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.

5. Incubate for 1 hour at the enzyme-specific appropriate temperature.

01:00:00

Can be decreased to 5-15 minutes by using a Time-Saver™ Qualified Restriction Enzyme
See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.

6. Adjust the salt concentration of the reaction (using a small volume of a concentrated salt solution) to approximate the reaction conditions of the second restriction endonuclease.

   Alternatively, a spin column can be used to isolate the DNA prior to the second reaction.

7. Add the second enzyme.

   ** Restriction Enzyme #2, 10 units is sufficient, generally **1 µl** is used

8. Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube.

9. Quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.

10. Incubate for 1 hour at the enzyme-specific appropriate temperature.

    Can be decreased to 5-15 minutes by using a Time-Saver™ Qualified Restriction Enzyme
    See the NEBuffer Activity/Performance Chart with Restriction Enzymes for the incubation temperatures.

Colony PCR 35S_NLSsplitGFP A_BAR; 35S_NLSsplitGFP_B_BAR; 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 18 Feb 2026 20:06:30 UTC
Entry Last Modified: 18 Feb 2026 21:03:18 UTC
Export Generated On: 05 Oct 2026 11:22:12 UTC

MITTWOCH, 18.2.2026

All plates showed succesfull growth of transformed e.coli
12 colonies per Plate were picked added onto an master plate and into 10 yl sterile dH20
1 yl of sterile H20 inoculated with e.coli was added as template
11 samples per plate plus one negative. 36 PCR samples total

Q5 colony PCR setup

	A	B	C
1		1x [µl]	13x [µl]
2	dH20	5.1	66.3
3	Q5 Buffer	2	26
4	5 mM dNTPs	0.4	5.2
5	Primer for	1	13
6	Primer rev	1	13
7	Q5 Pol	0.1	1.3
8	Template	1	

PCR programm

	A	B	C	D	E	F
1	1 cycle	45 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	55 °C	72 °C	72 °C	4 °C
4	05:00	00:30	00:15	02:30	02:00	endless

Primers

	Plasmid	Primer
1	35S_NLSsplit GFP A_BAR	oSNM 41 and I2602
2	35S_NLSsplit GFP_B_BAR	oSNM 41 and I2604
3	35S_lexA_TEV_XVE_BAR	oSNM 41 and I2608

Colony PCR 35S_NLSsplitGFP A_BAR; 35S_NLSsplitGFP_B_BAR; 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 19 Feb 2026 16:25:59 UTC
Entry Last Modified: 01 Apr 2026 14:24:04 UTC
Export Generated On: 05 Oct 2026 11:22:21 UTC

DONNERSTAG, 19.2.2026

6 colonies were picked per plate from 17.02.20206. Each picked colonie was inoculated on a fresh masterplate and in 10 yl sterile dH20.
For positive control 1 yl of PCR Clean up for each targeted insert was used as template.

Q5 Colony PCR setup

	A	B	C
1		1x [µl]	8.5 [µl]
2	C	5.8	49.3
3	Q5 Buffer	2.0	17
4	5 mM dNTPs	0.4	3.4
5	Primer for	0.5	4.25
6	Primer rev	0.5	4.25
7	Q5 Pol	0.4	3.4
8	Template	1	

Primers

	Sample	Primers	Targeted Insert
1	35S_NLSsplit GFP A_BAR	I2601 and I2602	Split GFP_A
2	35S_NLSsplit GFP_B_BAR	I2603 and I2604	Split GFP_B
3	35S_lexA_TEV_XVE_BAR	I2609 and I2610	TEV
4	35S_lexA_TEV_XVE_BAR	I2609 and I2610	XVE
5	35S_lexA_TEV_XVE_BAR	I2607 and I2608	lexA

PCR programm for Split GFP_A ,Split GFP_B, TEV, XVE

	A	B	C	D	E	F
1	1 cycle	40 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	58 °C	72 °C	72 °C	4 °C
4	05:00	00:30	00:15	02:30	02:00	endless

PCR programm for lexA

	A	B	C	D	E	F
1	1 cycle	40 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	64 °C	72 °C	72 °C	4 °C
4	05:00	00:30	00:15	00:20	02:00	endless

2nd colony PCR 35S_NLSsplitGFP A_BAR; 35S_NLSsplitGFP_B_BAR; 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 21 Feb 2026 23:55:40 UTC
Entry Last Modified: 22 Mar 2026 15:35:16 UTC
Export Generated On: 05 Oct 2026 11:22:30 UTC

DONNERSTAG, 19.2.2026

11 colonies were picked per plate from 17.02.20206.
For positive controls 1 µl each of plasmid used for insert amplification were used as template.

Q5 Colony PCR setup

	A	B	C
1		1x [µl]	8.5x [µl]
2	dH20	6.4	49.3
3	Q5 Buffer	2.0	17
4	5 mM dNTPs	0.4	3.4
5	10 µM Primer for	0.4	4.25
6	10 µM Primer rev	0.4	4.25
7	Q5 Polymerase	0.4	3.4

Primers

	Sample	Primers	Targeted Insert
1	35S_NLSsplit GFP A_BAR	I2601 and I2602	Split GFP_A
2	35S_NLSsplit GFP_B_BAR	I2603 and I2604	Split GFP_B
3	35S_lexA_TEV_XVE_BAR	I2609 and I2610	TEV

PCR programm

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	58 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	01:20	02:00	endless

3rd colony PCR 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project

Author: Julius Krupke

Status: In progress

Entry Created On: 22 Feb 2026 00:05:05 UTC

Entry Last Modified: 13 Mar 2026 16:58:18 UTC

Export Generated On: 05 Oct 2026 11:22:38 UTC

SONNTAG, 22.2.2026

36 colonies were picked from plate from 17.02.20206. The colonies were numbered 9 - 44, following the 8 colonies picked for the last colony PCR.

As template for the positive controle 1 µl of the pRK793 plasmid was used.

Q5 Colony PCR setup

	A	B	C
1		1x [µl]	8.5 [µl]
2	dH20	6.4	49.3
3	Q5 Buffer	2.0	17
4	5 mM dNTPs	0.4	3.4
5	10 mM Primer I2609	0.4	4.25
6	10 mM Primer I2610	0.4	4.25
7	Q5 Polymerase	0.4	3.4

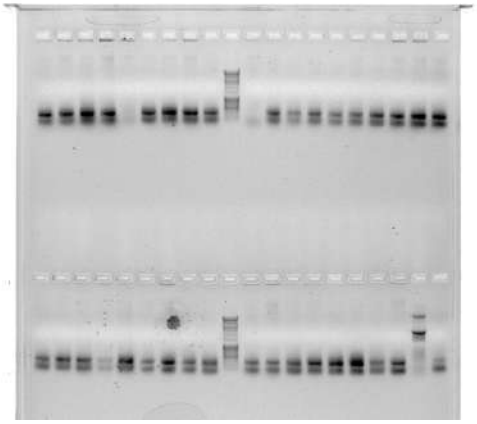
PCR programm

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	58 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	01:20	02:00	endless

Gel loading scheme

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	9	10	11	12	13	14	15	16	17	Marker	18	19	20	21	22	23	24	25	26	27
2	28	29	30	31	32	33	34	35	36	Marker	37	38	39	40	41	42	43	44	+	-

IGEM_Colony_PCR_21.02.26.png



Plasmid extraction 35S_NLSsplitGFP_A_BAR; 35S_NLSsplitGFP_B_BAR; 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 22 Feb 2026 00:14:47 UTC
Entry Last Modified: 20 Mar 2026 09:05:52 UTC
Export Generated On: 05 Oct 2026 11:21:08 UTC

FREITAG, 20.2.2026

A 5 ml dYT (spectinomicin 100 ng/µl) liquid culture each was inoculated with colony 1 from the 35S_NLSsplitGFP_A_BAR plate, colony 1 from the 35S_NLSsplitGFP_B_BAR plate and colony 9 from the 35S_lexA_TEV_XVE_BAR plate.
The cultures were grown over night at 37 °C, 188 rpm.

SAMSTAG, 21.2.2026

The cells grown in the 5 ml liquid culture were pelleted by centrifugation at 3000 xg for 10 minutes. The supernatant was discarded and 200 µl of BibDo1 buffer with 0.5% RNAseA freshly added was pipetted onto the pellet. The pellet was resuspended in the BibDo1 buffer by vortexing and transferred into a 1.5 ml reaction tube. After incubation of 10 minutes at RT 400 µl of BibDo2 buffer was added, the tube was inverted 10 times and incubated on ice for 5 minutes. After incubation 300 µl of BibDo3 buffer was added and the tube was inverted another 10 times before incubation on ice for 10 minutes. Afterwards the cell debris was pelleted by centrifugation at 13000 xg for 10 minutes. After centrifugation the supernatant was transferred into a new 1.5 ml reaction tube, filled to 1.5 ml with cold isopropanol and incubated at -20°C for 30 minutes. After incubation the Plasmid DNA was pelleted by centrifugation at 13000 xg and 4°C for 20 minutes. The resulting pellet was washed twice with 70% ethanol and resuspended in 50 µl deionized water.

Bibdo1 buffer

	Chemical	Concentration
1	Tris	25 mM
2	EDTA	10 mM
3	Glucose	50 mM

Bibdo2 buffer

	Chemical	Concentration
1	NaOH	0.2 M
2	SDS	1 %

Bibdo3 buffer

	Chemical	Concentration
1	potassium acetate	3 M
2	acetic acid	11.5 %

plasmid_a_b_TSV_cloning_2nd_round

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 23 Feb 2026 18:24:25 UTC
Entry Last Modified: 03 Mar 2026 12:13:20 UTC
Export Generated On: 05 Oct 2026 11:21:16 UTC

MONTAG, 23.2.2026

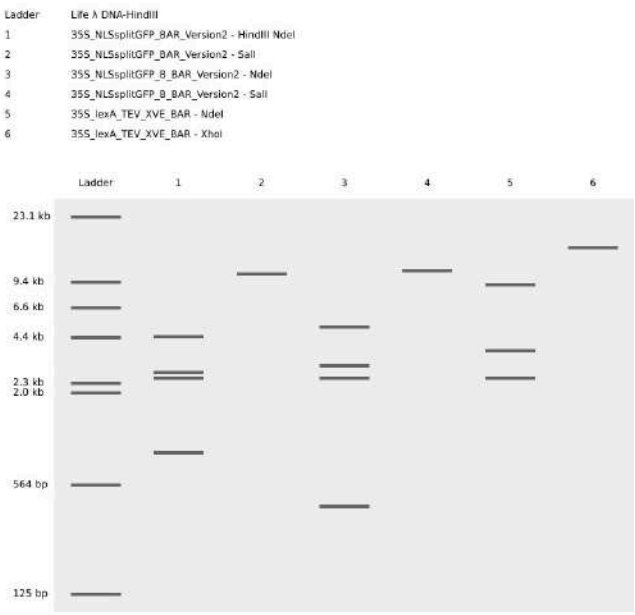
DIENSTAG, 24.2.2026

second try to digest

PCR clean up, assembly and E. coli transformation

these pasmids after the MP where used. first digest (not documented on benchling) was not good. Therefor we digested again.

virtual_digest_sequence_35S_lexA_TEV_XVE_BAR.png



see image on cloud (restriction_test_plasmid_a_b_tav_assembly_2026_02_24)
left is lamda hind III ladder and right ladder is 100 bp
No plasmid is usable.

This Protocoll was used to make a second try for a assembly.

Remeber, that **the insert have to be äquimolar**, and therefor if the conc. changes (e.g new PCR clean up), the volumen need to be calculated again.
As the Fragment where almost empty, we used another batch. Therefor the concentration changes and the volume.

Table 1: given amout used in assembly

	A	B	C	D	E	F
1	**GFP_A_BAR**		**B_BAR**		**lexA_TEV**	
2	Molecule	Volume	Molecule	Volume	Molecule	Volume
3	2 x NEB	5 µl	2 x NEB	5 µl	2 x NEB	5 µl
4	backbone 1-3	1.5 µl (each)	backbone 1-3	1.5 µl (each)	backbone 2	1.2 µl
5	NLSsplitGFP protein A	0.5 µl	NLSsplitGFP protein B	0.5 µl	backbone 1 w/o terminator	1.2 µl
6					backbone 3 w/o promotor	1.2 µl
7					XVE	0.6 µl
8					lexA	0.3 µl
9					TEV	0.5

PCR product was not cleaned up, but frozen away in PCR tubes. See in the could (fragments_backup) to use as a backup.
putification over gel (cutting out) is needed.
size is 3.3 kbp, all 3 fragments
Julius gave another, cleaned up PCR product, so we continues using this.
The Fragments where adjusted to 10 ng/µl as working solution.
Assembly and transpormation where performed accoring to the protocoll.

Table2: new Fragments 1-3 given by Julius 1

	A	B	C	D	E
1	**Fragments**	**conc**	**A260**	**A280**	**A260/A280**
2	Fragment 1	76,5	0,592	0,3	1,92
3	Fragment 2	146	2,921	1,8	1,58
4	Fragment 3	29,6	1,529	0,799	1,91

DONNERSTAG, 26.2.2026

no colonies on the plates.
Therefor the assembly and the transformation will be performed again. (same protocoll again)

2 µl where used for the transformation.

FREITAG, 27.2.2026

There are again no colonies. maybe the PCR product is too much contamined witch salts ? see Table2 A260/A280 ratios (the new fragments that julius gave to us), but these where 1:10 dialuted, so this should not be a issue ? maybe it is ?

MONTAG, 2.3.2026

The first assembly was tested again for plasmid A and plasmid B. Their inserts should be inside due to a positive colony PCR. Fragments 1 and 2 of the backbone include the antiobiotica resistance gene

35S_NLSsplitGFP_A_BAR_Assembly_details.dna (Archived)

Therefore we wanted to test for fragement 3 abundance:

Q5 PCR Mastermix

	A	1x [µl]	3.5x [µl]
1	dd H20	14.5	50.75
2	Q5 Buffer	5	17.5
3	5mM dNTPs	1	3.5
4	1 Assembly plasmid a/ b	1	
5	Q5	1	3.5
6	oSNM41	1.25	4.375
7	AP04	1.25	4.375

PCR programm

	A	B	C	D	E	F
1	1 cycle	45 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	95°C	95°C	58°C	72°C	72°C	4°C
4	05:00	00:15	00:30	02:00	02:00	endless

Gel loading scheme

	A	B	C	D	E	F	G
1	100 bp ext. ladder	assembly plasmid a test	assembly plasmid b test	neg. ctrl without template			

image.png



PCR for assembly confirmation for 35S_NLSsplitGFP_A_BAR and 35S_NLSsplitGFP_B_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 03 Mar 2026 21:11:48 UTC
Entry Last Modified: 03 Mar 2026 21:41:57 UTC
Export Generated On: 05 Oct 2026 11:21:46 UTC

DIENSTAG, 3.3.2026

Since the restriction digest yielded unexpected results a PCR was run for the backbone fragment 3, in order to confirm the presence or absence of this fragment in the assembled plasmid.

Backbone fragments 1 and 2 both contain half of the spectinomicin resistance and can thus be assumed to be contained in the plasmid. The presence of the inserts has been verified during the colony PCR.

PCR was performed using both the undiluted plasmid extraction and a 1:10 dilution as templates.
The expected amplicon size was 3.3 kBp.

.

PCR mix

	A	B	C
1		1x [µl]	5.5x [µl]
2	dH20	15	52.5
3	Q5 Buffer	5	27.5
4	5 mM dNTPs	1	5.5
5	10 µM Primer for	1	5.5
6	10 µM Primer rev	1	5.5
7	Q5 Polymerase	1	5.5

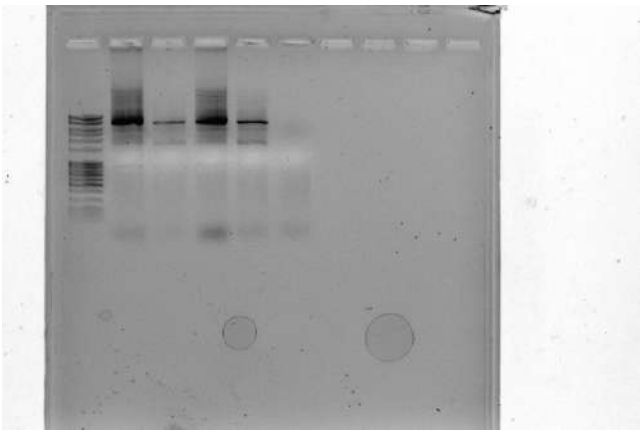
PCR programm

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	55°C	72°C	72°C	4°C
4	02:00	00:15	00:30	02:00	05:00	endless

Gel loading scheme

	A	B	C	D	E	F
1	100 bp ext. ladder	A	A 1:10	B	B 1:10	-

IGEM_PCR_fragment_3_03.03.2026.png



Untitled

Project: iGEM Bonn 2026 Shared Project

Author: Ruben Langenbacher

Status: In progress

Entry Created On: 08 Mar 2026 10:10:57 UTC

Entry Last Modified: 08 Mar 2026 10:12:02 UTC

Export Generated On: 05 Oct 2026 11:23:07 UTC

SONNTAG, 8.3.2026

Ligation Protocol WITH T4 DNA Ligase (M0202)

Introduction

Please see the NEB website for supporting information on this protocol.

Materials

- 10X T4 DNA Ligase Reaction Buffer
- T4 DNA Ligase
- Vector DNA (4kb)
- Insert DNA (1kb)
- Nuclease-free water

Procedure

Set up the T4 DNA Ligase Reaction

Note: T4 DNA Ligase should be added last. The table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes. *Use NEB calculator to calculate molar ratios.*

1. Thaw the T4 DNA Ligase Buffer and resuspend at room temperature.

   Tip: Alicuote the 10x buffer less concentrated so when thawing, the DTT gets soluble more easily.

2. Set up the following reaction in a microcentrifuge tube on ice:

Table1

	A	B
1	**Component**	**Volume (µl)**
2	10X T4 DNA Ligase Buffer	2
3	Vector DNA: 50 ng (0.020 pmol)	
4	Insert DNA: 37.5 ng (0.060 pmol)	
5	Nuclease-free water	17
6	T4 DNA Ligase	1
7	Total	20

3. Gently mix the reaction by pipetting up and down and microfuge briefly.

4. For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes. For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours.

5. Heat inactivate at 65 degrees C for 10 minutes.

00:10:00

6. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.

   Use 25 uL DH5α cells, and add 2 uL of reaction mixture.

35S_lexA_TEV_XVE_BAR, Plasmid Sequencing Analysis 2026-03-08

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 08 Mar 2026 10:31:19 UTC
Entry Last Modified: 08 Mar 2026 10:44:46 UTC
Export Generated On: 05 Oct 2026 11:21:25 UTC

SONNTAG, 8.3.2026

-35S_lexA_TEV_XVE_BAR (35S_lexA_TEV_XVE_BAR1.fasta), utg000001c LN:i:7820 RC:i:671 XC:f:1.000000 (35S_lexA_TEV_XVE_BAR.assembly.fasta) shows incomplete and highly variable Fragnment 1, no lexA Fragment, TEV Fragment, XVE, Fragmend and a incomplete Fragment 3 no promotor.
-utg000001c LN:i:7970 RC:i:382 XC:f:1.000000 (35S_NLSsplitGFP_B_BAR.assembly.fasta) (35S_NLSsplitGFP_B_BAR) shows incomplete and highly variable Fragnment 1 and NLSsplitGFP_B.
-utg000001c LN:i:7924 RC:i:393 XC:f:1.000000 (35S_NLSsplitGFP_A_BAR.assembly1.fasta) (35S_NLSsplitGFP_BAR) shows incomplete and highly variabke Fragment 1 and NLSsplitGFP_A is missing.
-PCR for Fragment 1 should be repeated.

Insert verification PCR for 35S_NLSsplitGFP_A_BAR and 35S_NLSsplitGFP_B_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 09 Mar 2026 16:00:52 UTC
Entry Last Modified: 11 Mar 2026 20:35:00 UTC
Export Generated On: 05 Oct 2026 11:21:57 UTC

MONTAG, 9.3.2026

PCR was performed to verifiy if the presence of the insert in these plasmids.
The primers used were BP12 and oSNM04.
Expected amplicon sizes were 1.6 kB for 35S_NLSsplitGFP_A_BAR and 2 kB for 35S_NLSsplitGFP_BAR.
As template the plasmids extracted on 20.02.2026 were used as well as their 1:10 dilutions.

PCR setup

	A	B	C
1		1x [µl]	5.5x [µl]
2	dH20	15	82.5
3	Q5 Buffer	5	27.5
4	5 mM dNTPs	1	5.5
5	10 µM Primer for	1	5.5
6	10 µM Primer rev	1	5.5
7	Q5 Polymerase	1	5.5

PCR programm

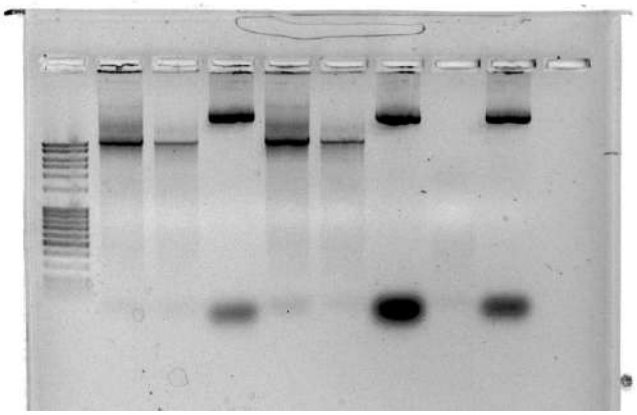
	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	55 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	02:00	02:00	endless

3 µl of each of the plasmid extractions were also loaded on the gel.

Gel loading scheme

	A	B	C	D	E	F	G	H	I
1	100 bp ext. ladder	A	A 1:10	A extraction	B	B 1:10	B extraction	-	TEV extraction

IGEM_Insert_PCR_9.3.26.png



Due to the unexpected band sizes the PCR was repeated using 1:50 and 1:100 dilutions of the plasmid as templates.

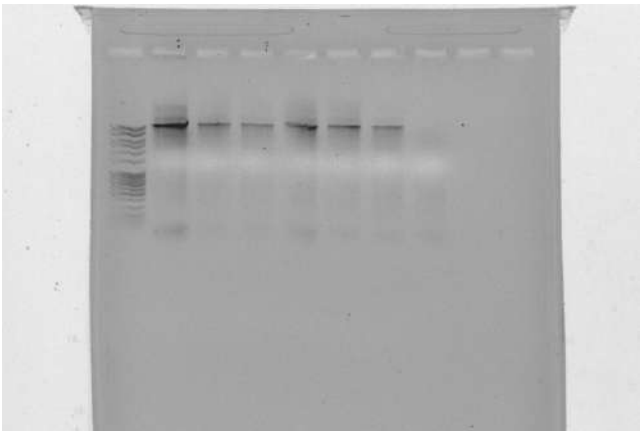
MITTWOCH, 11.3.2026

The PCR was repeated with the new plasmid extractions performed on 11.03.2026 as templates.

Gel loading scheme 2

	A	B	C	D	E	F	G	H	I
1	100 bp ext. ladder	A	A 1:10	A 1:50	B	B 1:10	B 1:50	-	

IGEM_insert_pcr_11.03.26.png



Copy of 35S_lexA_TEV_XVE_BAR, Plasmid Sequencing Analysis 2026-03-08

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 11 Mar 2026 15:02:03 UTC
Entry Last Modified: 11 Mar 2026 15:02:03 UTC
Export Generated On: 05 Oct 2026 11:21:35 UTC

SONNTAG, 8.3.2026

-35S_lexA_TEV_XVE_BAR (35S_lexA_TEV_XVE_BAR1.fasta), utg000001c LN:i:7820 RC:i:671 XC:f:1.000000 (35S_lexA_TEV_XVE_BAR.assembly.fasta) shows incomplete and highly variable Fragnment 1, no lexA Fragment, TEV Fragment, XVE, Fragmend and a incomplete Fragment 3 no promotor.
-utg000001c LN:i:7970 RC:i:382 XC:f:1.000000 (35S_NLSsplitGFP_B_BAR.assembly.fasta) (35S_NLSsplitGFP_B_BAR) shows incomplete and highly variable Fragnment 1 and NLSsplitGFP_B.
-utg000001c LN:i:7924 RC:i:393 XC:f:1.000000 (35S_NLSsplitGFP_A_BAR.assembly1.fasta) (35S_NLSsplitGFP_BAR) shows incomplete and highly variabke Fragment 1 and NLSsplitGFP_A is missing.
-PCR for Fragment 1 should be repeated.

3rd colony PCR for 35S_NLSsplitGFP_A_BAR and 35S_NLSsplitGFP_B_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 13 Mar 2026 16:48:07 UTC
Entry Last Modified: 13 Apr 2026 14:03:48 UTC
Export Generated On: 05 Oct 2026 11:22:09 UTC

DONNERSTAG, 12.3.2026

Colony PCR was performed for 20 new colonies of each plate (numbered 9 - 28).
For each colony 2 different primer combinations were used.
BP12 and oSNM04 flanking the insert, binding on the backbone and the gene specific primers (I2601 and I2602 for 35S_NLSsplitGFP_A_BAR and I2603 and I2604 for 35S_NLSsplitGFP_B_BAR) binding on the insert.
As positive controls the gene synthesis for NLSsplitGFP_A and NLSsplitGFP_B were supposed to be used but did not get added to the reaction tubes by accident.

Q5 Colony PCR setup

	A	B	C	D	E
1		1x [µl]	22.5x [µl]	41.5x [µl]	
2	dH20	6.4	144	265.6	
3	Q5 Buffer	2.0	45	83	
4	5 mM dNTPs	0.4	9	16.6	
5	10 µM Primer for	0.4	9	16.6	
6	10 µM Primer rev	0.4	9	16.6	
7	Q5 Polymerase	0.4	9	16.6	

PCR programm for primers BP12 and oSNM04

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	53 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	01:15	02:00	endless

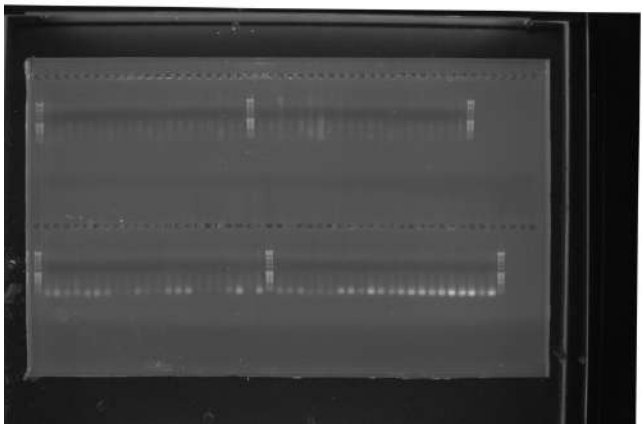
PCR programm for primers I2601, I2602, I2603 and I2604

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	62 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	00:45	02:00	endless

A = 35S_NLSsplitGFP_A_BAR
B = 35S_NLSsplitGFP_B_BAR
row 1 = flanking primers
row 2 = gene specific primers



IGEM_colony_pcr_12.03.26.png



FREITAG, 13.3.2026

The colony PCR was repeated, but this time only the primers flanking the insert were used.
As positive control a different plasmid with the same backbone carrying a 800 bp insert was used.
Also a new formulation of pre coloured Q5 buffer was used.
The reaction volume was scaled up to 25 µl.
Colonies 9 - 17 from yesterday were picked again as well as 9 new colonies (29 - 38) each.

