NEBuilder Hifi Mutagenesis

Introduction

LucidChart Overviews | DNA | Combined

Procedures List

Transformation Procedure

read this!!

https://www.neb.com/applications/cloning-and-synthetic-biology/dna-assembly-and-cloning/-/media/nebus/files/application-notes/improved-methods-for-site-directed-mutagenesis-using-nebuilder-hifi-dna-assembly-master-mix.pdf

Materials

- NEBuilder HiFi DNA Assembly Master Mix
- Q5® Hot Start High-Fidelity 2X Master Mix
- Overlapping Primers
- Template DNA (DNA to be mutagenized)
- DpnI
- NEB 5-alpha Competent E. coli
- LB-Amp plates

Procedure

PCR & Fragment Preparation

1. Use the following reaction conditions to amplify fragments with designed mutations using the designed primers. Assemble the reaction components and perform the PCR procedure. Determine the annealing temperature using the NEBTm calculator.

PCR Reaction Components

	A	B
1	**Foward Primer (10 µM)**	2.5 µL
2	**Reverse Primer (10 µM)**	2.5 µL
3	**Template DNA (5 ng/µL)**	1.0 µL
4	**MilliQ H2O**	19.0 µL
5	**Q5 Hot Start High-Fidelity 2X Master Mix**	25.0 µL
6	**Total Volume**	50 µL

PCR Cycles

	A	B	C
1	**Initial Denaturation**	98 °C	1 minute
2	**30 Cycles**	98 °C	10 seconds
3		X °C	15 seconds
4		72 °C	20-30 seconds/kb
5	**Final Extension**	72 °C	5 minutes
6	**Hold**	4 °C	∞

2. Following PCR, add 1 µl of DpnI was to each tube and incubated at 37°C for an additional 30 minutes.

3. After DpnI treatment, clean products using a PCR purification kit (we have GenScript).

Fragment Assembly

4. Determine the concentration of the fragments by Nanodrop™ instrument.

5. Thaw 2X NEBuilder HiFi DNA Assembly Master Mix at room temperature.

6. Set up the NEBuilder HiFi DNA Assembly reaction as follows:

   Here X is the total volume of the fragments & vector

Fragment Assembly Reaction

	A	B	C	D	E	F	G
1	**Component**	**Amount**					
2	Vector	0.05 pmols (note: your design likely does not have this)					
3	PCR products (for each fragment)	0.05 pmols					
4	2X NEBuilder HiFi DNA Assembly Master Mix	10 µl					
5	H2O	10-X µl					
6	Total Volume	20 µl					

7. Incubate the reaction at 50°C in a thermocycler for 1 hour.

8. Transform 2 µl of the reaction into 50 µl of NEB 5-alpha Competent E. coli (High Efficiency), plated on antibiotic selection plates, and incubate overnight at 37°C.

Analysis

9. After overnight, inoculate multiple colonies independently. Following the inoculation procedure.

10. Create a glycerol stock for each of the colonies to be stored at -80 °C.

11. Extract the plasmids using a plasmid purification kit.

12. Run a diagnostic gel to determine if mutagenesis was successful in any of the colonies.