

Danmarks
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BioBrick Workshop - Dry Lab exercise

Benchling – Primer and Plasmid Design

DTU BioBuilders 2025

Saturday 29/03/2025

Objective

Participants will learn how to design primers and construct a plasmid for cloning in Benchling, an online molecular biology tool.

Materials

- A. Laptop with an internet connection
- B. Benchling account (free version is sufficient)
- C. DNA sequences (provided or download manually)
- D. Protocol handout (in a digital form)

Workshop sections

1. Introduction to Benchling

- A. Overview of benchling tools
- B. Creating a new project and sequence file
- C. Understanding Benchling's sequence visualization
- D. Annotating features
- E. Select a gene of interest, along with a promoter, RBS and terminator

2. Primer design

- A. Setting primer design parameters
- B. Performing in silico PCR

3. Plasmid design

- A. Selecting a plasmid backbone
- B. Performing in silico digestion and cloning

4. Finalization and export

- A. Checking for errors
- B. Exporting the plasmid map

Step-by-step guide

💡 : This symbol indicates theoretical information that will help you understand the exercise's biological background.

🔧 : This symbol highlights exactly what you need to click on and provides all the instructions for the dry lab exercise.

🔍 : Other relevant information

Step 1: Introduction to Benchling – set up a new project

<https://benchling.com/>

🔧 Log in to your Benchling account and set up your project.

🔧 Click on “*Projects*” on the left-side panel. Give the new project a name (e.g., "Primer & Plasmid Design Workshop") and click on “*Create project*”.

🔍 In the wet lab exercise, the BBa_J04450 construct was provided pre-assembled in the pSB1C3 backbone as part of the iGEM Distribution Kit. We'll simulate the same procedure on Benchling by using Restriction Enzyme digestion & Cloning to mimic how the construct was probably inserted into pSB1C3.

Choose the desirable genetic construct (BBa_J04450).

💡 The BBa_J04450 construct is a well-characterized composite BioBrick device that consists of the following four standardized parts:

Promoter (BBa_R0010) – A lacI-regulated promoter (Plac) that allows for inducible expression in the presence of IPTG.

Ribosome Binding Site (BBa_B0034) – A strong, commonly used RBS that ensures efficient initiation of translation.

Reporter Gene (BBa_E1010) – Encodes the monomeric Red Fluorescent Protein (mRFP1), which allows for easy visual detection of gene expression.

Terminator (BBa_B0015) – A double terminator that effectively stops transcription, increasing the stability of the transcript and preventing read-through.

🔧 To get the desired part, go to the iGEM registry (https://parts.igem.org/Main_Page), search for the name and then click at “*Get part sequence*”. Copy the sequence.

Registry of Standard Biological Parts

tools catalog repository assembly protocols help search

main page design experience information part tools edit

Part:BBa_J04450
Designed by: Tamar Odle Group: iGEM2005 (2005-06-09)

RFP Coding Device

Released HQ 2013
Sample in stock
★ 1 Registry Star
69 Uses
9 Translations
Get This Part

Subparts | [Ruler](#) | [SS](#) | [DS](#) Scars: [Show](#) | [Hide](#) Vertical: [Show](#) | [Hide](#) Length: 1069 bp [View plasmid](#) [Get part sequence](#)

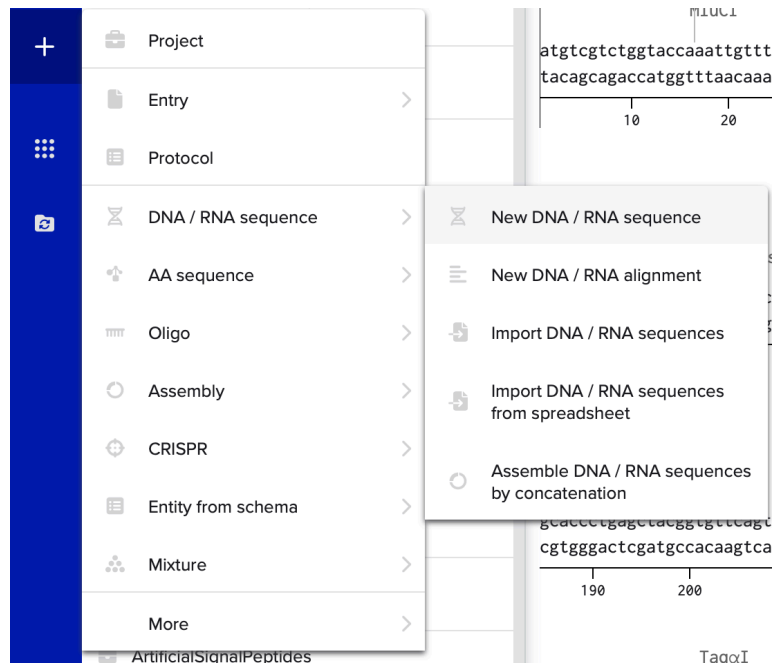
LacI R0010 B0034 mRFP1 E1010 B0015


Assembly Compatibility: [10](#) [12](#) [21](#) [23](#) [25](#) [1000](#)

The colonies are clearly red in color under natural light after about 18 hours. Smaller colonies are visibly red under UV. The RFP part does not contain a degradation tag and the RBS is strong.

- LacI sensitive
- CAP sensitive

🔧 Click “*Create*” → “*DNA/RNA Sequence*” → “*New DNA/RNA sequence*”, since we are pasting a DNA sequence from the registry.



