PreCyse

iGEM 2024

NOTEBOOK

iGEM Bielefeld-CeBiTec

Experiment 1

Characterization of Prime Editing Tools

iGEM BIELEFELD

Prologue

Introduction

All results and observations made during the proof of concept (PoC) of prime editing systems are documented in this lab book. This includes a detailed record of test conditions, test protocols, interim results and final analyses.

Prime editing technology offers significant advantages over conventional CRISPR/Cas systems, particularly in terms of precision and versatility. It allows targeted genetic modification without the introduction of double-stranded DNA breaks, potentially leading to fewer unwanted mutations. Proof of concept for prime editing is crucial to confirm the potential of this method. Testing these systems will help to understand the conditions under which they work most efficiently and how to overcome potential limitations. These results will be applied to our prime editing complex, PrimeGuide, over the course of the project.

Based on the results, adjustments are made to the protocols or experimental conditions to improve performance. This iterative process continues until reliable and reproducible results are achieved.

The aim of this Lab Book is to provide a comprehensive collection of experimental results and analysis of the Prime Editing technology. It serves as proof of the system's functionality and should form the basis of our further investigations. The documented cycles and results not only provide guidance for future experiments, but also valuable insights into the challenges and opportunities of this ground-breaking technology.

Overview: Research Project of Experiment 1

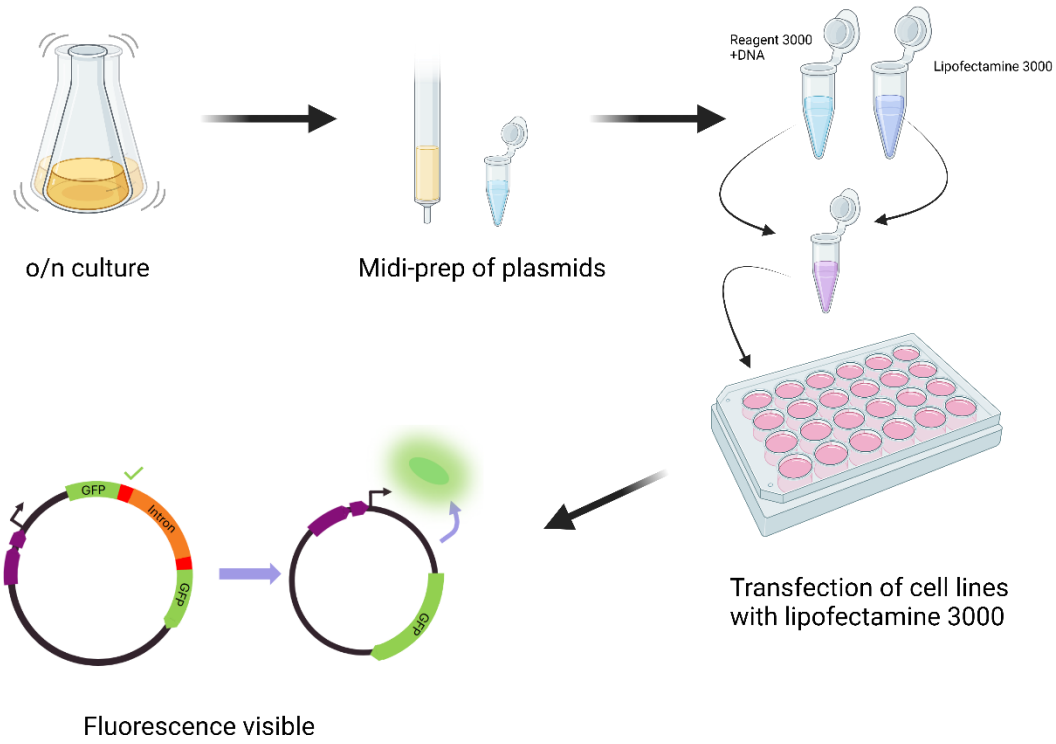

Figure Overview of our proof of concept experiment. Midi-preps were performed out of overnight cultures. DNA generated via Midi-prep was transfected with lipofectamine 3000 into HEK293 cell line. When the co-transfection with DNA and prime editor worked out fluorescence was visible.

Overall Information about this Notebook

[e.g. hyperlinks, protokolls, hints, etc]

Abbreviation	Meaning
o/n culture	Overnight culture
E. Coli	*Escherichia Coli*
Mini prep	Plasmid Preparation in Mini scale (7 ml)
Midi prep	Plasmid Preparation in Midi scale (200 ml)
DMEM	Dulbecco's Modified Eagle's Medium

FCS	Fetal calf serum
Pen	Penicillin
Strep	Streptomycin
Amp	Ampicillin
Acc	according

Table: *Name Abbrevations of experimentors*

Name	Abbreviations
Anna Baack	AB
Christian Michalek	CM
Isabell Alexandra Guckes	IG
Kai Kanthak	KK
Kathleen Susat	KS
Kaya Lange	KL
Lisa Wiesner	LW
Malte Lenger	ML
Philip Mundt	PM
Vera Köhler	VK

Table: *Overview of our timetable (in calendar weeks).*

Topic	**Protocols**	**Calendar weeks**
General lab preparations & midi-preps, mini preps	1-35	23-30
Transfection experiments	17-49	26-38

No	Date (DD/MM/YYYY)	Titel	Experimenters
01	07/06/2024	General Lab Preparations	ML, KK, VK, LW
02	10/06/2024	Plating & liquid cultures	KS
03	11/06/2024	Agarose Gels	PM
04	12/06/2024	Prepare LB+Agar	VK
05	13/06/2024	Pouring plates	KK

06	14/06/2024	OD-Measurement	KL, IG
07	16/06/2024	Measure concentration of mini-prep	CM
08	18/06/2024	Preparation of media and liquid cultures	IG, PM, ML
09	19/06/2024	Plasmid Isolation (Midi) & generation of chemically competent cells	IG, KS, AB, CM
10	20/06/2024	Passaging of HEK293, Plasmid Isolation (Midi) and Gel Extraction	IG, LW, CM, KS
11	21/06/2024	General Lab preparations	KS
12	22/06/2024	Midi Prep of LV-PE_CO-Mini and pDAS 12489	KK
13	24/06/2024	Plating & liquid cultures, passaging of HEK293, Transformation of *E. coli* DH5alpha with Mini Prep plasmids	KL, CM, AB, IG
14	25/06/2024	Transformation & Interlab preparations	PM, KL, KS
15	26/06/2024	planning cell culture experiments, checking transformation efficiency	IG, KK, KS
16	27/06/2024	Seeding for preliminary test proof of concept	IG
17	29/06/2024	Transfection for preliminary proof of concept, preparing media & plates	KS, SZ
18	30/06/2024	Microscopy of HEK293 transfection after 24h	LW
19	01/07/2024	Passaging of HEK293, Microscopy of HEK293 Transfection after 47h	IG, KS
20	02/07/2024	Microscopy of HEK293 transfection after 72h	KS
21	03/07/2024	Inoculation of liquid cultures	IG, KS, ML
22	04/07/2024	Passaging of HEK293	IG
23	05/07/2024	Preparing electrocompetent *E. coli* dH5a cells & glycerin stocks	KK, VK, AB
24	08/07/2024	Passaging of HEK293 and seeding for preliminary test proof of concept repetition	IG
25	10/07/2024	Transfection HEK, LB medium with Chloramphenicol	KS, AB
26	11/07/2024	Passaging of HEK293, Microscopy of transfected HEK cells	IG, KS
27	12/07/2024	Restriction of pDAS12124_PEAR-GFP-preedited & pDAS12489_PEAR-GFP_2in1_2.0, Microscopy of transfected HEK	VK, AB, KS
28	13/07/2024	Gel electrophoresis of digestion	PM
29	15/07/2024	Passing of HEK293, seed HEK293 for proof of concept	KS

30	16/07/2024	Transfection proof-of-concept, Microscopy of transfected cells	KS
31	17/07/24	Microscopy of transfected HEK cells	KS, CM
32	18/07/24	Microscopy, plasmid isolation	KS, VK, KK, AB
33	19/07/24	Microscopy of transfected cells, OD measurement, mini-prep, gel extraction	KL
34	22/07/24	Prepare LB medium	KS
35	29/07/24	Seeding cells for proof of concept test 2	KS
36	30/07/24	Transfection proof-of-concept test 2, microscopy	KS
37	31/07/24	Microscopy of proof of concept test 2	KS
38	01/08/24	Microscopy of proof of concept test 2	KS
39	29/08/2024	Passaging of HEK293	IG
40	02/09/2024	Passaging of HEK293, seeding of HEK293	KS
41	03/09/2024	Transfection, microscopy	KS
42	04//09/2024	Microscopy	KS
43	05/09/2024	Microscopy	KS
44	06/09/2024	Microscopy	KS
45	14/09/2024	Seed cells for Transfection	IG
46	16/09/2024	Transfection of HEK293	KS
47	17/09/2024	Microscopy	KS
48	18/09/2024	Microscopy	KS
49	19/09/2024	Microscopy & FACS measurement	KS

Laboratory Notebook

No	Date (DD/MM/YYYY)	Titel	Experimenters
01	07.06.2024	General Lab Preparations	ML, KK, VK, LW

Aims for the day

- Preparing liquid media
- Pouring LB-media plates with different antibiotics
- Sterilize lab materials

Protocols

Prepare LB medium acc. To P1

Prepare LB + agar acc. To P1

Prepare antibiotic stocks acc. To P1

Autoclaving acc. To P34

Calculations, Tipps/Hints, Outline/Illustration

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Results

- Obtained 2 L liquid LB-media, 1 L liquid LB-media with Ampicillin (final conc. 100 µg/ml), 1 L liquid LB-media with Kanamycin (final conc. 50 µg/ml), 1 L liquid LB-media with Chloramphenicol (final conc. 34 µg/ml)
- Obtained 50 LB-agar plates + 50 LB-agar plates per antibiotic used
- Obtained sterilized Lab materials

Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
02	10.06.2024	Plating & liquid cultures	KS

Aims for the day

- Inoculation of liquid cultures from stab cultures
- Plating of stab cultures

Protocols

Pouring plates acc. To P1

Inoculate from stab cultures

Calculations, Tipps/Hints, Outline/Illustration

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Results

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Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
03	11.06.2024	Agarose Gels	PM

Aims for the day

- Preparation for Agarose Gels for Gel Electrophoresis

Protocols

Preparing of agarose gel acc. To P13

- Preparation of 300 ml 1 % Agarose Solution (3 g Agarose + 300 ml TAE 1x Buffer)
- Pouring two gels for gel electrophoresis

Calculations, Tipps/Hints, Outline/Illustration

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Results

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Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
04	12.06.2024	Prepare LB+Agar	VK

Aims for the day

- Prepare LB+Agar

Protocols

LB+Agar acc. To P1

Calculations, Tipps/Hints, Outline/Illustration

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Results

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Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
05	13.06.2024	Pouring plates	KK

Aims for the day

- Pouring plates

Protocols

Pouring plates with LB+Agar acc. To P1

Calculations, Tipps/Hints, Outline/Illustration

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Results

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Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
06	14/06/2024	OD-Measurement	KL, IG

Aims for the day

- Determination of the optical density of the liquid *E. coli* cultures

Protocols

OD-Measurement performed

- Blank Measurement:
  - Fill a cuvette with 1 mL of sterile LB medium (or the appropriate blank medium)
  - Wipe the outside of the cuvette with a lint-free tissue to remove any fingerprints or droplets
  - Insert the cuvette into the spectrophotometer
  - Set the spectrophotometer to 600 nm
  - Zero the spectrophotometer with the blank
    - This sets the baseline absorbance to 0
- Sample Measurement:
  - Gently mix the *E. coli* culture by inverting the flask or using a vortex
  - Pipette 1 mL of the *E. coli* culture into a clean cuvette
  - Wipe the outside of the cuvette to remove any fingerprints or droplets
  - Insert the cuvette into the spectrophotometer
  - Measure the OD600
  - Record the value displayed by the spectrophotometer
  - For multiple samples repeat the steps above for each one
  - Always zero the spectrophotometer with the blank before measuring each new sample to ensure accuracy

  - The cultures were transferred into to refrigerator at 2pm

Calculations, Tipps/Hints, Outline/Illustration

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Results

Organism	Plasmid	Antibiotica	Dilution	Measured OD_{600}	Real OD_{600}
E. Coli DH5α	pDAS12124-PEAR-GFP-preedited	Kanamycin	20	0.203	4.06

E. Coli DH5α	pCMV-PE2	Ampicillin	2	0.836	1.672
E. Coli DH5α	pDAS12489-PEAR-GFP_2in1_2.0	Ampicillin	2	0.847	1.694
E. Coli DH5α	LV-PE_CO-Mini	Ampicillin	2	0.608	1.216
E. Coli DH5α	PU6-pegRNA-GG-acceptor	Ampicillin	4	0.83	3.32
E. Coli DH5α	PMJ326: pCMV-GtFz1	Ampicillin	4	0.757	3.028
E. Coli DH5α	pZMB938	Ampicillin	2	0.433	0.866
E. Coli DH5α	pVC19	Ampicillin	2	0.449	0.898

Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
07	16/06/2024	Measure concentration of mini-prep	CM

Aims for the day

- Concentration of Mini-Prep via Nanodrop (Mini Prep 12.6.24)

Protocols

Measure concentration of mini-preps acc. To P8

- Blank the Nanodrop with 1 µl nuclease-free water
- measure your samples by pipetting 1 µl on sensor

Calculations, Tipps/Hints, Outline/Illustration

Good lab practise: Dilute the DNA concentration to 1000 ng/µL

Results

Organism	Plasmid	C [ng/µL] Sample 1	C [ng/µL] Sample 2
E. Coli DH5α	pDAS12124-PEAR-GFP-preedited	290.4	-
E. Coli DH5α	pCMV-PE2	217.4	269.8
E. Coli DH5α	pDAS12489-PEAR-GFP_2in1_2.0	68.7	63.8

E. Coli DH5α	LV-PE_CO-Mini	212.7	87.5
E. Coli DH5α	PU6-pegRNA-GG-acceptor	128.7	-
E. Coli DH5α	PMJ326: pCMV-GtFz1	272.0	436.5

Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
08	18/06/2024	Preparation of media and liquid cultures	IG, PM, ML

Aims for the day

- General lab preparations - filling up the medium stock
- Start incubating liquid cultures for plasmid isolation (Midi) and generation of chemocompetent cells tomorrow

Protocols

Solid medium prepared acc. to P1 : 4x 0.5 L LB+ Agar

Liquid medium prepared acc. to P1 : 1x 0.5 L LB

Autoclaving of the prepared media acc. to P34

starting liquid cultures for midipreps tomorrow

- 200 mL LB each with corresponding antibiotic
- inoculated with pipette tip from glycerol stock
    - DH5a pDAS12489 (Amp)
    - DH5a pZMB938 (Amp)
    - DH5a pUC19 (Amp)
    - DH5a LV-PE-CO-Mini (Amp)
    - DH5a pMJ326 (Amp)
    - DH5a $pegRNA_A$ (Amp)
    - DH5a pDAS1212 (Kan)
    - DH5a DH5a pCMV_PE2 (Amp)

Starting liquid culture for production of chemically competent DH5a

- 100 mL LB without antibiotic
- inoculated with pipette tip from vial

Calculations, Tipps/Hints, Outline/Illustration

- creation of a sterile atmosphere using a Bunsen burner for inoculation of liquid cultures

Results

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Analysis & Interpretation

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Achievements of the Day

All aims achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
09	19/06/2024	Plasmid Isolation (Midi) & generation of chemically competent cells	IG, KS, AB, CM

Aims for the day

- Dry lab (planning of cell culture work)
- Preparing of LB and LB+Agar media
- Structuring and fill in of laboratory notebook
- Plasmid Preparation of yesterdays cultures (Midi)
- generation of chemically competent cells of yesterdays culture

Protocols

Solid medium prepared acc. to P1 : 2x 0.5 L LB+ Agar

Liquid medium prepared acc. to P1 : 1x 0.5 L LB, 1x 1 L LB

OD-Measurement and Plasmid Preparation with MN Kit NucleoBond® Xtra Midi Plus acc. to P35:

- DH5a pDAS12489 (Amp) from 18.6.
- DH5a LV-PE-CO-Min (Amp) from 18.6.
- DH5a pMJ326 (Amp) from 18.6.
- DH5a pegRNA$_A$ (Amp) from 18.6.
- DH5a pDAS1212 (Kan) from 18.6.
- DH5a DH5a pCMV_PE2 (Amp) from 18.6.

Re-inoculation of liquid cultures of DH5a pUC19 (Amp) with corresponding glycerin-stock for midiprep tomorrow

Plasmid digest acc. to P:

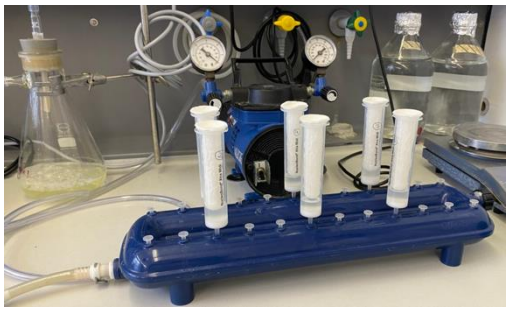
pDAS preedited	Volume [µL]	pCMV_PE2	Volume [µL]	PE-CO-Mini	Volume [µL]
H_2O	15.16	H_2O	14.46	H_2O	15.24
10x NEB Buffer	2	10x Tango	2	O Buffer	2
NheI	1	BamHI	1	NotI	2

XhoI	1	EcoRI	2	DNA	0.763
DNA	0.58	DNA	0.54		
pDAS 2 in 1	Volume [µL]	PMJ GtFz	Volume [µL]	PegRNA Acceptor	Volume [µL]
H_2O	14.85	H_2O	14.28	H_2O	16.16
10x Cut	2	10x cut	2	NEB Buffer	2
XbaI	1	HindIII	1	BsaI	1
XhoI	1	SpeI	2	DNA	0.84
DNA	2.15	DNA	0.71		

- Digest 1x with given volumes above and 1x with doubled volumes for each component
- incubation at 37°C for 5h

Calculations, Tipps/Hints, Outline/Illustration

- MN Kit NucleoBond® Xtra Midi Plus
  - 200 mL of OD>2 needed in best case (Volume=400/OD)
  - Rule of thumb: g ~ rcf for this protocol
  - Use suction device in room E0-213 for more rapid column flow (steps 9-11 in protocol) but elute in a new falcon (step 12)!



