# InterLab Study

**Project:** iGEM ETHZ 2018
**Authors:** Adriano Martinelli
**Date:** 2018-07-19

THURSDAY, 19/7/18

## Competent Cells:

- Prepared according to protocol of Tobias
- Pre-prepared buffers used from Tobias:

Table1

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | **TB1** | | | | | | |
| 2 | | | | g/mol | Desired volume (mL): | 50 | |
| 3 | RbCl | 100 | mM | 120.921 | | 0.6 | g |
| 4 | MnCl2·4H2O | 50 | mM | 197.91 | | 0.49 | g |
| 5 | Potassium acetate | 30 | mM | 98.15 | | 0.15 | g |
| 6 | CaCl2·2H2O | 10 | mM | 147.01 | | 0.07 | g |
| 7 | Glycerol | 15 | % | | | 7.5 | mL |
| 8 | | | | | | | |
| 9 | Adjust pH to 5.8 with 1 M acetic acid. Be very careful as you | | | | | | |
| 10 | approach 5.8; if the pH drops lower than 5.8, a black | | | | | | |
| 11 | precipitate may form. Filter sterilize (0.2µM) and store at room | | | | | | |
| 12 | temperature. | | | | | | |
| 13 | | | | | | | |
| 14 | **TB2** | | | | | | |
| 15 | | | | g/mol | Desired volume (mL): | 50 | |
| 16 | MOPS | 10 | mM | 209.263 | | 0.105 | g |
| 17 | RbCl | 10 | mM | 120.921 | | 0.06 | g |
| 18 | CaCl2·2H2O | 75 | mM | 147.01 | | 0.551 | g |
| 19 | Glycerol | 15 | % | | | 7.5 | mL |
| 20 | | | | | | | |
| 21 | Adjust the pH to 6.5 with 1 M KOH. Filter sterilize (0.2µM) and | | | | | | |
| 22 | store at room temperature. | | | | | | |

## Procedure:

- Inoculate 5mL LB media with a single DH5alpha colonie

- Grow o/n cluture at 30°C
- Inoculate 2x 50mL LB media with 0.5 mL o/n culture
- Grow at 37°C on the shaker
- Harvest at OD 0.425 (approx 3h)
- Put cells on ice for 10 minutes
- Centrifuge cells at 4°C for 7 minutes at 3200 x g
- Discarde supernatant
- Carefully resuspend pellet in buffer TFB1 buffer
- Incubate on ice for 90 minutes
- Centrifuge cells at 4°C for 7 minutes at 3200 x g
- Discard supernatant
- Carefully resuspend pellet in buffer TFB2
- Aliquot 100µL in pre-cooled 1.5mL Eppendorf Tubes
- Imerse tubes into liquid nitrogen
- Transfer frozen competent cells to -80°C

# Tranformation:

- The following transformations were performed according to the interlab protocol
    - Positive control
    - Negative control
    - Test Device 1-6
- In addition to that a positive transfection control was prepared with the test plasmid in the competent cell test kit
- For the negative transfection control pure LB media was used

## Procedure:

- For the tranformations of negative control, Test Device 1 - 6 the freshly made competent cells (see protocol above) were used unfrozen (!!!) to maximize efficiency
- For the transformations of the transfromation controls (positive and negative) as well as the negative control freshly made competent cells were thawed on ice for 10'
- 1µL of the corresponding DNA was transferred to the competent cells. This corresponds to 200-300pg DNA. For the positive transformation control 10 pg of the control plasmide provided in the competent cells test kit were used.
- Cells were incubated for 30 miutes on ice
- Heat shock the cells at 42°C in the heating block for 45 seconds
- Immediately transfer the cells back on ice for another 10 minutes
- Add 900µL prewarmed (37°C) LB media without any antibiotics
- Incubate cells for 40 minutes at 37°C on the shaker
- Plate 100µL of each transformation on LB agar plates with 25µg/mL chloramphenicole
- Spind the remaining 900µL transformation down at 6200 x g for 3 minutes
- Discarde supernatant by pipetting it off the cells (carefully!!)
- Add 100µL of prewaremd LB media
- Plate 100µL of each transformation on LB agar plates with 25µg/mL chloramphenicole
- Incubate o/n at 37°C for 14-16 hours (start time: 18:30)

# Split Luciferase

**Project:** iGEM ETHZ 2018
**Authors:** Adriano Martinelli
**Date:** 2018-07-30

MONDAY, 30/7/18

## Bubbling Experiment with vanillin

### Compouds:

Vanillin: Sigma-Aldrich, V1104-100G, Lot. BCBV5242

### Arithmetic serial dilution of vanillin:

Stock solutions:

1 mg/mL vanillin

- Weight 0.0100 g of Vanillin
- Flush in a 10 mL volumetric flask
- Fill up to the mark
- Transfer to 15mL Falcon tube