 Name your new sequence with an appropriate name that will help you distinguish it from other sequences, set the nucleotide type and project folder, and paste the sequence you copied from the registry and then “**Create**”.

Create DNA / RNA sequence ✕

[CREATE NEW](#)
[UPLOAD FILES](#)
[IMPORT FROM DATABASE](#)
[SELECT CHROMOSOMAL REGION](#)

Name*

Set nucleotide type*

DNA

RNA

Set topology

Linear
▼

Set project folder*


Primer & Plasmid Design Workshop
▼

Set schema

Select a schema...
▼

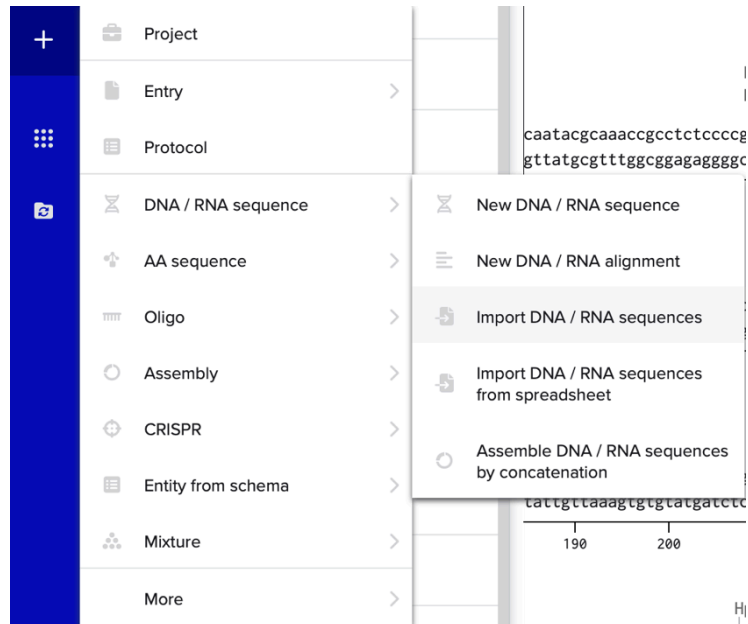
Bases

Close
Create

 For other genes, go to NCBI Genbank and get the sequence in a fasta or gb. format. By downloading the file you can click on “*Import DNA / RNA sequences*” > “*Upload file*”.

🔍 For all iGEM registered parts, there is the option to import directly the sequence by:

“Create” > “Import DNA / RNA sequences” > “Import from database” > write the part’s code



Create DNA / RNA sequence ✕

CREATE NEW UPLOAD FILES **IMPORT FROM DATABASE** SELECT CHROMOSOMAL REGION

Example searches:

- <https://www.addgene.org/browse/sequence/419908/> (Addgene URL)
- [BRCA2](#) (Gene name)
- [M62653](#) (NCBI Accession)
- [ENSMUSG00000041147](#) (ENSEMBL ID)
- [BBa_E0040](#) (Registry of Standard Biological Parts)
- [JPUB_001430](#) (JBEI Public Registry)

Import multiple sequences at once by entering space-separated or comma-separated accession numbers.

Sequence

It looks like Benchling faces some technical problems and currently this option doesn't lead to anywhere, so we'll annotate the sequence manually.

Create DNA / RNA sequence ×

CREATE NEW UPLOAD FILES **IMPORT FROM DATABASE** SELECT CHROMOSOMAL REGION

Sequence

BBa_E0040 Search × Clear

⚠ No results found for the accessions specified

Close Import


🔧 Since we have added our BBa_J04450 sequence, going back to the registry we can get the sequence for each subpart and manually annotate them in the sequence on Benchling. The genetic construct consists of the 4 different elements: LacI promoter (R0010), RBS (B0034), mRFP1 (E1010) and Terminator (B0015). We'll follow the same procedure to annotate each one.

First, click on each subpart's icon.

Part:BBa_J04450
 Designed by Tamar Odle Group: iGEM2005 (2005-06-09)

RFP Coding Device

Subpart [Ruler](#) | [SS](#) | [DS](#) Scars: [Show](#) | [Hide](#) Vertical: [Show](#) | [Hide](#) Length: 1069 bp



Assembly Compatibility: 10 12 21 23 25 1000

The colonies are clearly red in color under natural light after about 18 hours. Smaller colonies are visibly red under UV. 1 tag and the RBS is strong.

- LacI sensitive
- CAP sensitive

Scroll a bit further down, until you see the “*Get part sequence*” again.

Registry of Standard Biological Parts

tools catalog repository assembly protocols help search

main page design experience information part tools edit

Part:BBa_R0010
 Designed by: Group: Antiquity (2003-01-31)

promoter (lacI regulated)
 This part is an inverting regulator sensitive to LacI and CAP.
 It contains two protein binding sites. The first binds the CAP protein, which is generally present in E.coli and is associated with cell health and availability of glucose. The second binds LacI protein.

- In the absence of LacI protein and CAP protein, this part promotes transcription.
- In the presence of LacI protein and CAP protein, this part inhibits transcription.
- LacI can be inhibited by [http://openwetware.org/wiki/IPTG IPTG].
- LacI is coded by BBa_C0010

Intrinsic noise value: 0.0707 (compare with R0011: 0.0040; R0051: 0.0869). See [http://2015.igem.org/Team:William_and_Mary William_and_Mary IGEN 2015]

>Internal Priming Screening Characterization of BBa_R0010: Has 3 possible internal priming site between this BioBrick part and the VR primer.