  - For more rapid pellet drying put it in incubator in room E0-217
  - Use ice cold Isporopanol and 70 % (v/v) Ethanol for better plasmid precipitation, centrifuge at 4 °C!!
  - Solvation in sterile water, dependent on pellet size

Results

OD Measurement for plasmid preparation (Midi) 10 am

strain	OD_{600}
DH5a pDAS12489 inoculated on 18.6.	2.870
DH5a LV-PE-CO-Mini inoculated on 18.6.	1.515

DH5a pMJ326 inoculated on 18.6.	2.080
DH5a pegRNA$_A$ inoculated on 18.6.	2.575
DH5a pDAS12124 inoculated on 18.6.	1.850
DH5a pCMV_PE2 inoculated on 18.6.	>2.000

Concentration of plasmid preparation (Midi)

Organism	Plasmid	C [ng/µL]	260/280	260/230
E. Coli DH5α	LV-PE_CO-Mini	491.9	1.86	2.13
E. Coli DH5α	pDAS12489-PEAR-GFP_2in1_2.0	68.5		
E. Coli DH5α	pDAS12124-PEAR-GFP-preedited	3790.3	1.89	2.23
E. Coli DH5α	pDAS12124-PEAR-GFP-preedited	85.6	1.83	2.23
E. Coli DH5α	PU6-pegRNA-GG-acceptor	3457.8	1.85	2.16
E. Coli DH5α	PMJ326: pCMV-GtFz1	1100	1.86	2.21
E. Coli DH5α	PMJ326: pCMV-GtFz1	213.1	1.89	2.22
E. Coli DH5α	pCMV-PE2	1726		

Analysis & Interpretation

Repeat: pDAS12489-PEAR-GFP_2in1_2.0, LV-PE_CO-Mini

Did not grow: puC19 and pZMB

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
10	20/06/24	Passaging, Plasmid Isolation (Midi) and Gel Extraktion	IG, LW CM, KS

Aims for the day

- Passaging of HEK293 into a new T75 flask
- Plasmid Preparation of yesterdays cultures (Midi)

Protocols for the day

Passaging of HEK293 into a new T75 flask acc. To P17

- 100 % confluence
- Added 300 µL trypsin in DMEM and 5 mL DMEM to cells – volume of the cell suspension = 5.3 mL
- 1:20 dilution in 15 mL end volume: transfer of 265 µL cell suspension into new T75-flask and fill with DMEM to 15 mL

OD-Measurement and Plasmid Preparation with MN Kit NucleoBond® Xtra Midi Plus acc. to P35

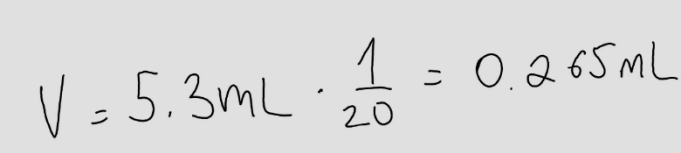
- DH5a pZMB (Amp) from 18.6.
- DH5a PUC19 (Amp) from 18.6.

Extraction of plasmids from digestion gel with NEB Kit acc. To P14

- Elution with 11 µl of elution buffer for 2 times!

Calculations, Tipps/Hints, Outline/Illustration

- Volume of cell suspension transferred into new T75-flask:



Results

OD Measurement for plasmid preparation (Midi)

Plasmid	OD_{600}
DH5a pZMB inoculated on 18.06	3.1
DH5a PUC19 inoculated on 18.6. (reinoculated on 19.06)	3.392

DNA concentration of plasmid preparation Midi

Plasmid	DNA c [ng/µl]	260/280	260/230
pZMB	457.7	1.92	2.05
puC19	1093.0	1.74	1,72

DNA concentration of gel extraction

Plasmid	DNA c [ng/µl]	260/280	260/230
1.1	24.6	1.87	1.46
1.2	12.5	1.95	0.19
2.1	4.7	1.86	0.45

3.1	8.6	1.96	0.62
3.2	3.0	3.73	0.26
4.1	12.5	1,93	0.97
5.1	17.7	1.83	0.74
5.2	19.5	1.81	1.02
6.1	24.9	1.81	1.02
6.2	8.5	1.91	0.25

Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
11	21/06/2024	General lab preparations	KS

Aims for the day

- Prepare LB and LB+agar media
- Autoclaving of the prepared media
- Pouring LB-media plates with different antibiotics

Protocols

Prepare LB medium acc. To P1

Prepare LB + agar acc. To P1

Prepare antibiotic stocks acc. To P1

Autoclaving acc. To P34

Calculations, Tipps/Hints, Outline/Illustration

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Results

Obtained 20 LB-agar plates with ampicillin + 20 LB-agar plates with kanamycin

Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
12	22/06/2024	Midi Prep of LV-PE_CO-Mini and pDAS12489	KK

Aims for the day

- Determine the OD of *E. coli* containing target plasmids
- Plasmid preparation (Midi)

Protocols

Plasmid preparation Midi acc. To P35

For the PECO Mini culture:

- Initial OD reading: ~0.4
- Calculated original OD: ~1.6 (0.4 * 4)

For the pDAS culture:

- Initial OD reading: >1
- Performed an additional 1:8 dilution.
- Second OD reading: ~0.5-0.6
- Calculated original OD: ~4.0-4.8 (0.5-0.6 * 8)
- Conducted measurements twice for each culture.
- Averaged the readings to obtain final OD values.

Calculations, Tipps/Hints, Outline/Illustration

/

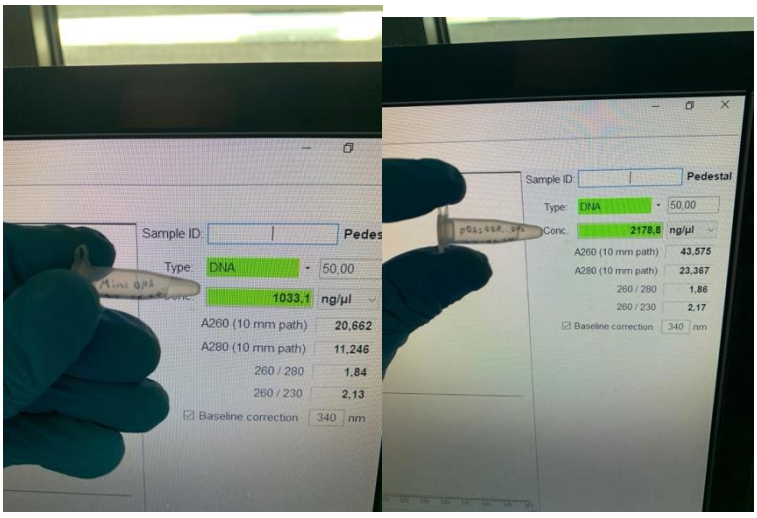
Results

OD measurement

Sample	Measured OD_{600}	Real OD_{600}
LV-PE_CO-Mini (1:4)	0.412	1.65
pDAS 12489 (1:8)	0.63	5.04

Plasmid concentration (Midi)

Sample	C[ng/µl]	A260/280	A260/220
LV-PE_CO-Mini	1033.1	1.84	2.13
pDAS 12489	2178.8	1.86	2.17



Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
13	24.06.24	Plating & liquid cultures, passaging of HEK293, Transformation of *E. coli* DH5alpha with Mini Prep plasmids	KL, CM, AB, IG

Aims for the day

- Inoculation of liquid cultures from stab cultures
- Plating of stab cultures with Amp
- Prepare LB-agar
- Transformation of *E. coli* DH5alpha with Mini Prep Plasmids (4 random Eppis of DH5alpha and 6 of 8 plasmids were used)
- Passaging of HEK293

Protocols

Inoculation & plating from stab culture

Prepare LB+agar acc. to P1

Transformation of *E. coli* acc to P3

- Take competent cells out of -80°C and thaw on ice (approximately 20-30 mins)
- Remove agar plates containing the appropriate antibiotic from storage at 4°C and incubate in 37°C incubator
- Mix 1 µl of DNA (100 pg) into 50 µL of competent cells in a 1.5 mL reaction tube
- gently mix by flicking the bottom of the tube with your finger a few times
- Incubate the competent cell/DNA mixture on ice for 20-30 mins
- Heat shock each transformation tube by placing the bottom of the tube into a 42°C water bath for 55 secs
- Put the tubes back on ice for 2 min
- Add 10 ml LB media (without antibiotic) to the bacteria
- grow in 37°C shaking incubator for 60 min
- centrifugate the cells in 15 ml reaction tubes (4 °C, 10 min, 4.000 x g)
- resuspend the pellet in 500 µl LB-Medium (without antibiotics)

Plate agar plates with antibiotics

- pipette 20 µl of transformant on LB agar plate containing the appropriate antibiotic
- immerse the plater in 70% Ethanol and enlighten it
- wait until all Ethanol is burned
- cool the plater down by carefully touching the LB agar plate on the edge
- plate the transformed *E. coli* by slightly running the plater over the plate
- wait until everything is dried

- Incubate plates upside down at 37°C overnight
    - Comment: just for testing the is no need to calculate the exact amount of dna, just use 1 µl of the plasmid-dna

Passaging of HEK293 into a new T75 flask acc. To P19

- 100 % confluence
- Added 300 µL trypsin in DMEM and 5 mL DMEM to cells – volume of the cell suspension = 5.3 mL
- 1:20 dilution in 15 mL end volume: transfer of 265 µL cell suspension into new T75-flask and fill with DMEM to 15 mL

Calculations, Tipps/Hints, Outline/Illustration

On W5 there is a microwave autoclave that can sterilize the medium in about 30 minutes (Anna has access to it). But lately it seems to autoclave perfectly in only 1 out of 3 cases.

The autoclave belt is not dyed in the process!

Organism	Plasmid
E. Coli DH5α	pDAS12124-PEAR-GFP-preedited
E. Coli DH5α	pCMV-PE2
E. Coli DH5α	pDAS12489-PEAR-GFP_2in1_2.0
E. Coli DH5α	LV-PE_CO-Mini
E. Coli DH5α	PU6-pegRNA-GG-acceptor
E. Coli DH5α	PMJ326: pCMV-GtFz1

PUC 19 and pZMB are not used in this experiment

Results

Plates overgrown

Contamination of pDAS12124-PEAR-GFP-preedited, PU6-pegRNA-GG-acceptor,

		
		

Analysis & Interpretation

Plates overgrown, amp resistance too old and wrong solution

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
14	25.06.2024	Transformation of *E. coli*	PM, KL, KS

Aims for the day

- Transformation of *E. coli* DH5alpha with Mini Prep Plasmids (4 reaction tubes of DH5alpha and 6 of 8 plasmids were used)

Protocols

Transformation of *E. coli* acc. to P3

- Take competent cells out of -80°C and thaw on ice (approximately 20-30 mins)
- Remove agar plates containing the appropriate antibiotic from storage at 4°C and incubate in 37°C incubator
- Mix 1 µl of DNA (100 pg) into 50 µL of competent cells in a 1.5 mL reaction tube
- gently mix by flicking the bottom of the tube with your finger a few times
- Incubate the competent cell/DNA mixture on ice for 20-30 mins
- Heat shock each transformation tube by placing the bottom of the tube into a 42°C water bath for 55 secs

- Put the tubes back on ice for 2 min
- Add 500 µl LB media (without antibiotic) to the bacteria
- grow in 37°C shaking incubator 450 rpm for 60 min

Plate out agar plates with antibiotics

- pipette 20 µl of transformant on LB agar plate containing the appropriate antibiotic
- immerse the plater in 70% Ethanol and enlighten it
- wait until all Ethanol is burned
- cool the plater down by carefully touching the LB agar plate on the edge
- plate out the transformed *E. coli* by slightly running the plater over the plate
- wait until everything is dried
- Incubate plates upside down at 37°C overnight

Calculations, Tipps/Hints, Outline/Illustration

Comment: just for testing the chemocompetence of the DH5alpha cells, there is no need to calculate the exact amount of DNA, just used 1 µl of the plasmid-dna

Organism	Plasmid
E. Coli DH5α	pDAS12124-PEAR-GFP-preedited
E. Coli DH5α	pCMV-PE2
E. Coli DH5α	LV-PE_CO-Mini
E. Coli DH5α	pDAS12489-PEAR-GFP_2in1_2.0
E. Coli DH5α	PMJ326: pCMV-GtFz1
E. Coli DH5α	PU6-pegRNA-GG-acceptor
E. Coli DH5α	pZMB
E. Coli DH5α	PUC19

Results

/

Analysis & Interpretation

/

Achievements of the Day

All goals achived.

No	Date (DD/MM/YYYY)	Titel	Experimenters
25	26/06/2024	planning cell culture experiments, checking transformation efficiency	IG, KK, KS

Aims for the day

- Checking Transformation efficiency on plates
- Development & planning of preliminary test for proof of concept

Protocols

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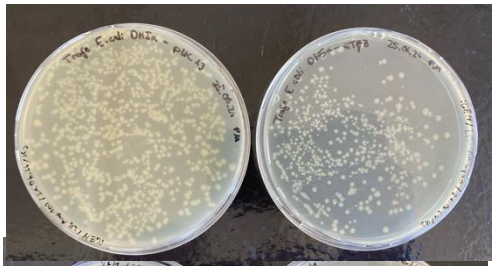
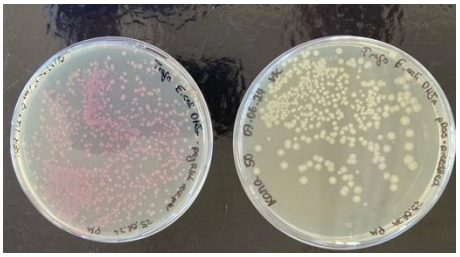
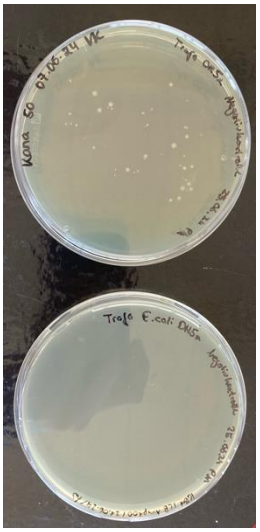
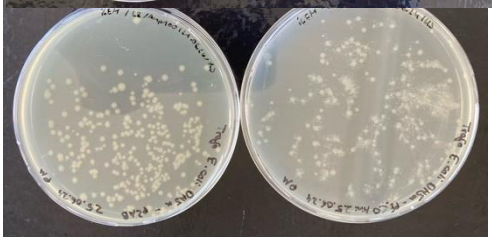
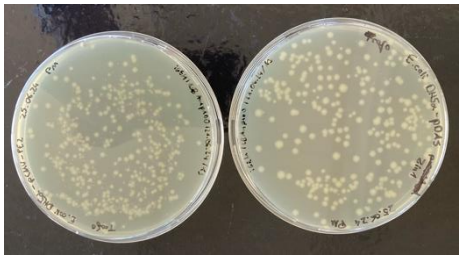
Calculations, Tipps/Hints, Outline/Illustration

/

Results


Analysis & Interpretation

- Negative control Amp: negative —> very good
- Negative control Kana: positive —> not good
- Positive control: positive —> very good
- All plasmids: positive —> very good!

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Title	Experimenters
16	27/06/2024	Seeding for preliminary test proof of concept	IG

Aims for the day

- Seeding of HEK293 cells for preliminary test for proof of concept

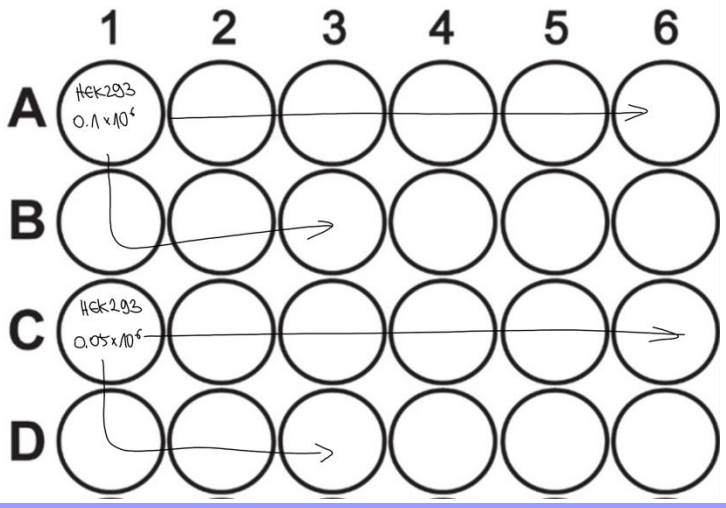
Protocols

Passaging of HEK293 acc. To P19

- Passaging from T75 to T75-flask at a 1:20 dilution 1x (detachment with 300 µL Trypsin, stopping with 5 mL DMEM, transfer of 265 µL into a new T75-flask and adding DMEM medium to a 15 mL volume)
- Seeding of $0.1x10^6$ and $0.05x10^6$ HEK293 cells into a 24 well plate, 9 wells for each concentration, adding DMEM medium up to a 0.5 mL volume

Calculations, Tipps/Hints, Outline/Illustration

- Layout of 24-well plate for preliminary test for proof of concept



Results

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Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
17	29/06/2024	Transfection for preliminary proof of concept, preparing media & plates	KS

Aims for the day

- Preparing media
- Pouring LB-media plates with different antibiotics
- Autoclave
- Transfection of HEK293 for preliminary proof of concept

Protocols

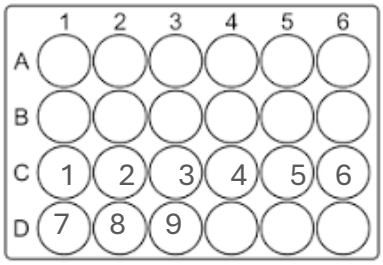
Prepare media acc. To P1

Prepare LB+agar acc. To P1

Transfection of HEK 293 with Lipofectamin 2000 acc. To P30

- Dilute lipofectamin 1:10 in DMEM media, incubate 5 min
- Dilute plasmid DNA if necessary in DMEM media, incubate 5 min
- Prepare Transfection mix: 750ng PE, 250ng pecRNA, same volume of diluted lipofectamine —> incubate 20 min
- Cells should have 60-80% confluence
- Remove media from wells
- Add 0.5 ml DMEM media and Transfection mix to each well
- Incubate 4h at 37°C
- Remove media and add fresh DMEM media
- Incubate at 37°C

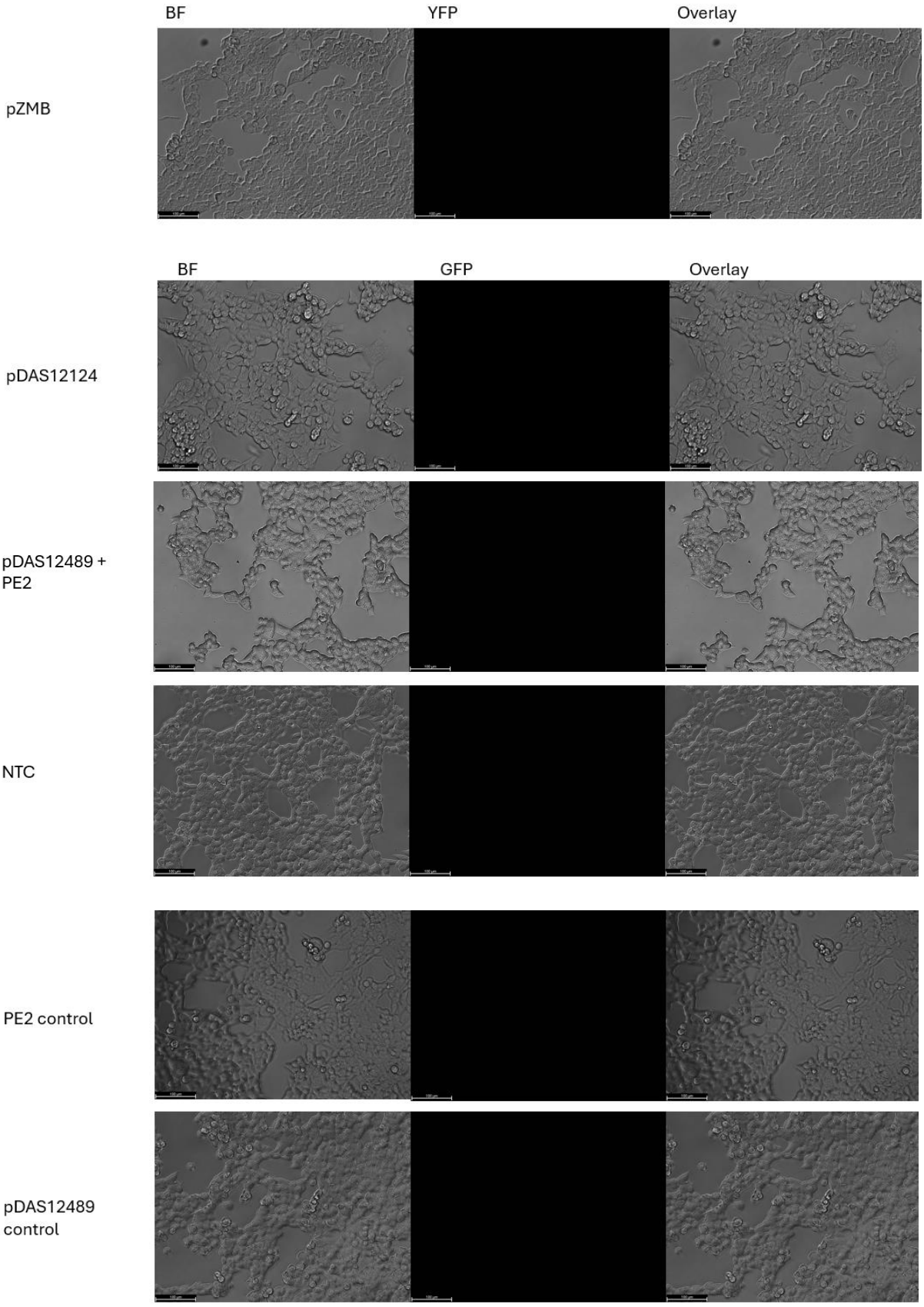
Calculations, Tipps/Hints, Outline/Illustration



Well	Number	Plasmids	Expected result
C1	1	pDAS12489-PEAR-GFP_2in1_2.0 (250 ng)	No Fluorescence
C2	2	pDAS12489-PEAR-GFP_2in1_2.0 (250 ng), pCMV-PE (750 ng)	Fluorescence (GFP)
C3	3	pCMV-PE (750 ng)	No Fluorescence
C6	6	pDAS12124-PEAR-GFP-preedited (1000 ng)	Fluorescence (GFP)
D1	7	pZMB (1000 ng)	Fluorescence (YFP)
D2	8	NTC	No Fluorescence

Results

Microscopy 4h post transfection



Analysis & Interpretation

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Achievements of the Day

All aims achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
18	30/06/2024	Microscopy of HEK293 transfection after 24h	LW

Aims for the day

- Screen cells for fluorescence

Protocols

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Calculations, Tipps/Hints, Outline/Illustration

/

Results

No fluorescence visible!

Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
19	01/07/2024	Passaging of HEK293, Microscopy of HEK293 Transfection after 47h	IG, KS

Aims for the day

- Passaging of HEK293
- Screen transfected cells for fluorescence

Protocols

Passaging of HEK293 acc. To P19

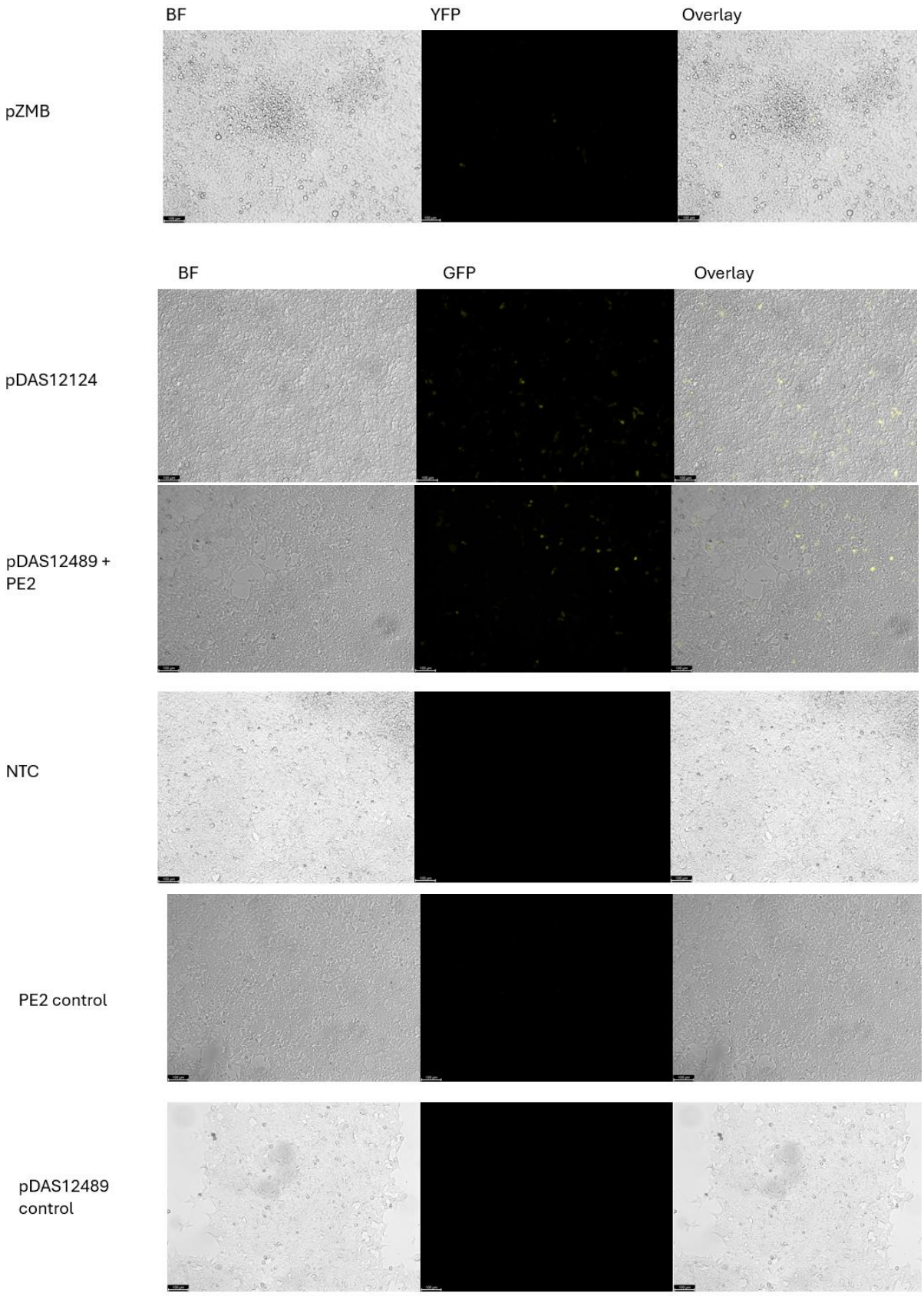
- Passaging from T75 to T75-flask at a 1:20 dilution 1x (detachment with 300 µL Trypsin, stopping with 5 mL DMEM, transfer of 265 µL into a new T75-flask and adding DMEM medium to a 15 mL volume)

Calculations, Tipps/Hints, Outline/Illustration

/

Results

Microscopy 47h post transfection


BF
YFP
Overlay
pZMB
BF
GFP
Overlay
pDAS12124
pDAS12489 +
PE2
NTC
PE2 control
pDAS12489
control

Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
20	02/07/2024	Microscopy of transfected HEK293 after 72h	KS

Aims for the day

- Screen transfected cells for fluorescence

Protocols

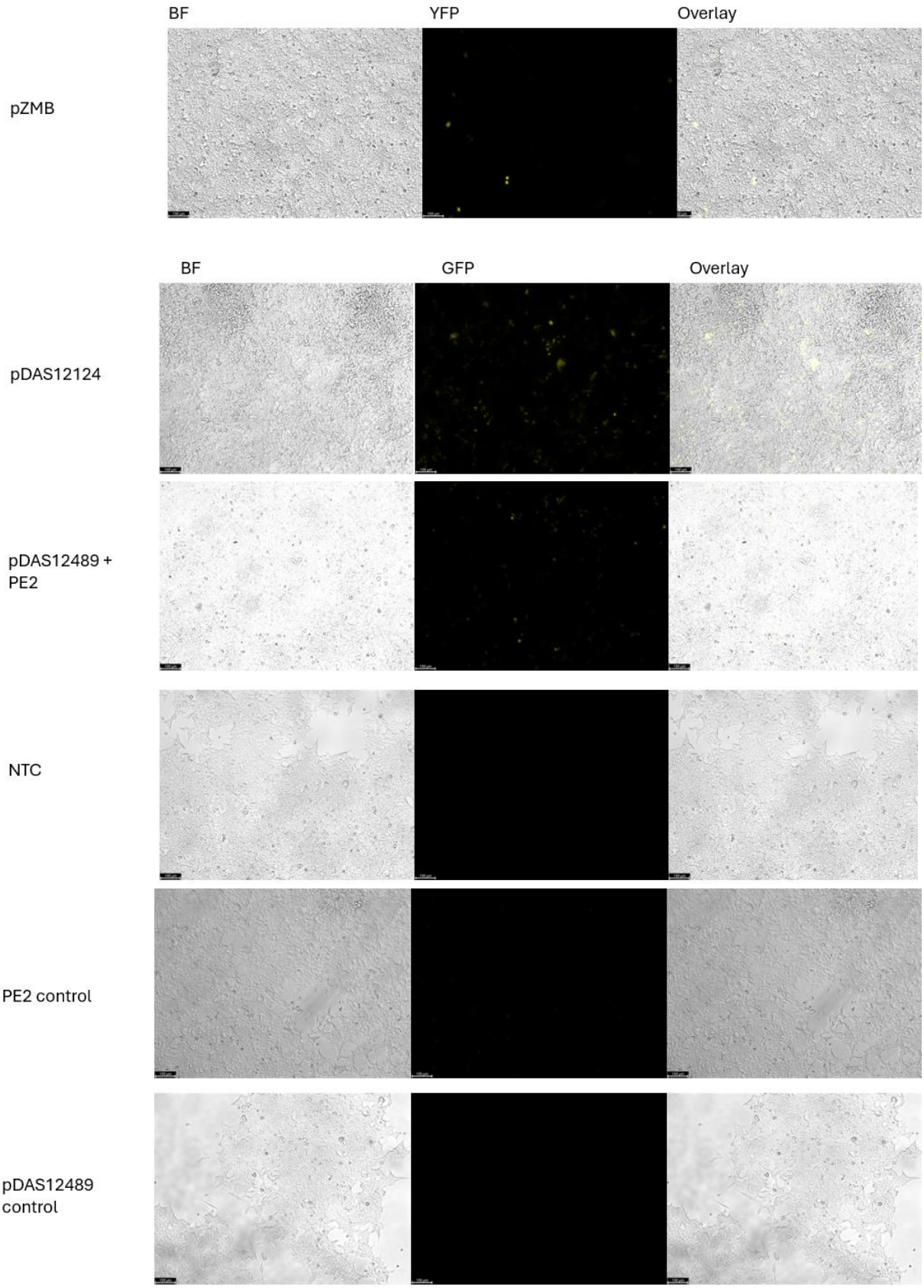
/

Calculations, Tipps/Hints, Outline/Illustration

/

Results

Microscopy 72h post transfection



Analysis & Interpretation

- Fluorescence as expected but transfection efficiency was very low

- Protocol has to be optimized!

Achievements of the Day

All aims achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
21	03/07/2024	inoculation of liquid cultures	IG, KS, ML

Aims for the day

- Start Preparation of electrocompetent DH5a

Protocols

Electrocompetent *E. coli* (needs to be finished tomorrow)

- Inoculation of 200 mL LB with DH5a to prepare electrocompetent *E. coli* tomorrow

Calculations, Tipps/Hints, Outline/Illustration

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Results

/

Analysis & Interpretation

/

Achievements of the Day

All aims achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
22	04/07/2024	Passaging of HEK93	IG

Aims for the day

- Passaging of HEK293

Protocols

Passaging of HEK293 acc. To P19

- Passaging from T75 to T75-flask at a 1:20 dilution 1x (detachment with 300 µL Trypsin, stopping with 5 mL DMEM, transfer of 265 µL into a new T75-flask and adding DMEM medium to a 15 mL volume)

Calculations, Tipps/Hints, Outline/Illustration

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Results

/

Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
23	05/07/2024	Preparing electrocompetent *E. coli* dH5a cells & glycerin stocks	KK, VK, AB

Aims for the day

- Prepare electrocompetent *E. coli* dH5a cells
- Prepare glycerin stocks of *E. coli* dH5a cells

Protocols

Preparing electrocompetent *E. coli* dH5a acc. To P2

- Measuring OD_{600} of o/n
- culture (actually 48 h culture), diluted if OD600 >1
- Prepare 500 ml culture with an OD_{600} of 0.2 (424 ml LB medium + 76 ml pre culture (OD 1.3))
- Incubate the culture for 1 h at 37°C, measuring OD_{600} again (aim 0.4-0.5).
- Prepare 170 ml 10 % glycerin solution: Mix 153 ml H2O with 17 ml 100 % glycerin
- Keep on ice for following wash steps (see below).
    - Centrifuge the cells at 4000 rpm / 20 min / 4°C
    - Wash the cell pellet with 1 volume of sterile 10 % glycerol
    - Centrifuge the cells at 4000 rpm / 20 min / 4°C
    - Wash the cell pellet with 0.5 volumes of sterile 10 % glycerol
    - Centrifuge the cells at 4000 rpm / 20 min / 4°C
    - Wash the cell pellet with 0.1 volumes of sterile 10 % glycerol
    - Centrifuge the cells at 4000 rpm / 10 min / 4°C
    - Add 0.5 mL of sterile 10 % glycerol (per 0.5 liter of liquid culture you started with)
    - resuspend the cell pellet
    - Transfer 100 µl Aliquots to 1.5 ml reaction tubes
- Store at –20 °C

Preparing glycerin stocks of *E. coli* dH5a acc. To P5

- Using the same o/n culture as for the preparation of the electrocompetent *E. coli* cells (see above) with an OD_{600} of 1
- Mix 500 µl cell culture with 1 ml 100 % glycerin in a 2 ml Eppi
- Store at –80 °C

Calculations, Tipps/Hints, Outline/Illustration

- To pipette the 100 % glycerol, cut off the tip of a 1 ml pipette tip or measure in a falcon tube

Results

OD_{600} of *E. coli* dH5a o/n culture:

- 1st measurement OD600 = 1.3
- 2nd measurement (after 1 h incubation, with start OD600 0.2) OD600 = 0.47

Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
24	08/07/2024	Passaging of HEK293 and seeding for preliminary test proof of concept repetition	IG

Aims for the day

- Seeding of HEK293 cells for preliminary test for proof of concept repetition

Protocols

Passaging of HEK293 acc. To P19

- Passaging from T75 to T75-flask at a 1:20 dilution 1x (detachment with 300 µL Trypsin, stopping with 5 mL DMEM, transfer of 265 µL into a new T75-flask and adding DMEM medium to a 15 mL volume)

Seed HEK293 acc. To 19

- Seeding of $0.05x10^6$ HEK293 cells into a 24 well (row C and D), adding DMEM medium up to a 0.5 mL volume

Calculations, Tipps/Hints, Outline/Illustration

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Results

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Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
25	10/07/2024	Transfection HEK, LB medium with Chloramphenicol	KS, AB

Aims for the day

- Transfection of HEK with pZMB to improve transfection skills (KS)
- Collecting Data, 4h after Transfection
- Prepare fresh LB-Medium with Chloramphenicol and distribute the media in 4x 250ml Shotflasks

Protocols

Transfection of HEK293 with Lipofectamine 2000 acc. To P30

- Dilute plasmid DNA in DMEM and Lipofectamine 2000 in DMEM & incubate 5 min

    - DNA in 50 µl DMEM
        - 1000 ng DNA: 2,18 µl DNA
        - 800 ng DNA: 1,75 µl DNA
    - Lipofectamine 2000 1:10: 7.5 µl (+ 67.5 µl DMEM)
    - Lipofectamine 2000 1:25: 3 µl (+ 72 µl DMEM)
- Mix diluted DNA with diluted Lipofectamine 2000, incubate 20 min
- Remove medium from cells, add 0,5 ml DMEM on cells
- Add transfection mix drop by drop (100 µl each well)
- Incubate 37°C

Microscopy of HEK293 4h after transfection

- collecting fluorescence data

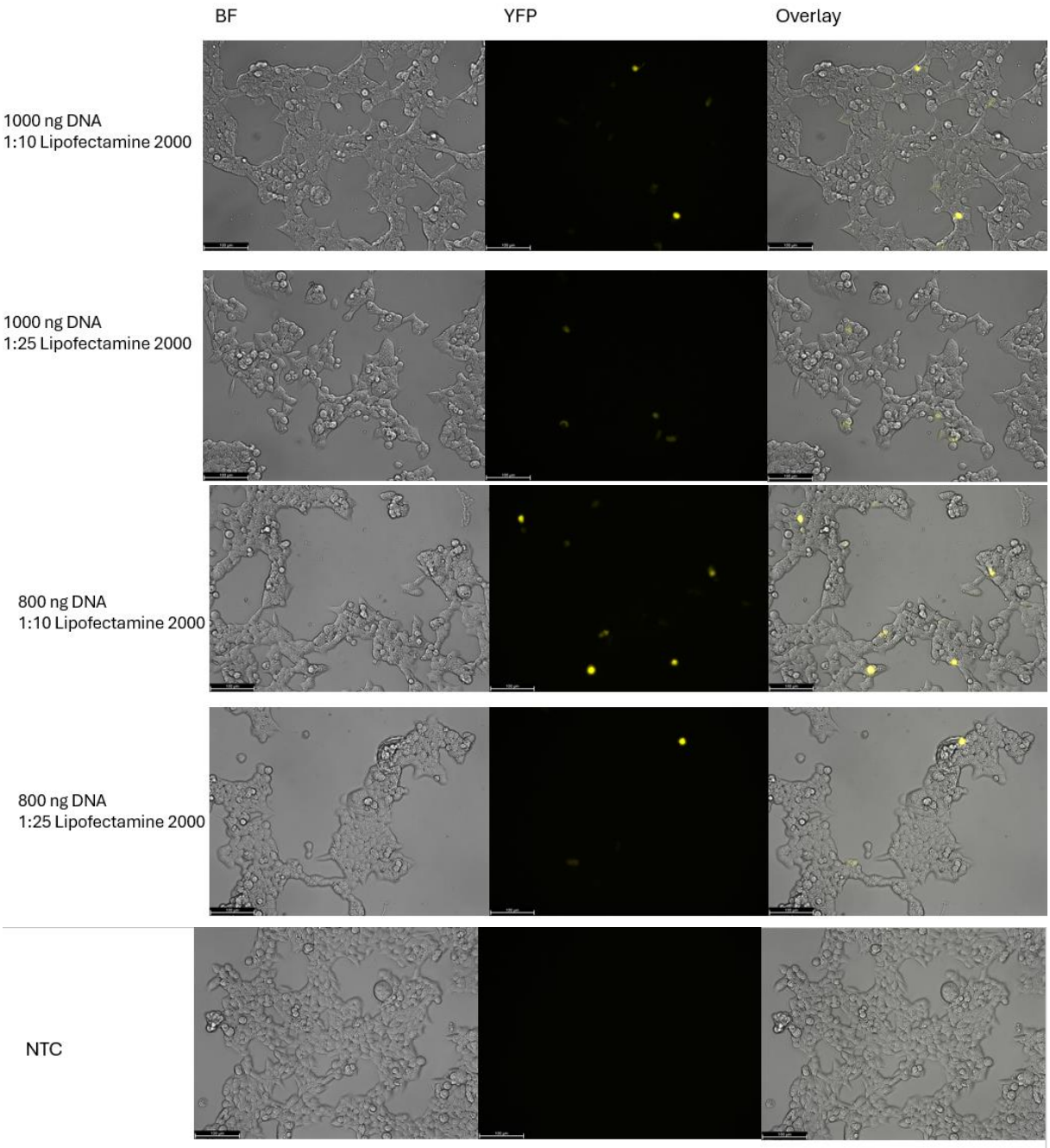
Prepare fresh LB-Media with Chloramphenicol à 1 L liquid LB-media with Chloramphenicol (final conc. 34 µg/ml) and distribute it to 4x 250ml flasks acc. To P1