1 µg/mL vanilin

- Add 9990µL of ddH20
- Add 10 µL of 1 mg/mL vanillin stock solution

1ng/mL vanillin

- Add 9990µL of ddH20
- Add 10 µL of 1 µg/mL vanillin stock solution

1pg/mL vanillin

- Add 9990µL of ddH20
- Add 10 µL of 1 ng/mL vanillin stock solution

Arithmetic serial dilution:

Table1

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | | 1 | 2 | 3 | 4 | 5 | 6 |
| 2 | Vanillin µg/mL | 500 | 400 | 300 | 200 | 100 | 75 |
| 3 | 1 mg/mL stock solution [mL] | 0.5 | 0.4 | 0.3 | 0.2 | 0.1 | 0.075 |
| 4 | 1 µg/mL stock solution [mL] | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 1 ng/mL stock solution [mL] | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 1 pg/mL stock solution [mL] | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | ddH2O [mL] | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 0.925 |

## Bubbling of vanillin

- Three spoons of vanillin were added in a 1L bucket and sealed with Parafilm.
- Air in the bucket was incubated. Start: 14:00

- GenElute Tube binding column is attachet to a 50mL syringe
- 600 mL ddH20 was added to the column
- 10, 30 and 50 mL of air was bubbled throught the column and the water. The air was taken from the normal lab environment for the negative control and from the bucket for the tests.
    10 mL was taken at 16:10. Air was incubated for 2h 10 minutes with vanilline.
    30 mL was taken at 16:20. Air was incubated for 2h 20 minutes with vanilline.
    50 mL was taken at 16:30. Air was incubated for 2h 30 minutes with vanilline.
- 500 µL of the bubbled water was transferred to a 1.5 mL Eppendorf Tube
- 100 µL of XXX o-toluidine was added to the bubbled water in the tubes

! According to the Paper 5 min incubation at RT, this incubation was skipped without purpose.

- Tubes were incubatet at 99°C in a heat block for 15 minutes. Start 16:25. End: 16:4.

# Untitled

## Introduction

Get started by giving your protocol a name and editing this introduction.

## Materials

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## Procedure

1.

# 07/08

**Project:** iGEM ETHZ 2018
**Authors:** Elisa Garulli
**Date:** 2018-08-07

TUESDAY, 7/8/18

Gibson Assembly

- For Elisa
- Used DNA: only the backbone of her Tar cloning vector, i.e. only one fragment, the backbone, was used to control for self-assembly. Minipreps B2, B3 and B4 were used.

Resistance: Chloramphenicol

Swimming Assay Agar

**Tryptone swimming agar**

- 1 % (w/v) bacto tryptone
- 0.5 % (w/v) NaCl
- 0.3 % (w/v) agar

Table1

| | A | B | C |
|---|---|---|---|
| 1 | | 500mL ddH2O | |
| 2 | Bacto tryptone | 5 | g |
| 3 | NaCl | 2.5 | g |
| 4 | Agar | 1.5 | g |

**M9 Swimming Agar**

- 1.25 % (v/v) glycerol
- 20 % (v/v) 5x M9 salt stock solution
- 0.1 % (v/v) of $CaCl_2$ x 2 $H_2O$ stock solution (20 mg/mL)
- 0.1 % (v/v) $MgSO_4$ stock solution (0.12 g/mL)
- 0.3 % (w/v) of agar

M9 Swimming Agar for 500mL total volume

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Material | Percentage | Volume/weight | volume from already existing stocks |
| 2 | Glycerol | 0.0125 | 6.25 | |
| 3 | M9 Salts | 0.2 | 100 | |
| 4 | CaCl2 * 2 H2O | 0.001 | 0.5 | 0.9 |
| 5 | MgSO4 | 0.001 | 0.5 | |
| 6 | Agar | 0.003 | 1.5 | |
| 7 | Total volume | 500 | | |

# Gibson Assembly® Protocol (E5510)

## Introduction

This is the protocol for Gibson Assembly using the Gibson Assembly® Cloning Kit (E5510). More information from NEB can be found here.

## Materials

- Gibson Assembly Cloning Kit
  - Gibson Assembly® Master Mix
  - NEBuilder® Positive Control
  - NEB® 5-alpha Competent *E. coli* (High Efficiency)
  - SOC Outgrowth Medium
  - pUC19 Transformation Control Plasmid
- DNA Polymerases (for generating PCR products)
  - Recommended: Q5® High-Fidelity DNA Polymerase, Q5 Hot Start High-Fidelity DNA Polymerase, or Q5 Hot Start High-Fidelity 2X Master Mix
- LB (Luria-Bertani) plates with appropriate antibiotic

## Procedure

### Set up the following reaction on ice:

1. Reaction volumes: *Use this table to calculate reaction volumes and set up the reaction. Remember to* ***input your total DNA fragment volume in cells B3 and C3*** *for assemblies with 2-3 fragments and 4-6 fragments, respectively.*