Q5 Colony PCR setup 13.03.2026

	A	B	C
1		1x [µl]	38.5x [µl]
2	dH20	16	616
3	Q5 Buffer	5	192.5
4	5 mM dNTPs	1	38.5
5	10 µM Primer for	1	38.5
6	10 µM Primer rev	1	38.5
7	Q5 Polymerase	1	38.5

PCR programm

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	53 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	01:30	02:00	endless

Gel loading scheme1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	100 bp ext.	A9	A10	A11	A12	A13	A14	A15	A16	A17	A29	A30	A31	A32	A33	A34	A35	A36	A37	+ control
2	100 bp ext.	B9	B10	B11	B12	B13	B14	B15	B16	B17	B29	B30	B31	B32	B33	B34	B35	B36	B37	- control

PCRs for second assembly of 35S_NLSsplitGFP_A_BAR, 35S_NLSsplitGFP_B_BAR and 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 16 Mar 2026 12:46:51 UTC
Entry Last Modified: 17 Mar 2026 17:09:59 UTC
Export Generated On: 05 Oct 2026 11:22:21 UTC

MONTAG, 16.3.2026

PCR was performed for all 10 fragments needed for assembly of the plasmids.

Amplified fragments

	Fragment	Primers	Template
1	35S_BAR backbone fragment 1	JK20 & oSNM38	35S_RUBY_BAR
2	35S_BAR backbone fragment 2	oSNM39 & oSNM40	35S_RUBY_BAR
3	35S_BAR backbone fragment 3	oSNM41 & AP04	35S_RUBY_BAR
4	35S_BAR backbone fragment 1 without terminator	oSNM38 & I2605	35S_RUBY_BAR
5	35S_BAR backbone fragment 3 without promotor	oSNM41 & I2606	35S_RUBY_BAR
6	NLSsplitGFP_A	I2601 & I2602	Gene synthesis
7	NLSsplitGFP_B	I2603 & I2604	Gene synthesis
8	lexA	I2607 & I2608	pMSR858
9	TEV	I2609 & I2610	pRK793
10	XVE	I2611 & I2612	pMSR858

PCR mix setup

	Substance	1x [µl]	2.5x [µl]
1	dH20	15	37.5
2	Q5 Buffer	5	12.5
3	5 mM dNTPs	1	2.5
4	10 mM Primer for	1	2.5
5	10 mM Primer rev	1	2.5
6	Q5 Polymerase	1	2.5

PCR programm backbone fragments

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	54 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	02:00	02:00	endless

PCR programm NLSsplitGFP_A, NLSsplitGFP_B & lexA

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	59 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	00:45	02:00	endless

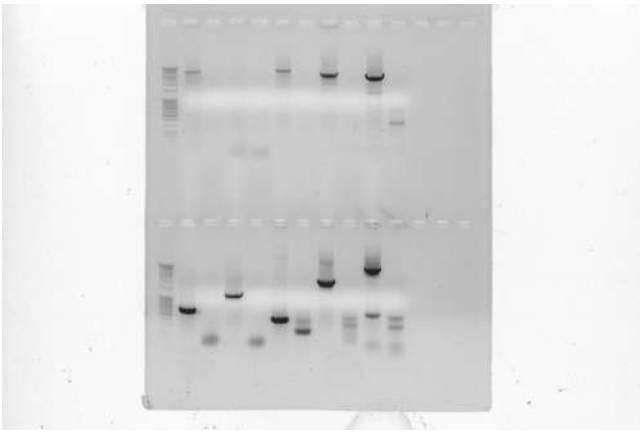
PCR programm TEV & XVE

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	60 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	01:45	02:00	endless

Gel loading scheme

	A	B	C	D	E	F	G	H	I	J	K
1	100 bp ext. ladder	35S_BAR backbone fragment 1	35S_BAR backbone fragment 1 -	35S_BAR backbone fragment 2	35S_BAR backbone fragment 2 -	35S_BAR backbone fragment 3	35S_BAR backbone fragment 3 -	35S_BAR backbone fragment 1 without terminator	35S_BAR backbone fragment 1 without terminator -	35S_BAR backbone fragment 3 without promotor	35S_BAR backbone fragment 3 without promotor -
2	100 bp ext. ladder	NLSsplitGFP_A	NLSsplitGFP_A -	NLSsplitGFP_B	NLSsplitGFP_B -	lexA	lexA -	TEV	TEV -	XVE	XVE -

IGEM_PCRs_16.03.26.png



Fragment 2 PCR 35S_Ruby_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 17 Mar 2026 18:41:05 UTC
Entry Last Modified: 17 Mar 2026 18:54:05 UTC
Export Generated On: 05 Oct 2026 11:22:32 UTC

DIENSTAG, 17.3.2026

Q5 PCR setup

	A	1x [µl]	5.5x [µl]
1	ddH20	15	82.5
2	dNTPs	1	5.5
3	osnM39 (1:10)	1	5.5
4	osnM40 (1:10)	1	5.5
5	Q5	1	5.5
6	Q5 Buffer 1:5	5	27.5
7	Template (35S_Ruby_BAR)	1	

PCR setup

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	elongation	elongation	hold
3	98 °C	98 °C	51°C, 52,4 °C, 56.5 °C, 58 °C	72 °C	72 °C	4 °C
4	2 min	00:15 min	00:30 min	2 min	5 min	hold
5						

Samples were run with diffrent annealing temperatures S1 51 °C, S2 52,4 °C, S3 56,5 °C, S4 58 °C, negative 51 °C.

Gel elution & assembly and transformation of 35S_NLSsplitGFP_A_BAR, 35S_NLSsplitGFP_B_BAR and 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 18 Mar 2026 17:13:58 UTC
Entry Last Modified: 18 Mar 2026 17:38:58 UTC
Export Generated On: 05 Oct 2026 11:21:03 UTC

MITTWOCH, 18.3.2026

Gel elution

Gel elution was performed for the PCR amplicons generated on 17.03.2026 and 18.03.2026.
Gel elution was performed using the Macherey-Nagel NucleoSpin PCR clean-up kit.
The kit's manual was followed for every step of the extraction and every optional step was performed.

Assembly

Utilising the extracted DNA fragments the plasmids 35S_NLSsplitGFP_A_BAR, 35S_NLSsplitGFP_B_BAR and 35S_lexA_TEV_XVE_BAR were assembled.
For the assembly of 35S_NLSsplitGFP_A_BAR the fragments 35S_BAR Backbone fragment 1, 35S_BAR Backbone fragment 2, 35S_BAR Backbone fragment 3 and NLSsplitGFP_A were used.

Assembly mix for 35S_NLSsplitGFP_A_BAR

	Reagent	Volue [µl]
1	2x NEBuilder master mix	5
2	35S_BAR Backbone fragment 1	1.5
3	35S_BAR Backbone fragment 2	1.5
4	35S_BAR Backbone fragment 3	1.5
5	NLSsplitGFP_A	0.5

For the assembly of 35S_NLSsplitGFP_B_BAR the fragments 35S_BAR Backbone fragment 1, 35S_BAR Backbone fragment 2, 35S_BAR Backbone fragment 3 and NLSsplitGFP_B were used.

Assembly mix for 35S_NLSsplitGFP_B_BAR

	Reagent	Volue [µl]
1	2x NEBuilder master mix	5
2	35S_BAR Backbone fragment 1	1.5
3	35S_BAR Backbone fragment 2	1.5
4	35S_BAR Backbone fragment 3	1.5
5	NLSsplitGFP_B	0.5

For the assembly of 35S_NLSsplitGFP_B_BAR the fragments 35S_BAR Backbone fragment 1 without terinator, 35S_BAR Backbone fragment 2, 35S_BAR Backbone fragment 3 without promotor, lexA, TEV and XVE were used.

Assembly mix for 35S_lexA_TEV_XVE_BAR

	Reagent	Volue [µl]
1	2x NEBuilder master mix	5
2	35S_BAR Backbone fragment 1 without terminator	1
3	35S_BAR Backbone fragment 2	2
4	35S_BAR Backbone fragment 3 without promotoror	1
5	lexA	0.1
6	TEV	0.5
7	XVE	0.4

The assemblys were incubated at 50 °C for 1 hour and immediately after incubation transformed into *E. coli*

Transformation

The assembled plasmids were each added to an aliquot of chemically competent XL1-Blue *E. coli* cells and incubated on ice for 15 minutes. After incubation, the cells were heat shocked for 90 seconds at 42 °C. After the heat shock, the cells were put back on ice for 5 minutes of regeneration. After 5 minutes, 850 µl of SOC medium, preheated to 37 °C, was added to the cells, and the cells were incubated at 37 °C, 850 rpm for 1 hour to express the antibiotic resistance. After incubation, the cells were plated on an LB (spectinomycin 100 ng/ml) plate and grown overnight at 37 °C.

Plasmid extraction 35S_NLSsplitGFP_A_BAR; 35S_NLSsplitGFP_B_BAR; 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 19 Mar 2026 17:05:55 UTC
Entry Last Modified: 24 Mar 2026 20:46:43 UTC
Export Generated On: 05 Oct 2026 11:21:14 UTC

DONNERSTAG, 19.3.2026

2 ml dYT (spectinomycin 100 ng/ml) cultures were inoculated with the colonies verified by colony PCR.
For 35S_NLSsplitGFP_A_BAR colonies 1 and 2 were picked; for 35S_NLSsplitGFP_B_BAR 3 and 4; and for 35S_lexA_TEV_XVE_BAR 2 and 3.
The cultures were grown overnight at 37 °C, 188 rpm.

FREITAG, 20.3.2026

Plasmid isolation

Single colonies were grown overnight in 2 mL selective medium (with the appropriate antibiotic) at 37 °C with shaking. The culture was transferred into a 1.5 mL tube and centrifuged at 3500 × g for 2 min to pellet the cells. The supernatant was discarded and the pellet resuspended in 100 µL MP Solution 1.

Next, 200 µL Buffer 2 was added and mixed gently by inverting the tube a few times (no vortexing!). At this step, care was taken to avoid harsh mixing, as genomic DNA is very fragile and can shear easily, which would lead to contamination. The lysate was incubated on ice for max. 2 min.

After that, 150 µL Buffer 3 was added and the tube was again gently inverted to mix. Optionally, a few drops of chloroform can be added here to improve purity, but too much can reduce yield. The sample was then centrifuged at 16,000 × g for 10 min.

About 400 µL of the clear supernatant was transferred into a fresh, pre-cooled tube. The sample was placed in the freezer for 5–10 min to precipitate the DNA, followed by centrifugation at max speed for 15 min (as cold as possible).

The supernatant was removed, and the pellet washed with 70% ethanol. After removing the ethanol completely, the pellet was briefly air-dried.. Finally, the DNA was resuspended in 30 µL H_2O.

virtual_digest_sequence_35S_NLSsplitGFP_BAR_Version2.png

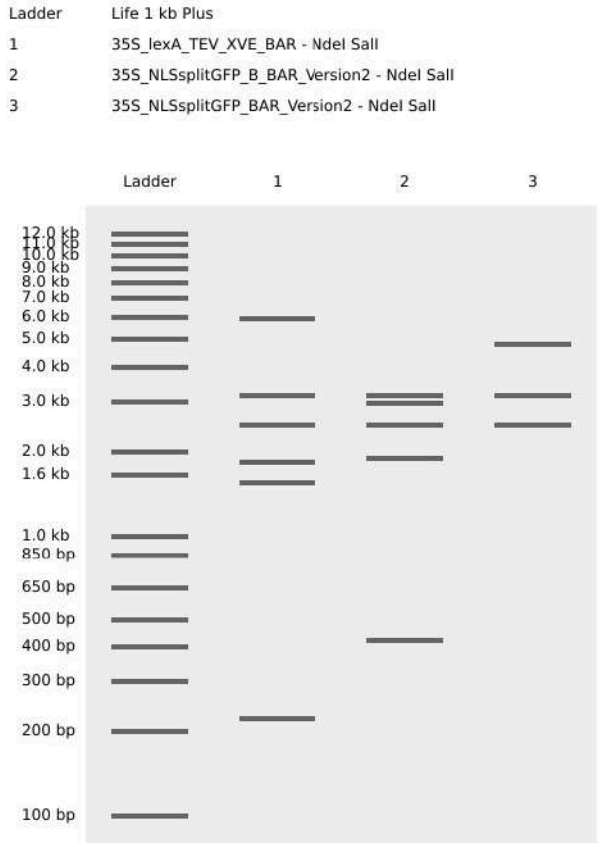
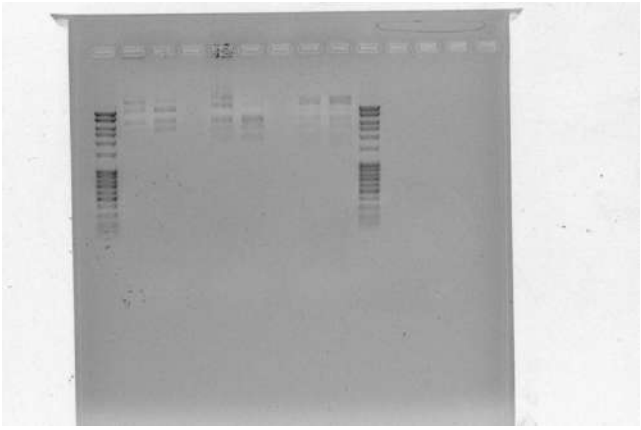


Table1

	A	B	C	D	E	F	G	H	I	J
1	100 bp ladder	GFP_A_C1	GFP_A_C2	empty	GFP_B_C4	GFP_B_C3	empty	TEV_C2	TEV_C31	100 bp ladder

20260320_test_digest_L_A1_A2_B24_B53_T2_T3_L.png



The upper bands are probably because of gDNA contamination.
The pellet was very big after spinning the bacteria down, therefor this was maybe the issue.
Next time if the pellet is big, the pellet will be resuspendet and alliq. into another Eppi. (alternatives: other cultivation method, e.g different media (LB), shorter incubation time, meassuring the OD, or taking less liquid culture volume)
While pippeting the MP sampels on the nanodrop, the liquid behaved like a sample wich is not "watery" (idk how to discribe more scientific). It was too viscosy.
Bands for A and B look good tho.
Due to the fact that the digest in both clones in all constructs look the same, the MP next time will be performed for one clone each construct.

For the next MP round:

- 750 µl liquid culture instead of 1,5 ml
- 100 µl MPS1 (0,5 mg/ml RNAseA), stays the same
- 300 µl MPS2
- 250
- µl MPS3
- ratio stays same (important for neutralization)
- spin down for 30 minutes in the precipitation step

DIENSTAG, 24.3.2026

The liquid culture was grown overnight in 2 mL selective medium (with the appropriate antibiotic) at 37 °C while shaking.
750 µl of the culture was transferred into a 1.5 mL tube and centrifuged at 3500 × g for 2 min to pellet the cells.
The supernatant was discarded and the pellet resuspended in 100 µL MP Solution 1 (0,5 mg/ml) and rest for 10 minutes at RT.

Next, 300 µL Buffer 2 (pH = 12.3) was added and mixed gently by inverting the tube a few times (no vortexing!).

At this step, care was taken to avoid harsh mixing, as genomic DNA is very fragile and can shear easily, which would lead to contamination.
The lysate was incubated on ice for max. 2 min.

After that, 250 µL Buffer 3 (pH = 5.3) was added and the tube was again gently inverted to mix for a few times.
Optionally, a few drops of chloroform can be added here to improve purity, but too much can reduce yield.
The sample was then centrifuged at fullsped, at -9°C for 10 min.

About 400 µL of the clear supernatant was transferred into a fresh, pre-cooled tube.
1 ml 100%, **ice cold** EtOH was added to the supernatant and the sample was vortext.
spin down at fullspeed, at -9°C for 1h

The supernatant was removed, and the pellet washed with 70% ethanol.
After removing the ethanol completely (P20 might help), the pellet was briefly air-dried.
Finally, the DNA was resuspended in 20 µL H_2O and the concentration was measured at the nanodrop

Table2

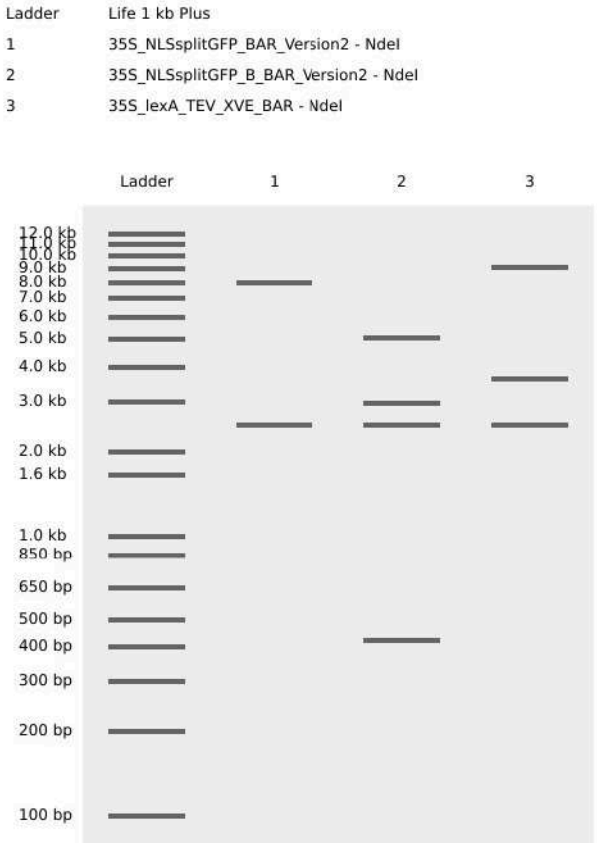
	A	B	C	D
1	sampel	conc (ng/µl)	260/280	260/280
2	A2	1454,6	2,02	2,51
3	B3	1309,6	1,95	2,54
4	TEV3	1727,6	1,96	2,27

A and B pippeted like water. TEV was again a little bit more viskosy.
Consider gDNA contamination for TEV.

analytic digest

All Plasmids where digested at 37°C for 1h with NdeI and subsequently seperated on a 0.8% agarose gel at 100 V for 1h

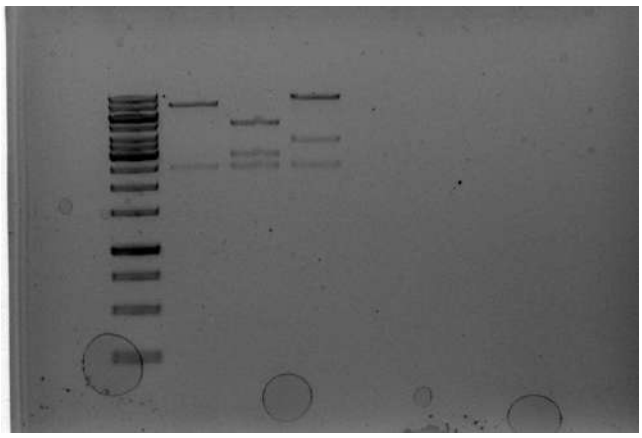
virtual_digest_sequence_35S_lexA_TEV_XVE_BAR.png



loading sheme after the analytic digest

	A	B	C	D
1	1kb ladder	GFP_A_C1	GFP_B_C3	TEV_C3

digest_A1_B3_T3_24032026.png



Colony PCR 35S_NLSsplitGFP_A_BAR; 35S_NLSsplzGFP_B_BAR; 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 22 Mar 2026 15:29:38 UTC
Entry Last Modified: 09 Apr 2026 19:29:25 UTC
Export Generated On: 05 Oct 2026 11:22:45 UTC

SONNTAG, 22.3.2026

12 colonies were picked for 35S_NLSsplitGFP_A_BAR
10 colonies were picked for 35S_NLSsplzGFP_B_BAR
12 colonies were picked for 35S_lexA_TEV_XVE_BAR

Q5 colony PCR setup

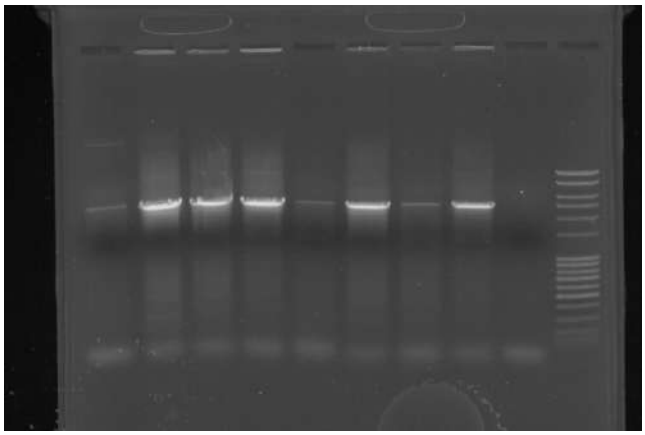
	A	1x [µl]	39,5x [µl]
1	dH20	16	632
2	Q5 Buffer	5	197,5
3	5 mM dNTPs	1	39.5
4	Q5 Pol	1	39.5
5	10 mM Primer for	1	
6	10 mM Primer rev	1	

Q5 Colony PCR setup for I2611 and osnM4

	A	B	C	D	E	F
1	1 cycle	40 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	72°C	72 °C	72 °C	4 °C
4	05:00	00:30	00:15	1 min	02:00	endless
5						

For 35S_lexA_TEV_XVE_BAR Primers I2611 and osnM4 were used.
For 35S_NLSsplitGFP_A_BAR and 35S_NLSsplitGFP_B_BAR Primers Bp12 and osnM4 were used.
For Bp12 a 1:10 dilution was prepared using 10 yl Primer 90 yl dH20.

Q5_PCR_lexA_TEV_23.03.2026.png



PCR succesfull. Expected size 2343 bp confirmed.

Q5 Colony PCR 35S_NLSsplitGFP_A_BAR; 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 08 Apr 2026 16:48:08 UTC
Entry Last Modified: 08 Apr 2026 16:58:02 UTC
Export Generated On: 05 Oct 2026 11:22:56 UTC

MITTWOCH, 8.4.2026

5 colonies were picked from 35S_NLSsplitGFP_A_BAR A.tumefaciens plate. Added to masterplate.
Primers I2607 and I2610 were used.

9 colonies were picked from 35S_lexA_TEV_XVE_BAR A.tumefaciens plate. Added to masterplate.
Primers Bp12 and oSNM4 were used.

Q5 Colony PCR setup

	A	B	C
1		1x [µl]	26x [µl]
2	dH20	16	416
3	Q5 Buffer	5	130
4	5 mM dNTPs	1	37,5
5	Primer for	1	
6	Primer rev	1	
7	Q5 Pol	1	26
8			

Q5 Colony PCR setup for Bp12 and oSNM4

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	53 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	01:15	02:00	endless
5						
6						

Q5 Colony PCR setup for I2607 and I2610

	A	B	C	D	E	F
1	1 cycle	45 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	71 °C	72 °C	72 °C	4 °C
4	05:00	00:30	00:15	01:10	02:00	endless
5						
6						

Electroporation of A.tumefaciens for 35S_NLSsplitGFP_A_BAR and 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 10 Apr 2026 14:45:01 UTC
Entry Last Modified: 17 Jul 2026 12:29:02 UTC
Export Generated On: 05 Oct 2026 11:21:16 UTC

FREITAG, 10.4.2026

-2 x 25 yl of Electrocompetent GV3101 pSoup BPL021 and 2 x 25 yl GV3101 A.tumefaciens were thawed on ice.
-1 yl of 35S_NLSsplitGFP_A_BAR or 35S_lexA_TEV_XVE_BAR Plasmid from miniprep were added.
-Cells+DNA were added into pre cooled electroporation cuvettes. Tapped to remove air bubbles and kept on ice for 10 min
-electroporation with 2.2 kV for 6 ms
-1 ml of roomtemperature YEP medium added to each sample immediatly after electroporation and transfered to 1.5 mL eppi
-Incubated for 2 h at 28 °C 1400 rpm
-cells pelleted by centrifuging 3500 g 8500 rpm r = 9.5 cm for 1 min. Removed supernatant, resuspended in return flow and plated on YEP+Rif+Gent+Spec plates.
-incubated at 28 °C
-succsfull growth on all plates except GV3101 pSoup BPL021 with 35S_lexA_TEV_XVE_BAR which was contaminated and therefore removed.

Q5 Colony PCR 35S_NLSsplitGFP_A_BAR; 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 13 Apr 2026 13:51:52 UTC
Entry Last Modified: 15 Apr 2026 18:17:11 UTC
Export Generated On: 05 Oct 2026 11:22:42 UTC

MONTAG, 13.4.2026

-plates from 2026-04-10 showed succesfull growth except for GV3101 pSoup with 35S_lexA_TEV_XVE_BAR which was contaminated.
-GV3101 35S_lexA_TEV_XVE_BAR and GV3101 35S_NLSsplitGFP_A_BAR were chosen for cPCR
-10 colonies were picke for each plate and a masterplate YEP+Rif+Gent+Spec for each plate was made.

Primers

	35S_NLSsplitGFP_A_BAR	35S_lexA_TEV_XVE_BAR
1	I2601 fwd	I2609 fwd
2	I2602 rev	I2610 rev
3		
4		

-expected amplicon size for 35S_lexA_TEV_XVE_BAR cPCR 1961 bp.
-expected amplicon size for 35S_NLSsplitGFP_A_BAR cPCR 627 bp.

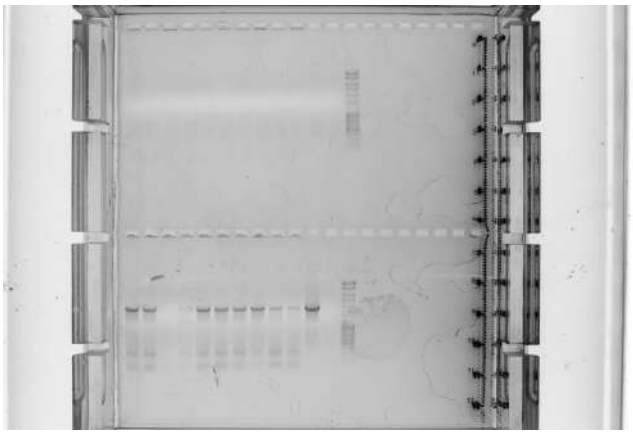
Q5 cPCR reagents

	A	B	C	D
1		1x [µl]	12.5x [µl]	
2	dH20	16	200	
3	Q5 Buffer	5	62.5	
4	5 mM dNTPs	1	12.5	
5	10 µM Primer for	1	12.5	
6	10 µM Primer rev	1	12.5	
7	Q5 Polymerase	1	12.5	

Q5 cPCR setup

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	72 °C	72 °C	72 °C	4 °C
4	05:00	00:30	00:30	1:10	02:00	endless

cPCR_2026-04-13_iGEM.png



-succesfull amplification for 35S_lexA_TEV_XVE_BAR. Amplicon size matches expected size of 1961 bp.