Calculations, Tipps/Hints, Outline/Illustration

Well	Concentration plasmid	Dilution Lipofectamine
C1/D1	1000 ng	1:10
C2/D2	1000 ng	1:25
C3/D3	800 ng	1:10
C4/D4	800 ng	1:25
C5/D5	NTC	1:10
C6/D6	NTC	1:25

Results

Microscopy 4h post transfection



Analysis & Interpretation

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Achievements of the Day

All goals achieved

No	Date (DD/MM/YYYY)	Titel	Experimenters
26	11/07/24	Passaging of HEK293, microscopy of transfected HEK cells	IG, KS

Aims for the day

- Passaging of HEK293 cells
- Pictures of transfected HEK cells from yesterday
- Collecting fluorescence data 24h after transfection with pZMB

Protocols

Passaging of HEK293 acc. To P19

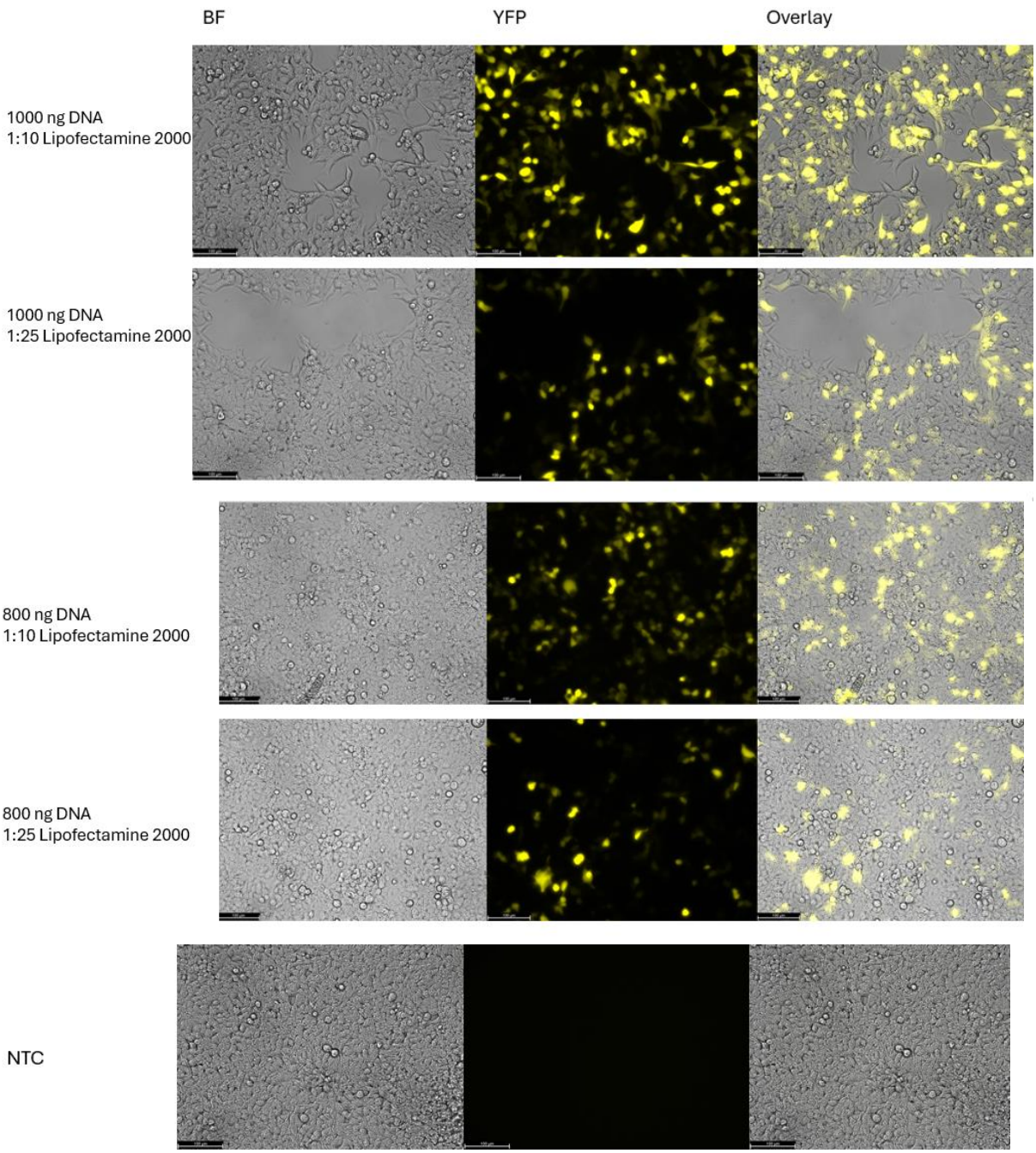
- Passaging from T75 to T75-flask at a 1:20 dilution 1x (detachment with 300 µL Trypsin, stopping with 5 mL DMEM, transfer of 265 µL into a new T75-flask and adding DMEM medium to a 15 mL volume)

Calculations, Tipps/Hints, Outline/Illustration

/

Results

Microscopy 24h post transfection



Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
27	12/07/24	Restriction of pDAS12124_PEAR-GFP-preedited & pDAS12489_PEAR-GFP_2in1_2.0, Microscopy of transfected HEK	VK, AB, KS

Aims for the day

- Restriction of pDAS12124_PEAR-GFP-preedited & pDAS12489_PEAR-GFP_2in1_2.0 with EcoRI & NheI for identification of both plasmids after double & single digestion via gel electrophoresis, as a control whether there was a risk of confusion before, and the transfection results from 29.06 explained.
- Collecting fluorescence data 48h after transfection with pZMB

Protocols

Digestion acc. To P12

Restriction approach for pDAS12124_PEAR-GFP-preedited (sample name "1"), double digestion:

Solution	Volumes for 1x approach (20 µl)
DNA (500 ng)	0.74 µl
EcoRI	1 µl
NheI	1 µl
CutSmart Buffer (10x)	2 µl
H2O	15.26 µl

Restriction approach for pDAS12489_PEAR-GFP_2in1_2.0 (sample name "2"), double digestion:

Solution	Volumes for 1x approach (20 µl)
DNA (500 ng)	2.2 µl
EcoRI	1 µl
NheI	1 µl
CutSmart Buffer (10x)	2 µl
H2O	13,8 µl

Restriction approach for pDAS12124_PEAR-GFP-preedited (sample name "3"), single digestion:

Solution	Volumes for 1x approach (20 µl)
DNA (500 ng)	0.74 µl
EcoRI	1 µl
CutSmart Buffer (10x)	2 µl
H2O	16.26 µl

Restriction approach for pDAS12489_PEAR-GFP_2in1_2.0 (sample name "4"), single digestion:

Solution	Volumes for 1x approach (20 µl)
DNA (500 ng)	2.2 µl

EcoRI	1 µl
CutSmart Buffer (10x)	2 µl
H2O	14.8 µl

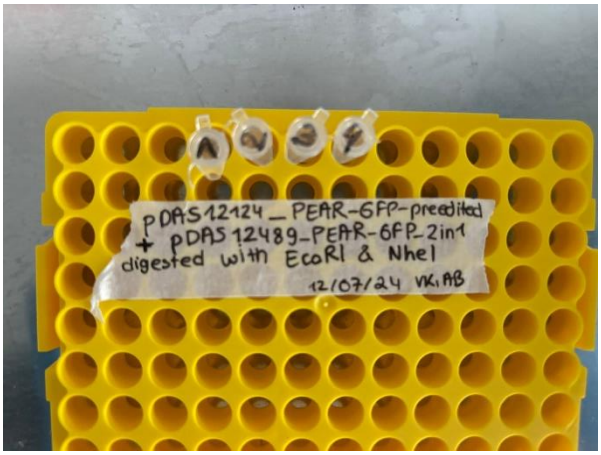
- Put the samples in the Thermocycler with the following program:
  - 3 h at 37 °C
  - 15 min at 80 °C
  - Cool down to 4°C
- Stored until gel electrophoresis at 4°C

Calculations, Tipps/Hints, Outline/Illustration

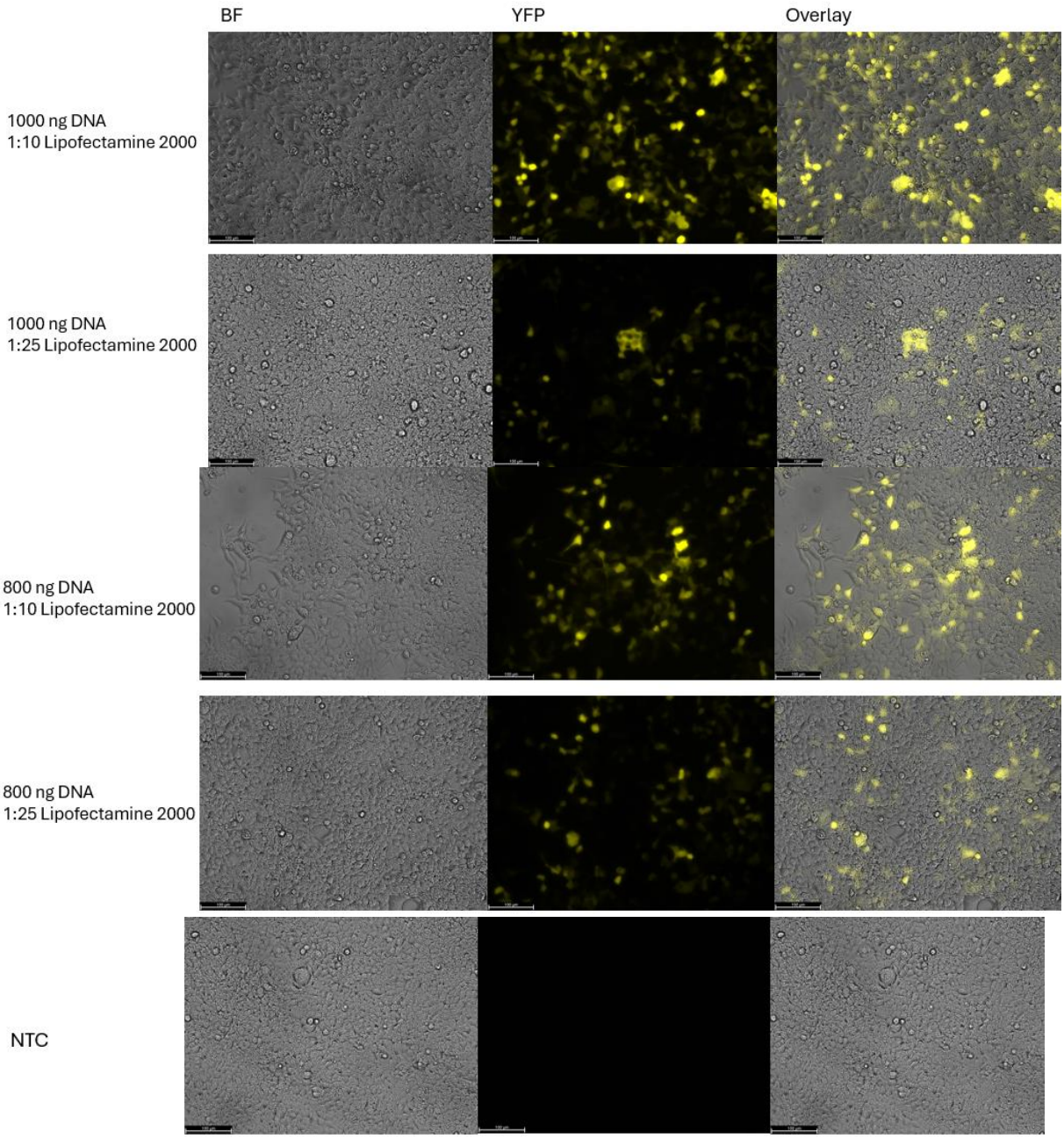
Calculation of the amount of DNA needed for a concentration of 500 ng in 20 µl approach:

- pDAS12489_PEAR-GFP_2in1_2.0: 224 ng/µl -> use 2,2 µl of plasmid
- pDAS12124_PEAR-GFP-preedited: 672 ng/µl -> use 0,74 µ of plasmid

Results



Microscopy 48h post transfection



Analysis & Interpretation

- the highest efficiency is when used 1:10 Lipofectamine 2000 and 1000 ng of DNA
- 1:10 Lipofectamine 2000 shows better transfection efficiency compared to 1:25 lipofectamine 2000

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
28	13/07/2024	Gel electrophoresis of digestion	PM

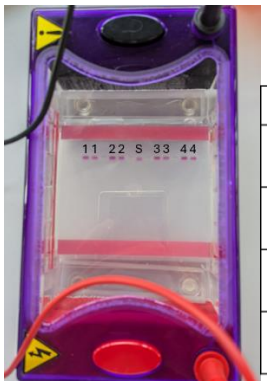
Aims for the day

- Making new Agarose solution for Gel Electrophoresis
- Gel Electrophoresis for Restriction of pDAS12124_PEAR-GFP-preedited & pDAS12489_PEAR-GFP_2in1_2.0 with EcoRI & NheI (see 27)

Protocols

Preparation of Agarose-Gel electrophoresis of digestion approach

- 300 ml of 1% Agarose Solution were prepared
- one gel was poured with a 16 teeth comb using 50 ml Agarose solution and 2.5 µl of RotiStain
- 1 µl of 1kB DNA Ladder was mixed with 1 µl of loading dye and 4 µl of H_20
- 20 µl of digestion solution were mixed with 4 µl of purple 6x loading dye
- 2x 10µl were loaded into the gel pockets for each of the samples
- the results were documented using UV gel documentation table



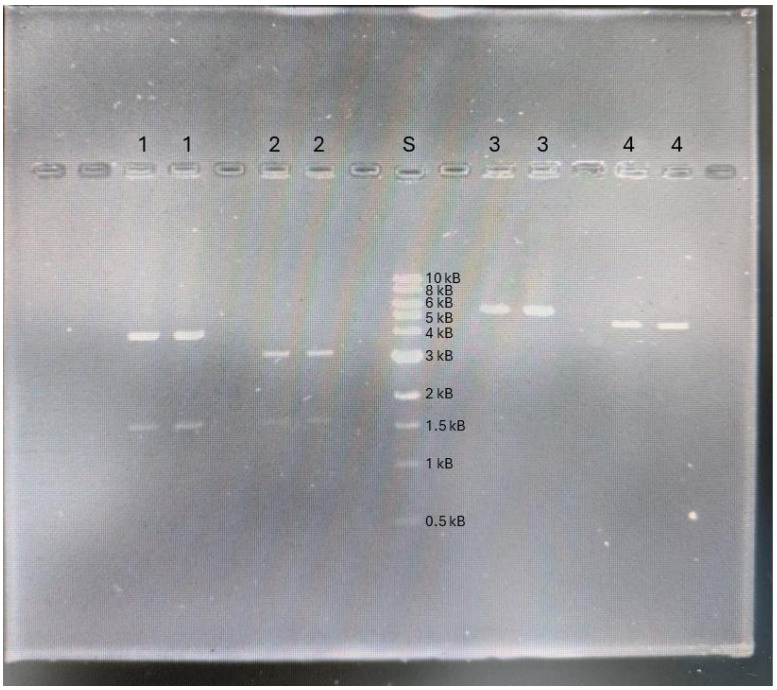
S	1 kB DNA Ladder
1	Double Digestion pDAS12124_PEAR-GFP-preedited (NheI and EcoRI)
2	Double Digestion pDAS12489_PEAR-GFP_2in1_2.0 (NheI and EcoRI)
3	Single Digestion pDAS12124_PEAR-GFP-preedited (EcoRI)
4	Single Digestion pDAS12489_PEAR-GFP_2in1_2.0 (EcoRI)

Calculations, Tipps/Hints, Outline/Illustration

/

Results

- Expected band sizes for double digested “pDAS12124_PEAR-GFP-preedited" with EcoRI & NheI: 1565 bp, 3963 bp
- Expected band sizes for double digested “pDAS12489_PEAR-GFP_2in1_2.0" with EcoRI & nheI: 1565 bp, 3118 bp



S	**1 kB DNA Ladder**
1	Double Digestion pDAS12124_PEAR-GFP-preedited (NheI and EcoRI)
2	Double Digestion pDAS12489_PEAR-GFP_2in1_2.0 (NheI and EcoRI)
3	Single Digestion pDAS12124_PEAR-GFP-preedited (EcoRI)
4	Single Digestion pDAS12489_PEAR-GFP_2in1_2.0 (EcoRI)

Analysis & Interpretation

- The results suggest, that the plasmids digested are indeed the ones they are supposed to be

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
28	15/07/24	Passaging of HEK293, seed HEK293 for proof of concept	KS

Aims for the day

- Passaging/expanding of HEK293 cells
- Seed cells for proof of concept

Protocols

Passaging/expanding of HEK293 cells acc. To P19

- Cell count: 3.15*10^6 (98% Viability)
- Passing: 1x T75 1:20
- Expanding: 1x T175, seeded 4.9*10^6 cells (1,56 ml)

Seed cells for proof of concept acc. To P19

- Seeding of $0.05x10^6$ HEK293 cells into a 24 well plate adding DMEM medium up to a 0.5 mL volume

Calculations, Tipps/Hints, Outline/Illustration

/

Results

/

Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
30	16/07/24	Transfection proof of concept, Microscopy of transfected cells	KS

Aims for the day

- Transfection proof of concept
- Microscopy of transfected cells

Protocols

Transfection of HEK293 with Lipofectamine 3000 acc. To P30

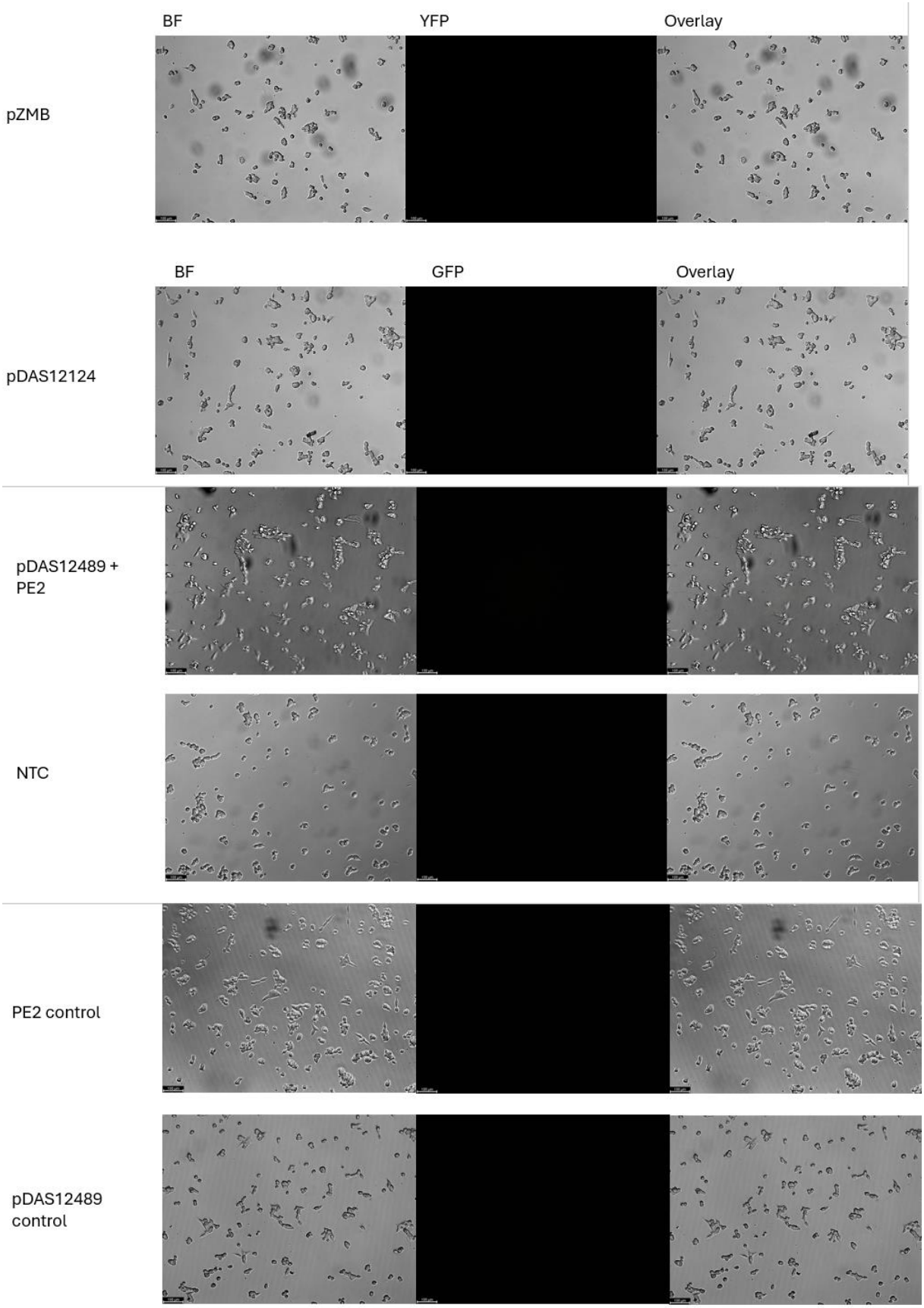
- Lipofectamine 3000: 1 µl in 25 µl DMEM
- DNA: your desired concentration in 50 µl DMEM
- Transfection mixture: 25 µl diluted Lipofectamine 3000 + 25 µl diluted DNA + 0.5 µl Reagent 3000