The 2018 Hawaii iGEM team evaluated the 40 most frequently used BioBricks and ran them through an internal priming screening process that we developed using the BLAST program tool. Out of the 40 BioBricks we evaluated, 10 of them showed possible internal priming of either the VF2 or VR primers and sometime even both. The data set has a range of sequence lengths from as small as 12 bases to as large as 1,210 bases. We experienced the issue of possible internal priming during the sequence verification process of our own BBa_K2574001 BioBrick and in the cloning process to express the part as a fusion protein. BBa_K2574001 is a composite part containing a VLP forming Gag protein sequence attached to a frequently used RFP part (BBa_E1010). We conducted a PCR amplification of the Gag-RFP insert using the VF2 and VR primers on the ligation product (pSB1C3 ligated to the Gag + RFP). This amplicon would serve as template for another PCR where we would add the NcoI and BamHI restriction enzyme sites through new primers for ligation into pET14b and subsequent induced expression. Despite gel confirming a rather large, approximately 2.1 kb insert band, our sequencing results with the VR primer and BamHI RFP reverse primer gave mixed results. Both should have displayed the end of the RFP, but the VR primer revealed the end of the Gag. Analysis of the VR primer on the Gag-RFP sequence revealed several sites where the VR primer could have annealed with ~9 - 12 bp of complementarity. Internal priming of forward and reverse primers can be detrimental to an iGEM project because you can never be sure if the desired construct was correctly inserted into the BioBrick plasmid without a successful sequence verification.

For the BioBrick part BBa_R0010, the first location of the internal priming site is on the 121-113 base number of the BioBrick and on the 12-20 base number of the VR primer. The second location of the internal priming site is on the 11-17 base number of the BioBrick and on the 4-10 base number of the VR primer. The third location of the internal priming site is on the 84-90 base number of the BioBrick and on the 14-20 base number of the VR primer.

Usage and Biology
 This is a direct copy of bases 0365739 through 0365540 of the E. coli K-12 MG1655 genome, Genbank NC_000913 in reverse complement form. It is the natural promoter for the LacZYA operon. It includes the tail end of the LacI gene coding region, but no promoter region for that partial gene.

Sequence and Features

Subparts | Ruler | SS | DS | Scars: Show | Hide | Vertical: Show | Hide | Length: 200 bp | View plasmid | Get part sequence

Assembly Compatibility: 10 12 21 23 25 1000

🔧 Copy the sequence and search for it in Benchling by Ctrl+ F, paste the sequence and see the highlighted bases. Select them, right click > "Create annotation". Fill out the info as shown:

ANNOTATIONS TRANSLATIONS

Visibility filter Create new

5 total 0 hidden

lacl - promoter 1-200

Name: lacl - promoter

Position: 1 - 200

Annotation type: Annotation type

Color:

Strand: Forward

Notes: Notes

Custom fields +

This annotation has no custom fields

Cancel Save

Follow the same procedure to annotate the rest of the parts.

💡 The mRFP1 sequence provided in the iGEM Registry does not include a stop codon, because it is designed to be part of a modular BioBrick system, allowing for flexible assembly with other genetic parts.

Choose a plasmid backbone (pSB1C3)

🔧 Download the backbone's sequence from Snapgene's database and upload the file:

https://www.snapgene.com/plasmids/basic_cloning_vectors/pSB1C3

“Create” > “DNA / RNA sequence” > “New DNA / RNA sequence” > “Upload from files”

Step 2: Primer design

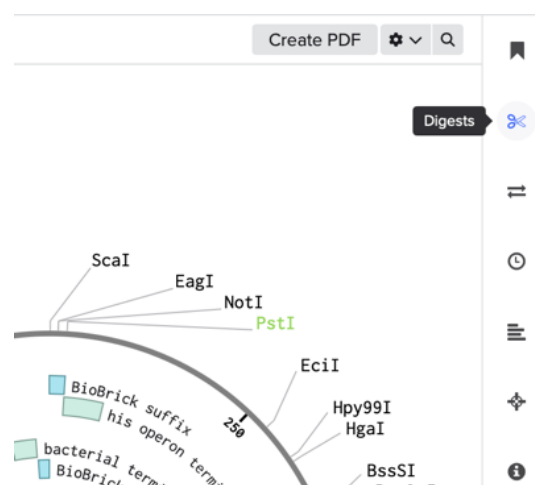
Restriction Enzyme digestion & cloning

For restriction enzyme (RE) cloning, the backbone will be digested with two different enzymes to generate sticky ends, and the corresponding RE recognition sites will be introduced at the ends of the insert using PCR primers.

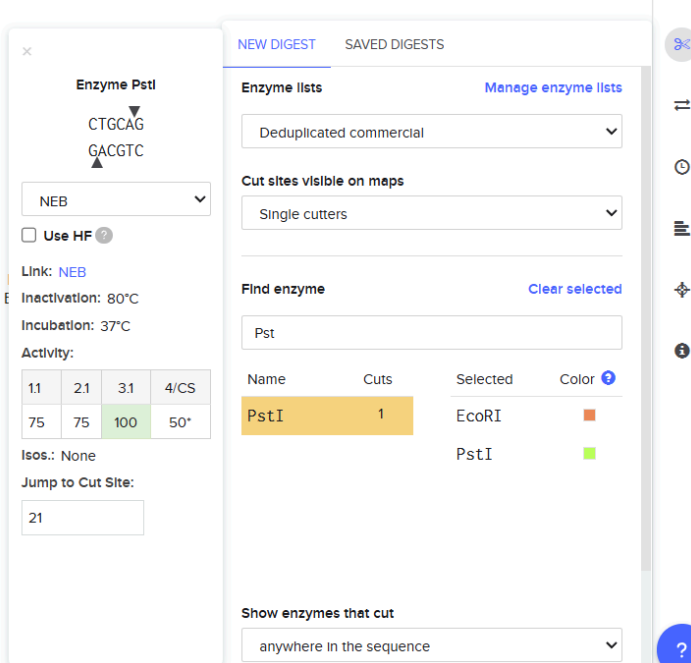
Specifically, the backbone will be cut with EcoRI and PstI.