-no amplifacation for 35S_NLSsplitGFP_A_BAR not even for positive control indication wrong PCR setup.

Loading scheme

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A+	A-	100 bp	
2	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X+	A-	100 bp	

Q5 Colony PCR 35S_NLSsplitGFP_A_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 15 Apr 2026 18:14:55 UTC
Entry Last Modified: 15 Apr 2026 18:39:32 UTC
Export Generated On: 05 Oct 2026 11:22:53 UTC

MITTWOCH, 15.4.2026

-10 colonies from Masterplate 2026-04-13 GV3101 YEP+Rif+Gent+Spec were repicked.
-positive controll using 1 yl 35S_NLSsplitGFP_A_BAR Plasmid was used.
-Primers BP12 and oSNM04 were used. Expected amplicon size 1572 bp.

Q5 Colony PCR setup

	A	B	C
1		1x [µl]	12.5x [µl]
2	dH20	16	200
3	Q5 Buffer	5	62.5
4	5 mM dNTPs	1	12.5
5	10 µM Primer for	1	12.5
6	10 µM Primer rev	1	12.5
7	Q5 Polymerase	1	12.5

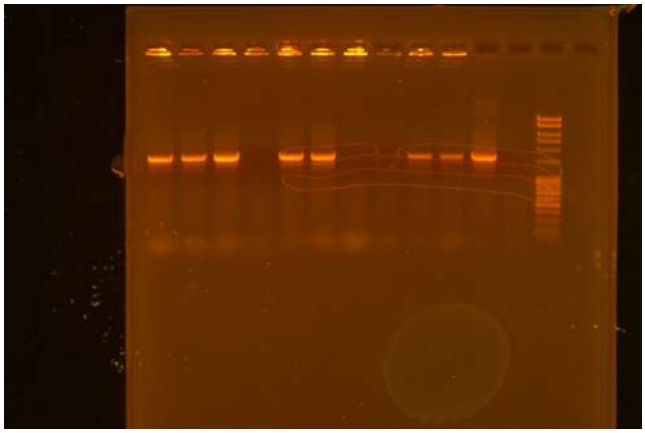
Table1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	55 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	02:00	02:00	endless

Loading scheme

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	pos control	neg control	100 bp ladder

cPCR_iGEM_2026-04-15.png



-succesfull cPCR for Colonies 1, 2, 3, 5, 6, 9 and 10.
-expected amplicon size matched.

Prepare YEP medium

Project: iGEM Bonn 2026 Shared Project
Author: Joana Heckmann
Status: In progress
Entry Created On: 17 Apr 2026 12:05:59 UTC
Entry Last Modified: 17 Apr 2026 12:14:40 UTC
Export Generated On: 05 Oct 2026 11:21:25 UTC

FREITAG, 17.4.2026

Prepared 1L of liquid YEP medium as following:
Fill bottle with less than 1L of deionized water
add while stirring:
10 g yeast extract
10 g peptone
5 g NaCl
fill up with deionized water to 1L mark

10 x PBS Buffer

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 17 Apr 2026 14:45:57 UTC
Entry Last Modified: 17 Apr 2026 14:56:05 UTC
Export Generated On: 05 Oct 2026 11:21:33 UTC

FREITAG, 17.4.2026

For 500 ml 10 x PBS:

Table1: Recepie for 500 ml PBS Buffer

	A	B	C	D
1	Chemical	Molarity	Mass	
2	NaCl	1.37 M	40 g	
3	KCl	27 mM	1 g	
4	Na_2HPO_4	100 mM	7.1 g	
5	K_2HPO_4	20mM	1,74 g	

Note: pH = 7.4

Use 400 ml dH_20 to dissolve the chemicals according to Tab. 1.
If the chemicals do not dissolve, try to heat the buffer on the heating stirrer.
Adjust the pH = 7.4 (NaOH or HCL). **Only measure and adjust pH when the buffer has RT !!!!!! (always!)**

Glycerol-Stocks of A.tumefaciens with 35S_NLSsplitGFP_A_BAR / B and 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Friedrich Nitschke
Status: In progress
Entry Created On: 17 Apr 2026 15:32:11 UTC
Entry Last Modified: 22 Apr 2026 16:24:30 UTC
Export Generated On: 05 Oct 2026 11:21:43 UTC

FREITAG, 17.4.2026

The Electroporation of A.tumefaciens for 35S_NLSsplitGFP_A_BAR and 35S_lexA_TEV_XVE_BAR (10.04.2026) resulted in colonies, which off for plasmid A colony one and for plasmid lexA colony one were verified by colony PCR (Q5 Colony PCR 35S_NLSsplitGFP_A_BAR).

Plasmid B was transformed into A. tumefaciens here (03.04.2026):

Electroporation of A.tumefaciens for 35S_NLSsplitGFP_A_BAR, 35S_lexA_TEV_XVE_BAR and 35S_NLSsplitGFP_B_BAR

The colony PCR verfied colony three and four ()

Glycerol stocks of were created off

- A.tumefaciens 35S_NLSsplitGFP_A_BAR colony 1
- A.tumefaciens 35S_lexA_TEV_XVE_BAR colony 1
- A.tumefaciens 35S_NLSsplitGFP_B_BAR colony 3 and 4

9 µL of each antibiotica (Rifampicin 100mg/mL, Gentanycin 25 mg/mL, Kanamycin 50 mg/mL) were added to 9 mL YEP media. 2 mL were aliquoted and inolated. Incubation at 28 °C for 48 hours.

MONTAG, 20.4.2026

- Liquid cultures showed no growth. Probably beacause Kanamycin is the the wrong antibiotik.
- 9 µL of each antibiotica (Rifampicin 100mg/mL, Gentanycin 25 mg/mL, Spectinomycin 30 mg/mL) were added to 9 mL YEP media. 3 ML were aliquoted and Inolated. Incubation at 28 °C for 48 hours.

MITTWOCH, 22.4.2026

Liquid Cultures showed succesfull growth.

Centrifuged 3500 rpm for 10 min. Discarded overflow. Resuspended pellet in return flow.

Added 500 yl of A.tumefaciens solution to 1.5 ml safelock eppi. Added 500 yl 50% Glycerol solution.

Frozen in Liquid Nitrogen and stored at -70 °C.

Electroporation of A.tumefaciens for 35S_NLSsplitGFP_A_BAR, 35S_lexA_TEV_XVE_BAR and 35S_NLSsplitGFP_B_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 17 Apr 2026 15:41:29 UTC
Entry Last Modified: 26 Aug 2026 13:04:16 UTC
Export Generated On: 05 Oct 2026 11:21:53 UTC

FREITAG, 3.4.2026

-3 x 25 yl of Electrocompetent BPL021 GV3101 A.tumefaciens were thawed on ice.
-1 yl of 35S_NLSsplitGFP_A_BAR or 35S_lexA_TEV_XVE_BAR or 35S_NLSsplitGFP_B_BAR Plasmid from miniprep were added.
-Cells+DNA were added into pre cooled electroporation cuvettes. Tapped to remove air bubbles and kept on ice for 10 min
-electroporation with 2.2 kV for 6 ms
-1 ml of roomtemperature YEP medium added to each sample immediatly after electroporation and transfered to 1.5 mL eppi

-Incubated for 2 h at 28 °C 1400 rpm
-cells pelleted by centrifuging 3500 g 8500 rpm r = 9.5 cm for 1 min. Removed supernatant, resuspended in return flow and plated on YEP+Rif+Gent+Spec plates.
-incubated at 28 °C

Colony PCR 35S_NLSsplitGFP_A_BAR; 35S_NLSsplzGFP_B_BAR; 35S_lexA_TEV_XVE_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Melda Bakir
Status: In progress
Entry Created On: 22 Apr 2026 14:15:49 UTC
Entry Last Modified: 22 Apr 2026 14:48:11 UTC
Export Generated On: 05 Oct 2026 11:23:04 UTC

FREITAG, 10.4.2026

Colony PCR was performed using A. tumefaciens colonies transformed using the 35S_NLSsplitGFP_A_BAR, 35S_NLSsplzGFP_B_BAR and 35S_lexA_TEV_XVE_BAR constructs.

1. **Colony PCR of 35S_lexA_TEV_XVE_BAR**
   Primers: I2611 and oSNM04
   PCR mix can be found in Tab. 1
   PCR program can be found in Tab. 2
   A total of 5 colonies were picked from the plate. dH2O was used as a negative control, isolated plasmid encoding for the construct was used as a positive control

Tab. 1: PCR mix for colony PCR of 35S_lexA_TEV_XVE_BAR

	Component	1x Mix [uL]	15x Mix [uL]
1	dH2O	16	240
2	Q5 buffer	5	75
3	dNTPs (5 mM)	1	15
4	Primer I2611 (10 mM)	1	15
5	Primer oSNM04 (10 mM)	1	15
6	Q5 polymerase	1	15
7			

Tab. 2: PCR program of colony PCR for 35S_lexA_TEV_XVE_BAR

	Step	Time	Temperature [°C]	D
1	Initial denaturation	5 min	98	
2	Denaturation	30 s	98	)
3	Annealing	15 s	72	) 30 repeats
4	Elongation	1 min	72	)
5	Final extension	2 min	72	
6	Hold	Unlimited	12	

2. **Colony PCR of 35S_NLSsplitGFP_A_BAR and 35S_NLSsplzGFP_B_BAR**
   Primers: Bp12 and oSNM04
   PCR mix can be found in Tab. 3 (the same one was used for both constructs)
   PCR program can be found in Tab. 4 (the same one was used for both constructs)
   A total of 9 colonies were picked from the plate for the 35S_NLSsplitGFP_A_BAR construct. 10 colonies were picked from the plate for the 35S_NLSsplitGFP_B_BAR construct. dH2O was used as a negative control, isolated plasmid encoding for the construct was used as a positive control

Tab. 3: PCR mix for colony PCR of 35S_NLSsplitGFP_A_BAR and 35S_NLSsplitGFP_B_BAR

	Component	1x Mix [uL]	15x Mix [uL]
1	dH2O	16	240
2	Q5 buffer	5	75
3	dNTPs (5 mM)	1	15
4	Primer Bp12 (10 mM)	1	15
5	Primer oSNM04 (10 mM)	1	15
6	Q5 polymerase	1	15
7			

Tab. 4: PCR program of colony PCR for 35S_NLSsplitGFP_A_BAR and 35S_NLSsplitGFP_B_BAR

	Step	Time	Temperature [°C]	D
1	Initial denaturation	5 min	98	
2	Denaturation	15 s	98	)
3	Annealing	30 s	53	) 30 repeats
4	Elongation	1:30 min	72	)
5	Final extension	2 min	72	
6	Hold	Unlimited	12	

Gel image:

Will be inserted as soon as the gel image is downloaded from the cloud

For all PCRs, the positive control shows a band at the expected size, whereas the negative control shows no band, therefore indicating no contamination. Moreover, for the samples of the colonies transformed using 35S_NLSsplitGFP_A_BAR and 35S_lexA_TEV_XVE_BAR, no bands are visible, which suggests that no positive clones were picked. For the colonies transformed using 35S_NLSsplitGFP_B_BAR, bands at the expected size are visible for two samples, which indicates the presence of the construct in the respective colonies. Hence, the colonies were picked for further use.

fixation of plant tissue

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 24 Apr 2026 06:51:50 UTC

Entry Last Modified: 24 Apr 2026 06:51:54 UTC

Export Generated On: 05 Oct 2026 11:22:02 UTC

FREITAG, 24.4.2026

260424_leaf_infiltration

Project: iGEM Bonn 2026 Shared Project

Author: dmoellmann

Status: In progress

Entry Created On: 25 Apr 2026 13:59:57 UTC

Entry Last Modified: 25 Apr 2026 14:08:34 UTC

Export Generated On: 05 Oct 2026 11:22:11 UTC

SAMSTAG, 25.4.2026

Header

Table1

	A	B
1	**Field**	**Value**
2	Date	24.4.2026
3	Experimenter	Melda, Daniel
4	Experiment ID	AGRO-001
5	Project	iGEM Bonn 2026
6	Objective	Transiently co-express NLS-tagged split GFP halves and a lexA-TEV-XVE construct in N. benthamiana leaves via agroinfiltration

Plant Material

Table2

	A	B
1	**Parameter**	**Value**
2	Species	Nicotiana benthamiana
3	Line / Ecotype	Wild-type (borrowed from external lab)
4	Age at infiltration	~4–6 weeks (estimated; sowing date unknown)
5	Growth conditions	16 h day
6	Leaves infiltrated	Marked by sample line (1, 2, 3); leaf number/position not recorded
7	Number of plants	2

⚠️ Plant age is an estimate; exact developmental stage was not confirmed. Future experiments should record leaf stage (true-leaf number) at time of infiltration.

Bacterial Strains & Constructs

Table3

	A	B	C	D	E
1	**Label**	**Construct**	**Agrobacterium strain**	**Selection**	**Purpose**
2	A	35S_NLSsplit GFP_A_BAR	GV3101	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin	NLS-tagged split GFP fragment A
3	B	35S_NLSplitGFP_B_BAR*	GV3101	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin	NLS-tagged split GFP fragment B
4	X	35S_lexA_TEV_XVE_BAR	GV3101	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin	Estradiol-inducible lexA-TEV-XVE driver

Liquid Culture (Day Prior — 2026-04-23)

Table4

	A	B	C	D	E	F
1	**Sample**	**Construct**	**Medium**	**Volume**	**Temp / Shaking**	**Antibiotics**
2	A1, A2, A3	35S_NLSsplit GFP_A_BAR	YEP	3 mL each	—	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin
3	B1, B2, B3	35S_NLSplitGFP_B_BAR	YEP	3 mL each	—	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin
4	X1, X2, X3	35S_lexA_TEV_XVE_BAR	YEP	3 mL each	—	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin

Note: Antibiotic concentrations for spectinomycin and gentamicin not recorded — add to future entries.

OD_{600} Measurements & Resuspension Calculation

Cultures were diluted **1:10** prior to measurement. Actual OD_{600} = measured value × 10. Pellet was resuspended in **10 mL** infiltration medium. Calculated final OD_{600} = (OD_actual × 3 mL) / 10 mL.

Table5

	A	B	C	D	E
1	**Sample**	**OD_{600} (measured, 1:10 dil.)**	**OD_{600} (actual)**	**OD_{600} (calc. after resuspension)**	**In target range (0.1–0.5)?**
2	A1	0.155	1.55	0.465	✓
3	A2	0.126	1.26	0.378	✓
4	A3	0.136	1.36	0.408	✓
5	B1	0.160	1.60	0.480	✓
6	B2	0.145	1.45	0.435	✓
7	B3	0.096	0.96	0.288	✓
8	X1	0.212	2.12	0.636	⚠️ above range
9	X2	0.148	1.48	0.444	✓
10	X3	0.138	1.38	0.414	✓

⚠️ Sample X1 calculated OD_{600} = 0.636, exceeding the 0.5 upper target. Final OD was not verified by re-measurement; this may have resulted in a higher bacterial density in sample line 1 mix.

Pellet Preparation Procedure

Table6

	A	B
1	**Step**	**Detail**
2	1. Centrifugation	3,000 × g, 10 min, RT
3	2. Wash	Supernatant discarded; pellet resuspended in infiltration medium (volume not recorded)
4	3. Re-pelleting	3,000 × g, 10 min, RT
5	4. Resuspension	Pellet resuspended in 10 mL infiltration medium per sample
6	5. OD_{600} verification	Not measured — final OD_{600} calculated from pre-pellet values (see table above)

Induction Conditions

Table7

	A	B
1	**Parameter**	**Value**
2	Infiltration medium	Standard (10 mM MES pH 5.6, 10 mM $MgCl_2$)
3	Acetosyringone	200 µM
4	Induction temperature	Room temperature
5	Induction in dark	Yes
6	Induction duration	— (not recorded)

Infiltration Mix (per sample line)

Each line was prepared by combining equal volumes of constructs A, B, and X from the corresponding replicate number.

Table8

	A	B	C
1	**Mix**	**Constructs combined**	**Leaf label**
2	Line 1	A1 + B1 + X1	Leaf marked "1"
3	Line 2	A2 + B2 + X2	Leaf marked "2"
4	Line 3	A3 + B3 + X3	Leaf marked "3"

Mix ratio A : B : X = 1 : 1 : 1 (equal volumes) Infiltration method: needleless syringe, abaxial surface, ca. 1 ml per leaf

260427_estrogen_treatment

Project: iGEM Bonn 2026 Shared Project
Author: dmoellmann
Status: In progress
Entry Created On: 27 Apr 2026 14:55:14 UTC
Entry Last Modified: 27 Apr 2026 15:00:36 UTC
Export Generated On: 05 Oct 2026 11:22:21 UTC

MONTAG, 27.4.2026

β-Estradiol Induction of XVE-Driven Constructs in Agroinfiltrated *N. benthamiana*

Header

Table1

	A	B
1	**Field**	**Value**
2	Date	27.4.2026
3	Experimenter	Daniel
4	Experiment ID	CHEM-IND-001
5	Project	Aurone biosynthesis / NbCHI silencing — thesis
6	Linked AGRO entry	260424_leaf_infiltration
7	Objective	Induce XVE-driven transgene expression in transiently agroinfiltrated N. benthamiana leaves by abaxial paintbrush application of β-estradiol (100 µM)

Plant Material at Time of Induction

Table2

	A	B
1	**Parameter**	**Value**
2	Species	Nicotiana benthamiana
3	Line	Wild-type
4	Age at induction	approx. 4 weeks
5	Days post-infiltration (dpi)	3
6	Number of plants treated	2
7	Leaves treated per plant	All infiltrated leaves (left half of each leaf)
8	Growth conditions at induction	16 h day

Constructs Present in Infiltrated Tissue

(Inherited from linked AGRO entry)

Table3

	A	B
1	**Agrobacterium strain**	**Construct**
2	GV3101	35S_NLSsplit GFP_A_BAR
3	GV3101	35S_NLSplitGFP_B_BAR
4	GV3101	35S_lexA_TEV_XVE_BAR

Induction Agent

Table4

	A	B
1	**Parameter**	**Value**
2	Compound	β-estradiol
3	Working solution concentration	100 µM
4	Solvent	0.1% (v/v) ethanol in dH_2O
5	Stock concentration	100 mM in 100% ethanol
6	Stock preparation date	27.04.2026
7	Working solution prepared	Same day as induction
8	Approximate volume used	250 µl

Induction Procedure

Table5

	A	B
1	**Step**	**Detail**
2	1. Working solution prep	100 µM β-estradiol in dH_2O (1:1000 dilution from 100 mM EtOH stock); prepared fresh
3	2. Application method	Soft paintbrush, hand-dipped in 100 µM solution
4	3. Leaf surface treated	Abaxial (lower) surface — left half of each infiltrated leaf only
5	4. Coverage	Single coat per application pass
6	5. Post-treatment	Leaves allowed to air-dry; plants returned to growth conditions
7	6. Mock control	None included in this experiment

Spatial Design

Table6

	A	B
1	**Leaf zone**	**Treatment**
2	Left half (abaxial)	100 μM β-estradiol — induced
3	Right half (abaxial)	No treatment — uninduced within-leaf reference

260509_estrogen_treatment

Project: iGEM Bonn 2026 Shared Project

Author: Julius Krupke

Status: In progress

Entry Created On: 09 May 2026 16:57:44 UTC

Entry Last Modified: 09 May 2026 17:03:14 UTC

Export Generated On: 05 Oct 2026 11:22:31 UTC

SAMSTAG, 9.5.2026

β-Estradiol Induction of XVE-Driven Constructs in Agroinfiltrated *N. benthamiana*

Header

Table1

	A	B
1	**Field**	**Value**
2	Date	09.05.2026
3	Experimenter	Julius
4	Project	IGEM 2026
5	Linked AGRO entry	missing
6	Objective	Induce XVE-driven transgene expression in transiently agroinfiltrated N. benthamiana leaves by abaxial paintbrush application of β-estradiol (100 µM)

Plant Material at Time of Induction

Table2

	A	B
1	**Parameter**	**Value**
2	Species	Nicotiana benthamiana
3	Line	Wild-type
4	Age at induction	approx. 4 weeks
5	Days post-infiltration (dpi)	1
6	Number of plants treated	3
7	Leaves treated per plant	All leaves infiltrated with all 3 plasmids (left half of each leaf)
8	Growth conditions at induction	16 h day

Constructs Present in Infiltrated Tissue

(Inherited from linked AGRO entry)

Table3

	A	B
1	**Agrobacterium strain**	**Construct**
2	GV3101	35S_NLSsplit GFP_A_BAR
3	GV3101	35S_NLSplitGFP_B_BAR
4	GV3101	35S_lexA_TEV_XVE_BAR

Induction Agent

Table4

	A	B
1	**Parameter**	**Value**
2	Compound	β-estradiol
3	Working solution concentration	100 µM
4	Solvent	0.1% (v/v) ethanol in dH_2O
5	Stock concentration	100 mM in 100% ethanol
6	Stock preparation date	09.05.2026
7	Working solution prepared	Same day as induction
8	Approximate volume used	150 µl

Induction Procedure

Table5

	A	B
1	**Step**	**Detail**
2	1. Working solution prep	100 µM β-estradiol in dH_2O (1:1000 dilution from 100 mM EtOH stock); prepared fresh
3	2. Application method	Soft paintbrush, hand-dipped in 100 µM solution
4	3. Leaf surface treated	Abaxial (lower) surface — left half of each infiltrated leaf only
5	4. Coverage	Single coat per application pass
6	5. Post-treatment	Leaves allowed to air-dry; plants returned to growth conditions
7	6. Mock control	None included in this experiment

Spatial Design

Table6

	A	B
1	**Leaf zone**	**Treatment**
2	Left half (abaxial)	100 μM β-estradiol — induced
3	Right half (abaxial)	No treatment — uninduced within-leaf reference

Untitled

Project: iGEM Bonn 2026 Shared Project

Author: Ruben Langenbacher

Status: In progress

Entry Created On: 11 May 2026 11:24:49 UTC

Entry Last Modified: 11 May 2026 11:24:49 UTC

Export Generated On: 05 Oct 2026 11:22:38 UTC

MONTAG, 11.5.2026

260518_inoculation_atumefaciens

Project: iGEM Bonn 2026 Shared Project
Author: dmoellmann
Status: In progress
Entry Created On: 18 May 2026 12:42:01 UTC
Entry Last Modified: 18 May 2026 12:45:41 UTC
Export Generated On: 05 Oct 2026 11:21:25 UTC

MONTAG, 18.5.2026

Table1

	A	B
1	**Field**	**Value**
2	Date	18.05.26
3	Experimenter	Daniel
4	Experiment ID	Incoulation 3
5	Project	GFP with Split NLS + TEV Protease

Inoculated *A. tumefaciens* GV3101 with Constructs A, B and TEVX for leaf infiltration the next day.

5 ml YEP medium
20 µg/ml Gentamycin
100 µg/ml Spectinomycin

incubation for 16-24 h at 28 ºC

Protocol for split eGFP cloning (do not use for now, this protocoll is in trial phase)

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 19 May 2026 10:26:27 UTC
Entry Last Modified: 29 May 2026 08:15:46 UTC
Export Generated On: 05 Oct 2026 11:23:23 UTC

DIENSTAG, 19.5.2026

eGFP_A:
https://benchling.com/igembonn2026/f/lib_TK020i1PMk-split_gfp/seq_mR3VZzEEnS-35s_egfp_a_bar/edit

To amplipy the eGFP_1_172 fragment + NLS use:
Template: NLS_split_B (https://benchling.com/igembonn2026/f/lib_ByZnmy8r40-split_nls/seq_XwMCt4DjHX-35s_nlssplitgfp_b_bar_version2/edit)
516 bp are expected

Tab.1: Q5 PCR setup (eGFP Insert amplification)

	A	1x [µl]
1	ddH20	15
2	dNTPs	1
3	I26026 (	1
4	I26036	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Tab.2: PCR programm for truncated N-eGFP

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	63 °C	72°C	72°C	4°C
4	05:00	00:15	00:20	00:15	02:00	endless

Amplified fragments1

	Fragment	Primers	Template
1	35S_BAR backbone fragment 1	I26025 & oSNM38	nlssplit_a
2	35S_BAR backbone fragment 2	oSNM39 & oSNM40	nlssplit_a
3	35S_BAR backbone fragment 3	oSNM41 & AP40	nlssplit_a

PCR programm backbone fragments

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	63 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	02:30	02:00	endless

for the EGFP_B construct:
https://benchling.com/igembonn2026/f/lib_TK020i1PMk-split_gfp/seq_mR3VZzEEnS-35s_egfp_a_bar/edit

To amplify the C-terminal fragment of the eGFP:
Template: NLS_split_B (https://benchling.com/igembonn2026/f/lib_ByZnmy8r40-split_nls/seq_XwMCt4DjHX-35s_nlssplitgfp_b_bar_version2/edit)

This generated the c-terminal truncation of the eGFP
241 bp are expected

Tab.1: Q5 PCR setup (eGFP Insert amplification)1

	A	1x [µl]
1	ddH20	15
2	dNTPs	1
3	I26028	1
4	I26029	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Tab.2: PCR programm for truncated N-eGFP1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	61 °C	72°C	72°C	4°C
4	05:00	00:15	00:20	00:15	02:00	endless

Amplified fragments

	Fragment	Primers	Template
1	35S_BAR backbone fragment 1	JK20 & oSNM38	nlssplit_b
2	35S_BAR backbone fragment 2	oSNM39 & oSNM40	nlssplit_b
3	35S_BAR backbone fragment 3	oSNM41 & I26052	nlssplit_b

PCR programm backbone fragments1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	55 °	72°C	72°C	4°C
4	05:00	00:15	00:30	02:30	02:00	endless

protocoll for split cre cloning (not done yet)

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 19 May 2026 12:03:57 UTC
Entry Last Modified: 19 May 2026 13:05:20 UTC
Export Generated On: 05 Oct 2026 11:22:49 UTC

DIENSTAG, 19.5.2026

This protocol aims to clone the split cre construct.

for the Cre_A construct:

https://benchling.com/igembonn2026/f/lib_yOBwNGcqxo-lsl_system/seq_uRsJxD4maG-35s_cre_nls_a_bar/edit

Template: Cre g-block
primers: **I26057** (+, w/ overhang, tm = 55.6°C) and **I26058** (-, w/ overhang, tm =55.3 °C)
This generates the N-truncated cre insert w/ overhangs (homologugus to F3 and the NLS-Intein)
174 **bp are expected**

Tab.1: Q5 PCR setup (eGFP Insert amplification)

	A	1x [µl]
1	ddH20	15
2	dNTPs	1
3	I26026 (1:10)	1
4	I26051 (1:10)	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplitgfp_b)	1

Consider upscaling the volume of the PCR, to ensure enough metrial is there for the downstream work. Tab.1 is for 25 µl. Do not more than 50 µl per reaction tube. If more then 50 µl total is wished, split it in mutiple tubes and pool prior purification (also check every sample on the gel, if the amplicon has the expected size)

Tab.2: PCR programm for truncated N-eGFP

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	56 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	00:15	02:00	endless

Subsequently open the Vector via PCR:
template: NLS_split_a (this ensures we have the cage, intein and NLS sequence already within the backbone. Subsequently, only the truncated N-terminal part (1-172) of the eGFP has to be inserted)
https://benchling.com/igembonn2026/f/lib_ByZnmy8r40-split_nls/seq_zeEisRYRJO-35s_nlssplitgfp_a_bar_version2/edit
primers: AP04 and I26050 (both 50°C annealing)
This opens the vector rv from the F3 and Fv from the NLS-NpuN_cage_F1
split GFP must be positioned there
The Fragments of the Backbone need to be amplified accrodingly.