Calculations, Tipps/Hints, Outline/Illustration

Well	Plasmids
A1, A2, C1, C2	pDAS12489-PEAR-GFP_2in1_2.0 (250 ng), pCMV-PE (750 ng)
A5, A6, C5, C6	pDAS12124-PEAR-GFP-preedited (1000 ng)
B1, D1	NTC
B2, D2	pZMB (1000 ng)
B3, D3	pDAS12489-PEAR-GFP_2in1_2.0 (250 ng),
B4, D4	pCMV-PE (750 ng)

Results

Microscopy 6h post transfection



Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
31	17/07/24	Microscopy of transfected HEK cells, prepare o/n cultures	KS, CM

Aims for the day

- Microscopy of the transfected HEK cell
- Prepare o/n cultures

Protocols

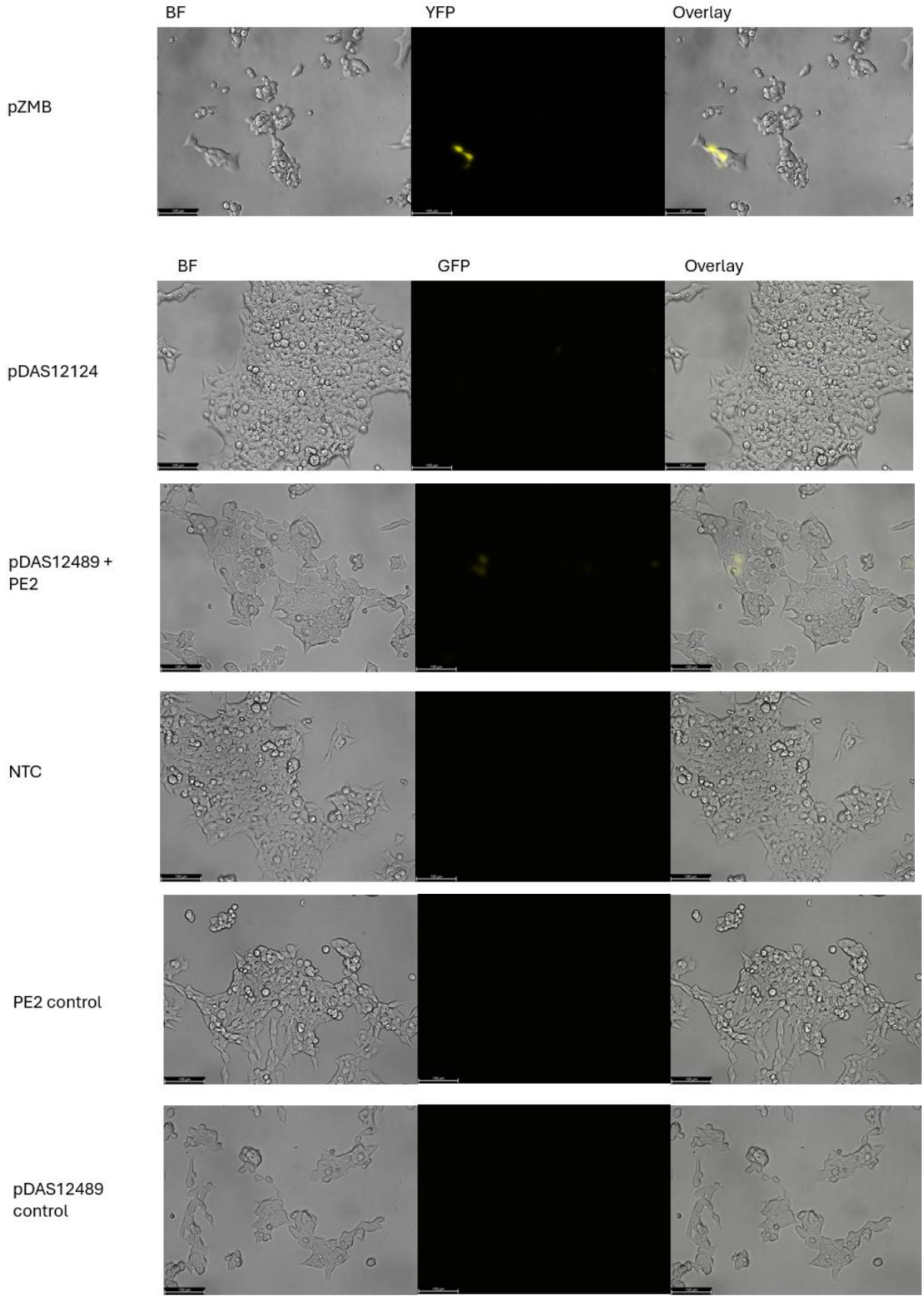
Prepare o/n cultures in 5 ml LB acc. To P36

Calculations, Tipps/Hints, Outline/Illustration

/

Results

Microscopy 26h post transfection



Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
32	18/07/2024	Microscopy of transfected HEK cells, plasmid isolation, prepare o/n cultures	KS, VK, KK, AB

Aims for the day

- Microscopy of transfected HEK cells
- Isolate plasmids pDAS12124-PEAR-GFP-preedited, pCMV-PE2, pU6-pegRNA-GG-acceptor
- Prepare o/n cultures

Protocols

Mini-prep acc. To P6

- Application of 5 ml *E. coli* dH5a o/n culture with plasmids pDAS12124-PEAR-GFP-preedited, pCMV-PE2, pU6-pegRNA-GG-acceptor diluted to an OD of 1
- Eluted with 50 µl DNase free water

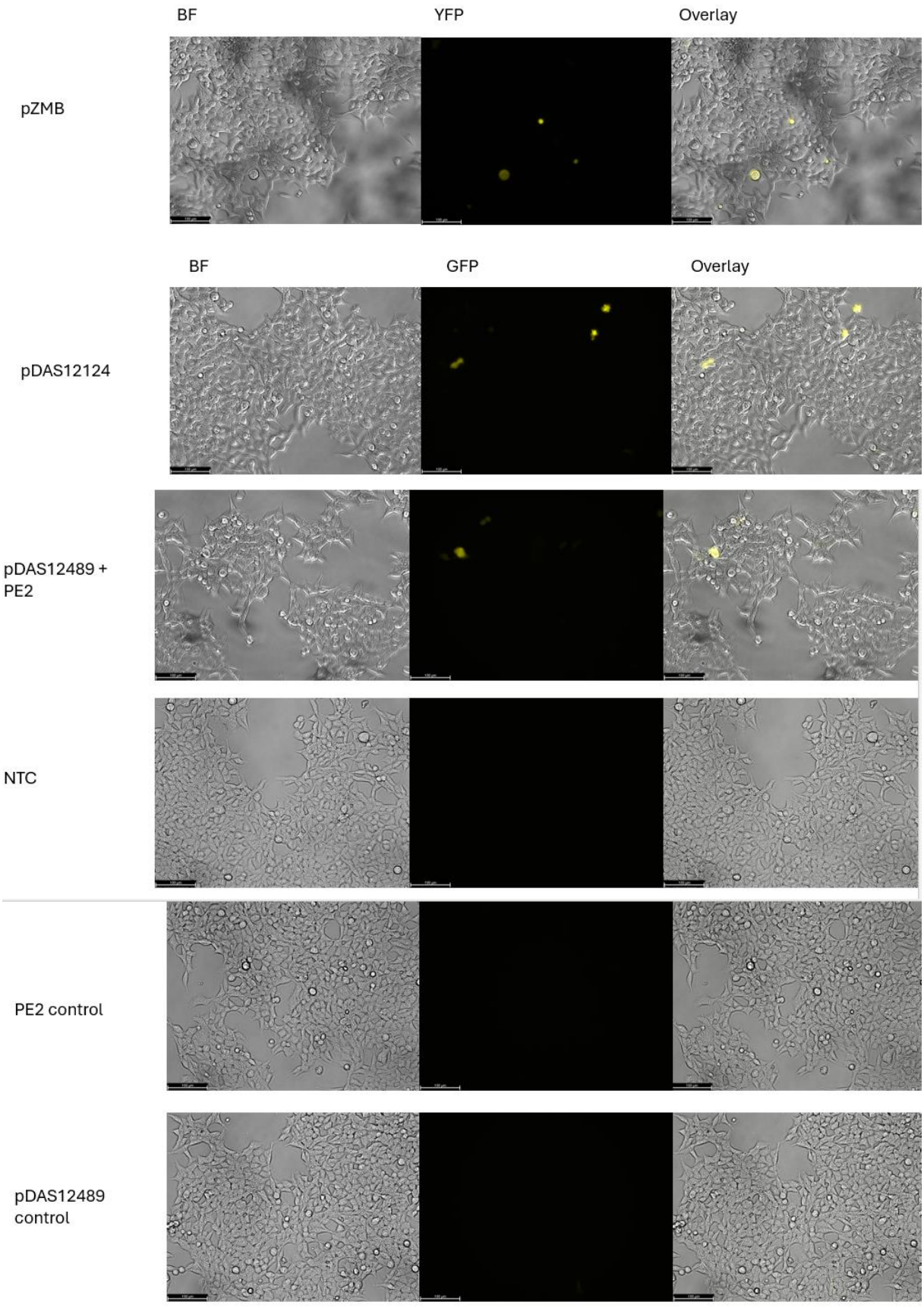
Prepare o/n cultures in 5 ml LB acc. To P36

Calculations, Tipps/Hints, Outline/Illustration

/

Results

Microscopy 48h post transfection



Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
33	19/07/2024	microscopy of transfected HEK cells, OD-measurement, mini-prep, gel extraction	KL

Aims for the day

- Microscopy of transfected HEK cells
- OD Measurement
- Mini prep
- Gel extraction
- PCR

Protocols

Mini-prep acc. To P6

Gel extraction acc. To P14

PCR with Q5 acc. To P9

Calculations, Tipps/Hints, Outline/Illustration

/

Results

OD Measurement

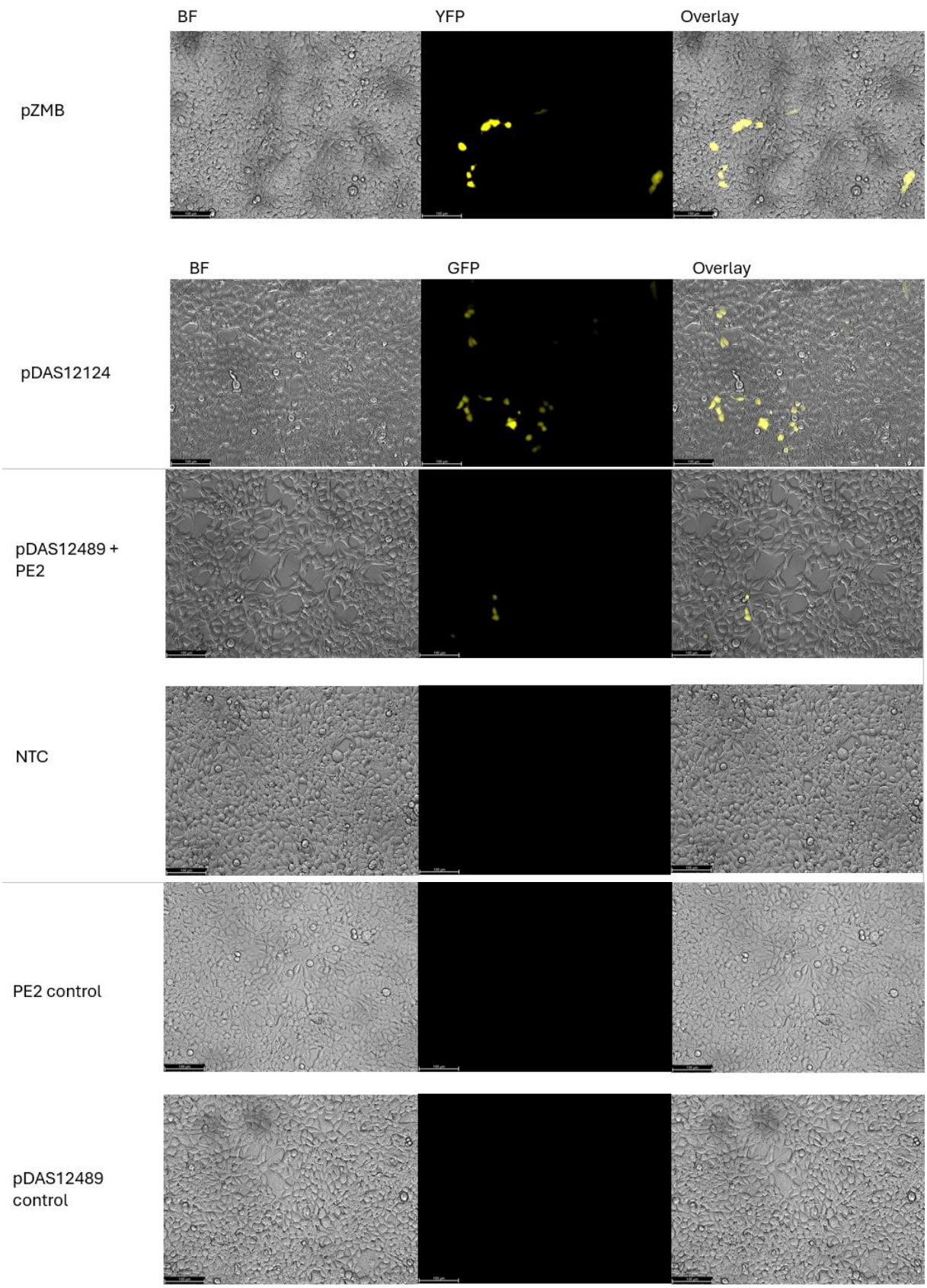
Plasmid	Measured OD_{600}	Dilution	Real OD_{600}
1	0.870	1	0.870
2	0.741	2	1.482
3	0.271	2	0.542

Concentration of Mini Prep

Plasmid	Concentration [ng/µl]	Volume eluted
pDAS12124 mini prep	324ng/µl	19µL
pDAS12124 mini prep	121,5 ng/µl	19µL
pDAS12124 mini prep	231,4 ng/µl	19µL
pegRNAacc mini prep	155,3 ng/µL	19µL
pegRNAacc mini prep	152,5	19

pegRNAacc mini prep	128,6	19
pCMVPE2 mini prep	478,9	19
pCMVPE2 mini prep	228,4	19
pCMVPE2 mini prep	154,5	19

Microscopy 72h post transfection



Analysis & Interpretation

- OD Measurement: not good growth, discarded
- Transfection worked out but efficiency was very bad → protocol must be optimized

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
34	22/07/2024	Prepare LB medium	KS

Aims for the day

- Prepare LB medium

Protocols

LB medium acc. To P1

Calculations, Tipps/Hints, Outline/Illustration

/

Results

/

Analysis & Interpretation

/

Achievements of the Day

All goals achieved

No	Date (DD/MM/YYYY)	Titel	Experimenters
35	29/07/2024	seeding cells for proof of concept test 2	KS

Aims for the day

- Seeding cells for proof-of-concept test

Protocols

Seeding cells on 24-well plate acc. To P19

Calculations, Tipps/Hints, Outline/Illustration

- HEK293 cells
    - 1x 24 well (5 wells each 0.1*10^6 cells)

Results

- HEK293: 1.47*10^6 cells (95.2% viability)

Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters

36	30/07/2024	Transfection proof-of-concept test 2, microscopy	KS

Aims for the day

- Transfection test 2 proof-of-concept with pZMB and lipofectamine 3000

Protocols

Transfected with Lipofectamine 3000 acc. To P30

Calculations, Tipps/Hints, Outline/Illustration

Concentration pZMB: 457.7 ng/µl

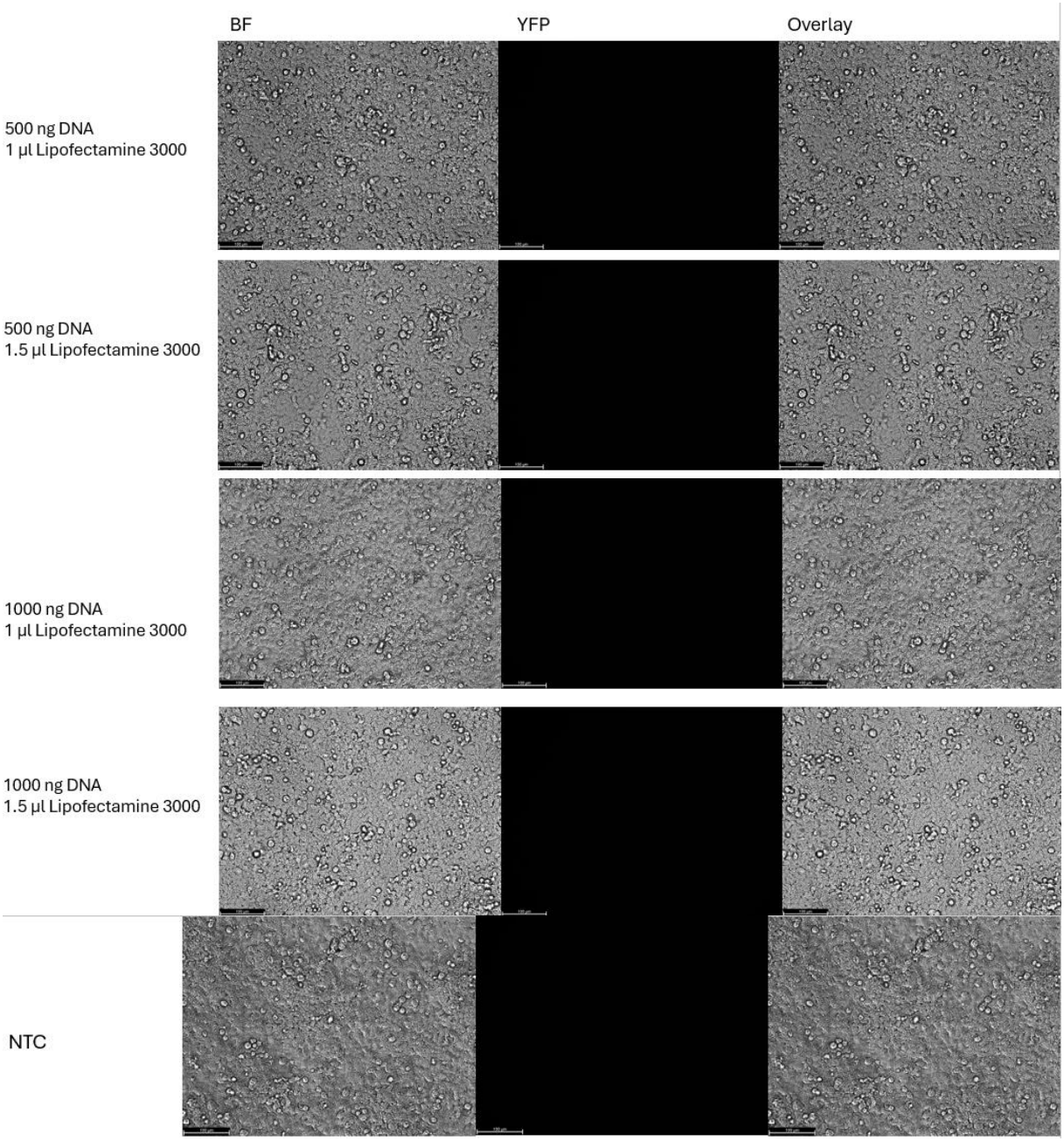
- DNA A1+A2: 2.18 µl
- DNA A3+A4: 4.37 µl
  - ➢ Dilute in 25 µl
- Lipofectamine 3000: 1 µl in 25 µl DMEM
- DNA: your desired concentration in 50 µl DMEM
- Transfection mixture: 25 µl diluted Lipofectamine 3000 + 25 µl diluted DNA + X µl Reagent 3000