💡 These enzymes were chosen because neither the pSB1C3 backbone nor the BBa_J04450 insert contains internal EcoRI or PstI recognition sites, avoiding unwanted fragmentation. Additionally, these sites are not located within any essential regions of pSB1C3, such as the origin of replication (ori) or the chloramphenicol resistance (CmR) gene. This careful selection of restriction enzymes ensures that BBa_J04450 can be accurately and efficiently inserted into pSB1C3.

🔧 In pSB1C3, click on “*Digests*” on the tool bar on the right side.



 Choose **EcoRI** & **PstI**.



Enzyme PstI

CTGCAG
GACGTC

NEB

Use HF ?

Link: [NEB](#)

Inactivation: 80°C

Incubation: 37°C

Activity:

1:1	2:1	3:1	4/CS
75	75	100	50*

Isos.: None

Jump to Cut Site:

21

NEW DIGEST SAVED DIGESTS

Enzyme lists [Manage enzyme lists](#)

Deduplicated commercial

Cut sites visible on maps

Single cutters


Find enzyme [Clear selected](#)


Pst


Name	Cuts	Selected	Color
PstI	1	<input checked="" type="checkbox"/>	EcoRI
		<input type="checkbox"/>	PstI

Show enzymes that cut

anywhere in the sequence

 Click “**Run digest**” to simulate cutting the vector.

 Click on the part you want to keep and “**Save**”.


LINEAR MAP PLASMID **DIGEST** × VIRTUAL DIGEST DESCRIPTION METADATA ... [Share](#) 

Digest [Save](#) NEB Use HF ?

Enzymes	Cuts	Temp.	1:1	2:1	3:1	4/CS
EcoRI	1	37°C	25	100*	50	50*
PstI	1	37°C	75	75	100	50*

Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
21	2049	2029	PstI	3'	EcoRI	5'
2050	20	41	EcoRI	5'	PstI	3'

To add the specific sticky ends to our BBA_J04450 construct, the restriction enzyme sequences have to be inserted by creating PCR primers, and then digested with the restriction enzymes.

 Highlight the 20 first bp of the J04450 sequence, right click > “**Create primer**” > “**Forward**”. On the pop-up window, click on “**Single Primer**” on the top left and change it to “**Primer Pair**”.

Add the EcoRI recognition site at the start of sequence, as overhang, to create the forward primer.

EcoRI (GAATTC) → The primary restriction enzyme for inserting the part.

Forward primer: 5' - GAATTC - 3'

For the reverse primer, highlight the 20 last bp of the J04450 sequence, right click > *“Copy special”* > *“Reverse complement”*. On the pop up window, copy the sequence on the right top box *“DNA reverse complement”*, to copy the reverse sequence of the one you just selected. This will be the first part of the reverse primer.

Copy special ✕


The following bases were copied from the sequence.
Click the sequence type you would like to copy.

<p>DNA sequence</p> <div style="border: 1px solid #ccc; padding: 5px; min-height: 40px;">tgggcctttctgcgtttata</div>	<p>DNA reverse complement</p> <div style="border: 1px solid #ccc; padding: 5px; min-height: 40px;">tataaacgcagaaaggccca</div>
<p>AA translation</p> <div style="border: 1px solid #ccc; padding: 5px; min-height: 40px;">WAFRLR</div>	<p>AA reverse translation</p> <div style="border: 1px solid #ccc; padding: 5px; min-height: 40px;">YKRRKA</div>
<p>RNA sequence</p> <div style="border: 1px solid #ccc; padding: 5px; min-height: 40px;">ugggccuuucugcguuuaua</div>	<p>RNA reverse complement</p> <div style="border: 1px solid #ccc; padding: 5px; min-height: 40px;">uauaaacgcagaaaggccca</div>

Additional options when copying the sequence or its reverse complement inside Benchling:

Include annotations/translations


Include annotations/translations not fully contained by selection

 Paste the sequence in the Design Primer window, and add the reverse primer's location.


Design

	Forward	Reverse
Strand		
Bases	5' GAATTCcaatagcaaacgcctc tc 3'	5' tataaacgcagaaaggccca 3'
3' Location	20	1050
Overhang	6	0 bp
Cut Site	AanI	

Use the dropdown above to look up restriction sites.

 Add the PstI recognition site at the 5' end of the reverse primer as an overhang, to create the reverse primer.

PstI (CTGCAG) → The second restriction enzyme for inserting the part.

 Since PstI recognizes the palindromic sequence 5'-CTGCAG-3', its reverse complement is identical to the original sequence. Therefore, when designing the reverse primer, the same sequence (CTGCAG) can be used directly without needing to reverse-complement it.

Reverse primer: 5' - CTGCAG - 3'

LINEAR MAP DESIGN PRIMER DESCRIPTION METADATA RELEVANT ITEMS Share

Primer Pair Jump to Primer Set from Selection

Design

Strand	Forward	Reverse
Bases	5' GAATTC caatagc aaaccgctc 3'	5' CTGCAC tataaacg cagaaggcc 3'
3' Location	20	1050
Overhang	6	6
Cut Site	AanI	

Use the dropdown above to look up restriction sites.

