DNA Amplification Mini Prep

Make LB plate

1. LB broth 12.5 g + Aga power 7.5 g + Water 500 ml
2. Autoclave for 1 hr in a bottle.
3. Wait until the LB media cools down to ~60oC. Add ampicilin (freezer) 500 ul and mix.
4. Pour LB media into the plate 15 ml/plate (~0.5 cm of thickness).
5. Leave the plates to cool down for ~30 min, they should be ready for transformation.

Make LB media

1. 500 ml water + 12.5 g LB broth
2. Autoclave, with no ampicilin

Make EB buffer

1. Dilute EB buffer with molecular water for 1x500 times, keep the stock in -20oC.

Day 1 Transformation (Late afternoon, duration: ~2 hours)

1. Get the ice bucket and keep DH5a chemical competent cells in ice.
2. Turn on the heat shock machine to low setting at 42oC, add water to the surface to make a water bath for even heat-up.
3. Add 1 ul DNA to DH5a, keep the vial in ice for 30 min; put 950 ul LB media to round bottom tube (14 ml)
4. Heat shock the vial of DH5a for 20 sec at 42oC. If the thermometer reads a higher temperature, add some water to cool it down first.
5. Cool down in ice for 2 min
6. Add 1 ul DNA mix to LB media, incubate in the warm room for 1 hour.

7. Dilute the DNA mix 20 times with LB media: 10 ul DNA mix + 190 ul LB media = 200 ul/dish, add 200 ul DNA mix to the plate
8. Soak spreader in ethonal, start gas fire, sterize the spreader using fire 2-3 times, let it cool before spreading DNA.
9. Spread the DNA mix with the spreader.
10. Put the plate upside down in the incubator for over night (16-18 hours).
11. Keep the round bottom tubes for a day in 4oC in case the baterial does not grow well.

Day 2 Amplification

Morning: Check the colonies. If good, seal with parafilm and put in frig to stop growing.

Afternoon:

1. Make LB media + Ampicilin (4ml LB media + 4 ul of ampicilin) and mix, add 4ml mix to each round bottom tube.
2. Use 10 ul pipette tip to pick colonies and leave the tips in round bottom tubes.
3. Incubate overnight in the warm room for 17-18 hours.

Day 3 Miniprep and Digestion

1. Transfer grown mixture to 2 ml tubes

2. Centrifuge at 8K RPM for 5 min

If you have 5ml grown mixture in the round bottom tubes, transfer 2 ml mixture at a time and centrifuge for 1 min, repeat this step 3 times to get DNA in one tube.

1. Use Quagent kit. Add 250 ul of P1 buffer (in frig) to the tube, re-suspend by scratch on the rack (3-4 times).

2. Add 250 ul of P2 buffer to the tubes, rock gently with hands (6-8 times), until the fluid turns blue.

3. Add 350 ul of N3 buffer to tubes, rock gently (6-10 times). Blue turns white and something can be seen in suspension.

4. Centrifuge at 13.2K RPM for 15 min.

5. Label the inner tubes. Apply the supernatant to the column (inner tube).

6. Centrifuge at 13.2 KRPM for 1.5 min, discard the liquid.

7. Add 750 ul PE buffer/ tube.

8. Centrifuge at 13.2K RPM for 1 min

9. Discard liquid

10. Centrifuge again at 13.2K RPM for 2 min.

11. Put the column into a clean vial (with label), elute with 50 ul diluted EB buffer (apply gently).

12. Wait for 2 min

13. Centrifuge at 13.2K RPM for 1 min.

14. Discard column and save the vials with DNA.