Amplified fragments1

	Fragment	Primers	Template
1	35S_BAR backbone fragment 1	I26050 & oSNM38	nlssplit_a
2	35S_BAR backbone fragment 2	oSNM39 & oSNM40	nlssplit_a
3	35S_BAR backbone fragment 3	oSNM41 & AP04	nlssplit_a

PCR programm backbone fragments

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	54 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	02:30	02:00	endless

for the EGFP_B construct:

https://benchling.com/igembonn2026/f/lib_TK020i1PMk-split_gfp/seq_mR3VZzEEnS-35s_egfp_a_bar/edit

To amplify the C-terminal fragment of the eGFP:
Template: NLS_split_B (https://benchling.com/igembonn2026/f/lib_ByZnmy8r40-split_nls/seq_XwMCt4DjHX-35s_nlssplitgfp_b_bar_version2/edit)
primers: I26053 (fv, w/ overhang, tm = 55.7 °C) and I26054 (rv, w/overhang, tm = 54.4°C)
This generated the c-terminal truncation of the eGFP
241 bp are expected

Use Tab. 1 as reagent mix. Consider using the right primers (**I26053, I26054**)
Use the same programm for the cycler in Tab.2.

Open the vector via PCR:
template: NLS_split_B
primers: JK20 (Fv, beginning of F1, tm = 47.7°C) and I26052 (Rv, anneals at NLS, goes over the Nnpu_C-cage-F3)

Amplified fragments

	Fragment	Primers	Template
1	35S_BAR backbone fragment 1	JK20 & oSNM38	nlssplit_b
2	35S_BAR backbone fragment 2	oSNM39 & oSNM40	nlssplit_b
3	35S_BAR backbone fragment 3	oSNM41 & I26052	nlssplit_b

PCR programm backbone fragments1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	54 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	02:30	02:00	endless

protocoll for canonical cre cloning (in the making)

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 19 May 2026 12:12:28 UTC
Entry Last Modified: 19 May 2026 12:12:32 UTC
Export Generated On: 05 Oct 2026 11:21:59 UTC

DIENSTAG, 19.5.2026

PCR of Backbones (F2, F1 and F3) for A and B (do not use for now)

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 20 May 2026 08:16:20 UTC
Entry Last Modified: 21 May 2026 08:19:24 UTC
Export Generated On: 05 Oct 2026 11:23:00 UTC

MITTWOCH, 20.5.2026

Amplified fragments1

	Fragment	Primers	Template
1	35S_BAR backbone fragment 2	oSNM39 & oSNM40	nlssplit_a
2	35S_BAR backbone fragment 3	oSNM41 & AP40	nlssplit_a

Amplified fragments

	Fragment	Primers	Template
1	35S_BAR backbone fragment 1	JK20 & oSNM38	nlssplit_b
2	35S_BAR backbone fragment 2	oSNM39 & oSNM40	nlssplit_b

Q5 PCR Mastermix

	A	1 x [50 µl]	C
1	dd H20	29	130.5
2	Q5 Buffer	10	45
3	5mM dNTPs	2	9
4	template	2	9
5	Q5	2	9
6	Primer for	2.5	11.25
7	Primer rev	2.5	11.25
8			

PCR programm2

	A	B	C	D	E	F
1	1 cycle	45 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	95°C	95°C	57 °C	72°C	72°C	4°C
4	05:00	00:15	00:45	02:00	02:00	endless

gel was empty :)))))

Infiltration of test construct

Project: iGEM Bonn 2026 Shared Project
Author: Friedrich Nitschke
Status: In progress
Entry Created On: 20 May 2026 14:04:56 UTC
Entry Last Modified: 20 May 2026 14:17:07 UTC
Export Generated On: 05 Oct 2026 11:22:08 UTC

DIENSTAG, 28.4.2026

Following constructs were infiltrated into N. benthamiana previously via Agrobacteriinfiltration:

35S_NLSsplitGFP_A_BAR_Assembly_details 35S_NLSsplitGFP_B_BAR_Assembly_details

35S_lexA_TEV_XVE_BAR_Assembly_details

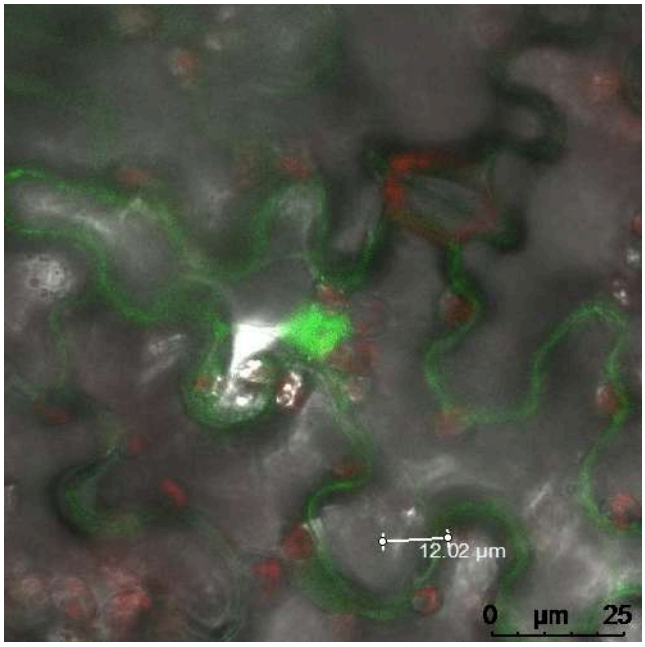
Agriinfiltration: 260424_leaf_infiltration

TEV induction: 260427_estrogen_treatment

For microscoping, a disk was stanced one day after estrogen-based TEV induction with the cover of an eppi. It as put onto a slide and imaged directly.

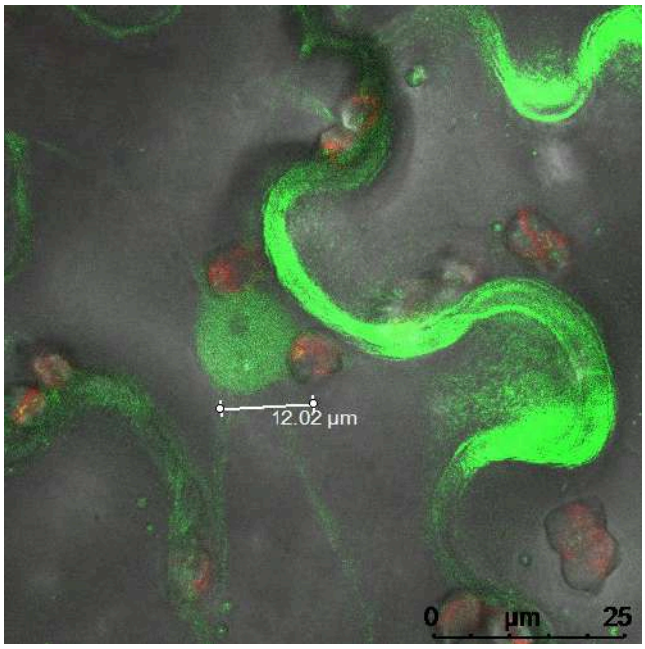
Non-induced:

Project_28.04.2026_Image014.tif



induced:

Project_28.04.2026_Image016_10X_Nbenth_A-GFP_489-532excitation_inducedestradiol.tif



Conclusion: GFP accumulates in nucleus nervertheless of the induction. Is it due to duiffusion into the nucleus? Next: Repeat with controls.

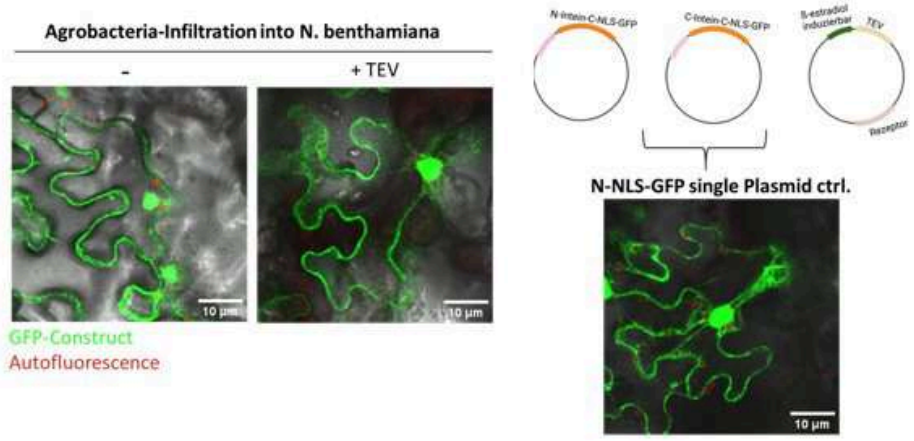
Infiltration of test construct

Project: iGEM Bonn 2026 Shared Project
Author: Friedrich Nitschke
Status: In progress
Entry Created On: 20 May 2026 14:17:33 UTC
Entry Last Modified: 20 May 2026 14:23:27 UTC
Export Generated On: 05 Oct 2026 11:22:18 UTC

DIENSTAG, 12.5.2026

Following constructs were infiltrated into N. benthamiana previously via Agrobacteriinfiltration:
35S_NLSsplitGFP_A_BAR_Assembly_details 35S_NLSsplitGFP_B_BAR_Assembly_details
35S_lexA_TEV_XVE_BAR_Assembly_details . Addtionally, we also checked a single plasmid infiltration control with plasmid B.
Agriinfiltration:
TEV induction: 260509_estrogen_treatment
For microscoping, a disk was stanced after estrogen-based TEV induction with the cover of an eppi. It as put onto a slide and imaged directly.

image.png



Conclusion: GFP accumulates in nucleus nervertheless of the induction. It is due to duiffusion into the nucleus

260508_leaf_Infiltration

Project: iGEM Bonn 2026 Shared Project

Author: Friedrich Nitschke

Status: In progress

Entry Created On: 20 May 2026 15:02:43 UTC

Entry Last Modified: 20 May 2026 15:21:34 UTC

Export Generated On: 05 Oct 2026 11:21:37 UTC

FREITAG, 8.5.2026

Header

Table1

	A	B
1	**Field**	**Value**
2	Date	24.4.2026
3	Experimenter	Ruben, Friedrich
4	Experiment ID	AGRO-002
5	Project	iGEM Bonn 2026
6	Objective	Transiently co-express NLS-tagged split GFP halves and a lexA-TEV-XVE construct in N. benthamiana leaves via agroinfiltration

Plant Material

Table2

	A	B
1	**Parameter**	**Value**
2	Species	Nicotiana benthamiana
3	Line / Ecotype	Wild-type (borrowed from external lab)
4	Age at infiltration	~4–6 weeks (estimated; sowing date unknown)
5	Growth conditions	16 h day
6	Leaves infiltrated	Marked by sample line
7	Number of plants	3

⚠️ Plant age is an estimate; exact developmental stage was not confirmed. Future experiments should record leaf stage (true-leaf number) at time of infiltration.

Bacterial Strains & Constructs

Table3

	A	B	C	D	E
1	**Label**	**Construct**	**Agrobacterium strain**	**Selection**	**Purpose**
2	A	35S_NLSsplit GFP_A_BAR	GV3101	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin	NLS-tagged split GFP fragment A
3	B	35S_NLSplitGFP_B_BAR*	GV3101	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin	NLS-tagged split GFP fragment B
4	X	35S_lexA_TEV_XVE_BAR	GV3101	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin	Estradiol-inducible lexA-TEV-XVE driver

Liquid Culture (Day Prior — 2026-05-23)

Table4

	A	B	C	D	E	F
1	**Sample**	**Construct**	**Medium**	**Volume**	**Temp / Shaking**	**Antibiotics**
2	A1, A2, A3	35S_NLSsplit GFP_A_BAR	YEP	10 mL each	—	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin
3	B1, B2, B3	35S_NLSplitG FP_B_BAR	YEP	10 mL each	—	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin
4	X1, X2, X3	35S_lexA_TE V_XVE_BAR	YEP	10 mL each	—	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin

Note: Antibiotic concentrations for spectinomycin and gentamicin not recorded — add to future entries.

OD_{600} Measurements & Resuspension Calculation

Cultures were diluted **1:10** prior to measurement. Actual OD_{600} = measured value × 10. Pellet was resuspended in **10 mL** infiltration medium. Calculated final OD_{600} = (OD_actual × 10 mL) / 10 mL.

All OD600 were between 0.3 and 0.4.

Pellet Preparation Procedure

Table6

	A	B
1	**Step**	**Detail**
2	1. Centrifugation	3,000 × g, 10 min, RT
3	2. Wash	Supernatant discarded; pellet resuspended in infiltration medium (volume not recorded)
4	3. Re-pelleting	3,000 × g, 10 min, RT
5	4. Resuspension	Pellet resuspended in 10 mL infiltration medium per sample
6	5. OD_{600} verification	Not measured — final OD_{600} calculated from pre-pellet values (see table above)

Induction Conditions

Table7

	A	B
1	**Parameter**	**Value**
2	Infiltration medium	Standard (10 mM MES pH 5.6, 10 mM $MgCl_2$)
3	Acetosyringone	200 µM
4	Induction temperature	Room temperature
5	Induction in dark	Yes
6	Induction duration	— (not recorded)

Infiltration Mix (per sample line)

Each line was prepared by combining equal volumes of constructs A, B, and X from the corresponding replicate number.

Table8

	A	B	C
1	**Mix**	**Constructs combined**	**Leaf label**
2	Line 1	A1 + B1 + X1	Leaf marked "1"
3	Line 2	A2 + B2	Leaf marked "2"
4	Line 3	B3	Leaf marked "3"

Mix ratio A : B : X = 1 : 1 : 1 (equal volumes) Infiltration method: needleless syringe, abaxial surface, ca. 1 ml per leaf

Untitled

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 21 May 2026 08:19:31 UTC

Entry Last Modified: 21 May 2026 08:19:31 UTC

Export Generated On: 05 Oct 2026 11:22:27 UTC

DONNERSTAG, 21.5.2026

PCR Backbones (F1, F2, F3, 35s standard, protocoll worked)

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 21 May 2026 09:08:50 UTC
Entry Last Modified: 29 May 2026 10:48:16 UTC
Export Generated On: 05 Oct 2026 11:23:10 UTC

DONNERSTAG, 21.5.2026

To esure we have enough Fragemnts for the assembly once the new primer arrive, amplification of the fragemnts, wich are as well needed to assembly the backbone, PCR was performed.
One day before, the PCR did not worked. Annealig is now lowered, as there where no products.
Other protocolls on benchling already showed amplicons for these Fragments with the given annealing tms (see tab below).

Amplified fragments

	Fragment	Primers	Template
1	35S_BAR backbone fragment 1	JK20 & oSNM38	35S_RUBY_BAR
2	35S_BAR backbone fragment 2	oSNM39 & oSNM40	35S_RUBY_BAR
3	35S_BAR backbone fragment 3	oSNM41 & AP04	35S_RUBY_BAR

PCR mix setup

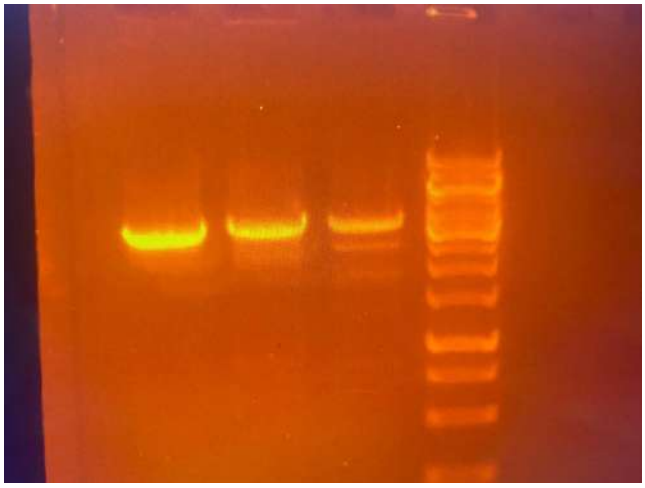
	Substance	1x [µl]	2.5x [µl]
1	dH20	15	52.5
2	Q5 Buffer	5	17.5
3	5 mM dNTPs	1	3.5
4	10 mM Primer for	1	3.5
5	10 mM Primer rev	1	3.5
6	Q5 Polymerase	1	3.5
7	Template (35s_ruby_bar)	1	3.5

PCR programm backbone fragments

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	54 °C	72°C	72°C	12°C
4	05:00	00:15	00:30	02:00	02:00	endless

1 kb ladder, F1, F2, F3, standard 35 Backbone

WhatsApp_Image_2026-05-29_at_12.47.15.jpeg



260521 Transformation mCherry-N1 plasmid

Project: iGEM Bonn 2026 Shared Project
Author: Friedrich Nitschke
Status: In progress
Entry Created On: 22 May 2026 07:37:17 UTC
Entry Last Modified: 29 May 2026 07:28:34 UTC
Export Generated On: 05 Oct 2026 11:21:50 UTC

DONNERSTAG, 21.5.2026

Table1

	A	B
1	**Field**	**Value**
2	Date	21.05.2026
3	Experimenter	Friedrich
4	Project	IGEM 2026
5	Objective	Transform new mCherry-N1 plasmid into E.coli for plasmid extraction; template for mCherry in later experiments

#

The plasmid mCHerry-N1 was derived from the AG Gruss (already sequenced). For producing more it was transformed into XL1-blue compis:

***E. coli* transformation**

Aliquot of chemically competent XL1-blue *E. coli* was thawed on ice. Once thawed one µL of plasmid was added and the bacteria were incubated on ice for 15 minutes. After incubation the bacteria were heat shocked at 42°C for 90 seconds. Following the heat shock the bacteria were put back on ice and incubated for another 5 minutes. Afterwards 850 µl of SOC medium preheated to 37°C were added and the bacteria were icubated at 37°C, 850 rpm for 1 hour to express the antibiotics resistance. After incubation the cells were pelleted by centrifugation at 3000x g for 5 minutes and 850 µl of the supernatant was discarded. The pellet was dissolved in the remaining supernatant and plated on a LB (Kanamycine 100 ng/µl) plate. The bacteria were grown overnight at 37°C.

retrafo of whole GRAPS kit

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 22 May 2026 11:02:54 UTC
Entry Last Modified: 25 May 2026 20:36:29 UTC
Export Generated On: 05 Oct 2026 11:22:06 UTC

FREITAG, 22.5.2026

The 43 plasmids from the kit where diluted in 10 µl ddH20.

E. coli transformation

Aliquot of chemically competent XL1-blue *E. coli* was thawed on ice. Once thawed one µL of plasmid was added and the bacteria were incubated on ice for 30 minutes. After incubation the bacteria were heat shocked at 42°C for 90 seconds. Following the heat shock the bacteria were put back on ice and incubated for another 5 minutes. Afterwards 850 µl of SOC medium preheated to 37°C were added and the bacteria were icubated at 37°C, 850 rpm for 1 hour to express the antibiotics resistance. After incubation the cells were pelleted by centrifugation at 3000x g for 5 minutes and the supernatant was discarded. The pellet was dissolved in the remaining supernatant and plated on a LB (Kanamycine 100 ng/µl) plate. The bacteria were grown overnight at 37°C.

GRASP Kit re-Transformation

Project: iGEM Bonn 2026 Shared Project

Author: Ruben Langenbacher

Status: In progress

Entry Created On: 22 May 2026 15:09:18 UTC

Entry Last Modified: 22 May 2026 15:15:54 UTC

Export Generated On: 05 Oct 2026 11:21:55 UTC

FREITAG, 22.5.2026

The GRASP Kit was delivered as dry Plasmid.

Dry Plasmid was redesolved in 5 yl of ddH20.

Heat Shock Transformation:

1. 45 aliquots of chemicaly competet XL1-Blue e.coli were thawed on ice.
2. on ice 1 yl of Plasmid solution was added per aliquot. Incubated on ice for 20 min.
3. heat shock 42 °C for 90 sek. Put back on ice
4. Icubate on ice for 5 min before adding 800 µl of 37°C preheated SOC medium.
5. Cells incubated 850 rpm 37 °C for 30 min
6. centrifuged 3500 rcf for 30 sec
7. 200 yl of resuspended pallet were plated on LB+Kan plates

mini preps GRASP-Kit clones 1.1 - 16.1 and 18.1-23.1

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 26 May 2026 08:55:52 UTC
Entry Last Modified: 29 May 2026 07:38:18 UTC
Export Generated On: 05 Oct 2026 11:22:16 UTC

DIENSTAG, 26.5.2026

i could not find the 17.1 (260525 Culture Inolation mCherry-N1 + Grasp kit plasmids)
two clones each construct was picked
otherwise, the plasmids from 1.1-23.1 (except 17.1 as i could not find it maybe it is still there) was preppen accoring to the protocoll
lower RNAseA concentration was used, as Boas said it is fine. Just let in incubate longer. idk.

The liquid culture was grown overnight in 2 mL selective medium (with the appropriate antibiotic) at 37 °C while shaking.
750 µl of the culture was transferred into a 1.5 mL tube and centrifuged at 3500 × g for 2 min to pellet the cells.
The supernatant was discarded and the pellet resuspended in 100 µL MP Solution 1 (0,05 mg/ml) and rest for 25 minutes at RT.

Next, 300 µL Buffer 2 (pH = 12.3) was added and mixed gently by inverting the tube a few times (no vortexing!).
At this step, care was taken to avoid harsh mixing, as genomic DNA is very fragile and can shear easily, which would lead to contamination.
The lysate was incubated on ice for **max. 2 min.**

After that, 250 µL Buffer 3 (pH = 5.3) was added and the tube was again gently inverted to mix for a few times.
Optionally, a few drops of chloroform can be added here to improve purity (this was not done)
The sample was then centrifuged at fullsped, at -9°C for 10 min.

About 400 µL of the clear supernatant was transferred into a fresh, pre-cooled tube.
1 ml 100%, **ice cold** EtOH was added to the supernatant and the sample was vortext.
(Note: u can also use isoprop, i dont as i have bad experince with it (salt contamination), EtOH worked always perfecly for me.)

spin down at fullspeed, at -9°C for 1h

The supernatant was removed, and the pellet washed with 70% ethanol.
After removing the ethanol completely (P20 might help), the pellet was briefly air-dried.
Finally, the DNA was resuspended in 20 µL H_2O and the concentration was measured at the nanodrop

Retransformation pAGM9121. Mini preps GRASP kit clones 17 and 43 and Nanodrop measurement. Preperation for Nanopore Sequencing

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 28 May 2026 15:28:39 UTC
Entry Last Modified: 28 May 2026 15:55:27 UTC
Export Generated On: 05 Oct 2026 11:22:26 UTC

DIENSTAG, 26.5.2026

The transformation of pAGM9121 (43) on 22.05.2026 was not succesfull because pAGM9121 contains a resistance for Spectinomycin and not Kanamycin but a Kanamycin plate was used.
Therefore the Transformation for pAGM9121 was repeated same as 22.05.2026.

MITTWOCH, 27.5.2026

pAGM9121 plate showed succesfull growth.
Clones were picked from plate 43 and 17 and 2 mL of SOC Medium per culture were inoculated.
After 9 hours at 37 °C and 250 rpm the cultures were centrifuged at 3500 g for 3 min to pellet the cells.
The supernatand was discarded. The pellet resuspendet in the return flow and 750 yl of this suspension were used for Mini Prep. The Mini Prep was conducted after the protocol from 26.05.2026 by Julia Hodakov.

Nanodrop Measurements were conducted for all 43 GRASP Plasmids and the mCherry Plasmid.

Table1

	Concentration [ng/yl]	B
1	3032,8	
2	3024,7	
3	2190,2	
4	2972,3	
5	2529,5	
6	3425,5	
7	2681,5	
8	2774,7	
9	2883,6	
10	2698,8	
11	3041,0	
12	2808,5	
13	2411,1	
14	2704,9	
15	3597,5	
16	2281,1	
17	4388,4	
18	2507,4	
19	2260,0	
20	2348,4	
21	2665,3	
22	2567,1	
23	3590,6	
24	891,1	
25	562,2	
26	1526,7	
27	1159,4	
28	2087,8	
29	456,9	
30	1112,6	
31	1074,8	
32	1044,7	
33	2509,7	

34	1071,5	
35	1004,6	
36	1061,6	
37	1949,1	
38	1504,8	
39	1873,2	
40	1686,0	
41	913,1	
42	2131	
43	5312,7	
44	2046,3	mCherry
45		

After nanodrop meassurement all samples were prepared for in house Nanopore sequencing.
10 yl with a concentration of 50 to maximum 200 ng/yl were expected.
Therefor for all samples with Concentration above 2000 ng/yl 9.5 yl dH20 and 0.5 yl Plasmid were used.
For samples with a concentration below 2000 ng/yl 9 yl dH20 and 1 yl Plasmid were used.
Samples 17 and 43 had concentrations way above 4000 ng/yl therefore 9.8 yl dH20 and 0.2 yl of Plasmid were used.

DONNERSTAG, 28.5.2026

As sequencing reference a Fasta datei was prepared containing all GRASP Kit Sequences as well as the mCherry Plasmid Sequence.