A1: 500 ng DNA + 1 µl Reagent 3000 + 1 µl Lipofectamin 3000
A2: 500 ng DNA + 1 µl Reagent 3000 + 1.5 µl Lipofectamin 3000
A3: 1000 ng DNA +2 µl Reagent 3000 + 1 µl Lipofectamin 3000
A4: 1000 ng DNA + 2 µl Reagent 3000 + 1.5 µl Lipofectamin 3000
A5: NTC

- 50 µl per well

Results

Microscopy after 6h



Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
37	31/07/2024	Microscopy of proof of concept test 2	KS

Aims for the day

- Microscopy proof-of-concept

Protocols

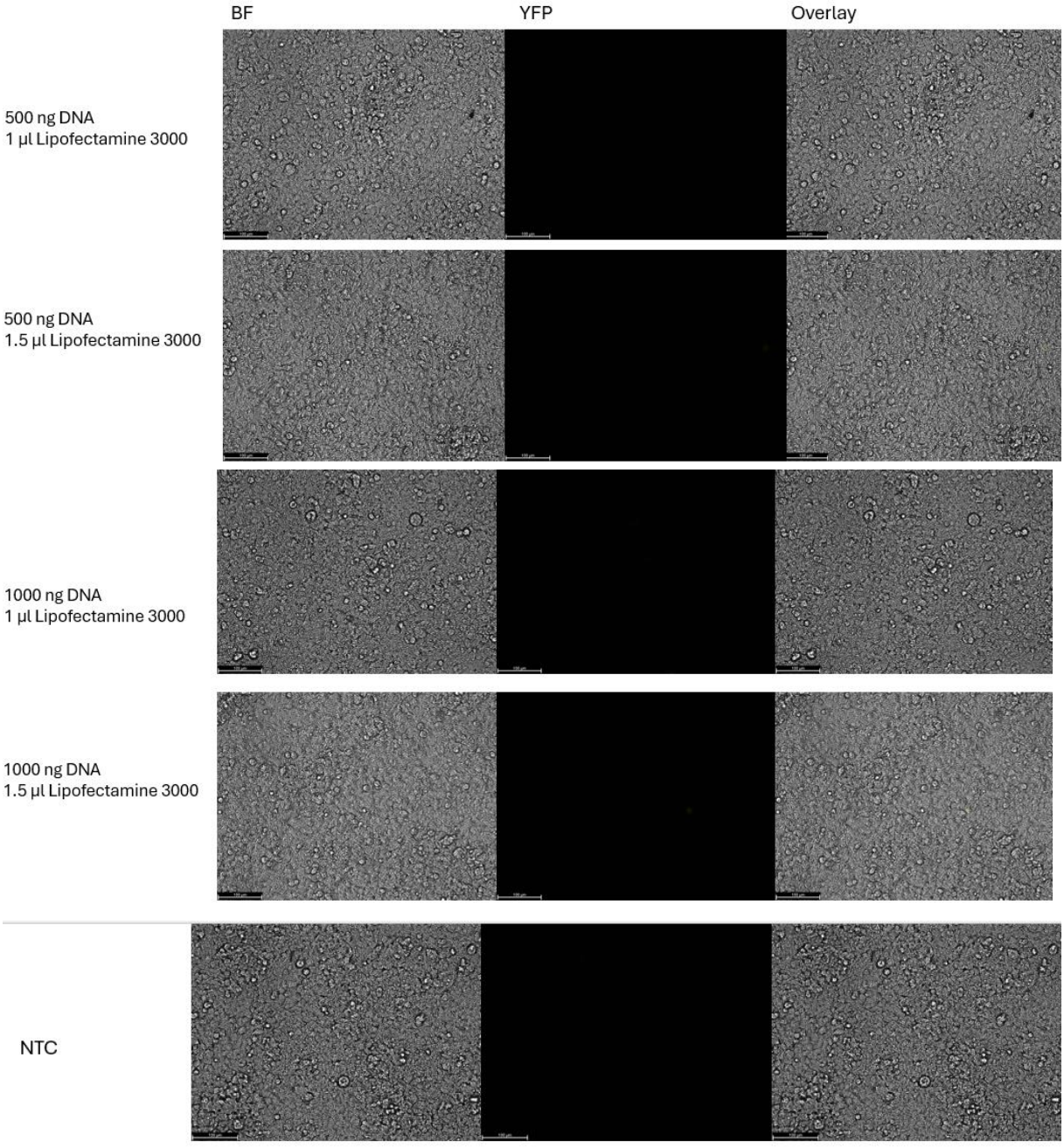
/

Calculations, Tipps/Hints, Outline/Illustration

/

Results

Microscopy after 24h



Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
38	01/08/2024	Microscopy of proof of concept test 3	KS

Aims for the day

- Microscopy proof-of-concept

Protocols

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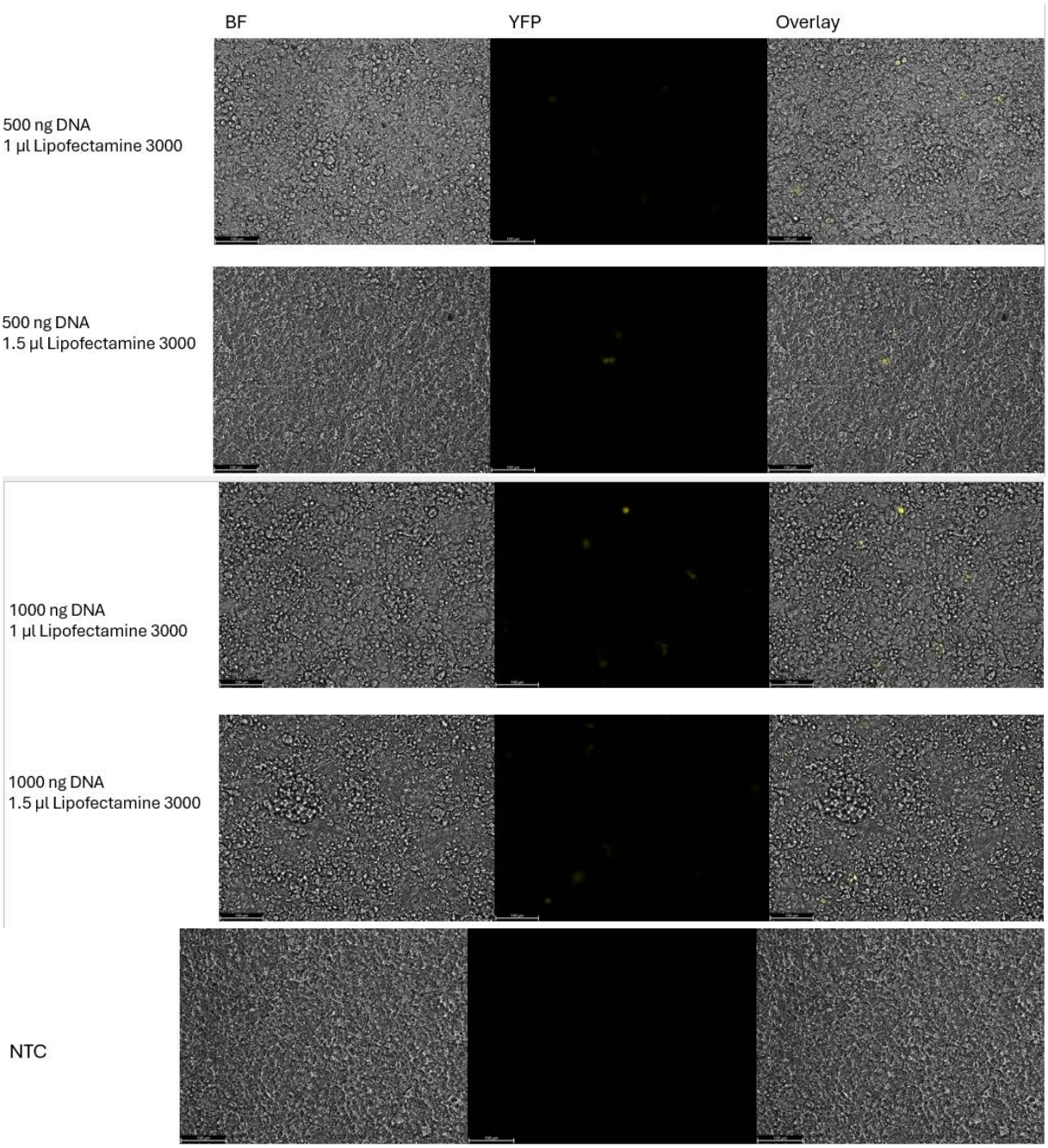
Calculations, Tipps/Hints, Outline/Illustration

/

Results

Microscopy after 48h



Analysis & Interpretation

- Efficiency was very bad
- Even worse compared to earlier transfections
- A reason could be that the cells were "too old" → new HEK cells should be thawed!

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
39	29/08/2024	Passaging of HEK293	IG

Aims for the day

- Passaging of HEK293 for proof of concept repetition

Protocols

Passaging of HEK293 acc. To P19: 1xT75 in 2xT75 flask

- Wash cells with 20 mL PBS
- Trypsinate with 300 µL DMEM + trypsin + EDTA for T75-flask
- Incubate for 5 minutes at 37 °C and 5 % CO_2
- Stop trypsination with 5 mL DMEM + 10 % FCS + Pen/Strep
- Transfer 265 µL of cell suspension in T75-flask (1:20 dilution)
- Add up DMEM + 10 % FCS + Pen/Strep to 10 mL

Calculations, Tipps/Hints, Outline/Illustration

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Results

/

Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
40	02/09/2024	Passaging of HEK293, seeding of HEK293	KS

Aims for the day

- Passaging of HEK293 for proof of concept repetition
- Seeding of HEK293 for proof of concept repetition

Protocols

Passaging of HEK293 acc. To P19: 1xT75 in 1xT75 flask

- Wash cells with 20 mL PBS
- Trypsinate with 300 µL DMEM + trypsin + EDTA for T75-flask
- Incubate for 5 minutes at 37 °C and 5 % CO_2
- Stop trypsination with 5 mL DMEM + 10 % FCS + Pen/Strep
- Transfer 265 µL of cell suspension in T75-flask (1:20 dilution)
- Add up DMEM + 10 % FCS + Pen/Strep to 8 mL

Seeding of HEK293 acc. To P19: in 24-well plate

- Wash cells with 20 mL PBS
- Trypsinate with 300 µL DMEM + trypsin + EDTA for T75-flask
- Incubate for 5 minutes at 37 °C and 5 % CO_2
- Stop trypsination with 5 mL DMEM + 10 % FCS + Pen/Strep
- Count cells

- Seed 0.05*10^6 cells/ml in each well (223 µl), add 277 µl DMEM

Calculations, Tipps/Hints, Outline/Illustration

/

Results

HEK293: 223333 cells/ml

➔ 0.05*10^6 cells/ml / 223333 cells/ml = 0.223 ml —> 223 µl

- Seeded 12 wells for proof of concept!

Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
41	03/09/2024	Transfection, microscopy	KS

Aims for the day

- Transfection with lipofectamine 3000
- Transfection with $CaCl_2$

Protocols

Transfection with Lipofectamine 3000 acc. To P19

- A: 25 µl Opti-MEM + 2 µl Reagent 3000 + 3.28 µl DNA
- B: 25 µl Opti-MEM + 1.5 µl Reagent 3000 + 2.18 µl DNA
- Well A1: A + 25 µl Opti-MEM + 1.5 µl
- Well A2: B + 25 µl Opti-MEM + 1.5 µl
- Well A3: 25 µl Opti-MEM + 1.5 µl

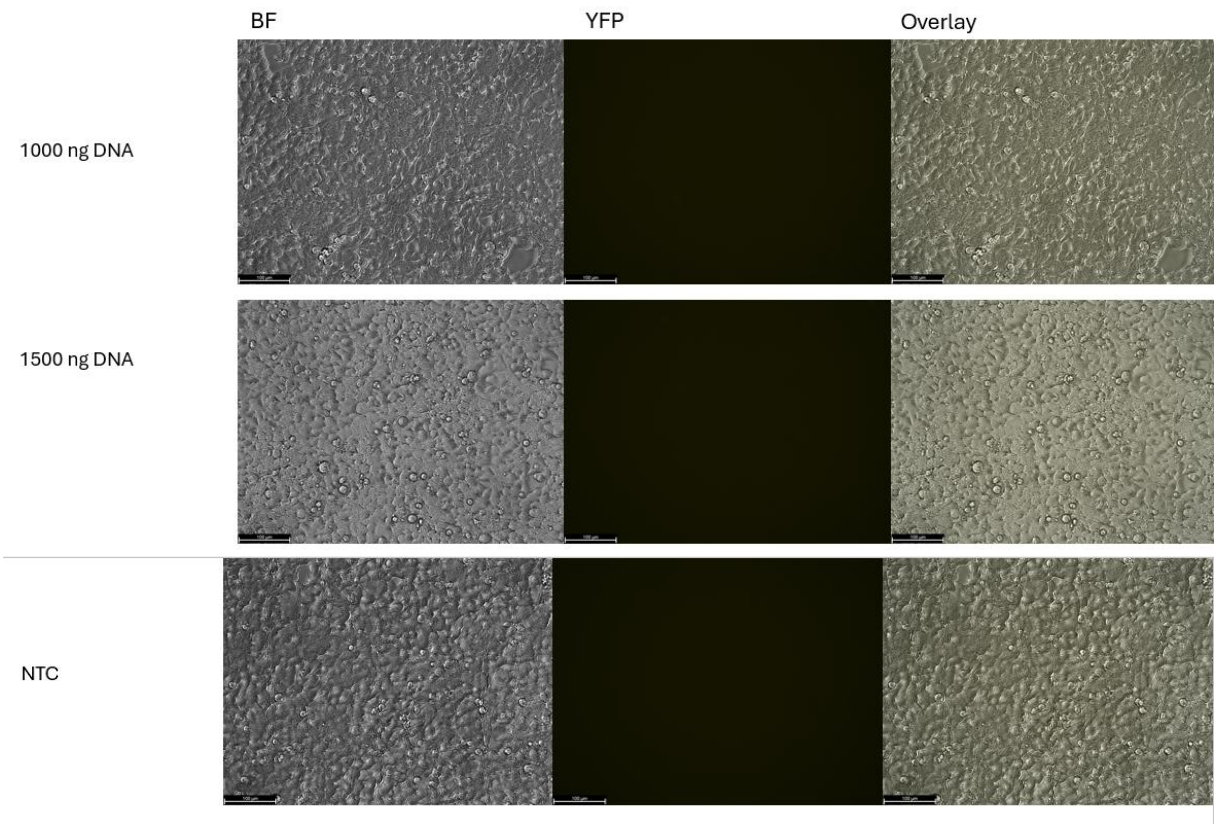
Transfection with $CaCl_2$ acc. To P53:

- A: 21.25 µl CaCl2 + x µl DNA
- B: 21.25 µl 2xHBS
- A dropwise into B
- Vortex (3 sec)
- Dropwise onto well
- Well A1: 0.5 µg DNA (1.09 µl)
- Well A2: 1 µg DNA (2.18 µl)
- Well A3: 1.5 µg DNA (3.28 µl)
- Well A4: NTC without DNA

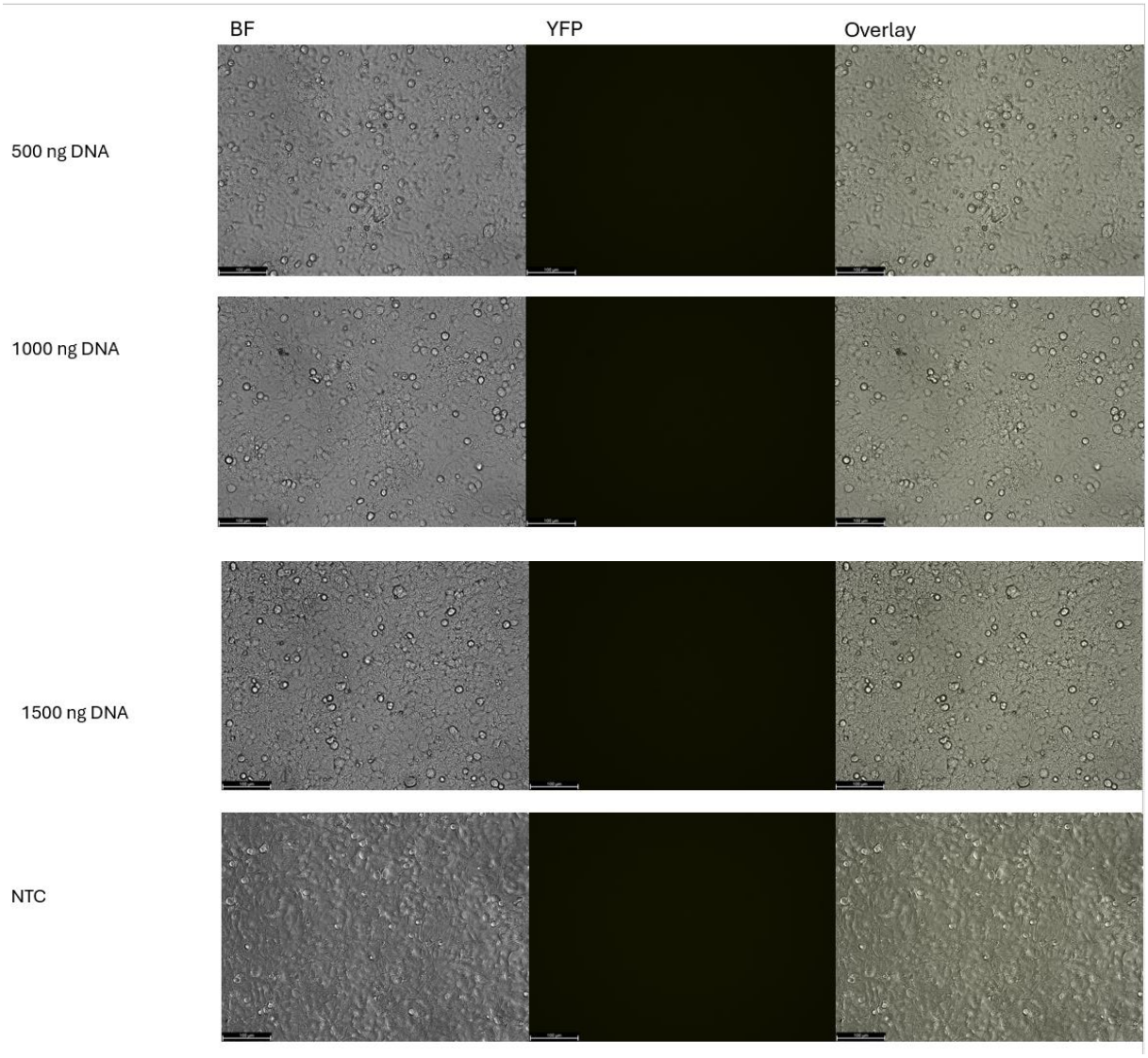
Results

Microscopy 6h post transfection

Lipofectamine 3000



$CaCl_2$



Analysis & Interpretation

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Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
42	04/09/2024	microscopy	KS

