Technische Universiteit
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Biomedical Engineering

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Gibson Assembly

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Table of contents

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1 NEBuilder HiFi DNA Assembly Reaction

Estimated bench time: 30 minutes
Estimated total time: 50 minutes
Purpose: The assembly of multiple inserts within the vector of choice.

When performing Gibson Assembly, you are working with DNA. It is essential to work with gloves at all times to protect your vector from DNase activity.

1.1 Materials

- Autoclaved dH_2O
- Bucket with ice
- dsDNA fragments which are to be inserted in the vector (e.g. gBlocks from IDT)
- Linearized vector in which the dsDNA fragments are to be inserted
- NEBuilder HiFi DNA Cloning Kit
- Thermal cycler

1.2 Setup & Protocol

- Look up the following parameters to complete the table. The molecular weight of the vector and gBlocks can be determined using a DNA molecular weight calculator online.

Component	Molecular weight [Da]	Concentration [ng/µl]
Linearized vector		
gBlock 1		10
Additional gBlocks		10

- Set up the following reaction on ice using gloves.

Component	Value in mixture	Volume [µl]
Linearized vector	50-100 ng	
gBlock 1 (10 ng/µl)	2 fold excess[1]	
Additional gBlocks (10 ng/µl)	2 fold excess[2]	
NEBuilder HiFi DNA Assembly Master Mix	10 µl	10
Autoclaved dH_2O	Till 20 µl	
Total volume		20

The volume of linearized vector for this reaction can be calculated with the following formula:

$$Volume\ [\mu l] = \frac{Value\ in\ mixture\ [ng]}{Concentration\ linearized\ vector\ [\frac{ng}{\mu l}]}$$

[1] A 2-fold excess is recommended for larger inserts. If the to be inserted dsDNA fragment is less than 200bp, a 5-fold excess is recommended for that particular insert.
[2] Note that efficiency goes down with increased numbers, and size of the gblcoks, plan accordingly

The volume of gBlock for this reaction can be calculated with the following formula:

$$Volume\ [\mu l] = 2 * \frac{\left(\frac{Value\ in\ mixture\ [ng]\ *\ Molecular\ weight\ gBlock\ [Da]}{Molecular\ weight\ linearized\ vector\ [Da]}\right)}{Concentration\ gBlock\ [\frac{ng}{\mu l}]}$$

- Incubate the samples at 50 °C for 15 minutes using the thermal cycler, or 60 minutes when 4-6 fragments are being assembled.
- After incubation, store the samples on ice for subsequent transformation.

2 Transformation into NEB 5-alpha competent E. Coli

Estimated bench time: 1 hour
Estimated total time: 2 hours
Purpose: Transformation of the assembled vector into competent cells. These cells can be used for subsequent plasmid amplification.

During transformation with NEB 5-alpha you are working with bacterial cells. Therefore, you need to work near the Bunsen burner flame, prohibiting the use of gloves during this step.

2.1 Materials

- Bucket with ice
- Bunsen Burner
- Heat/shaking-block
- Incubator
- LB-agar plates supplemented with the correct antibiotic
- NEB 5-alpha competent cells (these are supplied with the NEBuilder Hifi Assembly Cloning Kit)
- Pipettes and tips
- Plasmids to be transformed
- SOC solution (Super optimal broth with catabolite repression)
- Water bath

2.2 Setup & Protocol

- Thaw the NEB 5-alpha competent E. coli on ice such that all ice crystals disappear.
- Add 2 µl of the chilled samples to the competent cells. Mix gently by stirring with your pipette. Do not vortex nor pipette up and down.
- Place mixture on ice for 30 minutes. Do not mix.
- Heat shock the cells for exactly 30 seconds at 42 °C.
- Transfer the tubes directly to ice for 2 minutes.
- Add 950 µl of room-temperature SOC media to the tube.
- Incubate for 60 minutes at 37 °C and 300 rpm.

- Spread 100 µl of the cells onto the agar plates while working near the Bunsen burner flame. Incubate overnight at 37 °C.

3 References & Acknowledgements

This protocol was adapted from the NEBuilder HiFi Assembly Cloning Kit's manual.