   *- NEB recommends a total of* ***0.02–0.5 pmols of DNA fragments when 1 or 2 fragments*** *are being assembled into a vector and* ***0.2–1.0 pmoles of DNA fragments when 4–6 fragments*** *are being assembled. Efficiency of assembly decreases as the number or length of fragments increases. To calculate the number of pmols of each fragment for optimal assembly, based on fragment length and weight, we recommend using NEB's online tool, NEBioCalculator.*
   *- The mass of each fragment can be measured using the NanoDrop instrument, absorbance at 260 nm, or estimated from agarose gel electrophoresis followed by ethidium bromide staining.*

Table1

| | A | B |
|---|---|---|
| 1 | | **2-3 Fragments Assembly** |
| 2 | **Concentration Range of DNA fragments** | 02 - .5 pmols* |
| 3 | **Total Volume of Fragments (µl)** | 5 |
| 4 | **Gibson Assembly Master Mix (2x) (µl)** | 15 |
| 5 | **Deionized Water (µl)** | 10 |
| 6 | **Total Volume (µl) *** ** | 30 |

**Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts. Use 5 times more of inserts if size is less than 200 bps. Total volume of unpurified PCR fragments in Gibson Assembly reaction should not exceed 20%.*

*** Control reagants are provided for 5 experiments with the Gibson Asembly Kit.*

**** If greater numbers of fragments are assembled, additional Gibson Assembly Master Mix may be required.*

2. Incubate samples in a thermocycler at 50°C for 60 minutes when 2 or 3 fragments are being assembled or 60 minutes when 4-6 fragments are being assembled.

   *Note: Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases* (for further details see FAQ section).

   **Select the pencil icon and change the timer to 60 minutes if working with 4-6 fragments*

   01:00:00

3. Store samples on ice or at –20°C for subsequent transformation.

4. Transform Top10 Competent E. coli cells (provided with the kit) with 2 µl of the assembly reaction, following the chemcial transformation protocol or electro competent cells transformation protocol

# Transformation Chemically Comp. Cells

## Introduction

Jan's protocol for transformation of chemically competent cells

## Materials

- Chemically competent cells
- Ice
- Heat bath
- LB
- Agar plates with resistance
- Incubator

## Procedure

### Transformation procedure

- [x] 1. Thaw Bacteria from -80˚ on ice (10 min)
- [x] 2. Add 7.5 µl Plasmid (up to 10% of total volume) and mix gently with pipette, let it sit on ice for 30min

  1µl of 10pg/µl pSC1C3 as positive control
  7.5 µl of ddH2O as negative control
  7.5µl of backbone B2
  7.5µl of backbone B3
  7.5µl of backbone B4

  00:30:00

- [x] 3. Heat-shock tube in 42˚ for 45sec

  00:00:45

- [x] 4. Put tube on ice for 2min

  00:02:00

- [x] 5. Add 900µl LB
- [x] 6. Shake in incubator at 37˚ for 1 hour

01:00:00

- [x] 7. **Meanwhile**: Prepare appropriate agar plates with antibiotics and pre-heat in 37˚ (dry) incubator

    Plates with Chloramphenicol were used

- [x] 8. **After an Hour**, spin down bacteria to pellet in 4000g for 3 min, and discard of most of media, leaving only a-bit of liquid (~100ul), re-suspend pellet with the remained liquid and spread on plate)
- [x] 9. Incubate plates in 37˚ over-night