Aims for the day

- Microscopy 24h post transfection

Protocols

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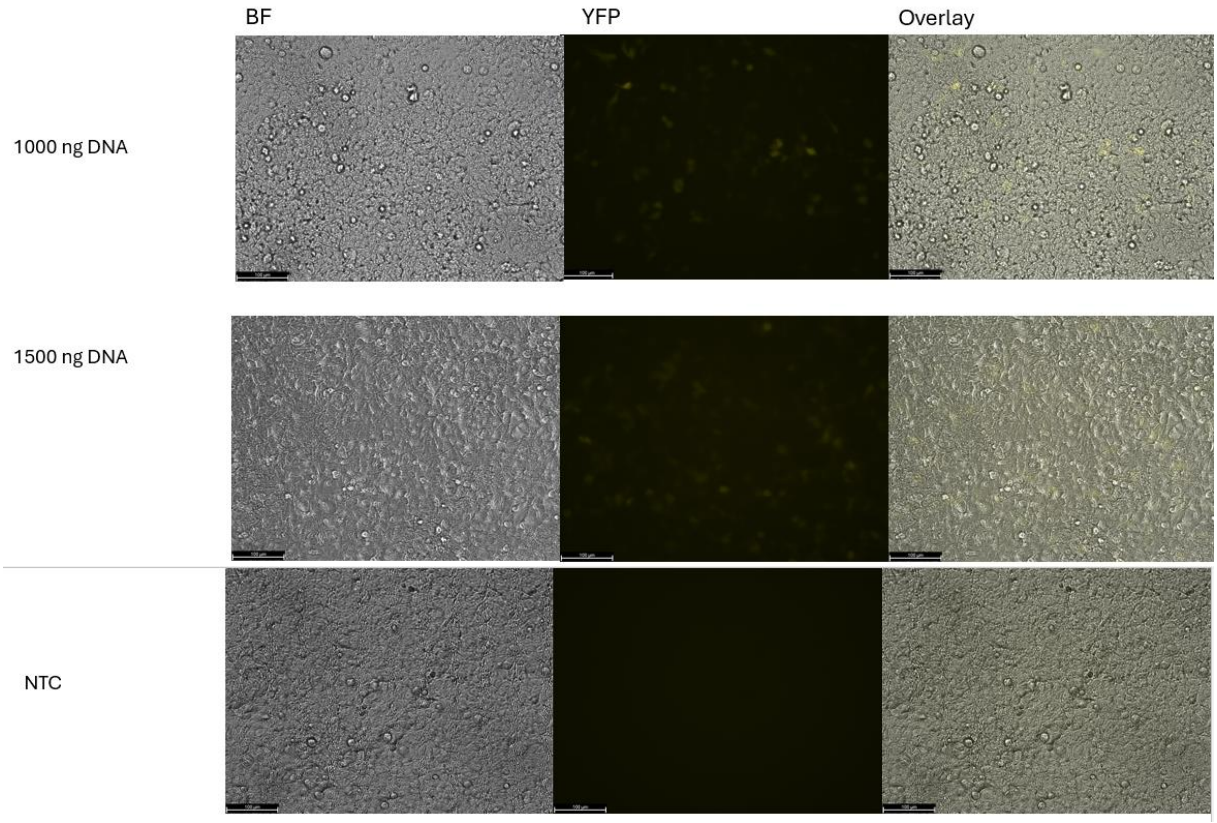
Results

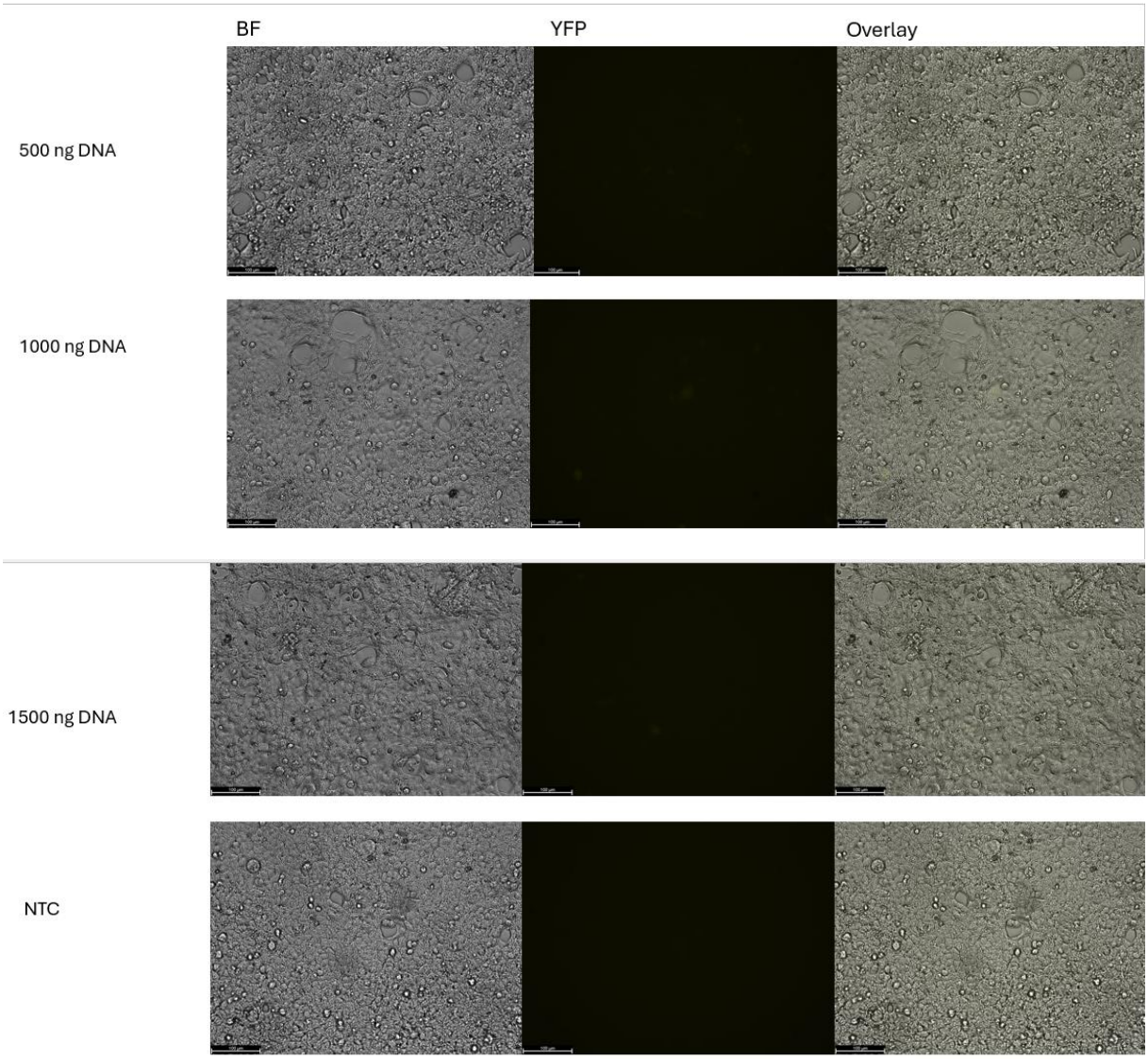
Microscopy 24h post transfection

Lipofectamine 3000

60



$CaCl_2$



Analysis & Interpretation

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Achievements of the Day

All goals achieved

No	Date (DD/MM/YYYY)	Titel	Experimenters
43	05/09/2024	microscopy	KS

Aims for the day

- Microscopy 48h post transfection

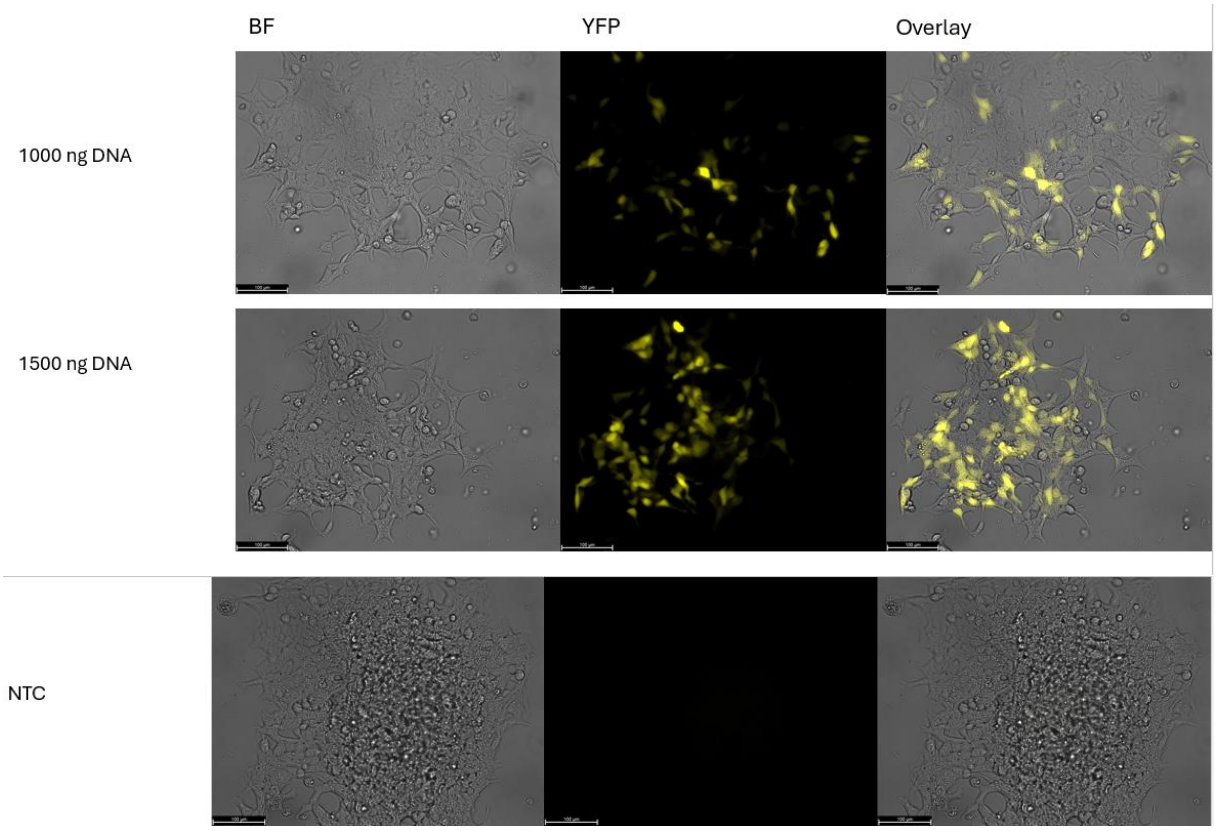
Protocols

/

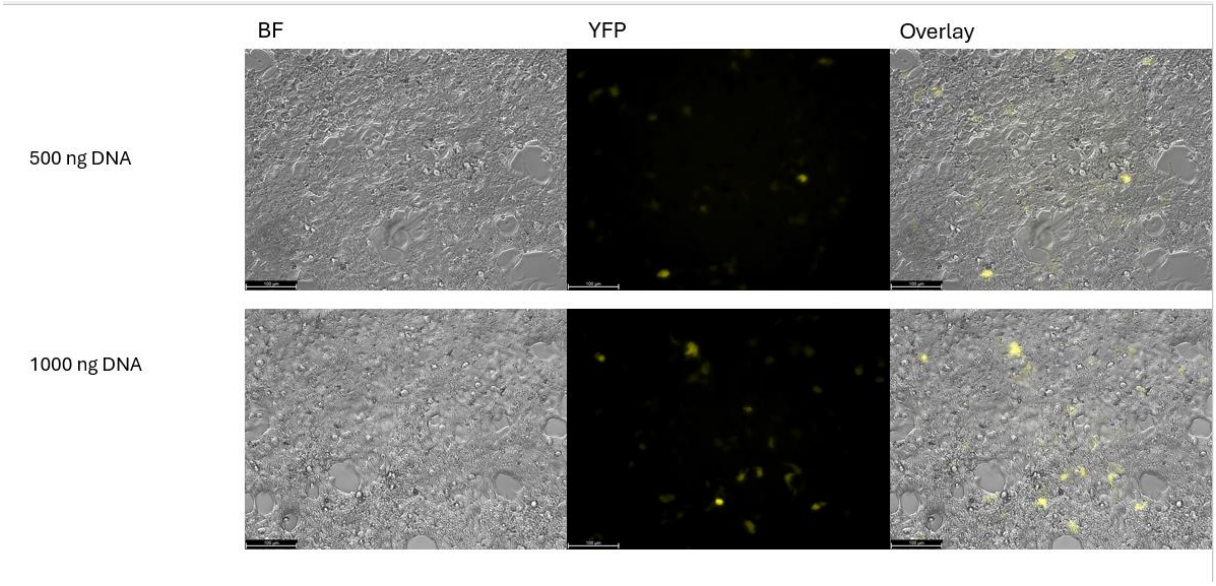
Results

Microscopy 48h post transfection

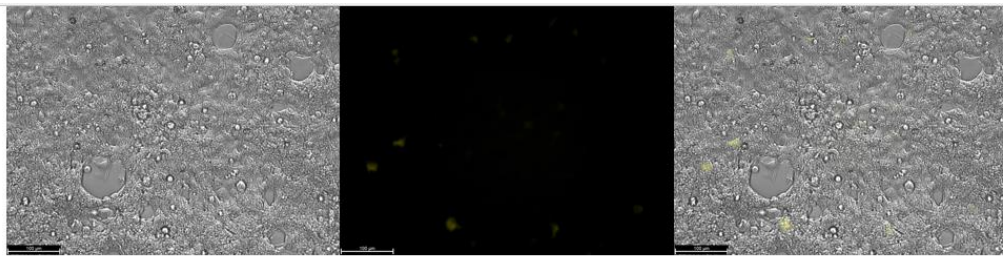
Lipofectamine 3000



$CaCl_2$



1500 ng DNA



NTC

Analysis & Interpretation

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Achievements of the Day

All goals achieved

No	Date (DD/MM/YYYY)	Titel	Experimenters
44	06/09/2024	Microscopy	KS

Aims for the day

- Microscopy 75h post transfection

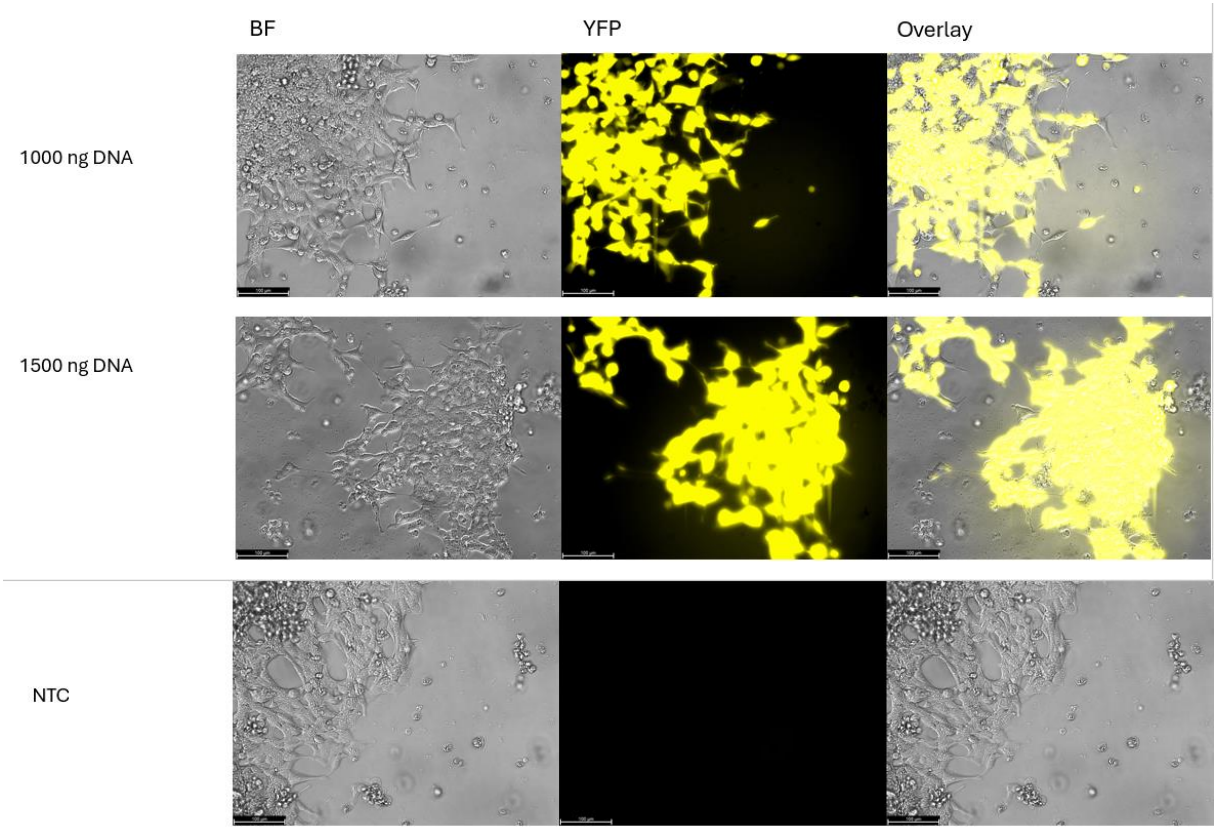
Protocols

/

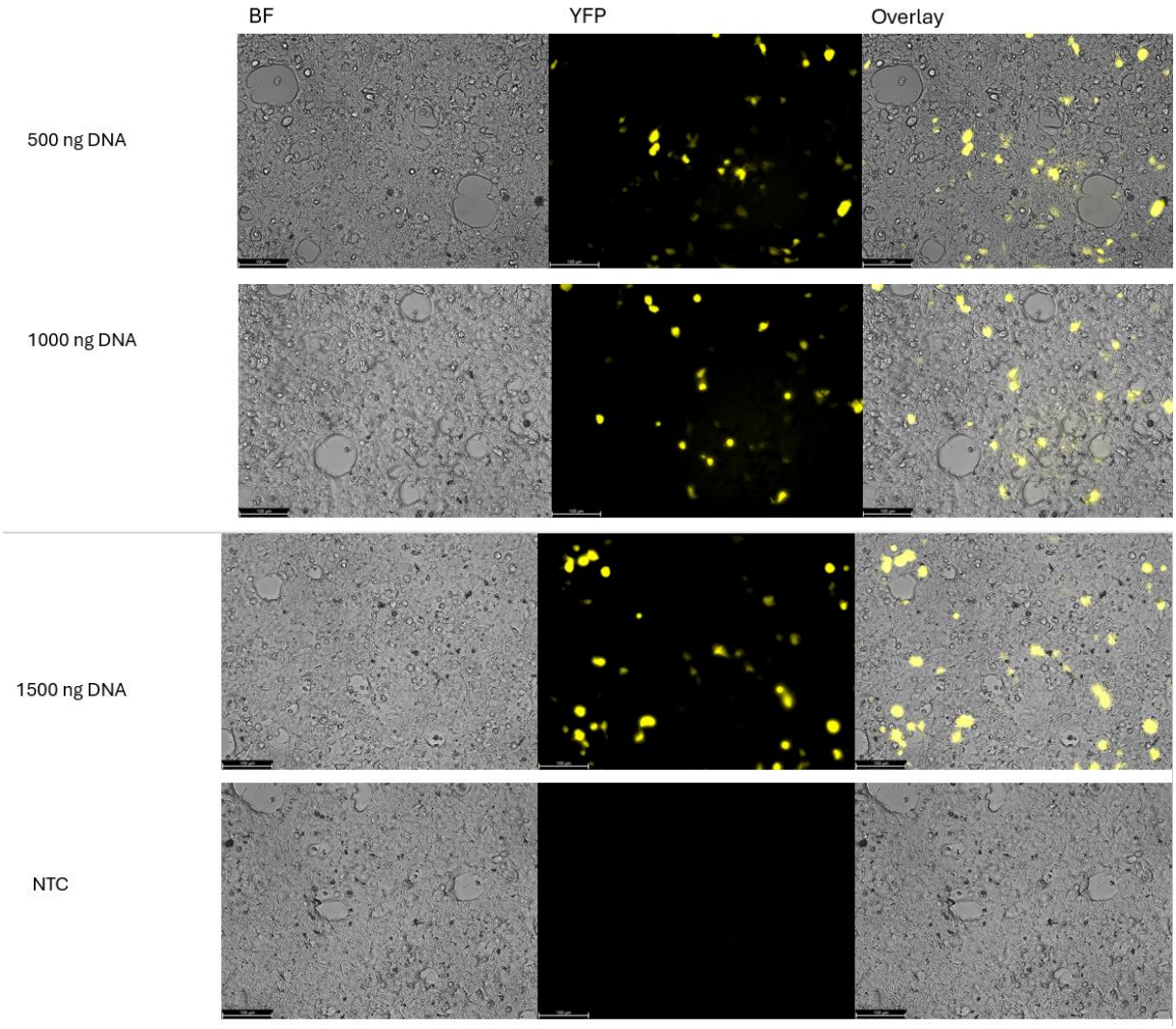
Results

Microscopy 75h post transfection

Lipofectamine 3000



$CaCl_2$



Analysis & Interpretation

- Transfection with Lipofectamine 3000 shows greater efficiency than transfection with $CaCl_2$
- Transfection with lipofectamine 3000 seems to have more or less the same efficiency if transfected with1000 ng DNA or 1500 ng
    - 1000 ng DNA will be used in further experiments!

