Cloning protocol

Using Life Technologies™ Topo® TA cloning® kit

Objectives

- Prepare PCR product for cloning
- Insert PCR product into cloning vector
- Practice microbiology techniques

Materials

from TOPO TA kit

- Salt solution
- TOPO vector
- OneShot chemically competent *E. coli* cells
- S.O.C. solution

Also

- PCR product

Equipment/Labware

- Water bath/heat block (at 42° C)
- Shaking incubator
- Ice bucket
- Selective plates: petri dishes with LB + ampicillin (50 µg/ml)
- Pipettes
- Spreader
- Bunsen burner
- Alcohol (for sterilizing)
- Microcentrifuge tube

Cleaning/Purifying

Why clean up? After a PCR reaction is finished, there are a lot of unused components like left over nucleotides, unused primers, salts and non-specific PCR products that may interfere with the cloning reaction.

There are many different ways to clean up PCR or other molecular reactions. Some of these work very much like the common membrane based protocols used to extract DNA.

Preparation

- Clean PCR product
- Prepare selective plates or let them come up to room temperature if made previously and frozen
- Set water bath or heat block to 42° C

Ligation

1. Prepare Ligation reaction.
   Mix the following reagents and volumes in a microcentrifuge tube:

Cleaned PCR product	4.0 ul
Salt solution	1.0 ul
TOPO vector	1.0 ul
FINAL VOL	6.0 ul

   Mix gently and incubate for 20 min at room temperature.

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Transformation

2. During the incubation:
   a. Thaw on ice 1 vial OneShot chemically competent E. coli cells for each transformation
   b. Warm vial of S.O.C. medium to room temp

3. After the 20 minute incubation, place the ligation reaction on ice.
   a. Add 2 µl cloning reaction into vial of chemically competent E coli and mix gently (don't mix by pipetting up and down).
   b. Incubate on ice 20 minutes.

4. Heat-shock cells by putting the microfuge tube from Step 3 in the 42°C waterbath or heat block for exactly 2 minutes.
   a. Immediately transfer to ice.

5. Add 250 ul of room temperature S.O.C. medium.
   a. Cap tube and shake horizontally (200 rpm) at 37°C 1 hour on shaker.
   b. Warm selective plates to 37° C in incubator for at least 30 minutes.

6. For each transformation spread 50 µl onto two separate pre-warmed selective plates and incubate overnight at 37° C.

Next day:

7. Pick colonies with sterile loops or pipette tips (don't reuse!) and place in a well of a 96 well plate with 50 µl of water. You will use this "clone pick plate" for the colony PCR reaction and add primers for the M13 vector and run the PCR.

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