# EPIC + OmpR + pSB3C5 Backbone PCR

**Project:** iGEM ETHZ 2018
**Authors:** Adriano Martinelli
**Dates:** 2018-08-16 to 2018-08-17

THURSDAY, 16/8/18

## Procedure

### PCR Amplification (PHUSION) of EPIC and OmpR

See 01_PCR_Amplification_Phusion protocol

### Agarose-Gel

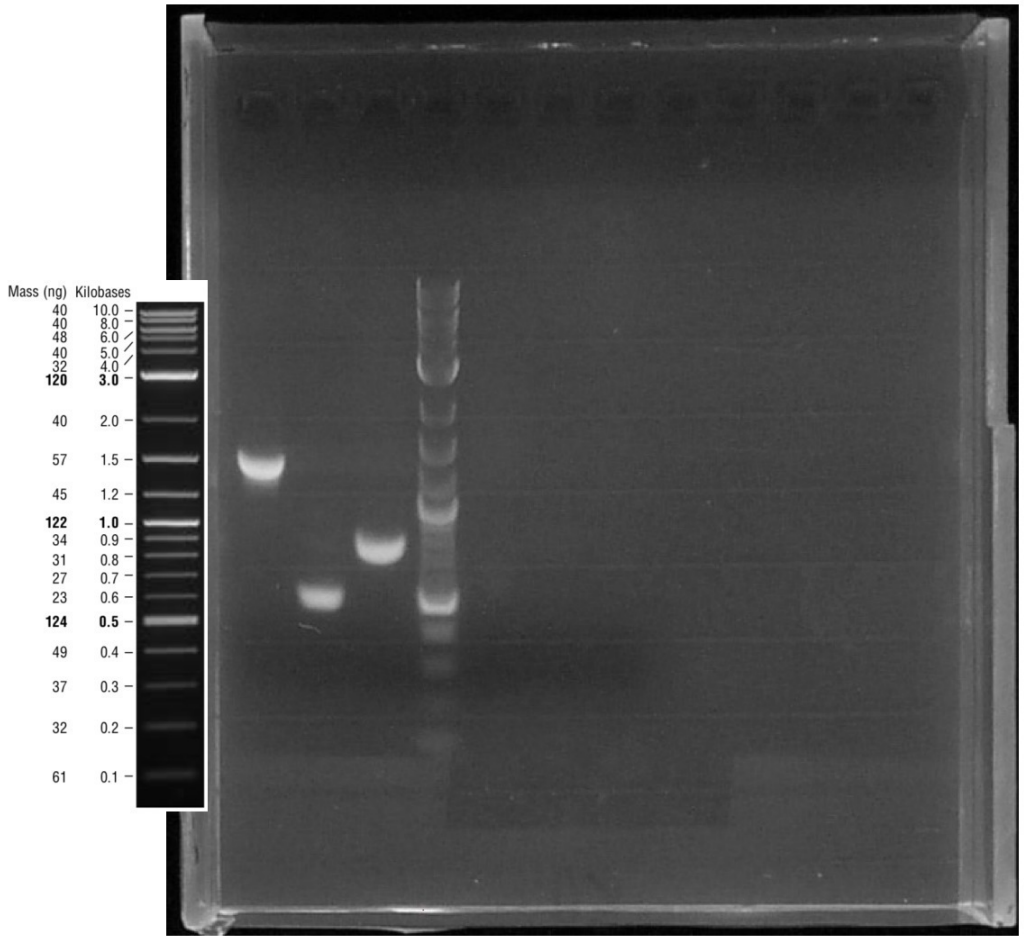
- 1% Agarose in 25mL TEA Buffer
- 1µL peqGreen
- Mix 15µL PCR product with 3µL Loading Buffer 6x
- Load gel:

Table1

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | **Line** | **1** | **2** | **3** | **4** | **5** | |
| 2 | **Sample** | EPIC Nluc | EPIC Cluc | OmpR Genome | Ladder | EPIC Nluc negativ control | EPIC Cluc negativ control |

- Run for 30' at 135V
- Take picture with Kodak machine in 3.50

Screen Shot 2018-08-16 at 4.02.37 PM.png




- Digest PCR with DPN1
    - Add 3.5µL Cut Smart Buffer
    - Add 1µL DPN1 enzyme
    - Diegest for 1h at 37°C with the PCR Cycler program IGEMDPN1

Nanodrop:
1: 21.5 ng/ul
2: 91.7 ng/ul
3: 8.3 ng/ul (note: PCR clean-up was performed before measuring the concentration. A kit from 2014 was used -> most likely the buffers don't work properly anymore !!!!)

## Gibson Assembly

See 02_GIBSON_ASSEMBLY protocol

1: 15 ul Gibson mix + 4 ul backbone (= 100 ng) + 11 ul pcr mix ( ~250 ng, could not at more as the final volume should be 30 ul)
2: 15 ul Gibson mix + 4 ul backbone (= 100 ng) + 3.27 ul pcr mix (~300 ng) + 7.77 ul ddH20
3: 30 ul Gibson mix + 4 ul backbone + 26 ul (~210 ng) pcr cleaned up mix

Run for 60 min. in Thermocycler.

# Evaluation

## PCR Amplification (PHUSION)

- Products were amplified:

Table2

| | A | B | C |
|---|---|---|---|
| 1 | **Pairs** | **Expected** | **Is** |
| 2 | EPIC Nluc | 1295 bp | ~1500 bp |
| 3 | EPIC Cluc | 512 bp | 500 bp |
| 4 | OmpR Genome | 767 bp | 800 bp |

- All negativ controls didn't show PCR products

FRIDAY, 17/8/18

# Procedure

## PCR Amplification (PHUSION) of pSB3C5 backbone

See 03_PCR_Amplification_Phusion protocol

## Agarose-Gel

- 1% Agarose in 25mL TEA Buffer
- Add 1µL peqGreen after cooling down to "touch-warm"
- Mix 10µL PCR product with 2µL Loading Buffer 6x
- Load gel with 10µL of each:

Table3

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | **Line** | **1** | **2** | **3** | **4** |
| 2 | **Sample** | pSV3C5 Miniprep 1 | pSV3C5 Miniprep 2 | Ladder | pSV3C5 Miniprep 3 |