Verify

Check Secondary Structure at 37 °C

T_m	56.1°C	53.6°C
GC Content	55.00%	45.00%
Length	26 bp	26 bp
Product Size	1081 bp	
T_m Diff.	-2.56°C	

Save the primers with names that clearly state what they are and click on “**Save Primers**”.

Save

Name J04450_F_EcoRI J04450_R_PstI

On the tool bar on the right, if you now click on the “**Primers**”, select “**Create PCR Product**”. Then, “**Copy**” and save the new DNA to your folder.

LINEAR MAP DESCRIPTION METADATA RELEVANT ITEMS Share

Create PDF

Ba_J04450_geneticconstruct (1069 bp)

PRIMERS PAIRS

Primer	Position	Product Size
J04450_F_EcoRI	+ / 20	1081
J04450_R_PstI	- / 1050	

Primer Pair Information Edit · Unlink

Link Primers

	Name	T_m
Forward Primer	J04450_F_EcoRI	56.1°C
Reverse Primer	J04450_R_PstI	53.6°C

Product Size 1081 bp
 T_m Difference -2.6° C

Create PCR Product Secondary Structure

Copy Selection to New DNA

Customize what gets copied over:

- Use primer bases instead of sequence (includes overhang if any)
- Annotations, translations, and primers
- Include annotations and translations not fully contained by selection
- Use reverse complement instead
- Preserve sequence indices
- Tags
- Description

🔧 “Run Digest” on the on the new J04450 part too to create sticky ends, compatible to the backbone’s.

🔧 Click on the part you want to keep and “Save”.

LINEAR MAP DIGEST × VIRTUAL DIGEST DESCRIPTION METADATA ... Share

Digest Save NEB Use HF

Enzymes	Cuts	Temp.	1.1	2.1	3.1	4/CS
EcoRI	1	37°C	25	100*	50	50*
PstI	1	37°C	75	75	100	50*

Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
1	1	1	None	blunt	EcoRI	5'
2	1080	1079	EcoRI	5'	PstI	3'
1081	1081	1	PstI	3'	None	blunt

🔧 Click on “ASSEMBLY” on the right bottom of your pSB1C3 plasmid map, to initiate the assembly.

Click on “**Backbone**” to set the backbone (pSB1C3).

SET FRAGMENT

Select an assembly fragment below.

Backbone Insert

Having selected the right backbone fragment you should be able to “**Set from Selection**”.

PREVIEW

Shift select two enzymes on the sequence map or run a digest and select a fragment.

Set from Selection

Backbone Insert + Hide Preview

Confirm that everything looks fine and click “**Done**”. Then, click on “**Insert**”.

PREVIEW

GTC TGG
ACGTCAG ACCTTAA

0 ERRORS AND 0 WARNINGS
✓ Looks like everything checks out

Reverse Orientation
Jump to Selection
View Enzyme Activity

pSB1C3
2.0 kb · PstI, EcoRI

Insert + Hide Preview

Done

SET FRAGMENT

Select an assembly fragment below.

pSB1C3
2.0 kb · PstI, EcoRI

Insert

Change tabs to your insert and click on the part you want to select to enable the “**Set from Selection**” option.

Check that everything checks out and click on “**Done**”.

PREVIEW

TGC AATTcCa taCTGCA GT
ACCTTAA Ggt atGACGTC A

0 ERRORS AND 0 WARNINGS
✓ Looks like everything checks out

Reverse Orientation
Jump to Selection
View Enzyme Activity

pSB1C3
2.0 kb · PstI, EcoRI

BBa_J04450_geneticconstruct [1-1069]
1.1 kb · EcoRI, PstI

+ Hide Preview

Done

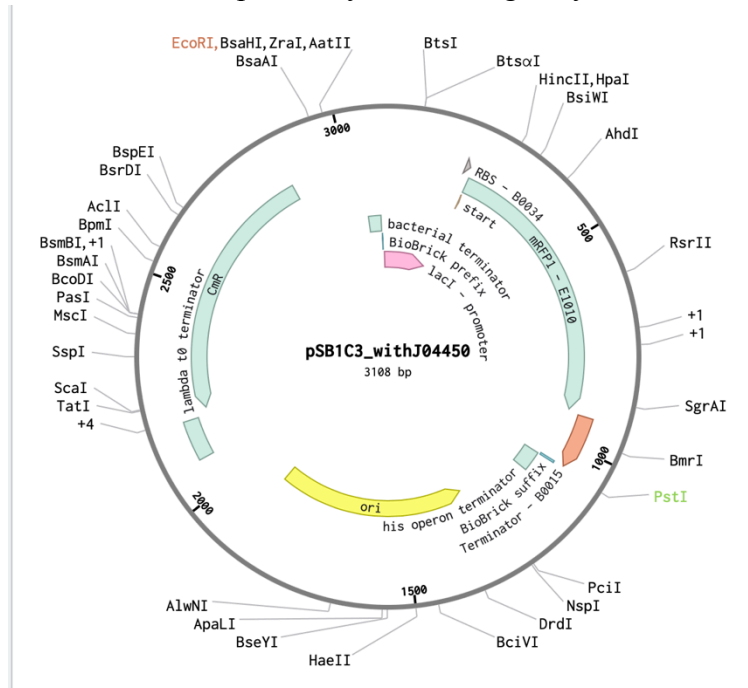
🔧 Name the assembly (e.g., pSB1C3_withJ04450) and “**Assemble**”.