A. tumefaciens transformation with 35S_RUBY_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 28 May 2026 20:04:15 UTC
Entry Last Modified: 09 Sep 2026 17:39:00 UTC
Export Generated On: 05 Oct 2026 11:21:32 UTC

DONNERSTAG, 28.5.2026

Table1

	A	B
1	**Field**	**Value**
2	Date	28.05.2026
3	Experimenter	Julius
4	Project	IGEM 2026
5	Objective	Transform A. tumefaciens with the 35S_RUBY_BAR plasmid for subsequent leaf infiltration of N. benthamiana
6		

-2 x 25 µl of Electrocompetent GV3101 A.tumefaciens were thawed on ice.
-1 µl of 35S_RUBY_BAR from different plasmid from minipreps was added.
-Cells+DNA were transferred into pre cooled electroporation cuvettes, tapped to remove air bubbles and kept on ice for 10 min
-The cells were pulsed at 2.2 kV
-Afterwards 1 ml of roomtemperature YEP medium added to each sample and the samples were transfered to 1.5 ml reaction tubes
-The bacteria were incubated for 2.5 h at 28 °C 850 rpm
-100 µl of the bacteria solution was plated on YEP+Rif+Gent+Spec plates, afterwards the remaining cells were pellted by centrifugation at 3000x g for 5 minutes. The supernatant was discarded and the pellet was resuspended in the remaining 100 µl of the medium. The remaining 100 µl were also plated on new YEP+Rif+Gent+Spec plates.
-The plates were grown at 28 °C for 2 days

260525 Culture Inolation mCherry-N1 + Grasp kit plasmids

Project: iGEM Bonn 2026 Shared Project
Author: Friedrich Nitschke
Status: In progress
Entry Created On: 29 May 2026 07:28:47 UTC
Entry Last Modified: 29 May 2026 07:36:24 UTC
Export Generated On: 05 Oct 2026 11:22:38 UTC

MONTAG, 25.5.2026

Table1

	A	B
1	**Field**	**Value**
2	Date	25.05.2026
3	Experimenter	Friedrich
4	Project	IGEM 2026
5	Objective	ONC inolation of new mCherry-N1 plasmid into E.coli for plasmid extraction; template for mCherry in later experiments

200 mL SOC media was supplemented with 200 µL of 50 mg/mL Kanamycine. Colonies from following plasmid transformations were picked (2 each) and transferred into 2 mL of the Kan-supplemented media for 37 °C incubation ON: mCherry-N1 (260521 Transformation mCherry-N1 plasmid) and GRASP Kit 1-16, and 18-42 (43 did not contain any colonies, 17 was forgotten) (retrafo of whole GRAPS kit).

split gfp pcr

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 29 May 2026 10:39:51 UTC
Entry Last Modified: 15 Jun 2026 15:18:30 UTC
Export Generated On: 05 Oct 2026 11:23:31 UTC

FREITAG, 29.5.2026

gfp a (n_gfp)

Tab.1: Q5 PCR setup (eGFP Insert amplification)

	A	1x [µl]
1	ddH20	15
2	dNTPs	1
3	I26026	1
4	I26029	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Tab.2: PCR programm for truncated N-eGFP

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	56 °C	72°C	72°C	4°C
4	05:00	00:15	00:20	00:20	02:00	endless

gfp b (c_gfp)

Tab.1: Q5 PCR setup (eGFP Insert amplification)1

	A	1x [µl]
1	ddH20	15
2	dNTPs	1
3	I26028	1
4	I26036	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Tab.2: PCR programm for truncated N-eGFP1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	61 °C	72°C	72°C	4°C
4	05:00	00:15	00:20	00:15	02:00	endless

loading

	A	B	C
1	1	2	3
2	100 bp ladder	n_gfp	c_gfp
3	size expected:	500 bp	200 bp

WhatsApp_Image_2026-05-29_at_12.42.59.jpeg



n_gfp has only side products (maybe a little bit of 510bp), increase annealing tm so make more specific
c_gfp has no side products. decrease the annealing tm.

260603_leaf_infiltration

Project: iGEM Bonn 2026 Shared Project

Author: Julius Krupke

Status: In progress

Entry Created On: 03 Jun 2026 16:07:54 UTC

Entry Last Modified: 07 Jun 2026 18:43:15 UTC

Export Generated On: 05 Oct 2026 11:21:43 UTC

DIENSTAG, 2.6.2026

A 5 ml culture of Yep (Rifampicin 100 ng/ml, Spectinomycin 100 ng/ml, Gentamicin 30 ng/ml) was inoculated with GV3101 *A. tumefaciens* carrying the 35S_RUBY_BAR plasmid. The cultures were grwon for 1 day at 28 °C, 188 rpm.

MITTWOCH, 3.6.2026

Table1

	A	B
1	**Field**	**Value**
2	Date	03.05.2026
3	Experimenter	Julius
4	Project	iGEM Bonn 2026
5	Objective	Transiently express RUBY construct in N. benthamiana leaves via agroinfiltration

Plant material

	A	B
1	**Parameter**	**Value**
2	Species	Nicotiana benthamiana
3	Line / Ecotype	Wild-type (borrowed from external lab)
4	Age at infiltration	6 weeks
5	Growth conditions	8 h day
6	Leaves infiltrated	Marked by sample line
7	Number of plants	2

Bacterial strain

	A	B	C	D
1	**Construct**	**Agrobacterium strain**	**Selection**	**Purpose**
2	35S_RUBY_BAR	GV3101	Rifampicin 50 µg/mL + Spectinomycin + Gentamicin	expression of RUBY

Pellet preparation procedure

	A	B
1	**Step**	**Detail**
2	1. Centrifugation	3,000 × g, 10 min, RT
3	2. Wash	Supernatant discarded; pellet resuspended in infiltration medium (volume not recorded)
4	3. Re-pelleting	3,000 × g, 10 min, RT
5	4. Resuspension	Pellet resuspended in 10 mL infiltration medium per sample

Infiltratoin conditions

	A	B
1	**Parameter**	**Value**
2	Infiltration medium	Standard (10 mM MES pH 5.6, 10 mM $MgCl_2$)
3	Acetosyringone	0 μM
4	Induction temperature	Room temperature
5	Induction in dark	Yes

Untitled

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 04 Jun 2026 10:43:55 UTC

Entry Last Modified: 04 Jun 2026 10:43:55 UTC

Export Generated On: 05 Oct 2026 11:22:49 UTC

DONNERSTAG, 4.6.2026

Untitled

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 04 Jun 2026 13:31:17 UTC

Entry Last Modified: 04 Jun 2026 13:31:17 UTC

Export Generated On: 05 Oct 2026 11:23:00 UTC

DONNERSTAG, 4.6.2026

Split GFP protocoll

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 05 Jun 2026 08:00:21 UTC

Entry Last Modified: 05 Jun 2026 08:31:54 UTC

Export Generated On: 05 Oct 2026 11:23:10 UTC

FREITAG, 5.6.2026

For GFP in component A:

Tab.1: Q5 PCR setup (eGFP Insert amplification)

	A	1x [µl]
1	ddH20	15
2	dNTPs	1
3	I26036 Fwd	1
4	I26026 Rv	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Tab.2: PCR programm for truncated N-eGFP

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	56 °C	72°C	72°C	4°C
4	05:00	00:15	00:20	00:20	02:00	endless

For GFP in component B:

Tab.1: Q5 PCR setup (eGFP Insert amplification)1

	A	1x [µl]
1	ddH20	15
2	dNTPs	1
3	I26054 Fwd	1
4	I26061 Rv	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Tab.2: PCR programm for truncated N-eGFP1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	56 °C	72°C	72°C	4°C
4	05:00	00:15	00:20	00:20	02:00	endless

Fragment 1 for component A

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 05 Jun 2026 08:33:02 UTC
Entry Last Modified: 05 Jun 2026 08:40:14 UTC
Export Generated On: 05 Oct 2026 11:23:20 UTC

FREITAG, 5.6.2026

Fragment 2 and Fragment 3 from the NLS split constructs can be used

find the working protocoll here:
https://benchling.com/igembonn2026/f/lib_RC2qrZTeWD-split-egfp-cloning/etr_8NShm4LGyn-pcr-backbones-f1-f2-f3-35s-standard-protocoll-worked/edit

Split eGFP PCR

Project: iGEM Bonn 2026 Shared Project

Author: Ruben Langenbacher

Status: In progress

Entry Created On: 05 Jun 2026 14:13:52 UTC

Entry Last Modified: 08 Jun 2026 09:33:40 UTC

Export Generated On: 05 Oct 2026 11:22:32 UTC

FREITAG, 5.6.2026

Q5 PCR setup for C-eGFP

	A	B
1	ddH20	15
2	dNTPs	1
3	I26055	1
4	I26061	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Q5 PCR running conditionsfor N-eGFP

	A	B	C	D	E	F
1	1 cycle	30 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	67 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	00:30	02:00	endless

Q5 PCR running conditions for C-eGFP

	A	B	C	D	E	F
1	1 cycle	30 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	59 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	00:30	02:00	endless

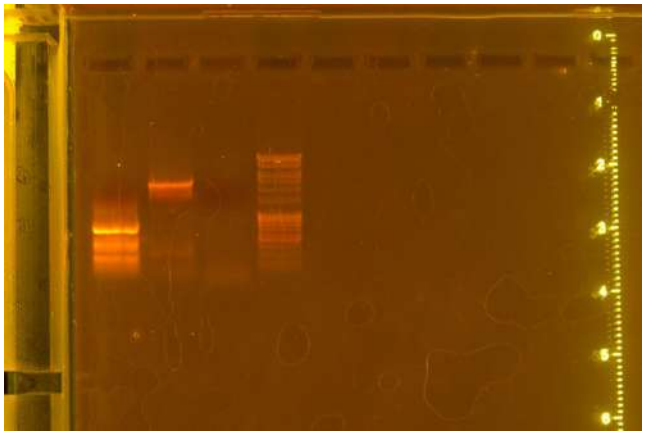
Q5 PCR setup for N-eGFP

	A	B
1	ddH20	15
2	dNTPs	1
3	I26036	1
4	I26059	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Loading scheme

	A	B	C	D
1	N-eGFP	C-eGFP	Negative controll	100 bp ladder
2				
3				

igem_segfp_2026_06_05.jpg



260607_leaf_infiltration

Project: iGEM Bonn 2026 Shared Project

Author: Julius Krupke

Status: In progress

Entry Created On: 07 Jun 2026 18:43:34 UTC

Entry Last Modified: 07 Jun 2026 18:46:43 UTC

Export Generated On: 05 Oct 2026 11:21:36 UTC

SAMSTAG, 6.6.2026

Two 5 ml cultures of Yep (Rifampicin 100 ng/ml, Spectinomycin 100 ng/ml, Gentamicin 30 ng/ml) were inoculated with GV3101 *A. tumefaciens* carrying the 35S_RUBY_BAR and the 35S_avGFP_BAR plasmid. The cultures were grwon for 1 day at 28 °C, 188 rpm.

SONNTAG, 7.6.2026

Table1

	A	B
1	**Field**	**Value**
2	Date	07.05.2026
3	Experimenter	Julius
4	Project	iGEM Bonn 2026
5	Objective	Transiently express RUBY and GFP in N. benthamiana leaves via agroinfiltration

Plant material

	A	B
1	**Parameter**	**Value**
2	Species	Nicotiana benthamiana
3	Line / Ecotype	Wild-type (borrowed from external lab)
4	Age at infiltration	6 weeks
5	Growth conditions	8 h day
6	Leaves infiltrated	Marked by sample line
7	Number of plants	3

Bacterial strain

	A	B	C	D
1	**Construct**	**Agrobacterium strain**	**Selection**	**Purpose**
2	35S_RUBY_BAR	GV3101	Rifampicin 100 µg/ml + Spectinomycin 100 µg/ml + Gentamicin 30 µg/ml	expression of RUBY
3	35S_avGFP_BAR	GV3101	Rifampicin 100 µg/ml + Spectinomycin 100 µg/ml + Gentamicin 30 µg/ml	expression of GFP

Pellet preparation procedure

	A	B
1	**Step**	**Detail**
2	1. Centrifugation	3,000 × g, 10 min, RT
3	2. Wash	Supernatant discarded; pellet resuspended in infiltration medium (volume not recorded)
4	3. Re-pelleting	3,000 × g, 10 min, RT
5	4. Resuspension	Pellet resuspended in 5 ml infiltration medium per sample

Infiltratoin conditions

	A	B
1	**Parameter**	**Value**
2	Infiltration medium	Standard (10 mM MES pH 5.6, 10 mM $MgCl_2$)
3	Acetosyringone	0 µM
4	Induction temperature	Room temperature
5	Induction in dark	Yes

Split eGFP PCR (F1+intein+cage, F1+intein+cage, N-eGFP, C-eGFP)

Project: iGEM Bonn 2026 Shared Project
Author: Joana Heckmann
Status: In progress
Entry Created On: 11 Jun 2026 15:46:57 UTC
Entry Last Modified: 11 Jun 2026 16:33:20 UTC
Export Generated On: 05 Oct 2026 11:22:46 UTC

DONNERSTAG, 11.6.2026

Q5 PCR setup for C-eGFP

	A	B
1	ddH20	15
2	dNTPs	1
3	I26055	1
4	I26061	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Q5 PCR running conditions for C-eGFP and F1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	61 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	00:30	02:00	endless

Q5 PCR setup for N-eGFP

	A	B
1	ddH20	15
2	dNTPs	1
3	I26036	1
4	I26059	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Q5 PCR running conditions for N-eGFP1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	69 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	00:30	02:00	endless

Q5 PCR setup for F1+Intein+cage

	A	B
1	ddH20	15
2	dNTPs	1
3	I26058	1
4	oSNM38	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_a)	1

Q5 PCR running conditions for F3+intake+cage

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	57 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	02:00	02:00	endless

Q5 PCR setup for F3+intein+cage

	A	B
1	ddH20	15
2	dNTPs	1
3	oSNM41	1
4	I26060	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Results:
Run 1: nothing
Run 2: testing with DNTPs 1 and DNTPs 2

C-eGFP PCR

Project: iGEM Bonn 2026 Shared Project
Author: Melda Bakir
Status: In progress
Entry Created On: 12 Jun 2026 12:44:48 UTC
Entry Last Modified: 12 Jun 2026 13:30:46 UTC
Export Generated On: 05 Oct 2026 11:22:57 UTC

FREITAG, 12.6.2026

To amplify the C-eGFP fragment, a gradient PCR was performed.
PCR mix can be found in Tab. 1
PCR Program can be found in Tab. 2
Template NLS_B (used for all 12 samples) was diluted to a final concentration of 1 ng/ul

Table1

	A	B	C
1	ddH2O	6 µl	90 µl
2	5 mM dNTPs	0.4 µl	6 µl
3	Primer I26055	0.4 µl	6 µl
4	Primer I26061	0.4 µl	6 µl
5	5x Q5 Buffer	2 µl	30 µl
6	Template	0.4 µl	
7	Q5 Polymerase	0.4 µl	6 µl
8	Final Volume	10 µl	
9			

Table2

	A	B	C
1	Initial Denaturation	98 °C	5 min
2	Denaturation	98 °C	15 s
3	Annealing	*	20 s
4	Elongation	72 °C	15 s
5	Final Extension	72 °C	2 min
6	Hold	12 °C	unlimited

*=Gradient PCR was performed. Annealing temperature per sample no.:

Table3

	A	B	C	D
1	1	59.4 °C	7	66.0 °C
2	2	59.6°C	8	67.7 °C
3	3	60.2 °C	9	68.9 °C
4	4	61.1 °C	10	69.8 °C
5	5	62.3 °C	11	70.4 °C
6	6	64.0 °C	12	70.6 °C

Gel will be run next week

F1/F3+intein+cage PCR

Project: iGEM Bonn 2026 Shared Project
Author: Melda Bakir
Status: In progress
Entry Created On: 12 Jun 2026 13:20:23 UTC
Entry Last Modified: 12 Jun 2026 13:30:38 UTC
Export Generated On: 05 Oct 2026 11:21:59 UTC

FREITAG, 12.6.2026

PCR was repeated using different diulutions of the respective templeate (1:10 and 1:200)
PCR mix for F1 can be found in Tab. 1
PCR programm for F1 can be found in Tab. 2

Table1

	A	B
1	ddH2O	15 µl
2	25 µM dNTPs	1 µl
3	Primer I26058	1 µl
4	Primer oSNM38	1 µl
5	5x Q5 Buffer	5 µl
6	Q5 Polymerase	1 µl
7		

Table2

	A	B	C
1	Initial denaturation	98 °C	5 min
2	Denaturation	98 °C	15 s
3	Annealing	61 °C	30 s
4	Elongation	72 °C	2 min
5	Final Extension	72 °C	2 min
6	Hold	12 °C	unlimited

PCR mix for F3 can be found in Tab. 3

PCR program can be found in Tab. 4 (reaction was not run today!)

Table3

	A	B
1	ddH2O	15 µl
2	25 µM dNTPs	1 µl
3	Primer l26060	1 µl
4	Primer oSNM41	1 µl
5	5x Q5 Buffer	5 µl
6	Q5 Polymerase	1 µl
7		

Table4

	A	B	C
1	Initial denaturation	98 °C	5 min
2	Denaturation	98 °C	15 s
3	Annealing	57 °C	30 s
4	Elongation	72 °C	2 min
5	Final Extension	72 °C	2 min
6	Hold	12 °C	unlimited

Gel will be run next week

Copy of Split eGFP PCR (F1+intein+cage, F1+intein+cage, N-eGFP, C-eGFP)

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 15 Jun 2026 12:09:00 UTC
Entry Last Modified: 15 Jun 2026 12:09:00 UTC
Export Generated On: 05 Oct 2026 11:22:11 UTC

DONNERSTAG, 11.6.2026

Q5 PCR setup for C-eGFP

	A	B
1	ddH20	15
2	dNTPs	1
3	I26055	1
4	I26061	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Q5 PCR running conditions for C-eGFP and F1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	61 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	00:30	02:00	endless

Q5 PCR setup for N-eGFP

	A	B
1	ddH20	15
2	dNTPs	1
3	I26036	1
4	I26059	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Q5 PCR running conditions for N-eGFP1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	69 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	00:30	02:00	endless

Q5 PCR setup for F1+Intein+cage

	A	B
1	ddH20	15
2	dNTPs	1
3	I26058	1
4	oSNM38	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_a)	1

Q5 PCR running conditions for F3+intake+cage

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	57 °C	72°C	72°C	4°C
4	05:00	00:15	00:30	02:00	02:00	endless

Q5 PCR setup for F3+intein+cage

	A	B
1	ddH20	15
2	dNTPs	1
3	oSNM41	1
4	I26060	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1

Results:
Run 1: nothing
Run 2: testing with DNTPs 1 and DNTPs 2

F3+intein+cage PCR

Project: iGEM Bonn 2026 Shared Project
Author: Melda Bakir
Status: In progress
Entry Created On: 15 Jun 2026 12:10:49 UTC
Entry Last Modified: 15 Jun 2026 12:12:37 UTC
Export Generated On: 05 Oct 2026 11:22:21 UTC

MONTAG, 15.6.2026

PCR mix from 12.06.2026 was accidentally loaded on gel without having been put in the cycler. The reaction was thus repeated today.
PCR mix and program can be found in Tab. 3 and 4, respectively.

Table3

	A	B
1	ddH2O	15 µl
2	25 µM dNTPs	1 µl
3	Primer I26060	1 µl
4	Primer oSNM41	1 µl
5	5x Q5 Buffer	5 µl
6	Q5 Polymerase	1 µl
7		

Table4

	A	B	C
1	Initial denaturation	98 °C	5 min
2	Denaturation	98 °C	15 s
3	Annealing	57 °C	30 s
4	Elongation	72 °C	2 min
5	Final Extension	72 °C	2 min
6	Hold	12 °C	unlimited

N and C GFP with DMSO

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 15 Jun 2026 12:16:07 UTC
Entry Last Modified: 15 Jun 2026 12:23:42 UTC
Export Generated On: 05 Oct 2026 11:23:08 UTC

MONTAG, 15.6.2026

Tab.1: Q5 PCR setup (N-GFP)

	A	1x [µl]
1	ddH20	13.75
2	dNTPs	1
3	I26036	1
4	I26059	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1
8	DMSO	1.25

Q5 PCR setup for C-eGFP

	A	B
1	ddH20	13.75
2	dNTPs	1
3	I26055	1
4	I26061	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1
8	DMSO	1.25

Q5 PCR running conditions

	A	B	C	D	E	F
1	1 cycle	30 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	gradient (see below)	72°C	72°C	12°C
4	05:00	00:15	00:30	00:20	02:00	endless

gradient

	A	B	C	D	E	F	G	H	I	J	K	L
1	65.7 °C	65.8 °C	66.1 °C	66.5°C	67.2 °C	68 °C	69 °C	69.8 °C	70.5 °C	70.9 °C	71.2 °C	71.3 °C

following tm where used and loaded with the same ordner on the gel :

Table1

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	tm												
2	N-GFP												
3	C-GFP												

two step nested PCR

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 15 Jun 2026 15:08:09 UTC
Entry Last Modified: 15 Jun 2026 15:42:44 UTC
Export Generated On: 05 Oct 2026 11:23:19 UTC

MONTAG, 15.6.2026

Due to the fact that the primers anneal very unspecific and that another primer desgin is not able to improve the PCR, we go now for a two step nested PCR.

This means, that we PCR the GFP out of the NLS_B and use this in a second PCR to split the GFP.

To PCR the downstream Template (whole GFP) out of the Plasmid:

Tab.1: Q5 PCR setup (eGFP Insert amplification)

	A	1x [µl]
1	ddH20	15
2	dNTPs	1
3	AP15	1
4	BP12	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (nlsplit_b)	1
8	size	1175 bp

AP15: ACAATCCCACTATCCTTC
28 nt
29% GC
Tm: 61°C

BP12: caccaaatatttcatcttcatcttcata
18 nt
44% GC
Tm: 58°C

Tab.2: PCR programm for GFP

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	59 °C	72°C	72°C	4°C
4	05:00	00:15	00:20	00:30	02:00	endless

The product of the right size (Tab.1) needs to be purifies via collum (or gel elution).
Dilute the PCR product down for the usage of downstream PCRs. (1:10)

Next, the GFP needs to be splittet.

Tab.1: Q5 PCR setup (N_eGFP Insert amplification)1

	A	1x [µl]
1	ddH20	15
2	dNTPs	1
3	I26036	1
4	I26059	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (GFP, 1:10, see above)	1
8	size	516 bp

following only the annealing part:

36: atgAGCAAGGGCGAGGAG
18 nt
61% GC
Tm: 68°C

59: GATCCGCCACAACATCGAG
19 nt
58% GC

Tm: 66°C

Tab.2: PCR programm for N_GFP

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	67 °C	72°C	72°C	4°C
4	05:00	00:15	00:20	00:20	02:00	endless

Tab.1: Q5 PCR setup (C_eGFP Insert amplification)

	A	1x [µl]
1	ddH20	15
2	dNTPs	1
3	I26032	1
4	I26061	1
5	Q5	1
6	Q5 Buffer 1:5	5
7	Template (GFP, 1:10, see above)	1
8	size	201 bp

61: GACGGCAGCGTGCAGC
16 nt
75% GC
Tm: 71°C

32: GCATGGACGAGCTGTACAAGTAA
23 nt
48% GC
Tm: 67°C

Tab.2: PCR programm for C_GFP1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	68 °C	72°C	72°C	4°C
4	05:00	00:15	00:20	00:20	02:00	endless

F3+cage+intein PCR

Project: iGEM Bonn 2026 Shared Project
Author: Melda Bakir
Status: In progress
Entry Created On: 19 Jun 2026 11:42:17 UTC
Entry Last Modified: 26 Jun 2026 13:01:50 UTC
Export Generated On: 05 Oct 2026 11:21:48 UTC

FREITAG, 19.6.2026

Gel from PCR of 15.06.2026 (run on 17.06.2026):
Both dilutions (1:10 and 1:200) show no PCR product
Neg control shows no PCR product
PCR will be reperated acccording to the follwing scheme:

Table1

	A	B
1	ddH2O	15 µl
2	25 µM dNTPs	1 µl
3	Primer I26058	1 µl
4	Primer oSNM38	1 µl
5	5x Q5 Buffer	5 µl
6	Q5 Polymerase	1 µl
7		

PCR was carried out in a total of four reactions: undiluted plasmid, 1:10 dilution and 1:100 dilution, neg control

split GFP PCR (A and B, WORKING PROTOKOLL)

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 25 Jun 2026 13:33:48 UTC
Entry Last Modified: 25 Jun 2026 13:34:41 UTC
Export Generated On: 05 Oct 2026 11:23:30 UTC

DONNERSTAG, 25.6.2026

split GFP A and B Colony PCR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 02 Jul 2026 15:02:13 UTC
Entry Last Modified: 02 Jul 2026 15:06:15 UTC
Export Generated On: 05 Oct 2026 11:23:40 UTC

DONNERSTAG, 2.7.2026

Q5 Mastermix was prepared 10,5x. Primers AP15 and BP12 were used.
Expected amplicon size for B 982 bp and for A 1200 bp.

Q5 cPCR running conditions

	A	B	C
1	Initial Denaturation	98 °C	5 min
2	Denaturation	98 °C	15 s
3	Annealing	59 °C	30 s
4	Elongation	72 °C	1:10 min
5	Final Extension	72 °C	2 min
6	Hold	12 °C	unlimited

Q5 cPCR 35S_eGFP_A_BAR and 35S_eGFP_B_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 06 Jul 2026 11:46:25 UTC
Entry Last Modified: 06 Jul 2026 11:55:00 UTC
Export Generated On: 05 Oct 2026 11:21:37 UTC

MONTAG, 6.7.2026

For B Colonies 12, 13 and 15 were repicked.
For A Colonies 1 to 6 were repicked.

Q5 cPCR setup

	A	B	C
1		1x [µl]	10.5 [µl]
2	dH20	16	168
3	Q5 Buffer	5	52.5
4	5 mM dNTPs	1	10.5
5	10 mM Primer BP12	1	10.5
6	10 mM Primer oSNM04	1	10.5
7	Q5 Polymerase	1	10.5

Table1

	A	B	C	D	E	F
1	1 cycle	30 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	58 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:15	01:10	02:00	endless

Expected Amplicon size for 35S_eGFP_A_BAR is 2085 bp

For 35S_eGFP_B_BAR it is 1850 bp

Loading scheme

	A	B	C	D	E	F	G	H
1								

F3+intein and F2

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 13 Jul 2026 11:24:42 UTC

Entry Last Modified: 13 Jul 2026 11:24:47 UTC

Export Generated On: 05 Oct 2026 11:22:54 UTC

MONTAG, 13.7.2026

PCR purification

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 13 Jul 2026 11:26:38 UTC

Entry Last Modified: 13 Jul 2026 11:26:43 UTC

Export Generated On: 05 Oct 2026 11:23:02 UTC

MONTAG, 13.7.2026

35S_splitGFP_bar_A and B Q5 cPCR

Project: iGEM Bonn 2026 Shared Project

Author: Ruben Langenbacher

Status: In progress

Entry Created On: 14 Jul 2026 13:46:46 UTC

Entry Last Modified: 14 Jul 2026 16:02:06 UTC

Export Generated On: 05 Oct 2026 11:21:45 UTC

DIENSTAG, 14.7.2026

Transformations from 13.07.2026 done by Daniel M. showed succesfull growth.
9 colonies were picked per plate for A and B. One Positive Control using 35S_SplitNLS_B as Template and one negative control. were used.