- Run for 30' at 135V
- Take picture with Kodak machine in 3.50

## Transformation of Gibson Assemblies from 16/08 and pSEVA 471

See 04_Transformation_Chemically_Comp. Cells protocol

# 01_PCR_Amplification_Phusion

## Introduction

PCR amplification of fragments or backbones

## Materials

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## Procedure

### Mix

1 = Epic Nluc
2 = Epic Cluc
3 = OmpR_Genome

Table1

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | | | | Master Mix | Number of PCRs | 6 |
| 2 | mQH2O | 32.5 | 48.75 | 214.5 | | |
| 3 | 5X HF buffer | 10 | 15 | 66 | 44 | |
| 4 | 10 mM dNTPs | 1 | 1.5 | 6.6 | | |
| 5 | 10 uM F | 2.5 | 3.75 | | | |
| 6 | 10 uM R | 2.5 | 3.75 | | | |
| 7 | (~10 ng/ul) DNA | 1 | 1.5 | | | |
| 8 | Phusion | 0.5 | 0.75 | 3.3 | | |
| 9 | | 50 | 75 | 290.4 | | |

Set the following protocol on a thermocycler:

- [x] 1. 98°C 30"
- [x] 2. 98 °C 10"
- [x] 3. Tm 10"
- [x] 4. 72 °C 15-30" /Kb
- [x] 5. Go to step 2 for 29 additional times
- [x] 6. 72 °C 10'

- ✔ 7. 10°C ∞
- ✔ 8.

# 02_GIBSON_ASSEMBLY

## Introduction

This is the protocol for Gibson Assembly using the Gibson Assembly® Cloning Kit (E5510). More information from NEB can be found here.

## Materials

- Gibson Assembly Cloning Kit
  - Gibson Assembly® Master Mix
  - NEBuilder® Positive Control
  - NEB® 5-alpha Competent *E. coli* (High Efficiency)
  - SOC Outgrowth Medium
  - pUC19 Transformation Control Plasmid
- DNA Polymerases (for generating PCR products)
  - Recommended: Q5® High-Fidelity DNA Polymerase, Q5 Hot Start High-Fidelity DNA Polymerase, or Q5 Hot Start High-Fidelity 2X Master Mix
- LB (Luria-Bertani) plates with appropriate antibiotic

## Procedure

### Set up the following reaction on ice:

1. Reaction volumes: *Use this table to calculate reaction volumes and set up the reaction. Remember to* ***input your total DNA fragment volume in cells B3 and C3*** *for assemblies with 2-3 fragments and 4-6 fragments, respectively.*

   *- NEB recommends a total of* ***0.02–0.5 pmols of DNA fragments when 1 or 2 fragments*** *are being assembled into a vector and* ***0.2–1.0 pmoles of DNA fragments when 4–6 fragments*** *are being assembled. Efficiency of assembly decreases as the number or length of fragments increases. To calculate the number of pmols of each fragment for optimal assembly, based on fragment length and weight, we recommend using NEB's online tool, NEBioCalculator.*
   *- The mass of each fragment can be measured using the NanoDrop instrument, absorbance at 260 nm, or estimated from agarose gel electrophoresis followed by ethidium bromide staining.*

Table1

| | A | B | C | |
|---|---|---|---|---|
| 1 | | **2-3 Fragments Assembly** | **4-6 Fragments Assembly** | **Posit** |
| 2 | **Concentration Range of DNA fragments** | 02 - .5 pmols* | .2 - 1.0 pmols* | 0 pm |
| 3 | **Total Volume of Fragments (µl)** | 7.27 | | |
| 4 | **Gibson Assembly Master Mix (2x) (µl)** | 15 | 10 | |
| 5 | **Deionized Water (µl)** | 7.73 | #VALUE! | |
| 6 | **Total Volume (µl) *** ** | 30 | 20 | |

**Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts. Use 5 times more of inserts if size is less than 200 bps. Total volume of unpurified PCR fragments in Gibson Assembly reaction should not exceed 20%.*

*** Control reagants are provided for 5 experiments with the Gibson Asembly Kit.*

**** If greater numbers of fragments are assembled, additional Gibson Assembly Master Mix may be required.*

2. Incubate samples in a thermocycler at 50°C for 15 minutes when 2 or 3 fragments are being assembled or 60 minutes when 4-6 fragments are being assembled.

   *Note: Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases* (for further details see FAQ section).

   **Select the pencil icon and change the timer to 60 minutes if working with 4-6 fragments*

   00:15:00

3. Store samples on ice or at –20°C for subsequent transformation.

4. Transform NEB 5-alpha Competent E. coli cells (provided with the kit) with 2 µl of the assembly reaction, following the chemcial transformation protocol or electro competent cells transformation protocol

# 03_PCR_Amplification_Phusion

## Introduction

PCR amplification of fragments or backbones

## Materials

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## Procedure

### Mix

Table1

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | | | | Master-Mix | Number of PCR's | 6 |
| 2 | mQH2O | 32.5 | 48.75 | 204.75 | ok | |
| 3 | 5X HF buffer | 10 | 15 | 63 | ok | |
| 4 | 10 mM dNTPs | 1 | 1.5 | 6.3 | ok | |
| 5 | 10 uM F | 2.5 | 3.75 | 15.75 | ok | |
| 6 | 10 uM R | 2.5 | 3.75 | 15.75 | ok | |
| 7 | (~10 ng/ul) DNA | 1 | 1.5 | | | |
| 8 | Phusion | 0.5 | 0.75 | 3.15 | | |
| 9 | | 50 | 75 | 308.7 | 48.5 | |

Set the following protocol on a thermocycler:

- 1. 98°C 30"
- 2. 98 °C 10"
- 3. Tm 10"
- 4. 72 °C 15-30" /Kb
- 5. Go to step 2 for 29 additional times
- 6. 72 °C 10'
- 7. 10°C ∞

- ✔ 8.