pSB1C3_withJ04450

🗑️

Assemble

Congratulations, this is the plasmid you are using for your wet lab exercise 🎉



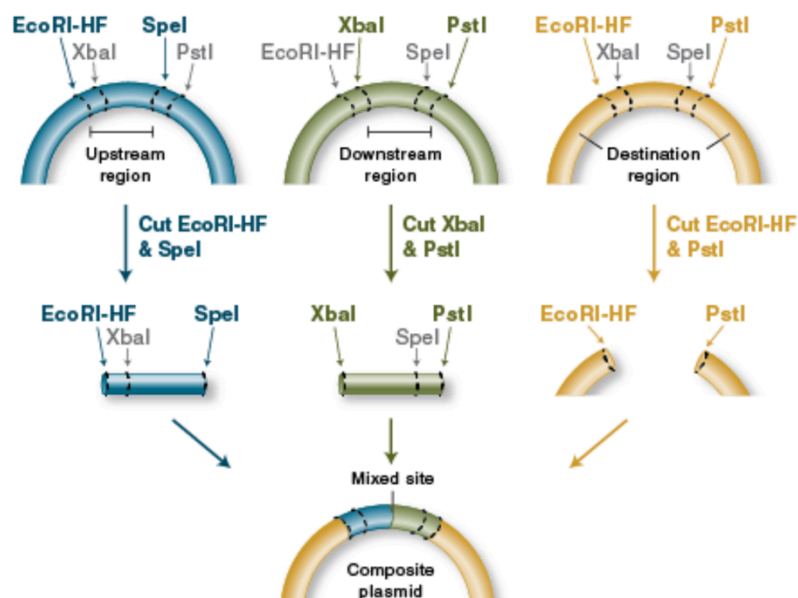
💡 In plasmid designs, it's common for the antibiotic resistance cassette to be on the opposite strand, since it's under its own control and not part of the assembled genetic device.

BioBrick Assembly

Another useful technique that could be followed, is Biobrick Assembly to incorporate all different J04450 subparts into the backbone. For this exercise, we divide our construct into 2 subparts instead of 4 (promoter + RBS/mRFP1/dterminator), but a similar procedure can be followed to incorporate more Biobricks into a plasmid.

💡 BioBrick assembly is a standardized method used in synthetic biology to combine DNA parts—called BioBricks—into larger genetic constructs. Each BioBrick part is flanked by specific restriction enzyme sites (EcoRI, XbaI, SpeI, and PstI), allowing them to be cut and ligated together in a predictable and modular way. This makes it easy to mix and match promoters, coding sequences, RBSs, and terminators to build custom genetic circuits without needing to redesign the cloning strategy for each combination.

If you are not familiar with the technique, pay attention to the figure below.



New England Biolabs. (n.d.). *BioBrick®* assembly. <https://www.neb.com/en/applications/cloning-and-synthetic-biology/dna-assembly-and-cloning/biobrick-assembly>

💡 In this method, three plasmids/sequences are involved: one containing the **upstream** BioBrick part, one containing the **downstream** part, and one serving as the **destination vector**. Each of these plasmids contains specific restriction sites that allow for precise cutting and ligation.

The upstream part is excised from its plasmid by digesting with EcoRI and SpeI. This releases the DNA fragment with sticky ends compatible with ligation. Simultaneously, the downstream part is cut using XbaI and PstI, generating another fragment with complementary overhangs. SpeI and XbaI produce compatible overhangs that can ligate together, but their ligation forms a mixed site that cannot be cleaved again by either enzyme—this feature ensures stability in the resulting composite part.

The destination plasmid is opened using EcoRI and PstI, creating a site where the upstream and downstream parts can be inserted. After the three fragments are prepared, they are ligated together to form a composite plasmid. The final construct contains both BioBrick parts joined in order, flanked by the original prefix and suffix, and ready for further use or additional rounds of assembly. This standardized method allows for predictable and modular construction of genetic circuits.

💡 The BBa_J04450 construct is a well-characterized composite BioBrick device that consists of the following four standardized parts:

- A. **Promoter (BBa_R0010)**
- B. **Ribosome Binding Site (BBa_B0034)**
- C. **Reporter Gene (BBa_E1010)**
- D. **Terminator (BBa_B0015)**

💡 Each of these components is a BioBrick, meaning they can conform to the BioBrick standard assembly rules. This allows to easily replace or combine them with other BioBrick parts using the BioBrick Assembly method or other compatible cloning techniques. For example, you could swap the promoter to test different expression levels or replace mRFP1 with another reporter gene to suit your experimental needs.

💡 The BBa_J04450 subparts on the registry don't contain the standard prefix and suffix that are essential to the BioBrick assembly, only the coding region. So, they must be added, again by creating PCR primers.