Expected amplicon size: A=2085 bp, B=1850 bp, pos=2043 bp

Q5 cPCR setup

	A	B	C
1		1x [µl]	20.5x [µl]
2	dH20	16	328
3	Q5 Buffer	5	102,5
4	5 mM dNTPs	1	20,5
5	10 µM Primer oSNM04	1	20,5
6	10 µM Primer BP12	1	20,5
7	Q5 Polymerase	1	20,5

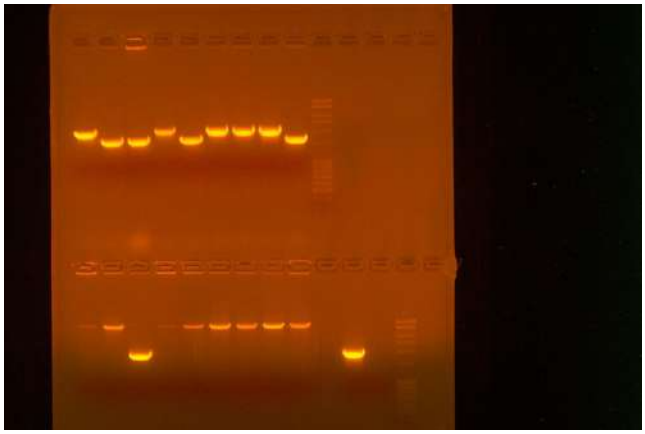
Q5 cPCR running conditions

	A	B	C	D	E	F
1	1 cycle	30 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	58 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	1 min	02:00	endless

Loading scheme

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	A1	A2	A3	A4	A5	A6	A7	A8	A9	100 bp				
2														
3	B1	B2	B3	B4	B5	B6	B7	B8	B9	pos.	neg.	100 bp		
4														
5														

cPCR_igem170726.jpg



mini prep of plasmid split_GFP_A and split_GFP_B

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 15 Jul 2026 09:07:51 UTC
Entry Last Modified: 30 Sep 2026 09:42:41 UTC
Export Generated On: 05 Oct 2026 11:22:21 UTC

MITTWOCH, 15.7.2026

The liquid culture was grown overnight in 2 mL selective medium (with the appropriate antibiotic) at 37 °C while shaking.

750 µl of the culture was transferred into a 1.5 mL tube and centrifuged at 3500 × g for 2 min to pellet the cells.
The supernatant was discarded and the pellet resuspended in 100 µL MP Solution 1 (0,5 mg/ml RNAseA) and rest for 25 minutes at RT.

Next, 300 µL Buffer 2 (pH = 12.3) was added and mixed gently by inverting the tube a few times (no vortexing!).
At this step, care was taken to avoid harsh mixing, as genomic DNA is very fragile and can shear easily, which would lead to contamination.
The lysate was incubated on ice for **max. 2 min.**

After that, 250 µL Buffer 3 (pH = 5.3) was added and the tube was again gently inverted to mix for a few times.
Optionally, a few drops of chloroform can be added here to improve purity (this was not done)
The sample was then centrifuged at fullsped, at -9°C for 10 min.

About 400 µL of the clear supernatant was transferred into a fresh, pre-cooled tube.

1 ml 100%, **ice cold** EtOH was added to the supernatant and the sample was vortext.
(Note: u can also use isoprop, i dont as i have bad experince with it (salt contamination), EtOH worked always perfecly for me.)
ps. voretexing these samples is essential. idk why but the yield increases by that :)

Important:
as it is very warm these days, keep the samples on in the freezer and let the cetrifuge cool down until -9 °C

spin down at fullspeed, at -9°C for 1h

The supernatant was removed, and the pellet washed with 70% ethanol.
After removing the ethanol completely (P20 might help), the pellet was briefly air-dried.
Finally, the DNA was resuspended in 20 µL H_2O and the concentration was measured at the nanodrop
Next, the plasmids will be digested via REs.

F3+intein+cage Q5 PCR

Project: iGEM Bonn 2026 Shared Project

Author: Ruben Langenbacher

Status: In progress

Entry Created On: 15 Jul 2026 14:09:01 UTC

Entry Last Modified: 15 Jul 2026 14:12:46 UTC

Export Generated On: 05 Oct 2026 11:23:12 UTC

MITTWOCH, 15.7.2026

Q5 PCr setup

	A	1x [µl]	3.5x [µl]
1	dH20	15	52.5
2	Q5 Buffer	5	17.5
3	5 mM dNTPs	1	3.5
4	10 mM Primer I26060	1	3.5
5	10 mM Primer oSNM41	1	3.5
6	Q5 Polymerase	1	3.5
7	Template (35s_splitNLS_B_bar)	1	3.5

Table1

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	96°C	96°C	58 °C	72°C	72°C	12°C
4	02:00	00:15	00:30	02:00	02:00	endless

analytic digest of GFP_A and GFP_N

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 16 Jul 2026 09:41:57 UTC

Entry Last Modified: 16 Jul 2026 10:05:38 UTC

Export Generated On: 05 Oct 2026 11:22:29 UTC

DONNERSTAG, 16.7.2026

Table1

	A	B	C
1	reagent	x 1	x 6.5
2	H2O	7 µl	45,5
3	10 x HiFI Buffer (accroding to enzyme)	1 µl	6.5 µl
4	RE1	0.5 µl	3.25 µl
5	RE2	0.5 µl	3.25 µl
6	template	1 µl	per tube
7	total	10 µl	65 µl

NEVER ADD MORE THAN 10% VOLUME OF RESTRICTIONSENZYME AS THE GLYREOL CAN INHIBIT PLLSSS DONT DO THAT

only 1 µl per 10 µl reaction .. if u need to use multilpe RE to digest, then split them equal so both make up the volume of 1 µl (per reaction, 10 µl)

Electroporation of AGL1 A.tumefaciens with 35S_splitGFP_A_bar and 35S_splitGFP_B_bar

Project: iGEM Bonn 2026 Shared Project

Author: Ruben Langenbacher

Status: In progress

Entry Created On: 17 Jul 2026 12:29:21 UTC

Entry Last Modified: 17 Jul 2026 12:43:37 UTC

Export Generated On: 05 Oct 2026 11:21:55 UTC

FREITAG, 17.7.2026

-2 x 25 yl of Electrocompetent A.tumefaciens AGL1 were thawed on ice.

-1 yl of 35S_splitGFP_A_BAR or 35S_splitGFP_B

_BAR or 35S_NLSsplitGFP_B_BAR Plasmid from miniprep were added.

-Cells+DNA were added into pre cooled electroporation cuvettes. Tapped to remove air bubbles and kept on ice for 10 min

-electroporation with 2.2 kV for 6 ms

-1 ml of roomtemperature YEP medium added to each sample immediatly after electroporation and transfered to 1.5 mL eppi

-Incubated for 2 h at 28 °C 1400 rpm

-cells pelleted by centrifuging 3500 g 8500 rpm r = 9.5 cm for 1 min. Removed supernatant, resuspended in return flow and plated on YEP+Rif+Gent+Spec plates.

-incubated at 28 °C

Preperation for Nanoporesequencing of 35S_eGFP_A_BAR and 35S_eGFP_B_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 21 Jul 2026 09:30:55 UTC
Entry Last Modified: 21 Jul 2026 11:46:30 UTC
Export Generated On: 05 Oct 2026 11:22:46 UTC

DONNERSTAG, 16.7.2026

-nanodrop measurements: 35S_eGFP_B_BAR conc. 1510.2 ng/yl and 35S_eGFP_A_BAR conc. 1812.9 ng/yl
-for in house Nanopore Sequencing 10 yl with a conc. of around 50 ng/yl were required.
-The Plasmids used here came from miniprep done by Julia Hokadov 14.07.2026. Plasmids used for sequencing are from colonies A1 and B3.
-For 35S_eGFP_A_BAR 0.28 yl of Plasmid solution were added to 9.72 yl of dH20.
-For 35S_eGFP_B_BAR 0.33 yl of Plasmid solution were added to 9.67 yl of dH20.

DIENSTAG, 21.7.2026

cPCR of A.tumefaciens (35S_splitEGFP_A_bar and 35S_splitEGFP_B_bar)

Project: iGEM Bonn 2026 Shared Project

Author: Ruben Langenbacher

Status: In progress

Entry Created On: 22 Jul 2026 13:23:30 UTC

Entry Last Modified: 22 Jul 2026 13:33:27 UTC

Export Generated On: 05 Oct 2026 11:22:12 UTC

MONTAG, 20.7.2026

-transformation from 17.07.2026 plates showed growth for both constructs.

-4 Colonies were picked for 35S_splitEGFP_B_bar and 3 for 35S_splitEGFP_A_bar

-picked colonies were out on a fresh YEP+Spec+Carb+Ace Masterplate

Table1

	A	B	C
1		1x [µl]	8.5x [µl]
2	dH20	16	136
3	Q5 Buffer	5	42,5
4	5 mM dNTPs	1	8,5
5	10 µM Primer oSNM04	1	8,5
6	10 µM Primer BP12	1	8,5
7	Q5 Polymerase	1	8,5

Table2

	A	B	C	D	E	F
1	1 cycle	30 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	58 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	1 min	02:00	endless

cPCR was not succesfull the gel showed no bands at all.

DIENSTAG, 21.7.2026

The cPCR from 20.07.2026 was repeated. Although 2 of the 3 Colonies for A did not grow on the Masterplate.
Positive controll for both plasmids was used by addin 1 yl of Plasmid solution to the PCR mix.

Table3

	A	B	C
1		1x [µl]	8.5x [µl]
2	dH20	16	136
3	Q5 Buffer	5	42,5
4	5 mM dNTPs	1	8,5
5	10 µM Primer oSNM04	1	8,5
6	10 µM Primer BP12	1	8,5
7	Q5 Polymerase	1	8,5

Table4

	A	B	C	D	E	F
1	1 cycle	30 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	58 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	2 min	02:00	endless

Untitled

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 05 Aug 2026 07:24:22 UTC

Entry Last Modified: 05 Aug 2026 07:24:22 UTC

Export Generated On: 05 Oct 2026 11:22:04 UTC

MITTWOCH, 5.8.2026

improved cystein region GFP

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 05 Aug 2026 07:35:15 UTC
Entry Last Modified: 05 Aug 2026 08:06:06 UTC
Export Generated On: 05 Oct 2026 11:22:38 UTC

MITTWOCH, 5.8.2026

PCR (Q5)

1. Objective

The B plasmid of out intein constucts where not having a cysteine, which in needed for the transsplicing. Therefor we take the NLS-B (encodes whole GFP) and use primers to the GFP (we only need the C-termnal truncated part) and indtroduce the cysteine via the primers. For this, the GFP and the intein needs to be splitted (see here to understand: https://benchling.com/igembonn2026/f/lib_xRI2Jvmm9n-improved_intein/seq_Ab2XuUcS5A-copy-of-cage_intein_split_gfp/edit). Therefor the Insert will be two fragments in the assembly.
The stragety is, that the cystein is introduced via the overlap in the primers (therefor we also need to split the insert)

2. Reaction Setup Parameters

Two Reactions where performed. Both runned at the same extension time.

first:
Intein with Cys overlap: I26097 and AP14
annealing at 63 °C

second:
GFP with cys n-terminal: I26098 and AP15
annealing at 62 °C

3. Reactions Overview

Reactions Overview

	Reaction #	Target / Fragment	Fwd Primer	Rev Primer	Template	Expected size (bp)
1	1	Intein	AP14	I26097	NLS-B	351
2	2	GFP	I26098	AP15	NLS-B	579
3						

4. Q5 PCR Reaction Mix

Standard 25 µl reaction. The Mastermix column = per-reaction volume × number of reactions (from the Parameters table).

PCR Mix

	Component	Per reaction [µl]	Mastermix [µl]
1	dH2O	15	37.5
2	5x Q5 Buffer	5	12.5
3	5 mM dNTPs	1	2.5
4	10 µM Primer fwd	1	2.5
5	10 µM Primer rev	1	2.5
6	Q5 Polymerase	1	2.5
7	Template	1	2.5
8	Total	25	60

5. PCR Program

PCR Program

	Step	Initial Denat.	Denaturation	Annealing	Elongation	Final Elongation	Hold
1	Cycles	1x	30x	30x	30x	1x	1x
2	Temperature (°C)	98	98		72	72	12
3	Time (mm:ss)	05:00	00:15	00:30	00:30	02:00	∞

6. Gel Electrophoresis

Gel Loading Scheme

	Lane	Sample	Expected size (bp)
1	1	DNA ladder	
2	2	intein	
3	3	GFP	
4			

Agarose gel: 1.5 %, run at 100 V for 40 min. Attach gel image below.

7. Results & Notes

Record outcome (successful/failed amplification per reaction), band sizes observed, and any troubleshooting or deviations from the protocol.

P1-P2, lox-term., GFP, mCherry

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 05 Aug 2026 08:11:58 UTC
Entry Last Modified: 05 Aug 2026 08:26:50 UTC
Export Generated On: 05 Oct 2026 11:25:40 UTC

MITTWOCH, 5.8.2026

PCR (Q5)

1. Objective

To see, if out cre contruct is working, we need a Reporter plasmid.
The GFP is floxed and upon the trans-splicing the cre is recombining and removes the signal from the reporter plasmid.

2. Reaction Setup Parameters

four Reactions where performed. Both runned at the same extension time.

first:
P1-P2: I26094, I26093
annealing: 57 °C

second:
GFP: I26091, I26092
annealing: 62 °C

thrid:
lox-term.: AP14, I26096
annealing: 61 °C

fourth:
mCherry: I26098, I26090
annealing: 62 °C

3. Reactions Overview

Reactions Overview

	Reaction #	Target / Fragment	Fwd Primer	Rev Primer	Template	Expected size (bp)
1	1	Barstar	I26094	I26093	barstar gBlock	312
2	2	GFP	I26091	I26092	NLS-B	768
3	3	lox / term.	AP14	I26096	lox. term gBlock	1216
4	4	mcherry	I26089	I26090	mCherry plamid genetics	739

4. Q5 PCR Reaction Mix

Standard 25 µl reaction. The Mastermix column = per-reaction volume × number of reactions (from the Parameters table).

PCR Mix

	Component	Per reaction [µl]	Mastermix [µl]
1	dH2O	15	
2	5x Q5 Buffer	5	
3	5 mM dNTPs	1	
4	10 µM Primer fwd	1	
5	10 µM Primer rev	1	
6	Q5 Polymerase	1	
7	Template	1	
8	Total	25	

5. PCR Program

PCR Program

	Step	Initial Denat.	Denaturation	Annealing	Elongation	Final Elongation	Hold
1	Cycles	1x	30x	30x	30x	1x	1x
2	Temperature (°C)	98	98		72	72	12
3	Time (mm:ss)	05:00	00:15	00:30	00:30	02:00	∞

6. Gel Electrophoresis

Gel Loading Scheme

	Lane	Sample	Expected size (bp)
1	1	DNA ladder	
2	2	other sample (ingnore)	---
3	3	other sample (ignore)	---
4	4	empty	---
5	5	P1-P2	312
6	6	GFP	768
7	7	lox term.	1216
8	8	mcherry	739

Agarose gel: 1.5 %, run at 100 V for 40 min. Attach gel image below.

7. Results & Notes

Record outcome (successful/failed amplification per reaction), band sizes observed, and any troubleshooting or deviations from the protocol.

Assembly & Transformation of splitGFP_A & splitGFP_B

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 06 Aug 2026 15:28:21 UTC
Entry Last Modified: 06 Aug 2026 15:48:26 UTC
Export Generated On: 05 Oct 2026 11:25:20 UTC

SAMSTAG, 27.6.2026

Assembly

The plasmids were assembled using previosly amplified parts of the 35S_BAR backbone containing the intein domains and the amplified halves of the GFP CDS.

Parts and quantitys used for assembly (all parts are concentrated equimolar for assembly)

	A	B	C	D	E	F	G	H
1	Plasmid	35S_BAR Fragment 1	35S_BAR Fragment 2	35S_BAR Fragment 3	35S_BAR Fragment 1 + intein	35S_BAR Fragment 3 + intein	GFP N-term	GFP C-term
2	splitGFP_A	x	1 µl	1 µl	2 µl	x	1 µl	x
3	splitGFP_B	1 µl	1 µl	x	x	2 µl	x	1 µl

The assemblys were performed used the NEBuilder kit at a final reaction volume of 10 µl.
After 1 h of incubation at 50 °C the plasmids were immediately transformed into chemically competent XL1-blue *E. coli*.

E. coli transformation

Aliquot of chemically competent XL1-blue *E. coli* was thawed on ice. Once thawed 10 µl of assembled plasmid was added and the bacteria were incubated on ice for 15 minutes. After incubation the bacteria were heat shocked at 42°C for 90 seconds. Following the heat shock the bacteria were put back on ice and incubated for another 5 minutes. Afterwards 850 µl of SOC medium preheated to 37°C were added and the bacteria were icubated at 37°C, 850 rpm for 1 hour to express the antibiotics resistance. After incubation the cells were pelleted by centrifugation at 3000x g for 5 minutes and 850 µl of the supernatant was discarded. The pellet was dissolved in the remaining supernatant and plated on a LB (Spectinomycin 100 ng/µl) plate. The bacteria were grown overnight at 37°C.

Assembly & Transformation of splitGFP_B_Cys

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 06 Aug 2026 15:43:15 UTC
Entry Last Modified: 06 Aug 2026 15:48:20 UTC
Export Generated On: 05 Oct 2026 11:25:30 UTC

DONNERSTAG, 6.8.2026

Assembly

Parts and quantitys used for assembly (all parts are concentrated equimolar for assembly)

	A	B	C	D	E
1	35S_BAR Fragment 1	35S_BAR Fragment 2	35S_BAR Fragment 3	Intein Cys	GFP Cys
2	1.33 µl	1.99 µl	1.33 µl	0.1 µl	0.25 µl

The assembly was performed used the NEBuilder kit at a final reaction volume of 10 µl.
After 1 h of incubation at 50 °C the plasmids were immediately transformed into chemically competent XL1-blue *E. coli*.

***E. coli* transformation**
Aliquot of chemically competent XL1-blue *E. coli* was thawed on ice. Once thawed 10 µl of assembled plasmid was added and the bacteria were incubated on ice for 15 minutes. After incubation the bacteria were heat shocked at 42°C for 90 seconds. Following the heat shock the bacteria were put back on ice and incubated for another 5 minutes. Afterwards 850 µl of SOC medium preheated to 37°C were added and the bacteria were icubated at 37°C, 850 rpm for 1 hour to express the antibiotics resistance. After incubation the cells were pelleted by centrifugation at 3000x g for 5 minutes and 850 µl of the supernatant was discarded. The pellet was dissolved in the remaining supernatant and plated on a LB (Spectinomycin 100 ng/µl) plate. The bacteria were grown overnight at 37°C.

Colony PCR for splitGFP_B_cys

Project: iGEM Bonn 2026 Shared Project

Author: Julius Krupke

Status: In progress

Entry Created On: 12 Aug 2026 16:49:31 UTC

Entry Last Modified: 12 Aug 2026 17:19:39 UTC

Export Generated On: 05 Oct 2026 11:26:35 UTC

SONNTAG, 9.8.2026

Colony PCR was performed for 11 colonies.

Primers BP12 and I26071 were used for amplification.

As positive control the plasmid NLSsplitGFP_B was used.

Q5 Colony PCR setup

	A	B	C
1		1x [µl]	13.5x [µl]
2	dH20	16	202.5
3	Q5 Buffer	5	67.5
4	5 mM dNTPs	1	13.5
5	10 µM BP12	1	13.5
6	10 µM I26071	1	13.5
7	Q5 Polymerase	1	13.5

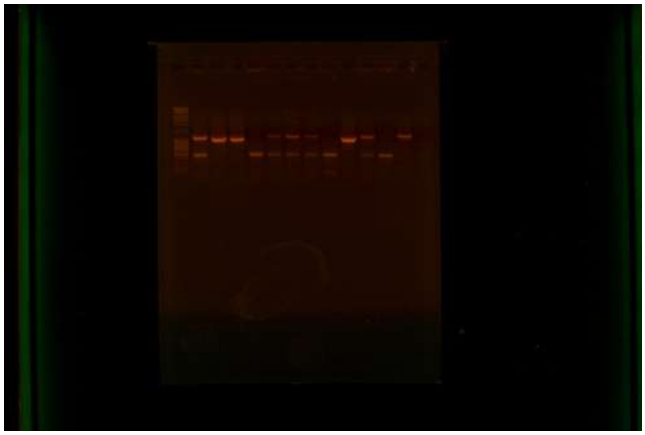
PCR programm

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	57°C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	00:40	02:00	endless

Gel loading scheme

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	100 bp ext.	1	2	3	4	5	6	7	8	9	10	11	+	-

IGEM_cPCR_09.08.26.jpg



Plasmid extraction for splitGFP_B_cys

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 12 Aug 2026 17:22:04 UTC
Entry Last Modified: 12 Aug 2026 17:24:32 UTC
Export Generated On: 05 Oct 2026 11:25:10 UTC

DIENSTAG, 11.8.2026

Two 5 ml dYT (spectinomicin 100 ng/µl) liquid culture was inoculated with colony colonies number 3 and 9 from the colony PCR.
The cultures were grown over night at 37 °C, 188 rpm.

MITTWOCH, 12.8.2026

The cells grown in the 5 ml liquid culture were pelleted by centrifugation at 3000 xg for 10 minutes. The supernatant was discarded and 200 µl of BibDo1 buffer with 0.5% RNAseA freshly added was pipetted onto the pellet. The pellet was resuspended in the BibDo1 buffer by vortexing and transferred into a 1.5 ml reaction tube. After incubation of 10 minutes at RT 400 µl of BibDo2 buffer was added, the tube was inverted 10 times and incubated on ice for 5 minutes. After incubation 300 µl of BibDo3 buffer was added and the tube was inverted another 10 times before incubation on ice for 10 minutes. Afterwards the cell debris was pelleted by centrifugation at 13000 xg for 10 minutes. After centrifugation the supernatant was transferred into a new 1.5 ml reaction tube, filled to 1.5 ml with cold isopropanol and incubated at -20°C for 30 minutes. After incubation the Plasmid DNA was pelleted by centrifugation at 13000 xg and 4°C for 20 minutes. The resulting pellet was washed twice with 70% ethanol and resuspended in 50 µl deionized water.

Bibdo1 buffer

	Chemical	Concentration
1	Tris	25 mM
2	EDTA	10 mM
3	Glucose	50 mM

Bibdo2 buffer

	Chemical	Concentration
1	NaOH	0.2 M
2	SDS	1 %

Bibdo3 buffer

	Chemical	Concentration
1	potassium acetate	3 M
2	acetic acid	11.5 %

N-cre and C-cre MP

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 19 Aug 2026 09:00:10 UTC
Entry Last Modified: 25 Sep 2026 08:32:44 UTC
Export Generated On: 05 Oct 2026 11:25:51 UTC

MITTWOCH, 19.8.2026

The liquid culture was grown overnight in 2 mL selective medium (with the appropriate antibiotic) at 37 °C while shaking.

750 µl of the culture was transferred into a 1.5 mL tube and centrifuged at 3500 × g for 2 min to pellet the cells.
The supernatant was discarded and the pellet resuspended in 100 µL MP Solution 1 (0,5 mg/ml RNAseA) and rest for 25 minutes at RT.

Next, 300 µL Buffer 2 (pH = 12.3) was added and mixed gently by inverting the tube a few times (no vortexing!).
At this step, care was taken to avoid harsh mixing, as genomic DNA is very fragile and can shear easily, which would lead to contamination.
The lysate was incubated on ice for **max. 2 min.**

After that, 250 µL Buffer 3 (pH = 5.3) was added and the tube was again gently inverted to mix for a few times.
Optionally, a few drops of chloroform can be added here to improve purity (this was not done)
The sample was then centrifuged at fullsped, at -9°C for 10 min.

About 400 µL of the clear supernatant was transferred into a fresh, pre-cooled tube.

1 ml 100%, **ice cold** EtOH was added to the supernatant and the sample was vortext.
(Note: u can also use isoprop, i dont as i have bad experince with it (salt contamination), EtOH worked always perfecly for me.)
ps. voretexing these samples is essential. idk why but the yield increases by that :)

Important:
as it is very warm these days, keep the samples on in the freezer and let the cetrifuge cool down until -9 °C

spin down at fullspeed, at -9°C for 1h

The supernatant was removed, and the pellet washed with 70% ethanol.
After removing the ethanol completely (P20 might help), the pellet was briefly air-dried.
Finally, the DNA was resuspended in 20 µL H_2O and the concentration was measured at the nanodrop
Next, the plasmids will be digested via REs.

PCR fragments split Cre

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 19 Aug 2026 09:01:47 UTC
Entry Last Modified: 19 Aug 2026 09:05:52 UTC
Export Generated On: 05 Oct 2026 11:26:13 UTC

MITTWOCH, 19.8.2026

C-Cre:
I26069 and I26062
anneal at 62 °C
1235 bp size
elongation 30s
tamplate: gen synthese C-Intein-Cre

N-Cre:
I26063 and I26013
anneal at 67 °C
797 bp size
elongation 30s
tamplate: gen synthese N-Intein-Cre

PCR MM:

Table1

	A	B
1	H2O	15 µl
2	diy Q5 buffer	5 µl
3	Rv	1 µl
4	Fw	1 µl
5	dNTPs	1 µl
6	Q5	1 µl
7	template	see above

PCR fragments for the reporter plasmid

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 19 Aug 2026 09:06:00 UTC
Entry Last Modified: 19 Aug 2026 09:11:29 UTC
Export Generated On: 05 Oct 2026 11:26:24 UTC

MITTWOCH, 19.8.2026

Table1

	A	B	C	D	E
1	primer pair	tm annealing	expected size	template	fragment name
2	94/93	57 °C	312 bp	bastar gen synthese	P1-P2
3	91/92	62 °C	768 bp	NLS_B	GFP
4	AP14/96	61 °C	1216 bp	barstar gen syntese	lox / term
5	89/90	62 °C	739 bp	mch (gruss)	mcherry
6					

all of the fragments had a elongation of 30s and 30 cycels. (35 would be also fine and should be done the next time)

Table2

	A	B
1	H2O	15 µl
2	diy Q5 buffer	5 µl
3	Rv	1 µl
4	Fw	1 µl
5	dNTPs	1 µl
6	Q5	1 µl
7	template	see above

Untitled

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 24 Aug 2026 08:56:05 UTC

Entry Last Modified: 24 Aug 2026 08:56:05 UTC

Export Generated On: 05 Oct 2026 11:24:47 UTC

MONTAG, 24.8.2026

Untitled

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 24 Aug 2026 09:00:02 UTC

Entry Last Modified: 24 Aug 2026 09:00:02 UTC

Export Generated On: 05 Oct 2026 11:24:58 UTC

MONTAG, 24.8.2026

reporter plasmid MP

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 25 Aug 2026 07:51:30 UTC
Entry Last Modified: 03 Sep 2026 10:35:47 UTC
Export Generated On: 05 Oct 2026 11:26:02 UTC

DIENSTAG, 25.8.2026

The liquid culture was grown overnight in 2 mL selective medium (with the appropriate antibiotic) at 37 °C while shaking.