# 04_Transformation_Chemically_Comp. Cells

## Introduction

Transformation of the the Gibson Assemblies done in 02_GIBSON_ASSEMBLY Protocol and pSEVA 471

1 = Epic Nluc
2 = Epic Cluc
3 = OmpR_Genome
5 = pSEVA 471

## Materials

- Chemically competent cells
- Ice
- Heat bath
- LB
- Agar plates with resistance
- Incubator

## Procedure

### Transformation procedure

- [x] 1. Thaw Bacteria from -80˚ on ice (10 min)
- [x] 2. Add 7.5 µl of each Gibson Assembly and 2 µl of pSEVA 471 Plasmid (up to 10% of total volume) and mix gently with pipette, let it sit on ice for 30min

00:30:00

- [x] 3. Heat-shock tube in 42˚ for 45sec

00:00:45

- [x] 4. Put tube on ice for 2min

00:02:00

- 5. Add 900µl LB
- 6. Shake in incubator at 37˚ for 1 hour

01:00:00

- 7. **Meanwhile**: Prepare appropriate agar plates with antibiotics and pre-heat in 37˚ (dry) incubator
- 8. **After an Hour -** Spin down bacteria to pellet in 4000g for 3 min, and discard of most of media, leaving only a-bit of liquid (~100ul), re-suspend pellet with the remained liquid and spread on plate
- 9. Incubate plates in 37˚ over-night

# FRET

**Project:** iGEM ETHZ 2018
**Authors:** Adriano Martinelli
**Dates:** 2018-07-13 to 2018-07-16

FRIDAY, 13/7/18

How to choose FRET-pair based on distance?
How to introduce linkers?
How to choose linkers?
Would LRET be an alterantive? LERT with the luminescence of lanthanide ions could overcome several shortcomings of standard FRET because (i) the Förster radius is larger, allowing energy transfer over a larger distance; (ii) the donor emission is not polarized as in FRET, which provides for a greater topological flexibility between donor and acceptor; (iii) the luminescence of lanthanides does not bleach and is long-lived (up to the millisecond range)[6]; thus, the short-lived (up to nanoseconds) background fluorescence, which is due to the direct fluorescence of the acceptor, a common issue with FRET, can therefore be circumvented with a time gate of 10–100 µsec.

FRET with previous iGEM Teams:

- http://2014.igem.org/Team:Aachen/Project/FRET_Reporter
- http://2017.igem.org/Team:AshesiGhana/fret

Microscope

1. Switch on current
2. Turn on computer
3. Open NIS elements
4. Filter: use D to make the light diffused. Additional filters are GIF takes out blue, NCB takes out red
5. Invert slides with bacteria on it. Das Deckglas muss unten sein.
6. Fluorescence:
    a. Need to turn on back next to the switches
    b. On the wall right to the microscope there is a list of which filters are used for which wavelength
    c. No auto exposure
    d. Switch of LED, close Shutter but start EPI, open shutter on the control, select cube (filter)
7. Software:
    a. Right click in the empty window and add right and left control
    b. Add acquisition control, filters and shutters, flash4.0Settings, Manual
    c. On the objective: Vergrösserung/N.A. and WD = working distance
    d. Filter4.0 Setting: No binning
    e. Filters and Shutter: Apply filter
    f. OC Panel: You can set up microscope setting and save them
    g.
8. Adjust itensity of light for the brightfiel on the right side of the microscope with the CoolLED. Usually you don't need to exeed a few percent
9. Use the other controller for the fluroescence intensity.
10. Right click: Visualization control open LUT. Adjust exposure time accordingly
11. Save images as tiff since this is lossless
    a. Folder: Microscopy data
12. ND Aquisition:
    a. Time lapse pictures
    b. Lambda scan frames

MONDAY, 16/7/18

Gespräch mit Christen

Welche Strategie wurde verwendet um FRET sensoren herzustellen
- Mikroskopie das problem
- Luca PhD
- Linker optimierung eher sehr schweirig
- Ansatz: Mehrere Rezeptoren und einfach die die Chromophore anhängen
- Wie wollen wir signal vom ligand in messbares Sigal
- Schwierigkeit bei zwei Proteinen: Kalibrieren von zwei einzelmolekülen A und B. Zusätzlich verkompliziert.
- 10 Moleküle die dimerisieren pro Zelle würd ausreichen um Signal zu messen
- Diemerisierung von Rezeptor können wir auch mit luziferase machen
- Ligand Domäne
- P. seud
-

- Tilmann Schirmer
- Grescheck, Stefan

- Megneto Taxis
- Valdi,

Existiert das Konstrukt für den c-di-GMP Sensor noch?
- Existiert, können wir verwenden.