💡 The BioBrick format follows a standardized structure:

BioBrick Prefix (before the gene starts)

This sequence ensures compatibility with **EcoRI** and **XbaI** digestion:

5' - GAATTC GCGGCCGC T TCTAGA G XXXXXXXX -3'
(EcoRI - NotI - XbaI)

- a) **EcoRI (GAATTC)** → The primary restriction enzyme for inserting the part.
- b) **NotI (GCGGCCGC)** → Useful for verification but not needed for standard cloning.
- c) **XbaI (TCTAGA)** → Used for ligation with a SpeI-cut plasmid.
- d) **XXXXXXX** → The first bases of your gene

The prefix sequence will match the beginning of the forward primer:

(1) Forward primer: 5' - GAATTC GCGGCCGC T TCTAGA G XXXXXXXX -3'

BioBrick Suffix (after the gene ends)

This sequence ensures compatibility with **SpeI** and **PstI** digestion:

5' - XXXXXXXX T ACTAGT A GCGGCCG CTGCAG - '3
(SpeI - NotI - PstI)

- a) **SpeI (ACTAGT)** → Compatible with XbaI for assembly.
- b) **NotI (GCGGCCGC)** → Again, for verification purposes.
- c) **PstI (CTGCAG)** → Used for cutting the plasmid downstream.

💡 The suffix (SpeI and PstI sites) must be included at the 5' end of the reverse primer, but because reverse primers bind to the complementary strand in the opposite direction, you must use the reverse complement of the suffix sequence as part of your reverse primer.

(2) Reverse primer: 5' - CTGCA GCGGCCGC T ACTAGT A XXXXXXXX - '3

(PstI - NotI - SpeI)

The pSB1C3 backbone already contains the prefix and suffix sequence. PCR primers need to be created for the promoter (BBa_R0010) and the rest of the J04450 construct (in this case, BBa_B0034+BBa_E1010+BBa_B0015).

🔧 Open the first BioBrick - the promoter's sequence, from the registry.

https://parts.igem.org/Part:BBa_R0010.

Copy the sequence, and "Create" > "DNA / RNA sequence" > "New DNA / RNA sequence". Name it as "lacI promoter - R0010".

You can annotate it again, if you want.

🔧 As before, select the 20 first bp of the sequence, right click > "Create primer" > "Forward". Paste the provided forward primer sequence (1) as a 22 bases overhang.

Design

Strand	Forward
Bases	5' GAATTCGCGGCCGCTTCTAGAGcaat acgcaaaccgcctctc 3'
3' Location	<input type="text" value="20"/>
Overhang	<input type="text" value="22"/>

🔧 For the reverse primer, select the 20 last bp of your sequence, right click > "Copy special" > "Reverse Complement". Copy the reverse complement sequence and paste it at your Reverse primer.

🔧 Add the provided 21 bp overhang reverse primer sequence (2) as the 5' end of the reverse primer, at sequence position 181.

Reverse
5' CTGCAGCGGCCGCTACTAGTAtgtgt gaaattggttatccgc 3'
<input type="text" value="181"/>
<input type="text" value="21"/>

🔧 Save the primers under appropriate names (e.g., R0010_promoter_F_prefix & R0010_promoter_R_suffix) and “*Create PCR product*”.

Save

Name

PRIMERS PAIRS

Primer	Position	Product Size
R0010_promoter_F_prefix	+ / 20	243
R0010_promoter_R_suffix	- / 181	

Primer Pair Information [Edit - Unlink](#)

[Link Primers](#)

	Name	T _m
Forward Primer	R0010_promoter_F_prefix	56.1°C
Reverse Primer	R0010_promoter_R_suffix	51.1°C

Product Size 243 bp
T_m Difference -5.0° C

[Create PCR Product](#) [Secondary Structure](#)

🔧 Now, to get hold of the second BioBrick (BBa_B0034 + BBa_E1010 + BBa_B0015), search for the promoter's sequence in the previously annotated J04450 construct sequence, copy and paste the rest of the construct's sequence (promoter's sequence excluded).

https://parts.igem.org/Part:BBa_J04450

Once you have the right sequence for the rest of the parts, you can annotate them.

🔧 Create primers for this part too. You should follow the same procedure as shown before. The “*Design*” table should look like this:

Primer Pair Jump to Primer Set from Selection

Design

Editing this saved primer and adding a binding site:

You can also [remove it](#).

Strand: Forward

Bases:

3' Location:

Overhang:

Cut Site:

Editing this saved primer and adding a binding site:

You can also [remove it](#).

Strand: Reverse

Bases:

3' Location:

Overhang:

Use the dropdown above to look up restriction sites.

Verify Check Secondary Structure at °C

T _m	45.9°C	53.6°C
GC Content	35.00%	45.00%
Length	42 bp	41 bp
Product Size	912 bp	
T _m Diff.	+77.2°C	

Save

Name

🔧 Save the primer pair and “*Create PCR product*”.

Since Benchling doesn't offer a built-in option for BioBrick assembly, perform “*Digest and Ligate*” assembly.

🔧 “*Digest*” the promoter’s sequence from the PCR product with EcoRI and SpeI, and save.

Enzyme SpeI

ACTAGT
TGATCA

NEB

Use HF

Link: [NEB](#)

Inactivation: 80°C
Incubation: 37°C

Activity:

1.1	2.1	3.1	4/CS
25	50	10	100

Isos.: [BcuI](#)

Jump to Cut Site:
225

NEW DIGEST SAVED DIGESTS

Enzyme lists [Manage enzyme lists](#)

Deduplicated commercial

Cut sites visible on maps
Single cutters

Find enzyme [Clear selected](#)

spe

Name	Cuts	Selected	Color
BspEI	0	EcoRI	■
FspEI	22	SpeI	■
SpeI	1		

Show enzymes that cut
anywhere in the sequence

Highlight enzymes with compatible sticky ends

[Run digest](#)

LINEAR MAP **DIGEST** × VIRTUAL DIGEST DESCRIPTION METADATA RELEVANT ITEMS [Share](#)

Digest [Save](#) NEB Use HF

Enzymes	Cuts	Temp.	1.1	2.1	3.1	4/CS
EcoRI	1	37°C	25	100*	50	50*
SpeI	1	—	Not available for this vendor			

Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
1	1	1	None	blunt	EcoRI	5'
2	224	223	EcoRI	5'	SpeI	5'
225	243	19	SpeI	5'	None	blunt

🔧 “*Digest*” the subpart (RBS_mRFP1_dterminator) PCR product with XbaI and PstI, and save.