750 µl of the culture was transferred into a 1.5 mL tube and centrifuged at 3500 × g for 2 min to pellet the cells.
The supernatant was discarded and the pellet resuspended in 100 µL MP Solution 1 (0,5 mg/ml RNAseA) and rest for minutes at RT.

Next, 300 µL Buffer 2 (pH = 12.3) was added and mixed gently by inverting the tube a few times (no vortexing!).
At this step, care was taken to avoid harsh mixing, as genomic DNA is very fragile and can shear easily, which would lead to contamination.
The lysate was incubated on ice for **max. 2 min.**

After that, 250 µL Buffer 3 (pH = 5.3) was added and the tube was again gently inverted to mix for a few times.
Optionally, a few drops of chloroform can be added here to improve purity (this was not done)
The sample was then centrifuged at fullsped, at -9°C for 10 min.

About 400 µL of the clear supernatant was transferred into a fresh, pre-cooled tube.

1 ml 100%, **ice cold** EtOH was added to the supernatant and the sample was vortext.
(Note: u can also use isoprop, i dont as i have bad experince with it (salt contamination), EtOH worked always perfecly for me.)
ps. voretexing these samples is essential. idk why but the yield increases by that :)

Important:
as it is very warm these days, keep the samples on in the freezer and let the cetrifuge cool down until -9 °C

spin down at fullspeed, at -9°C for 1h

The supernatant was removed, and the pellet washed with 70% ethanol.
After removing the ethanol completely (P20 might help), the pellet was briefly air-dried.
Finally, the DNA was resuspended in 20 µL H_2O and the concentration was measured at the nanodrop
Next, the plasmids will be digested via REs.

Untitled

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 26 Aug 2026 07:50:28 UTC

Entry Last Modified: 26 Aug 2026 07:50:28 UTC

Export Generated On: 05 Oct 2026 11:26:47 UTC

MITTWOCH, 26.8.2026

Electroporation of A.tumefaciens with 35S_splitCre_A 35S_splitCre_B and 35S_lox_mCherry_eGFP

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 26 Aug 2026 14:37:03 UTC
Entry Last Modified: 26 Aug 2026 14:40:06 UTC
Export Generated On: 05 Oct 2026 11:30:18 UTC

MITTWOCH, 26.8.2026

-3 x 25 yl of Electrocompetent GV3101 A.tumefaciens were thawed on ice.
-1 yl of 35S_splitCre_A ,35S_splitCre_B and 35S_lox_mCherry_eGFP Plasmid from miniprep were added.
-Cells+DNA were added into pre cooled electroporation cuvettes. Tapped to remove air bubbles and kept on ice for 10 min
-electroporation with 2.2 kV for 6 ms
-1 ml of roomtemperature YEP medium added to each sample immediatly after electroporation and transfered to 1.5 mL eppi
-Incubated for 2 h at 28 °C 1400 rpm
-cells pelleted by centrifuging 3500 g 8500 rpm r = 9.5 cm for 1 min. Removed supernatant, resuspended in return flow and plated on YEP+Rif+Gent+Spec plates.
-incubated at 28 °C

PCR for Verification of P2 in 35S_lox_mCherry_eGFP

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 26 Aug 2026 14:40:23 UTC
Entry Last Modified: 27 Aug 2026 13:12:36 UTC
Export Generated On: 05 Oct 2026 11:30:30 UTC

MITTWOCH, 26.8.2026

-As template 1 yl of 1:10 diluted 35S_lox_mCherry_eGFP was used. A negative control was used.
-Expected amplicon size 265 bp.

PCR setup

	A	B	C
1		1x [µl]	2.5x [µl]
2	dH20	15	37.5
3	Q5 Buffer	5	12.5
4	5 mM dNTPs	1	2.5
5	10 µM Primer for I26050	1	2.5
6	10 µM Primer rev I26053	1	2.5
7	Q5 Polymerase	1	2.5

PCR running conditions

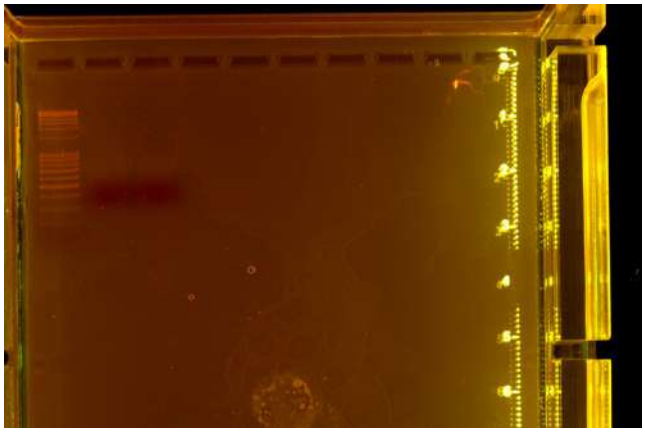
	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	60 °C	72 °C	72 °C	4 °C
4	02:00	00:15	00:30	00:20	02:00	endless

-A 1.6% 50 ml agarose gel was prepared. Run at 120 V for 30 min.

Loading scheme

	A	B	C
1	100 bp ladder	1	neg
2			

igem_2026_08_25_p2.tiff



-PCR was no succesfull

-new primer were used for second setup

PCR setup

	A	B	C
1		1x [µl]	2.5x [µl]
2	dH20	15	37.5
3	Q5 Buffer	5	12.5
4	5 mM dNTPs	1	2.5
5	10 µM Primer for I26093	1	2.5
6	10 µM Primer rev I26094	1	2.5
7	Q5 Polymerase	1	2.5

-expected ampicon size 305 bp

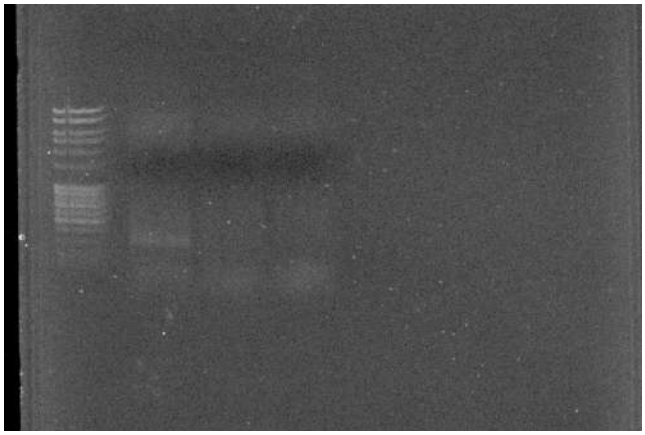
PCR running conditions

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	68 °C	72 °C	72 °C	4 °C
4	02:00	00:15	00:30	00:20	02:00	endless

Loading scheme

	A	B	C	D
1	100 bp ladder	1:10	1:100	neg.
2				

igem_pcr_27.08_veri.jpg



-even tough the resolution of this geldoc picture is bad we concluded that the band visible for PCR with 1:10 dilution is at 300 bp.

Q5 PCR for backbone F1, F2 and F3 from 35S_splitNLS_B

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 28 Aug 2026 13:16:51 UTC
Entry Last Modified: 28 Aug 2026 15:53:57 UTC
Export Generated On: 05 Oct 2026 11:30:42 UTC

FREITAG, 28.8.2026

PCR setup

	A	1x [µl]	7.5x [µl]
1	ddH20	15	112.5
2	dNTPs	1	7.5
3	Primer for 1:10	1	
4	Primer rev 1:10	1	
5	Q5	1	7.5
6	Q5 Buffer 1:5	5	37.5
7	Template (35S_splitNLS_B 1:10)	1	

Used Primers

	A	B	C
1	F1	oSNM38	JK20
2	F2	oSNM39	oSNM40
3	F3	oSNM41	AP04
4			

PCR setup for F1/F3

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	elongation	elongation	hold
3	98 °C	98 °C	57 °C	72 °C	72 °C	4 °C
4	2 min	00:15 min	00:30 min	1:30 min	5 min	hold

PCR setup for F2

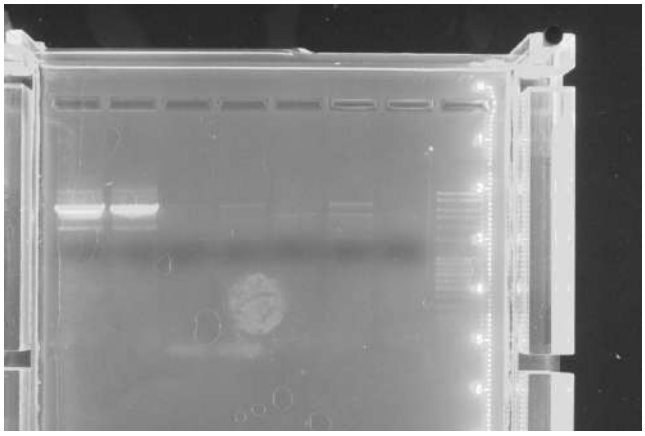
	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	elongation	elongation	hold
3	98 °C	98 °C	61 °C	72 °C	72 °C	4 °C
4	2 min	00:15 min	00:30 min	1:30 min	5 min	hold

-1 % Agarose Gel run at 100 V for 45 min.

Loading scheme

	A	B	C	D	E	F	G	H
1	F1.1	F1.2	F2.1	F2.2	F3.1	F3.2	neg.	100 bp
2								

f123_igem_28.08.26.tiff



Q5 PCR for GFP+Intein_B and only GFP

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 28 Aug 2026 13:25:57 UTC
Entry Last Modified: 28 Aug 2026 15:53:27 UTC
Export Generated On: 05 Oct 2026 11:30:58 UTC

FREITAG, 28.8.2026

PCR setup

	A	1x [µl]	5.5x [µl]
1	ddH20	15	82.5
2	dNTPs	1	5.5
3	Primer for 1:10	1	
4	Primer rev 1:10	1	
5	Q5	1	5.5
6	Q5 Buffer 1:5	5	27.5
7	Template	1	

-as Template 35S_splitGFP_B for GFP+Intein_B and 35S_splitGFP_A_Cys for only GFP were used. 1 yl each of 1:10 dilution.

Used Primers

	A	B	C
1	GFP+intein	I26103	I26071
2	only GFP	AP14	I26105

PCR setup for GFP+Intein B

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	elongation	elongation	hold
3	98 °C	98 °C	65 °C	72 °C	72 °C	4 °C
4	2 min	00:15 min	00:30 min	00:30 min	5 min	hold

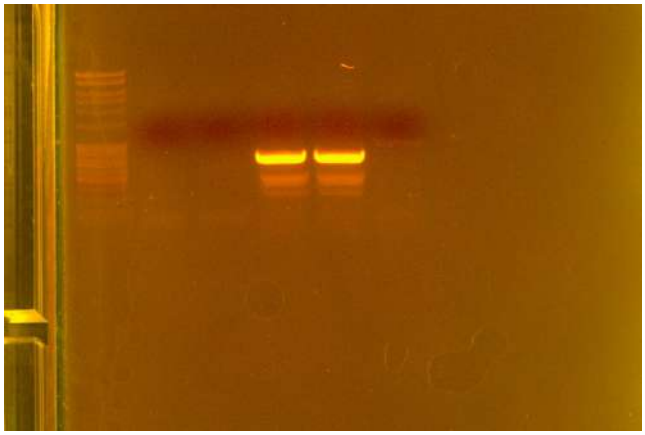
PCR setup for only GFP (A)

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	elongation	elongation	hold
3	98 °C	98 °C	64 °C	72 °C	72 °C	4 °C
4	2 min	00:15 min	00:30 min	00:30 min	5 min	hold

Table1

	A	B	C	D	E	F
1	100 bp ladder	A1	A2	B1	B2	neg.
2						

igem_gfp_inteinpcr_2_28.08.26.tiff



Untitled

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 31 Aug 2026 14:05:42 UTC

Entry Last Modified: 31 Aug 2026 14:05:42 UTC

Export Generated On: 05 Oct 2026 11:32:11 UTC

MONTAG, 31.8.2026

Q5 cPCR improved 35S_splitGFP_A, Miniprep of improved 35S_splitGFP_A

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 02 Sep 2026 11:57:33 UTC
Entry Last Modified: 03 Sep 2026 14:46:44 UTC
Export Generated On: 05 Oct 2026 11:31:14 UTC

MITTWOCH, 2.9.2026

-9 colonies were picked from plate 1 from the Assembly (01.09.2026). Negative controll was introduced.

-Expected amplicon size 872 bp.

Q5 cPCR setup

	A	B	C
1		1x [µl]	10.5x [µl]
2	dH20	16	168
3	Q5 Buffer	5	52.5
4	5 mM dNTPs	1	10.5
5	10 µM Primer AP15	1	10.5
6	10 µM Primer BP12	1	10.5
7	Q5 Polymerase	1	10.5

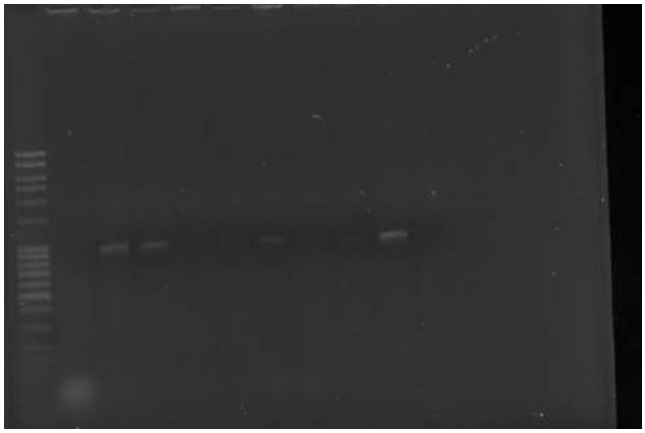
Q5 cPCR running conditions

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	59 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	1 min	02:00	endless

Loading scheme

	A	B	C	D	E	F	G	H	I	J	K
1	100 bp ladder	1	2	3	4	5	6	7	8	9	neg.
2											

igem_cpcr_02.09.26_.tiff



-succesfull cPCR for C2, C3, C6, C8 and C9
-colonies 2,3 and 9 were picked again and used to inoculate 3x 2 ml LB Media with 100 yg/ml Spec for Miniprep the next day. Incubated at 37 °C, 144 rpm over night.

DONNERSTAG, 3.9.2026

-for miniprep C9 was chosen.
- 750 µl of the culture was transferred into a 1.5 mL tube and centrifuged at 3500 × g for 2 min to pellet the cells.
The supernatant was discarded and the pellet resuspended in 100 µL MP Solution 1 (0,5 mg/ml RNAseA) and rest for 25 minutes at RT.

Next, 300 µL Buffer 2 (pH = 12.3) was added and mixed gently by inverting the tube a few times (no vortexing!).
At this step, care was taken to avoid harsh mixing, as genomic DNA is very fragile and can shear easily, which would lead to contamination.
The lysate was incubated on ice for **max. 2 min.**

After that, 250 µL Buffer 3 (pH = 5.3) was added and the tube was again gently inverted to mix for a few times.
The sample was then centrifuged at fullsped, at -9°C for 10 min.

About 400 µL of the clear supernatant was transferred into a fresh, pre-cooled tube.

1 ml 100%, **ice cold** EtOH was added to the supernatant and the sample was vortext.

spin down at fullspeed, at -9°C for 1h

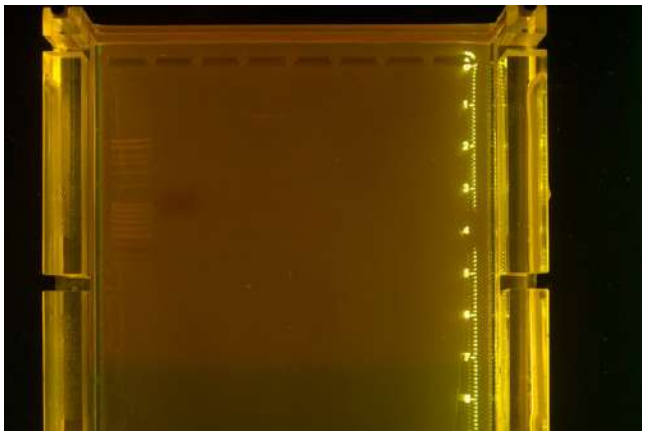
The supernatant was removed, and the pellet washed with 70% ethanol.
After removing the ethanol completely (P20 might help), the pellet was briefly air-dried.
Finally, the DNA was resuspended in 20 µL H_2O and the concentration was measured at the nanodrop
Nanodrop measurement showed a concentration of 260 ng/yl.

1 yl was added to 2 yl of 3x Loading dye and loaded for Gel Elecrophoresis. 120 V for 45 min.

Table1

	A	B	C	D
1	100 bp ladder	F3 PCR		35S_splitGFP_A improved

f3_u_plasmi_03092026_igem.tiff



-Only one band is visible indicating a succesfull Miniprep without gDNA contamination.

Q5 PCR for F3

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 03 Sep 2026 11:20:20 UTC
Entry Last Modified: 03 Sep 2026 14:46:21 UTC
Export Generated On: 05 Oct 2026 11:31:30 UTC

DONNERSTAG, 3.9.2026

-due to limited amounts of PCR reagents no negative controll was used and the PCR mix was prepared 1x instead of the usual 1.5x.

Q5 PCr setup

	A	1x [yl]	C
1	dH20	15	
2	Q5 Buffer	5	
3	5 mM dNTPs	1	
4	10 mM Primer AP04	1	
5	10 mM Primer oSNM41	1	
6	Q5 Polymerase	1	
7	Template (35S_splitGFP_B) 1:10	1	

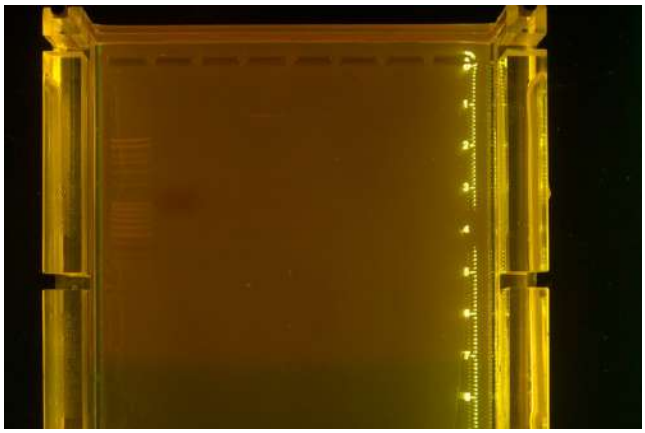
Q5 cPCR running conditions

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	Annealing	Elongation	Elongation	hold
3	98°C	98°C	54 °C	72°C	72°C	12°C
4	02:00	00:20	00:30	02:00	02:00	endless

Table1

	A	B	C	D
1	100 bp ladder	F3		35S_splitGFP_A improved

f3_u_plasmi_03092026_igem.tiff



-PCR was not succesfull

assembly strategy for gfp A split

Project: iGEM Bonn 2026 Shared Project
Author: Julia Hodakov
Status: In progress
Entry Created On: 03 Sep 2026 14:13:55 UTC
Entry Last Modified: 14 Sep 2026 08:00:30 UTC
Export Generated On: 05 Oct 2026 11:30:07 UTC

DONNERSTAG, 3.9.2026

take the template GFP A_Intei_Cage

without cage
109 and 104
at 65 °C, 536 bp

with cage
109 and 013
at 68 °C, 803 bp

fpr both use the fuckes up GFP_A plasmid lmao

Electroporation of GV3101 A.tumefaciens for 35S_splitCre_A, 35S_splitCre_B and 35S_lox_GFP_mCherry

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 04 Sep 2026 15:53:20 UTC

Entry Last Modified: 04 Sep 2026 15:53:24 UTC

Export Generated On: 05 Oct 2026 11:31:46 UTC

FREITAG, 4.9.2026

Electroporation of GV3101 A.tumefaciens for 35S_splitCre_A, 35S_splitCre_B and 35S_lox_GFP_mCherry

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 04 Sep 2026 15:57:00 UTC
Entry Last Modified: 04 Sep 2026 15:58:27 UTC
Export Generated On: 05 Oct 2026 11:31:59 UTC

FREITAG, 4.9.2026

-3 x 25 µl of Electrocompetent GV3101 A.tumefaciens were thawed on ice.
-1 µl off S_splitCre_A, 35S_splitCre_B and 35S_lox_GFP_mCherry were added to seperat Electrocompetent GV3101 A.tumefaciens aliquots.
-Cells+DNA were transferred into pre cooled electroporation cuvettes, tapped to remove air bubbles and kept on ice for 10 min
-The cells were pulsed at 2.2 kV for 6 ms
-Afterwards 1 ml of roomtemperature YEP medium added to each sample and the samples were transfered to 1.5 ml reaction tubes
-The bacteria were incubated for 3 h at 28 °C 1400 rpm
-100 µl of the bacteria solution was plated on YEP+Rif+Gent+Spec plates, afterwards the remaining cells were pellted by centrifugation at 3000x g for 5 minutes. The supernatant was discarded and the pellet was resuspended in the remaining 100 µl of the medium. The remaining 100 µl were also plated on new YEP+Rif+Gent+Spec plates.
-The plates were grown at 28 °C for 2 days

split_cre_a_uncaged

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 05 Sep 2026 16:52:43 UTC

Entry Last Modified: 05 Sep 2026 16:58:06 UTC

Export Generated On: 05 Oct 2026 11:32:23 UTC

SAMSTAG, 5.9.2026

template: gBlock N-Cre

primers: 63 Fv / 104 Rv

annealing: 65°C

size: 530 bp

split_cre_b_uncaged

Project: iGEM Bonn 2026 Shared Project

Author: Julia Hodakov

Status: In progress

Entry Created On: 05 Sep 2026 16:58:12 UTC

Entry Last Modified: 07 Sep 2026 11:22:05 UTC

Export Generated On: 05 Oct 2026 11:32:33 UTC

SAMSTAG, 5.9.2026

template: gBlock C-Cre

primers: 62 Fv / 103 Rv

annealing: 65°C

size: 1012 bp

Q5 PCR for N- and C-Cre without inein cages

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 08 Sep 2026 13:58:27 UTC
Entry Last Modified: 08 Sep 2026 17:54:14 UTC
Export Generated On: 05 Oct 2026 11:30:45 UTC

DIENSTAG, 8.9.2026

Q5 PCR setup

	A	1x [µl]	3.5x [µl]
1	ddH2O	15 µl	52.5
2	25 µM dNTPs	1 µl	3.5
3	Primer for	1 µl	
4	Primer rev	1 µl	
5	5x Q5 Buffer	5 µl	17.5
6	Q5 Polymerase	1 µl	3.5
7	Template	1µl	

Table2

	Primer for	Primer rev	Template
1	I26063	I26104	N-Cre (g-block 1:10)
2	I26062	I26103	C-Cre (g-block 1:10)
3			

Q5 cPCR running conditions

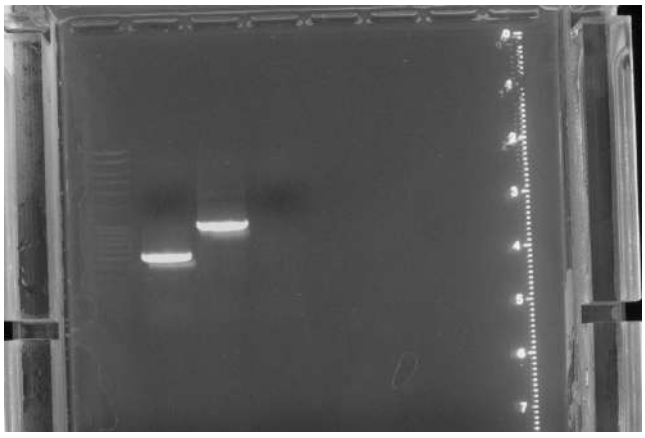
	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	elongation	elongation	hold
3	98 °C	98 °C	65 °C	72 °C	72 °C	4 °C
4	2 min	00:15 min	00:30 min	1 min	2 min	hold

-run at 100 V 0.8 % Agarose gel for 45 min.

Loading scheme

	A	B	C	D
1	100 bp ladder	N-Cre	C-Cre	neg.
2				

pcr_cre_one_int_08.09.26.tiff



Fragments match expected amplicon size. Fragments were exciced and stored in the freezer for Gel elution the following day.

Q5 cPCR A.tumefaciens GV3101 for 35S_splitCre_N and 35S_splitCre_C

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 09 Sep 2026 11:57:38 UTC
Entry Last Modified: 09 Sep 2026 17:44:24 UTC
Export Generated On: 05 Oct 2026 11:30:56 UTC

MITTWOCH, 9.9.2026

-Colonies C4, C5, N1, N2, N3, N4 were picked for cPCR. Colonies were replated on a fresh YEP+Rif+Gent+Spec plate. Neg. controll was used. Pos. controll was used with 35S_split_GFP_B 1:10 as template.