- Marc Folcher, light inducible
- Dimerisierung von Cylasen
- Rezeptor mit DGC engineeren, cytosolisch oder membran
- Mehrere Zielmoleküle
- Beliebige dimerisierungen als Startpunkt

Aaron Ponti, plate reader.

# Gibson Assembly® Protocol (E5510)

## Introduction

This is the protocol for Gibson Assembly using the Gibson Assembly® Cloning Kit (E5510). More information from NEB can be found here.

## Materials

- Gibson Assembly Cloning Kit
  - Gibson Assembly® Master Mix
  - NEBuilder® Positive Control
  - NEB® 5-alpha Competent *E. coli* (High Efficiency)
  - SOC Outgrowth Medium
  - pUC19 Transformation Control Plasmid
- DNA Polymerases (for generating PCR products)
  - Recommended: Q5® High-Fidelity DNA Polymerase, Q5 Hot Start High-Fidelity DNA Polymerase, or Q5 Hot Start High-Fidelity 2X Master Mix
- LB (Luria-Bertani) plates with appropriate antibiotic

## Procedure

### Set up the following reaction on ice:

1. Reaction volumes: *Use this table to calculate reaction volumes and set up the reaction. Remember to* ***input your total DNA fragment volume in cells B3 and C3*** *for assemblies with 2-3 fragments and 4-6 fragments, respectively.*

   *- NEB recommends a total of* ***0.02–0.5 pmols of DNA fragments when 1 or 2 fragments*** *are being assembled into a vector and* ***0.2–1.0 pmoles of DNA fragments when 4–6 fragments*** *are being assembled. Efficiency of assembly decreases as the number or length of fragments increases. To calculate the number of pmols of each fragment for optimal assembly, based on fragment length and weight, we recommend using NEB's online tool, NEBioCalculator.*
   *- The mass of each fragment can be measured using the NanoDrop instrument, absorbance at 260 nm, or estimated from agarose gel electrophoresis followed by ethidium bromide staining.*

Table1

| | A | B | C | |
|---|---|---|---|---|
| 1 | | **2-3 Fragments Assembly** | **4-6 Fragments Assembly** | **Posit** |
| 2 | **Concentration Range of DNA fragments** | 02 - .5 pmols* | .2 - 1.0 pmols* | 0 pm |
| 3 | **Total Volume of Fragments (µl)** | | | |
| 4 | **Gibson Assembly Master Mix (2x) (µl)** | 10 | 10 | |
| 5 | **Deionized Water (µl)** | #VALUE! | #VALUE! | |
| 6 | **Total Volume (µl) *** ** | 20 | 20 | |

**Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts. Use 5 times more of inserts if size is less than 200 bps. Total volume of unpurified PCR fragments in Gibson Assembly reaction should not exceed 20%.*

*** Control reagants are provided for 5 experiments with the Gibson Asembly Kit.*

**** If greater numbers of fragments are assembled, additional Gibson Assembly Master Mix may be required.*

2. Incubate samples in a thermocycler at 50°C for 15 minutes when 2 or 3 fragments are being assembled or 60 minutes when 4-6 fragments are being assembled.

   *Note: Extended incubation up to 60 minutes may help to improve assembly efficiency in some cases* (for further details see FAQ section).

   **Select the pencil icon and change the timer to 60 minutes if working with 4-6 fragments*

   00:15:00

3. Store samples on ice or at –20°C for subsequent transformation.

4. Transform NEB 5-alpha Competent E. coli cells (provided with the kit) with 2 µl of the assembly reaction, following the chemcial transformation protocol or electro competent cells transformation protocol

# Gibson Assembly® Protocol (E5510)

## Introduction

This is the protocol for Gibson Assembly using the Gibson Assembly® Cloning Kit (E5510). More information from NEB can be found here.

## Materials

- Gibson Assembly Cloning Kit
  - Gibson Assembly® Master Mix
  - NEBuilder® Positive Control
  - NEB® 5-alpha Competent *E. coli* (High Efficiency)
  - SOC Outgrowth Medium
  - pUC19 Transformation Control Plasmid
- DNA Polymerases (for generating PCR products)
  - Recommended: Q5® High-Fidelity DNA Polymerase, Q5 Hot Start High-Fidelity DNA Polymerase, or Q5 Hot Start High-Fidelity 2X Master Mix
- LB (Luria-Bertani) plates with appropriate antibiotic

## Procedure

### Set up the following reaction on ice:

1. Reaction volumes: *Use this table to calculate reaction volumes and set up the reaction. Remember to* ***input your total DNA fragment volume in cells B3 and C3*** *for assemblies with 2-3 fragments and 4-6 fragments, respectively.*

