Plasmid Expansion

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ABSTRACT

This is the protocol for plasmid expansion.

ATTACHMENTS

Plasmid_Expansion_ALI_Ning.pdf

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ATTACHMENTS

Plasmid_Expansion_ALI_Ning.pdf

1 Thaw the competent cells on ice

2 Chill approximately 5 µg (2 µl) of Plasmid DNA in 1.5 mL microcentrifuge tube

3 Add 50 µl of competent cells to the DNA. Mix gently by pipetting up and down or flicking the tube 4–5 times to mix the cells and DNA. Do not vortex.

4 Place the mixture on ice for 30 minutes. Do not mix.

5 Heat shock at 42°C for 30 seconds*. Do not mix.

6 Add 950 µl of room temperature media* to the tube.

7 Place tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.

8 Warm selection plates to 37°C.

9 Spread 50–100 µl of the cells and ligation mixture onto the plates.

10 Incubate overnight at 37°C.

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10.1 Please note: For the duration and temperature of the heat shock step as well as for the media to be used during the recovery period,
please follow the recommendations provided by the competent cells' manufacturer.

Plasmids and antibiotics

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A	B
PLVX mCherry (HLA-A2)	Amp 50µg/mL
Ploc MLANA (MART1)	Blasticidin (50µg/mL)
psPAX2	Amp 50µg/mL
VSV.G	Amp 50µg/mL

Preparation of Liquid Media - LB-Ampicillin Broth and LB-Blasticidin

12 Add 20g of LB broth Lennox into 1 liter of distilled water in an Erlenmeyer flask

13 Autoclave the LB broth in the Flask on a wet cycle for 30min

14 Storage of LB in 4ºC is recommended and is good for up to 1 year

15 Ensure that the LB broth has come to room temperature before you add ampicillin

16 The final concentration of ampicillin in the broth should be 50µg/mL

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17 LB broth containing ampicillin should be stored at 4ºC for up to 1 month

Preparation of Solid Media

18 LB-Amp Agar and LB-Blasticidin Agar: Each Petri Dish takes about 10mL of LB-Agar so scale the volume accordingly.

1 LB agar tablet makes 50mL

19 Take 1 tablet and dissolve it in 50mL of distilled water in an autoclaved flask

20 Autoclave the LB agar for 30min on a wet cycle

21 Once the cycle is complete check to make sure that all the agar is dissolved

22 Allow the LB agar to cool until it is comfortable to the touch (50ºC), a water batch set to 50ºC is useful for this step

23 While the agar is cooling label the plates using sharpie for each antibiotic

23.1 a. Ampicillin = Red
b. Blasticidin = Blue

24 The final concentration of ampicillin and Blasticidin should be 50µg/mL

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25 Make sure that the LB agar has cooled to 50ºC as excessive heat will degrade the antibiotics

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