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
45	14/09/2024	Seed cells for transfection	IG

Aims for the day

- Seed cells for Transfection

Protocols

Seed HEK293 acc. To P19

- Wash cells in T175-flask with PBS
- Trypsinate with 700 µl DMEM + trypsin + EDTA for T25 flask
- Incubate for 5 minutes at 37 °C and 5 % CO_2
- Stop trypsination with 5 ml DMEM + 10 % FCS + Pen/Strep
- Count cells with trypan blue: 1,25 x 10^6 cells/ml
- Seed 0.05 x 10^6 cells/ml
    - Seed 40 µl of cells per well
    - Add DMEM until final volume of 500 µ
    - Incubate at 37 °C and 5 % CO_2

Results

/

Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
46	16/09/2024	Transfection of HEK293	KS

Aims for the day

- Microscopy 72h post transfection

Protocols

Transfection with Lipofectamine 3000 acc. To P30

pDas2in1 + PE2	pDasPre	pDAS2in1	PE2	Technical positive control (pZMB)	NTC

- A total of 1000 ng DNA was used: 100 ng of PEAR target plasmid, 300 ng of pegRNA, and 600 ng of PE coding plasmid
  For 1 well:
- Dilute concentration needed in 25 µl opti-MEM + 1 µl Reagent 3000
- Dilute 1 µl Lipofectamine 3000 in 25 µl opti-MEM

Results

/

Analysis & Interpretation

/

Achievements of the Day

All goals achieved

No	Date (DD/MM/YYYY)	Titel	Experimenters
47	17/09/2024	Microscopy	KS

Aims for the day

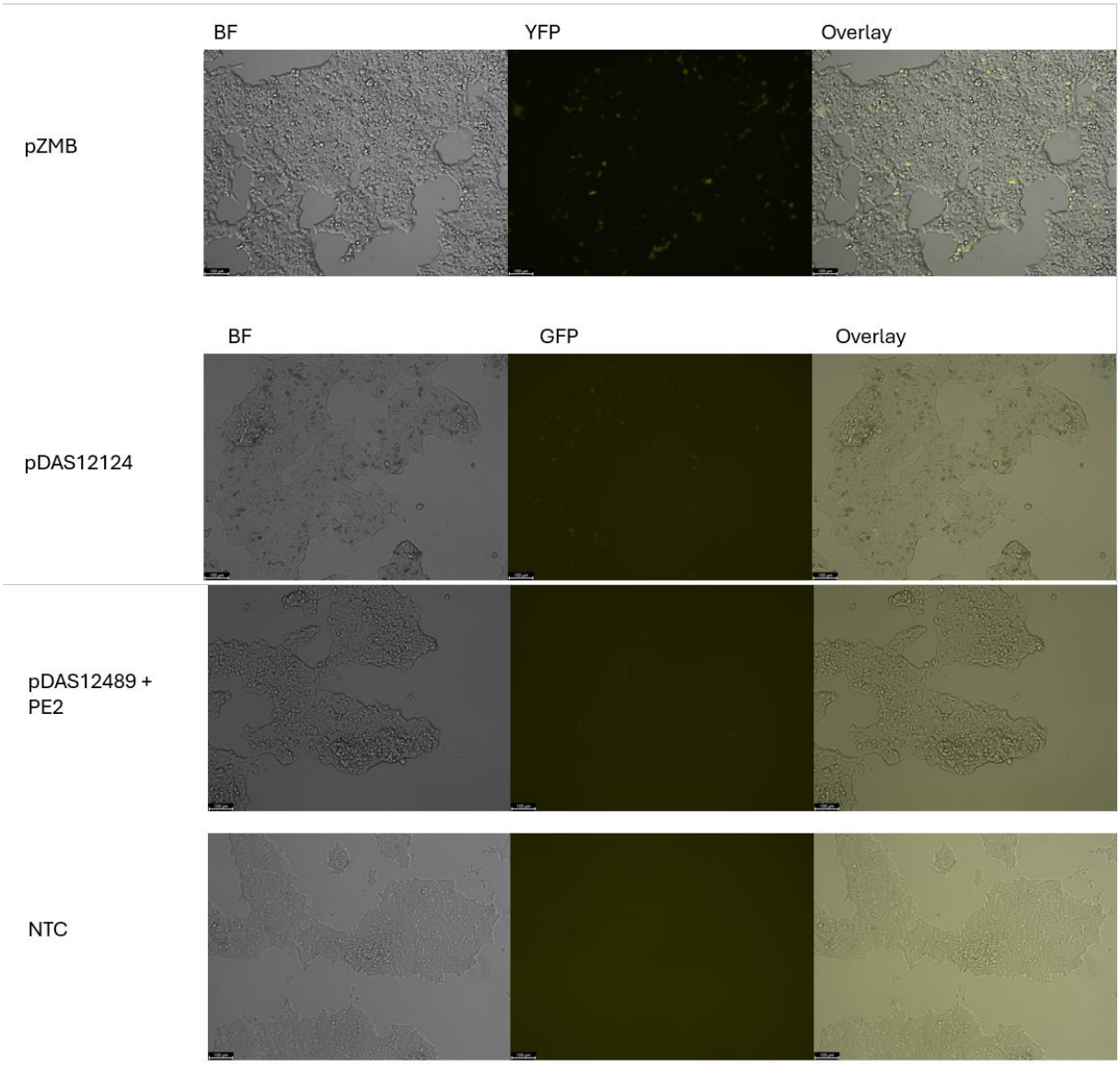
- Microscopy 24h post transfection

Protocols

/

Results

Microscopy 24h post transfection



Analysis & Interpretation

- Transfection with opti-mem medium shows fluorescence of medium (seen in all samples, also in the NTC!)

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters

48	18/09/2024	microscopy	KS

Aims for the day

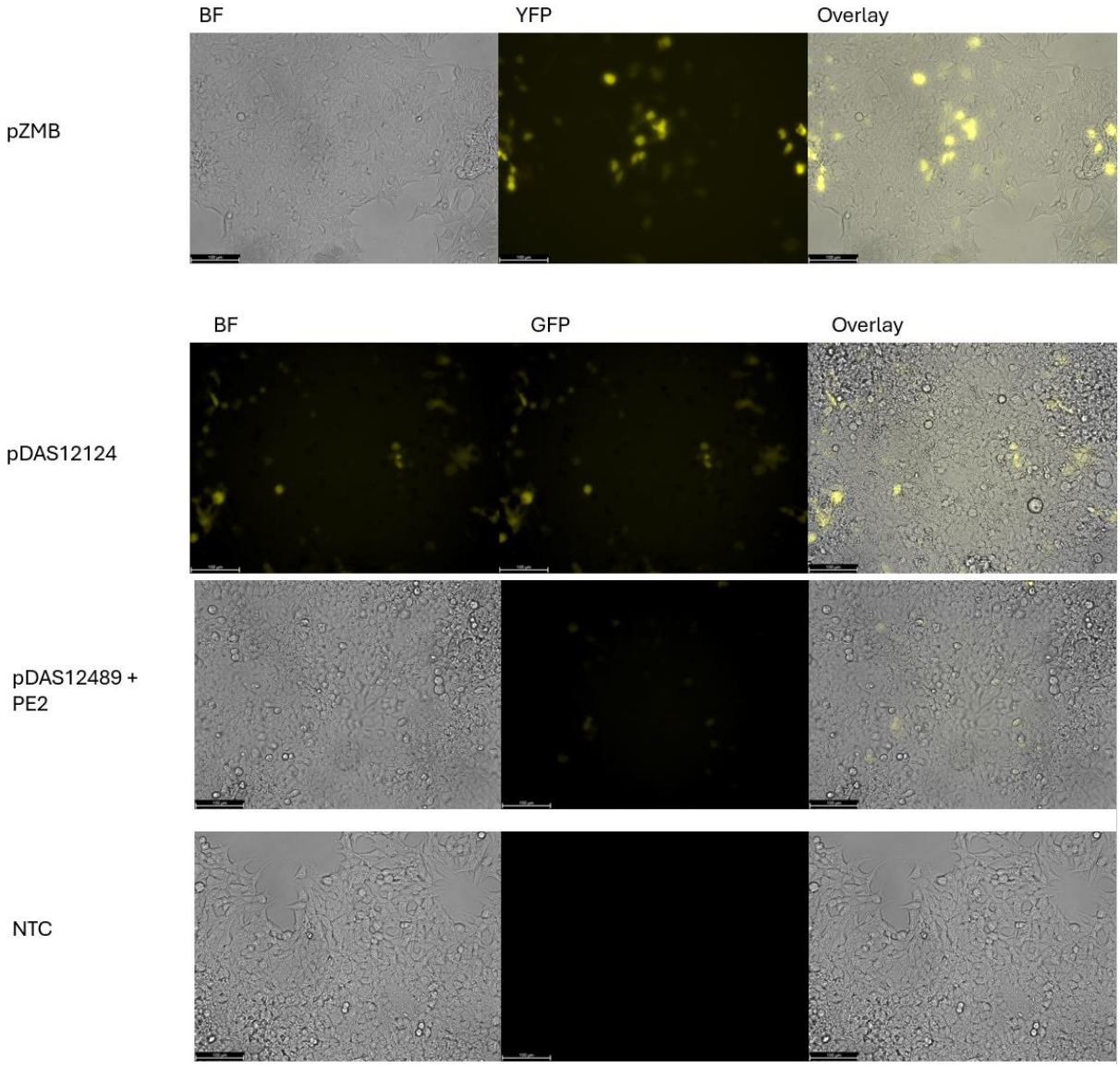
- Microscopy 48h post transfection

Protocols

/

Results

Microscopy 48h post transfection



Analysis & Interpretation

/

Achievements of the Day

All goals achieved.

No	Date (DD/MM/YYYY)	Titel	Experimenters
49	19/09/2024	Microscopy & FACS measurement	KS

Aims for the day

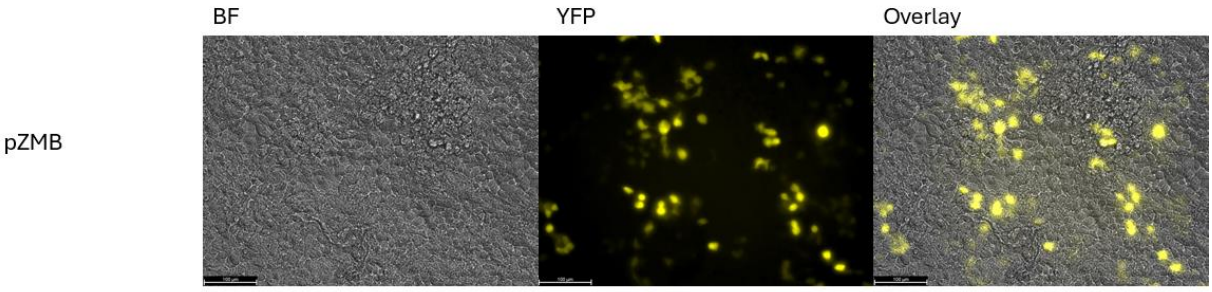
- Microscopy 67h post transfection

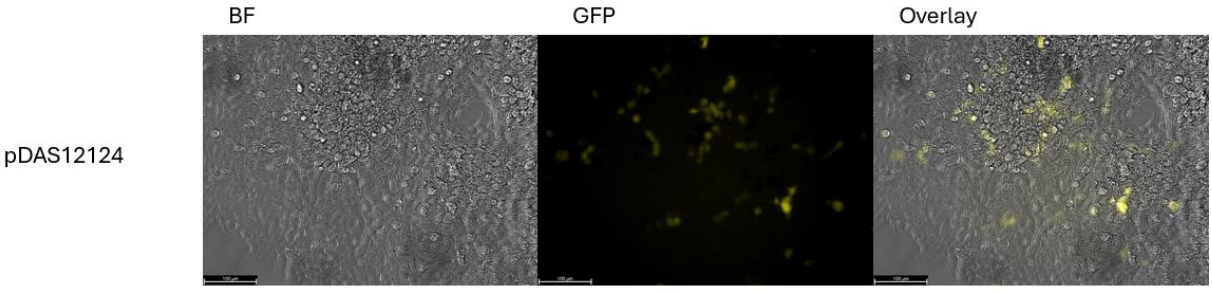
Protocols

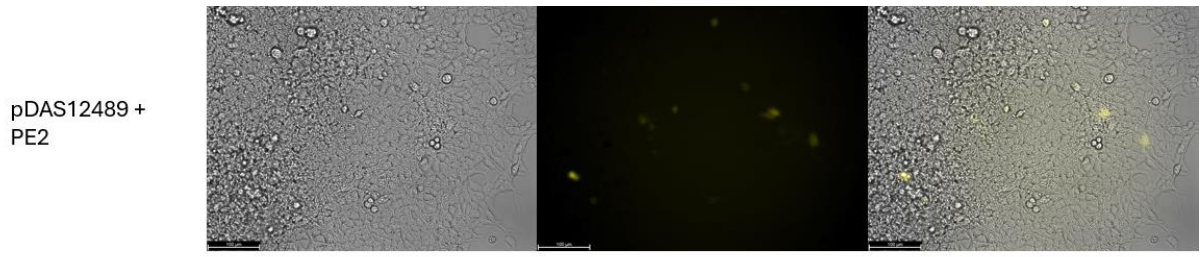
/

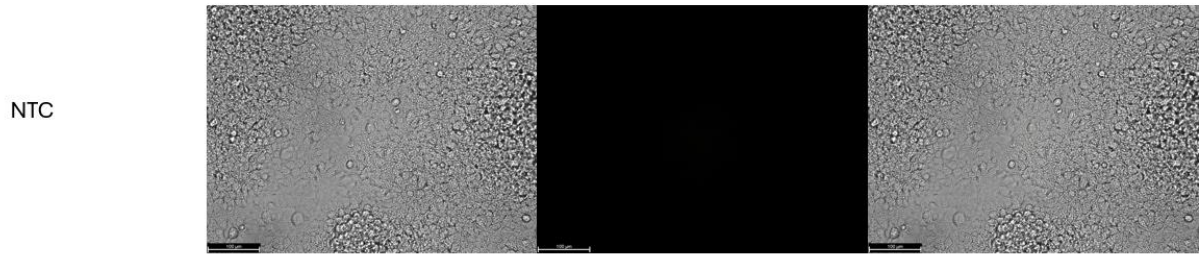
Results

Microscopy 67h post transfection



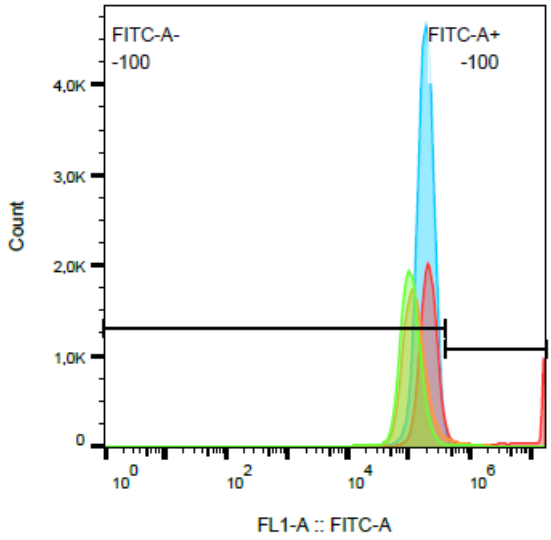






FACS results

sample	Statistic (%)	#Cells
A01 HEK293 Negative.fcs		100000
A01 HEK293 Negative.fcs/healthy	59	58953
A01 HEK293 Negative.fcs/healthy/FITC-A+	1,02	604
A01 HEK293 Negative.fcs/healthy/FITC-A-	99	58349
A02 HEK293 pos Control.fcs		69420
A02 HEK293 pos Control.fcs/healthy	42,7	29623
A02 HEK293 pos Control.fcs/healthy/FITC-A+	15,5	4588
A02 HEK293 pos Control.fcs/healthy/FITC-A-	84,5	25035
B08 pre.fcs		74426
B08 pre.fcs/healthy	40,1	29876
B08 pre.fcs/healthy/FITC-A+	5,52	1649
B08 pre.fcs/healthy/FITC-A-	94,5	28227
B10 pe2.fcs		62901
B10 pe2.fcs/healthy	47,4	29814
B10 pe2.fcs/healthy/FITC-A+	2,92	871
B10 pe2.fcs/healthy/FITC-A-	97,1	28943



	Sample Name	Subset Name	Count	Freq. of Total	Freq. of Parent
	B10 pe2.fcs	healthy	29814	47,4	2,92
	B08 pre.fcs	healthy	29876	40,1	5,52
	A02 HEK293 pos Control.fcs	healthy	29623	42,7	15,5
	A01 HEK293 Negative.fcs	healthy	58953	59,0	1,02

Analysis & Interpretation

/

Achievements of the Day

All goals achieved.