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   *- The mass of each fragment can be measured using the NanoDrop instrument, absorbance at 260 nm, or estimated from agarose gel electrophoresis followed by ethidium bromide staining.*

Table1

| | A | B | C | |
|---|---|---|---|---|
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| 3 | **Total Volume of Fragments (µl)** | | | |
| 4 | **Gibson Assembly Master Mix (2x) (µl)** | 10 | 10 | |
| 5 | **Deionized Water (µl)** | #VALUE! | #VALUE! | |
| 6 | **Total Volume (µl) ***** | 20 | 20 | |

**Optimized cloning efficiency is 50–100 ng of vectors with 2–3 fold of excess inserts. Use 5 times more of inserts if size is less than 200 bps. Total volume of unpurified PCR fragments in Gibson Assembly reaction should not exceed 20%.*
*** Control reagants are provided for 5 experiments with the Gibson Asembly Kit.*
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   **Select the pencil icon and change the timer to 60 minutes if working with 4-6 fragments*

   00:15:00

3. Store samples on ice or at –20°C for subsequent transformation.

4. Transform NEB 5-alpha Competent E. coli cells (provided with the kit) with 2 µl of the assembly reaction, following the chemcial transformation protocol or electro competent cells transformation protocol

# InterLab Marburg

**Project:** iGEM ETHZ 2018
**Authors:** Adriano Martinelli
**Date:** 2018-09-12

WEDNESDAY, 12/9/18

Media Preparation:

- Prepared by Toni

Electrocompetent cells

- 10mL LB + v2 media was inoculated with some Vibro natriegens from the provided plate
- Incubate o/n at 37°C
- Overnight cultur was grown to a density of 5.62 OD600
- Day culture was prepared by inculating 2x 250mL LB + v2 media with overnight culture with a final OD of 0.05 (1:112 dilution)
- Culture was grown at 37°C

Table1

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Hours | 0h (17:15) | 0.5h (17:47) | 1h (18:11) | 1.3h (18:32) |
| 2 | OD Flask1 /OD Flask2 | 0.05 / 0.05 | 0.132 / 0.129 | 0.266 / 0.267 | 0.583 / 0. 574 |

- Culture was divided into 10 x 50mL Falcon tubes (pre-chilled on ice) and kept on ice for 15 minutes
- Cells were centrifuged at 3000 x g for 20 minutes at 4°C
- Supernatant was discared and cells resuspended in chilled electroporation buffer
- Resuspended cells were pooled and split in two 50mL Falcon tubes, filled up with electroporation buffer
- Cells were centrifuged at 3000 x g for 15 minutes at 4°C, supernatant discared and tubes refilled with chilled electrophoration buffer
- Repeat washing step for a total of 3 washes
- After the last washing step discard supernatant and resuspend cells in the remaining electroporation buffer. Cells are now in approximately 1mL.
- Measure OD600 against electrophoration buffer after 1:100 dilution. OD600(1:100) = 0.416, i.e. OD600 = 41.6
- Fill up to 2.5mL with chilled electrophoration buffer to adjust OD600 to 16.
- Aliquots of 80µL of electrocompetent cells in 1.5mL Eppendorf tubes were prepared
- 9 aliquotes were kept on ice for the following transformation, the remaining (20) aliquots were immersed in liquid nitrogen and stored at -80°C in the freeezer on floor 3.

Transformation

- Add 1µL of the DNA (100ng/µL) provided by the iGEM Marburg team to the electrocompetent cells (80µL)

Table2

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | Tube | 1 | 2 | 3 | 4 | 5 | |
| 2 | DNA | Test Device 1 | Test Device 2 | Test Device 3 | Test Device 4 | Test Device 5 | Test Device 6 |

- Electroporation is performed with a BioRad MicroPulser electroporator in a 0.1cm gap electroporation cuvette

Table3

| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | Tube | 1 | 2 | 3 | 4 | 5 | |
| 2 | DNA | Test Device 1 | Test Device 2 | Test Device 3 | Test Device 4 | Test Device 5 | Test Device |
| 3 | Voltage [kV] | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | |
| 4 | Impulse Time [ms] | 1.2 | 1.3 | 6.4 | 1.3 | 1.3 | |

- After pulse, cells are resuspended in recovery media pre-heated at 45°C
- Cells were incubated for 1.5h at 37°C
- Cells were centrifuged at 3000 x g for 1 minute and most of the supernatant was discarded
- Cells were respuspended in the remains of supernatant and plated out

Cell measurement

- Pick two colonies from each plate and inoculate 5mL of LB + v2 + 2µg/mL Chloramphenicol media
- Incubate cultures at RT for 24h
- Incubate cultures at 37°C for 45 minutes
- Measure OD600 of each culture by diluting the culture 1:2 in a grainer black transparent flat bottom 96 well plate
- !! Colony 2 for the negativ control did not grow
- Dilute cultures to a final OD600 of 0.02
- Take 500µL sample of each culture
- Grow cultures for 3h at 37°C
- Take 500µL sample of each culture
- Plate out samples in 96-well plate and