Hifi DNA assembly and Transformation Protocol

Introduction

Adapted by Jacob Mejlsted & Joen Haahr Jensen from IDT's HiFi assembly protocol

Materials

- (X is the number of reactions)
- Consumables
  - X PCR tubes for each reaction + 1 for positive control
  - X eppendorf tupe for each reaction + 1 for postive control
  - X selection plate for each reaction
  - 1 Amp plate for positive control
- Chemical
  - Hifi DNA assembly Master mix
  - Steril MilliQ water
  - Competent E. coli
  - Prepared DNA fragments for assembly (See information on primer construction)

Procedure

Assembly protocol

1. Set up the following reaction on ice:

Reagants

	A	B	C	D
1		Recommended amount of fragments used for assembly		
2		2-3 Fragments*	4-6 Fragements	Postive control
3	Recommended DNA Molar Ratio	Vector:insert = 1:2	Vector:insert = 1:1	
4	Total amount of DNA fragments	0.03-0.3pmols X uL	0.2-0.5 pmols X uL	10 uL
5	NEB Hifi Assembly master mix	10 uL	10 uL	10 uL
6	MilliQ water	10-X uL	10-x uL	0 uL
7	Total volume	20 uL	20 uL	20 uL

* If the inserts are less less than 200 bp, use a 5 fold excess of inserts instead of a 2 fold excess
** If a greater number of fragments are assembled, increase the volumen of the reaction and use additional HiFi DNA assembly master mix

2. Incubate the reaction samples in a thermocycler at 50°C for 15 minutes (when 2-3 fragments are assembled) or 60 minutes (when 4-6 fragments are assembled). Following incubation, store the reaction samples at -20°C for subsequent transformation

   Note: Extended incubation up to 60 minutes can in some cases improve transformation efficiency

Transformation protocol

3. Thaw chemically-competent cells on ice
4. Add 2 uL of the chilled assembly product to the competent cells. Mix gently by pipetting up or down or by flicking the tube 4-5 times. Do NOT vortex
5. Place the mixture on ice for 30 minutes. Do not mix
6. Heat shock at 42°C for 30 seconds. Do not mix
7. Transfer tubes to ice for 2 minutes
8. Add 950 uL of room temperature SOC media to the tubes
9. Incubate the tube for 37°C for 60 minutes. shake vigerously (250 rpm) or rotate
10. Warm selection plates to 37°C
11. Spread 100uL of the cells onto the selection plates.

    Note: Use Amp plates for the positive control

12. Incubate overnight at 37°C.