TRANSFORMATION, COLONY PICK UP, MINIPREP, DIGESTION

Transformation of Bacteria by heatshock method

Note, it is not correct to say “transformation of plasmid”
TA will do up to 2 for you

1. Prepare ice in ice bucket
2. Thaw competent cell (bacteria) on ice. Always keep on ice.
3. Transfer 90 µl bacteria in precooled 15 ml falcon tubes.
4. Add ~10 µl (whatever left in tube) of ligation product
5. Incubate on ice for 30 min.
6. Warm at 42 °C for 45 sec and return on ice.
7. Add 1 ml of LB.
8. Incubate at 37 °C for 1 hour with shaking.
9. While waiting, leave the kanamycin plates (one for each reaction) at 37 °c with the rid half open.
10. Apply 200 µl on to kanamycin plate.
11. Add 10-20 glass beads
12. Shake the plate horizontally and let the beads roll around to spread the bacteria.
13. Incubate at 37 °C for overnight. If the plate is wet, leave the rid open until it dries.

Pick-up colonies

1. Prepare terrific-broth (TB medium)+50 µg/ml kanamycin in 15 ml culture tubes (approx. 1.5 ml/tube).
2. Identify a well-separated colony.
3. Touch with pipette tip or sterile forceps and drop it in the tube.
4. Shake in 37 °C incubator for O/N.

Miniprep

1. Grow minipreps in TB medium+50 µg/ml kanamycin, from single colonies in 15 ml culture tubes (approx. 1.5 ml/tube). Each 4 per group.

2. Simply pour bacteria from the Falcon tubes into the epp tubes. Pour 1-1.5 ml. Don’t spill b/c it will cross contaminate. Keep original culture tubes.

3. Spin epp. tubes at max. speed for 15 sec.
   -Place the epp tubes in the centrifuge with the cap opening facing down.
   -Speed maximum is at 13.0

4. Open the tubes carefully, watching for cross contamination. Dump supernatant by pouring it into the waste beaker from a sort of high height (you don’t want any contamination). Make sure not to mix the contents of different tubes.

5. add 200 µl of solution 1 to bacterial pellet.
   -Solution 1 contains the following:

4.5 grs glucose
12.5 ml of Tris pH 8
10 ml EDTA pH 8
RNase
fill with distilled water up to 500 ml.

-EDTA eliminates Ca and Mg so DNAse won't be active and therefore will not degrade DNA. Thus, the DNA is stable.

-Tris is a buffer.

6. Close eppendorf tubes and vortex them (until pellet is dissolved).

7. Add 200 µl of solution 2 and close tubes. Invert each tube gently to mix the contents well.

-Solution 2 contains the following:

10 ml of NaOH 10N
50 ml of 10% SDS
fill up to 500 ml with water

-SDS is a detergent that lyses cells

-NaOH is a strong base that works with the strong acid in Solution 3 to cause genomic DNA to precipitate leaving the plasmid DNA in solution.

8. Open tubes and add 200 µl of solution 3. Mix vigorously by shaking with your hand.

-Solution 3 contains the following:

89 grs of potassium acetate
58 ml of acetic acid
fill up to 500 ml with distilled water

9. Spin at max. speed for 3-5 minutes at RT.

-The plasmid DNA remains in the supernatant. The genomic DNA is the pellet.

10. During the spin, prepare new epp. tubes, and add 1 ml of isopropanol to each tube.

-DNA precipitates with isopropanol. The minimum volume necessary for this is 0.7x your solution.

11. Take 500 µl of the supernatant from the spin tubes and add to the tubes containing 1 ml of isopropanol.

-Try to get just the liquid, and leave behind the gunk from the tube. Mix vigorously by hand.

-Pipet gingerly to avoid the pellet. Tilt the tube nearly horizontally and run the pipet down the wall of the tube.

-The DNA is very stable in alcohol.

12. Spin max. speed RT for 5-10 minutes

13. Dump supernatant. (See notes for dumping the supernatant for step 4).

14. Add ~500 µl of 70% ethanol. Spin for 1 minute.

15. Dump ethanol. Keep tubes inverted over paper towel to drain the remnants of ethanol.

-Make sure to set the tubes far enough apart to avoid cross contamination.

16. After DNA is dry, resuspend in 50 µl 10 mM Tris HCl (pH 8.0).

Digestion of Miniprep DNA

1. Prepare digestion mix:

Reagent	Quantity 5X (µL)	Quantity 1X (µL)
Buffer 2	5	1
EcoRI	2.5	0.5
XhoI	2.5	0.5
(bovine serum albumin) BSA	5	1
dH_2O	20	4

Measure out each reagent to the 5X amount. Then use this solution to aliquot one-fifth of the solution (1X) into each tube for your digestion.

NOTE: The enzymes are not stable at room temperature: keep them out for as short as it is possible, or put them in a cooler, or on ice.

-Centrifuge the mix about 10 seconds in its epp tube labeled “digestion mix”

2. Prepare tubes for digestion: aliquot 7 µl of the digestion mix/tube

3. Vortex and add 3 µl of the DNA into each 7 µl digestion tube. Mix well by pipetting.

4. Digest the 4 DNA’s that you purified.

5.Incubate at 37C for at least 40 minutes (some enzymes require longer).

6.Run entire sample for each tube on 0.8% agarose gel along with PCR product and vector DNA you prepared last week and along with the DNA ladder.

-Do not forget to add BPB to samples if you have not done so previously.