Enzyme PstI

CTGCAG
GACGTC

NEB

Use HF

Link: [NEB](#)

Inactivation: 80°C

Incubation: 37°C

Activity:

11	21	31	4/CS
75	75	100	50'

Isos.: None

Jump to Cut Site:

890

NEW DIGEST SAVED DIGESTS

Enzyme lists [Manage enzyme lists](#)

Deduplicated commercial

Cut sites visible on maps

Single cutters

Find enzyme [Clear selected](#)

pst

Name	Cuts	Selected	Color
PstI	1	<input checked="" type="checkbox"/>	■
XbaI		<input type="checkbox"/>	■

Show enzymes that cut

anywhere in the sequence

Highlight enzymes with compatible sticky ends

Run digest

LINEAR MAP DIGEST VIRTUAL DIGEST DESCRIPTION METADATA RELEVANT ITEMS [Share](#)

Digest Save NEB Use HF

Enzymes	Cuts	Temp.	11	21	31	4/CS
PstI	1	37°C	75	75	100	50'
XbaI	1	37°C	<10	100	75	100

Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
1	16	16	None	blunt	XbaI	5'
17	889	873	XbaI	5'	PstI	3'
890	890	1	PstI	3'	None	blunt

“Digest” the the pSB1C3 vector with EcoRI and PstI, and save.

Enzyme PstI

CTGCAG
GACGTC

NEB

Use HF

Link: [NEB](#)

Inactivation: 80°C

Incubation: 37°C

Activity:

11	21	31	4/CS
75	75	100	50'

Isos.: None

Jump to Cut Site:

21

NEW DIGEST SAVED DIGESTS

Enzyme lists [Manage enzyme lists](#)

Deduplicated commercial

Cut sites visible on maps

Single cutters

Find enzyme [Clear selected](#)

pst

Name	Cuts	Selected	Color
PstI	1	<input checked="" type="checkbox"/>	■
EcoRI		<input type="checkbox"/>	■
PstI		<input type="checkbox"/>	■

Show enzymes that cut

anywhere in the sequence

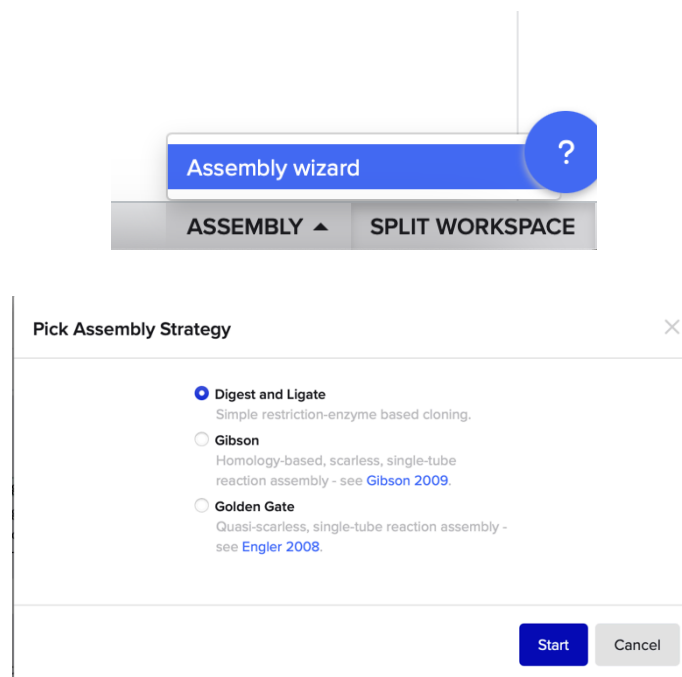
Highlight enzymes with compatible sticky ends

Run digest

DIGEST						
Enzymes	Cuts	Temp.	11	2.1	3.1	4/CS
EcoRI	1	37°C	25	100*	50	50*
PstI	1	37°C	75	75	100	50*

Start	End	Length	Left Cutter	Left Overhang	Right Cutter	Right Overhang
21	2049	2029	PstI	3'	EcoRI	5'
2050	20	41	EcoRI	5'	PstI	3'

Go to your digested backbone sequence click on “**ASSEMBLY**” > “**Assembly wizard**” and start “**Digest and Ligate**” assembly.



As before, begin by setting the fragments you want to ligate, making sure to choose the correct fragment resulting from the digestion.

- 1) Use pSB1C3 as backbone.
- 2) For insert, select the promoter, again ensuring you’ve chosen the appropriate fragment generated by the restriction digest.
- 3) Add another insert tab by clicking on the “+” sign. The second insert will be the subparts sequence.

Something like this:

PREVIEW

aTCTAGAGa CGCTGCA GT
tATGATCTct GCGACGTCa

0 ERRORS AND 0 WARNINGS
✓ Looks like everything checks out

Reverse Orientation
Jump to Selection
View Enzyme Activity

+ Hide Preview

pSB1C3 2.0 kb · PstI, EcoRI

promoter_R0010 [1-200] 223 bp · EcoRI, SpeI

RBS_mRFP1_terminator [1-847] 873 bp · XbaI, PstI