Q5 cPCR setup

	A	1x	8.5x
1	ddH2O	8.5 µl	72.25
2	25 µM dNTPs	0.5 µl	4.25
3	Primer oSNM04	0.5 µl	4.25
4	Primer BP12	0.5 µl	4.25
5	5x Q5 Buffer	2.5 µl	21.25
6	Q5 Polymerase	0.5 µl	4.25
7			

Q5 cPCR running conditions

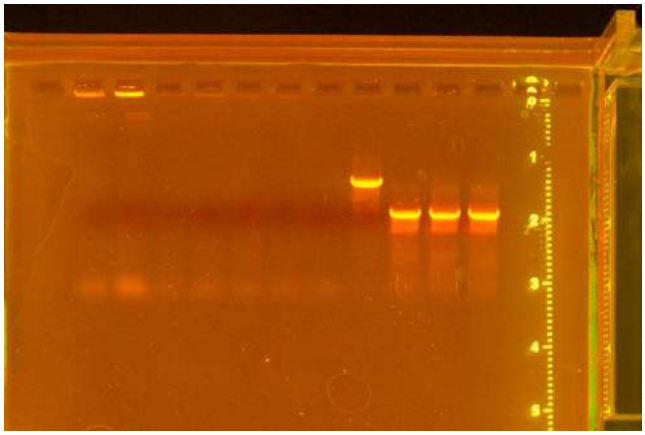
	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	elongation	elongation	hold
3	98 °C	98 °C	65 °C	72 °C	72 °C	4 °C
4	2 min	00:15 min	00:30 min	1 min	2 min	hold

Loading scheme

	A	B	C	D	E	F	G	H	I	J	K	L
1	100 bp	C4	C5	N1	N2	N3	N4	pos.	neg.	1	2	3
2												
3												

0.8% Agarose was run at 100 V for 45 min.

cpcr_09.09_.jpg



-no positve colonies. Pos. controll worked. 1, 2 and 3 PCR were tests for 3 different newly made Q5 buffers
-the ladder was forgotten.

Electroporation of A.tumefaciens with 35S_splitCre_A 35S_splitCre_B and 35S_lox_mCherry_eGFP

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 09 Sep 2026 17:39:29 UTC
Entry Last Modified: 09 Sep 2026 17:39:53 UTC
Export Generated On: 05 Oct 2026 11:30:07 UTC

MITTWOCH, 9.9.2026

-3 x 25 yl of Electrocompetent GV3101 A.tumefaciens were thawed on ice.
-1 yl of 35S_splitCre_A ,35S_splitCre_B and 35S_lox_mCherry_eGFP Plasmid from miniprep were added.
-Cells+DNA were added into pre cooled electroporation cuvettes. Tapped to remove air bubbles and kept on ice for 10 min
-electroporation with 2.2 kV for 6 ms
-1 ml of roomtemperature YEP medium added to each sample immediatly after electroporation and transfered to 1.5 mL eppi
-Incubated for 2.5 h at 28 °C 850rpm
-cells pelleted by centrifuging 3500 g 8500 rpm r = 9.5 cm for 1 min. Removed supernatant, resuspended in return flow and plated on YEP+Rif+Gent+Spec plates.
-incubated at 28 °C

Q5 PCR GFP_A with Intein

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 10 Sep 2026 15:53:37 UTC
Entry Last Modified: 11 Sep 2026 14:14:34 UTC
Export Generated On: 05 Oct 2026 11:31:10 UTC

DONNERSTAG, 10.9.2026

pcr_10.09.2026.tiff

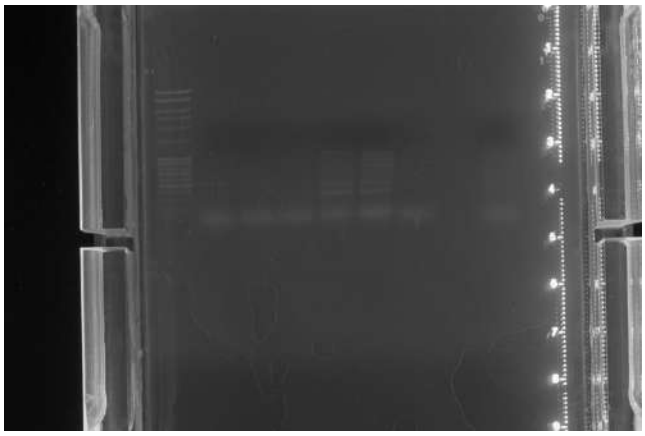


Table1

	A	B	C
1		1x [µl]	7.5x [µl]
2	dH20	15	112.5
3	Q5 Buffer	5	37.5
4	5 mM dNTPs	1	
5	10 µM Primer for	1	
6	10 µM Primer rev	1	
7	Q5 Polymerase	1	7.5
8	Template (35S_		

Used Primers an expected amplicon length

	A	B	C
1	I26109	I26104	536 bp
2	I26109	I26113	803 bp
3			
4			

Q5 PCR running conditions for I26109/I26113

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	68 °C	72 °C	72 °C	4 °C
4	02:00	00:15	00:30	00:40	02:00	endless

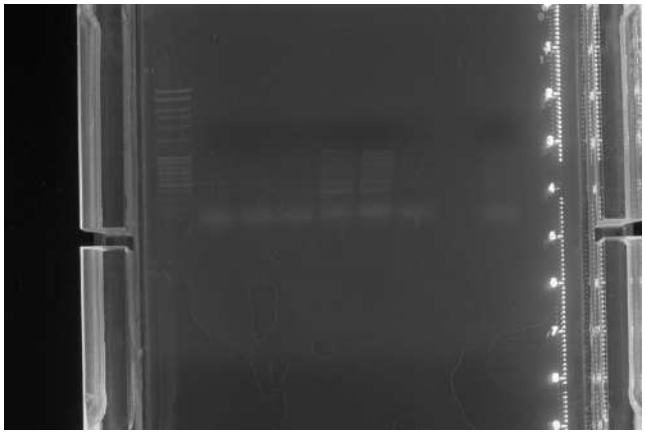
Q5 PCR running conditions for I26109/I26104

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	65 °C	72 °C	72 °C	4 °C
4	02:00	00:15	00:30	00:30	02:00	endless
5						

Table2

	A	B	C	D	E	F	G	H	I
1	100 bp ladder	4.1	4.2	4.3	13.1	13.2	13.3		-
2									

pcr_10.09.2026.tiff



Q5 cPCR A.tumefaciens for 35S_splitCre_N, 35S_splitCre_C and 35S_lox_eGFP_mCherry

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 11 Sep 2026 13:58:53 UTC
Entry Last Modified: 11 Sep 2026 14:08:31 UTC
Export Generated On: 05 Oct 2026 11:31:22 UTC

FREITAG, 11.9.2026

-Colonies C4,C5, N1, N2, N3, N4 were repicked from MP 08.09.2026.
-Transformation plates from 09.09.2026 showed a few colonies. From Cre_N and Cre_C 3 Colonies were picked. From 35S_lox_eGFP_mCherry 5 Colonies were picked.
-For positive Controll improved 35S_splitGFP_A from miniprep 03.09.2026 1:10 was used.

Table1

	A	B	C
1		1x [µl]	20.5x [µl]
2	dH20	16	328
3	Q5 Buffer	5	102.5
4	5 mM dNTPs	1	29.5
5	Primer AP15 1:10	1	20.5
6	Primer BP12 1:10	1	20.5
7	Q5 Pol	1	20.5

Table2

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	58 °C	72 °C	72 °C	4 °C
4	05:00	00:30	00:30	01:00	02:00	endless
5						

Table3

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	100 bp ladder	C4	C5	N1	N2	N3	N4	R1	R2	R3	R4	R5	c1	c2	c3
2	100 bp ladder	n1	n2	n3	+	-									
3															

Agrobacterium cPCR troubleshoot

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 16 Sep 2026 14:57:02 UTC
Entry Last Modified: 21 Sep 2026 12:30:07 UTC
Export Generated On: 05 Oct 2026 11:30:20 UTC

MITTWOCH, 16.9.2026

-We saw in multiple attempts to do a cPCR on Agrobacteria GV3101 no positive results. Even though the bacteria grew on selective plates containing Spec for the transformed Plasmid indicating succesfull transfrmation at first. No cPCR was succesfull.
-In order to asses if the bacteria itself are a Problem in our selfmade Q5 Mastermix we set out to test 2 things.

1. Bibdo extraxtion of 35S Plasmid from A.tumefaciens GV3101 with following PCR on the extraction
2. Heating normaly picked A.tumefaciens GV3101 diluted in water at 105 °C for 10 min and using 1 yl of this as Template afterwards.

Bibdo Extraction:
Liquid cultures of A.tumefaciens with 35S_ backbone containing Plasmid was grown overnight in 2 mL selective medium (with the appropriate antibiotic) at 37 °C while shaking.

750 µl of the culture was transferred into a 1.5 mL tube and centrifuged at 3500 × g for 2 min to pellet the cells.
The supernatant was discarded and the pellet resuspended in 100 µL MP Solution 1 no RNAse A was added and rest for 25 minutes at RT.

Next, 300 µL Buffer 2 (pH = 12.3) was added and mixed gently by inverting the tube a few times (no vortexing!).
At this step, care was taken to avoid harsh mixing, as genomic DNA is very fragile and can shear easily, which would lead to contamination.
The lysate was incubated on ice for **max. 2 min.**

After that, 250 µL Buffer 3 (pH = 5.3) was added and the tube was again gently inverted to mix for a few times.
Optionally, a few drops of chloroform can be added here to improve purity (this was not done)
The sample was then centrifuged at fullsped, at -9°C for 10 min.

About 400 µL of the clear supernatant was transferred into a fresh, pre-cooled tube.

1 ml 100%, **ice cold** EtOH was added to the supernatant and the sample was vortext.
(Note: u can also use isoprop, i dont as i have bad experince with it (salt contamination), EtOH worked always perfecly for me.)
ps. voretexing these samples is essential. idk why but the yield increases by that :)

spin down at fullspeed, at -9°C for 1h

The supernatant was removed, and the pellet washed with 70% ethanol.

After removing the ethanol completely (P20 might help), the pellet was briefly air-dried.

-After BiBdo extraction a PCR was done with the heated A.tumefaciens and the Bibdo extraction
-The PCR setup was done as showed below. Exact details and Geldoc picture were lost. The Geldoc Picture showed no amplifcation. Not for pos controll aswell.

Table1

	A	B	C
1		1x [µl]	
2	dH20	15	
3	Q5 Buffer	5	
4	5 mM dNTPs	1	
5	10 µM Primer oSNM04	1	
6	10 µM Primer BP12	1	
7	Q5 Polymerase	1	
8	Template	1	

MONTAG, 21.9.2026

-GV3101 Agrobacteria Transformed on 17.09.2026 overgrew the plate. To obtain single colonies a new plate was incoculated. Agrobacteria were picked used to inoculate 50yl od steril ddH20 and then immediatly used to inocculate another 50 yl without picking additional bacteria. The samples with more bacteria added now refered as + and with less as - .

Table2

	A	B	C
1		1x [µl]	
2	dH20	15	
3	Q5 Buffer	5	
4	5 mM dNTPs	1	
5	10 µM Primer oSNM04	1	
6	10 µM Primer BP12	1	
7	Q5 Polymerase	1	
8	Template	1	

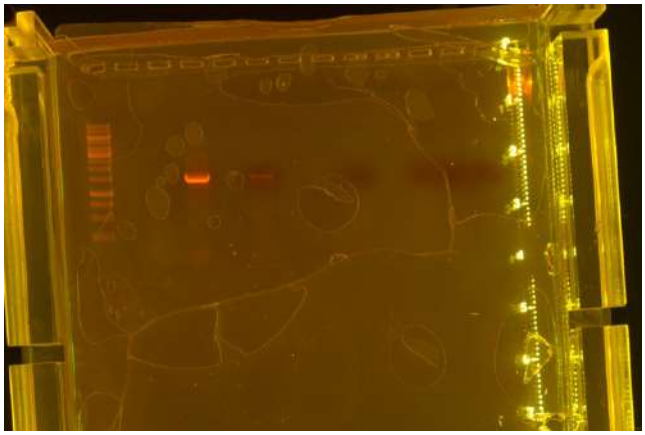
Table3

	A	B	C	D	E	F	G	H	I	J	K	L
1	1 kb ladder	R+	R-	C+	C-	N+	N-	C1	C2	C3	C4	C5
2												

cPCR running conditions

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	58 °C	72 °C	72 °C	4 °C
4	05:00	00:30	00:30	1:00	02:00	endless

igem_cpcr_21.09.tiff



Succesfull amplification for C+ and N+.

Electroporation of A.tumefaciens with 35S_splitCre_A 35S_splitCre_B and 35S_lox_mCherry_eGFP

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 16 Sep 2026 15:06:44 UTC
Entry Last Modified: 16 Sep 2026 15:07:02 UTC
Export Generated On: 05 Oct 2026 11:30:33 UTC

MITTWOCH, 16.9.2026

-3 x 25 yl of Electrocompetent GV3101 A.tumefaciens were thawed on ice.
-1 yl of 35S_splitCre_A ,35S_splitCre_B and 35S_lox_mCherry_eGFP Plasmid from miniprep were added.
-Cells+DNA were added into pre cooled electroporation cuvettes. Tapped to remove air bubbles and kept on ice for 10 min
-electroporation with 2.2 kV for 6 ms
-1 ml of roomtemperature YEP medium added to each sample immediatly after electroporation and transfered to 1.5 mL eppi
-Incubated for 2.5 h at 28 °C 850rpm
-cells pelleted by centrifuging 3500 g 8500 rpm r = 9.5 cm for 1 min. Removed supernatant, resuspended in return flow and plated on YEP+Rif+Gent+Spec plates.
-incubated at 28 °C

35S_splitGFP_a_no_cage colony PCR

Project: iGEM Bonn 2026 Shared Project

Author: Julius Krupke

Status: In progress

Entry Created On: 19 Sep 2026 13:00:20 UTC

Entry Last Modified: 19 Sep 2026 15:42:55 UTC

Export Generated On: 05 Oct 2026 11:31:36 UTC

SAMSTAG, 19.9.2026

Colony PCR was performed on the 4 colonies resulting form the transformation of the previos day.

Colony PCR was performed using 2 primer combinations (BP12 & I26104 and BP12 & oSNM04).

Q5 Colony PCR setup

	A	B	C
1		1x [µl]	5.5x [µl]
2	dH20	16	88
3	Q5 Buffer	5	27.5
4	5 mM dNTPs	1	5.5
5	10 µM Primer for	1	5.5
6	10 µM Primer rev	1	5.5
7	Q5 Polymerase	1	5.5

PCR programm

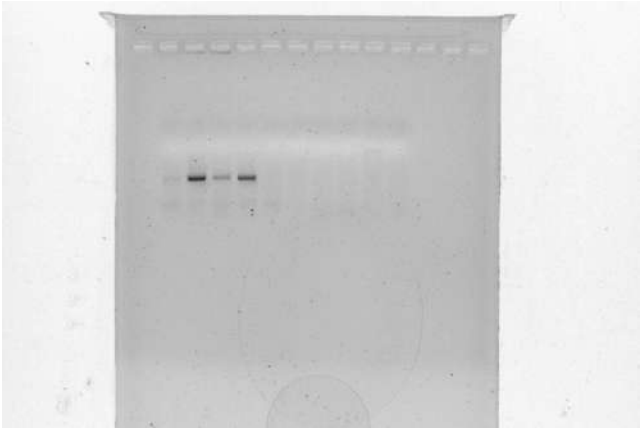
	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	57 °C	72 °C	72 °C	4 °C
4	05:00	00:15	00:30	01:30	02:00	endless

Gel loading scheme

	A	B	C	D	E	F	G	H	I	J	K
1	1kb ladder	Colony 1 I26104	Colony 2 I26104	Colony 3 I26104	Colony 4 I26104	- control I26104	Colony 1 oSNM04	Colony 2 oSNM04	Colony 3 oSNM04	Colony 4 oSNM04	- control oSNM04

The ladder was forgotten while loading the gel.

IGEM_190926_colony_pcr_splitgfp_a_nocage.png



Assembly & Transforamtion of splitGFP_A_caged & split GFP_B_nocage

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 21 Sep 2026 16:28:52 UTC
Entry Last Modified: 28 Sep 2026 10:54:22 UTC
Export Generated On: 05 Oct 2026 11:32:15 UTC

MONTAG, 21.9.2026

Assembly

The plasmids were assembled using the three standard backbone fragments for the 35S_BAR backbone.
The three backbone fragments and the insert were used in equimolar quantities.
The assemblys were performed used the Geneart kit (Thermo Fischer Scientific) at a final reaction volume of 10 µl.
After 1 h of incubation at 50 °C the plasmids were immediately transformed into chemically competent XL1-blue *E. coli*.

E. coli transformation

One aliquot of chemically competent XL1-blue *E. coli* was thawed on ice. Once thawed 10 µl of assembled plasmid was added and the bacteria were incubated on ice for 15 minutes. After incubation the bacteria were heat shocked at 42°C for 90 seconds. Following the heat shock the bacteria were put back on ice and incubated for another 5 minutes. Afterwards 850 µl of SOC medium preheated to 37°C were added and the bacteria were icubated at 37°C, 850 rpm for 1 hour to express the antibiotics resistance. After incubation the cells were pelleted by centrifugation at 3000x g for 5 minutes and 850 µl of the supernatant was discarded. The pellet was dissolved in the remaining supernatant and plated on a LB (Spectinomycin 100 ng/µl) plate. The bacteria were grown overnight at 37°C.

cPCR for 35S_splitGFP_A_no_cage

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 23 Sep 2026 13:12:42 UTC
Entry Last Modified: 23 Sep 2026 13:20:31 UTC
Export Generated On: 05 Oct 2026 11:31:47 UTC

MITTWOCH, 23.9.2026

-7 colonies were picked
Primers oSNM04 and BP12 were used

Q5 cPCR setup

	A	B	C
1		1x [µl]	7.5x [µl]
2	dH20	16	120
3	Q5 Buffer	5	37.5
4	5 mM dNTPs	1	7.5
5	10 µM Primer for	1	7.5
6	10 µM Primer rev	1	7.5
7	Q5 Polymerase	1	7.5

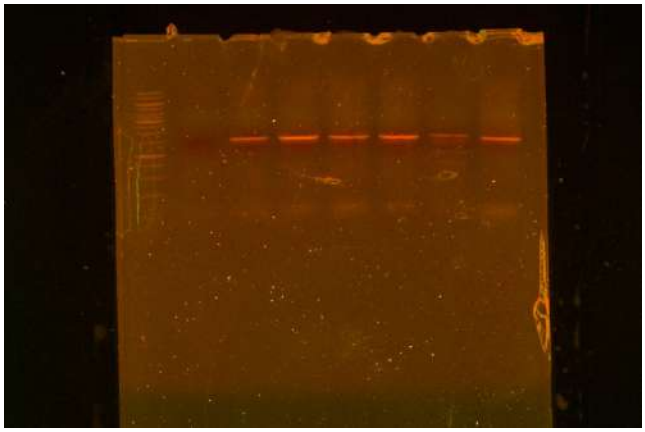
Q5 cPCR running conditions

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	58 °C	72 °C	72 °C	4 °C
4	05:00	00:30	00:30	1:00	02:00	endless

Table1

	A	B	C	D	E	F	G	H
1	1 kb ladder	C1	C2	C3	C4	C5	C6	C7
2								

cpcr_igem_23.09.tiff



-succewsfull amplification for C2, C3, C4, C5, C6 ,C7

-C2, C3, C4 and C5 were used to inoculate 2 ml each of LB+Spec for Miniprep the next day. Grew at 37 °C overnight.

Q5 cPCR GV3101 A.tumefaciens. 35S_lox_eGFP_mCherry, 35S_splitCre_N, 35S_splitCre_C

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 25 Sep 2026 13:53:53 UTC
Entry Last Modified: 25 Sep 2026 13:58:24 UTC
Export Generated On: 05 Oct 2026 11:32:00 UTC

FREITAG, 25.9.2026

3 colonies were picked for each plate. Plated on a fresh YEP+Rif+Gent+Spec plate and used to inoculate 50 yl of sterile ddH20.
The 50 yl with A.tumefaciens were heated to 98 °C for 15 min.
Primers oSNM04 and BP12 were used.

Table1

	A	B	C
1		1x [µl]	11.5x [µl]
2	dH20	15	172.5
3	Q5 Buffer	5	57.5
4	5 mM dNTPs	1	11.5
5	10 µM Primer for	1	11.5
6	10 µM Primer rev	1	11.5
7	Q5 Polymerase	1	11.5
8	Template	1	

Table2

	A	B	C	D	E	F
1	1 cycle	35 cycles			1 cycle	1 cycle
2	denaturation	denaturation	annealing	Elongation	elongation	hold
3	98°C	98 °C	58 °C	72 °C	72 °C	4 °C
4	05:00	00:30	00:30	1:30	02:00	endless

Assembly and transformation of splitGFP_A_caged

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 28 Sep 2026 10:51:57 UTC
Entry Last Modified: 28 Sep 2026 10:54:37 UTC
Export Generated On: 05 Oct 2026 11:32:26 UTC

MITTWOCH, 23.9.2026

Assembly

The plasmid was assembled using the three standard backbone fragments for the 35S_BAR backbone.
The three backbone fragments and the insert were used in equimolar quantities.
The assemblys were performed used the Geneart kit (Thermo Fischer Scientific) at a final reaction volume of 10 µl.
After 1 h of incubation at 50 °C the plasmids were immediately transformed into chemically competent XL1-blue *E. coli*.

E. coli transformation

One aliquot of chemically competent XL1-blue *E. coli* was thawed on ice. Once thawed 10 µl of assembled plasmid was added and the bacteria were incubated on ice for 15 minutes. After incubation the bacteria were heat shocked at 42°C for 90 seconds. Following the heat shock the bacteria were put back on ice and incubated for another 5 minutes. Afterwards 850 µl of SOC medium preheated to 37°C were added and the bacteria were icubated at 37°C, 850 rpm for 1 hour to express the antibiotics resistance. After incubation the cells were pelleted by centrifugation at 3000x g for 5 minutes and 850 µl of the supernatant was discarded. The pellet was dissolved in the remaining supernatant and plated on a LB (Spectinomycin 100 ng/µl) plate. The bacteria were grown overnight at 37°C.

Assembly & Transformation of 35S_N-Cre_uncaged_BAR and 35S_C-Cre_uncaged_BAR

Project: iGEM Bonn 2026 Shared Project
Author: Julius Krupke
Status: In progress
Entry Created On: 28 Sep 2026 10:56:24 UTC
Entry Last Modified: 28 Sep 2026 10:58:23 UTC
Export Generated On: 05 Oct 2026 11:30:29 UTC

MITTWOCH, 23.9.2026

Assembly

The plasmids were assembled using the three standard backbone fragments for the 35S_BAR backbone.
The three backbone fragments and the insert were used in equimolar quantities.
The assemblys were performed used the Geneart kit (Thermo Fischer Scientific) at a final reaction volume of 10 µl.
After 1 h of incubation at 50 °C the plasmids were immediately transformed into chemically competent XL1-blue *E. coli*.

E. coli transformation

One aliquot of chemically competent XL1-blue *E. coli* was thawed on ice. Once thawed 10 µl of assembled plasmid was added and the bacteria were incubated on ice for 15 minutes. After incubation the bacteria were heat shocked at 42°C for 90 seconds. Following the heat shock the bacteria were put back on ice and incubated for another 5 minutes. Afterwards 850 µl of SOC medium preheated to 37°C were added and the bacteria were icubated at 37°C, 850 rpm for 1 hour to express the antibiotics resistance. After incubation the cells were pelleted by centrifugation at 3000x g for 5 minutes and 850 µl of the supernatant was discarded. The pellet was dissolved in the remaining supernatant and plated on a LB (Spectinomycin 100 ng/µl) plate. The bacteria were grown overnight at 37°C.

Electroporation of GV3101 A.tumefaciens with 35S_splitGFP_A_uncaged, 35S_splitGFP_B_uncaged, 35S_splitCre_N_uncaged, 35S_splitCre_C_uncaged

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 28 Sep 2026 12:26:23 UTC
Entry Last Modified: 28 Sep 2026 12:29:42 UTC
Export Generated On: 05 Oct 2026 11:30:11 UTC

MONTAG, 28.9.2026

-4 x 25 yl of Electrocompetent GV3101 A.tumefaciens were thawed on ice.
-1 yl of 35S_splitGFP_A_uncaged, 35S_splitGFP_B_uncaged, 35S_splitCre_N_uncaged, 35S_splitCre_C_uncaged Plasmid from miniprep 1:10 dilution were added.
-Cells+DNA were added into pre cooled electroporation cuvettes. Tapped to remove air bubbles and kept on ice for 10 min
-electroporation with 2.2 kV for 6 ms
-1 ml of roomtemperature YEP medium added to each sample immediatly after electroporation and transfered to 1.5 mL eppi
-Incubated for 3 h at 28 °C 850 rpm
-cells pelleted by centrifuging 3500 g 8500 rpm r = 9.5 cm for 1 min. Removed supernatant, resuspended in return flow and plated on YEP+Rif+Gent+Spec plates.
-incubated at 28 °C

minprep of 35S_splitGFP_A_caged and Electroporation of GV3101 A.tumefaciens

Project: iGEM Bonn 2026 Shared Project
Author: Ruben Langenbacher
Status: In progress
Entry Created On: 30 Sep 2026 14:19:34 UTC
Entry Last Modified: 30 Sep 2026 14:23:18 UTC
Export Generated On: 05 Oct 2026 11:30:20 UTC

MITTWOCH, 30.9.2026

The liquid culture was grown overnight in 2 mL selective medium with 2 yl Spectinomycin (Stock 100 mg/ml) at 37 °C while shaking.

750 µl of the culture was transferred into a 1.5 mL tube and centrifuged at 3500 × g for 2 min to pellet the cells.
The supernatant was discarded and the pellet resuspended in 100 µL MP Solution 1 (0,5 mg/ml RNAseA) and rest for 25 minutes at RT.

Next, 300 µL Buffer 2 (pH = 12.3) was added and mixed gently by inverting the tube a few times (no vortexing!).
At this step, care was taken to avoid harsh mixing, as genomic DNA is very fragile and can shear easily, which would lead to contamination.
The lysate was incubated on ice for **max. 2 min.**

After that, 250 µL Buffer 3 (pH = 5.3) was added and the tube was again gently inverted to mix for a few times.
The sample was then centrifuged at fullsped, at -9°C for 10 min.

400 µL of the clear supernatant was transferred into a fresh, pre-cooled tube.

1 ml 100%, **ice cold** EtOH was added to the supernatant and the sample was vortext.

spin down at fullspeed, at -9°C for 1h

The supernatant was removed, and the pellet washed with 70% ethanol.
After removing the ethanol completely , the pellet was briefly air-dried.
Finally, the DNA was resuspended in 20 µL H_2O and the concentration was measured at the nanodrop
480,4 ng/yl

25 yl of Electrocompetent GV3101 A.tumefaciens were thawed on ice.
-1 yl of 35S_NLSsplitGFP_A_caged Plasmid from miniprep 1:10 dilution was added.
-Cells+DNA were added into pre cooled electroporation cuvettes. Tapped to remove air bubbles and kept on ice for 10 min
-electroporation with 2.2 kV for 6 ms
-1 ml of roomtemperature YEP medium added to each sample immediatly after electroporation and transfered to 1.5 mL eppi
-Incubated for 3 h at 28 °C 1400 rpm

-cells pelleted by centrifuging 3500 g 8500 rpm r = 9.5 cm for 1 min. Removed supernatant, resuspended in return flow and plated on YEP+Rif+Gent+Spec plates.
-incubated at 28 °C