Transformation Procedure

1. Take ice, put DH5a, wait to thaw
2. Aliquote 50 ul to prechilled 600 ul tubes
3. Add 1 ul DNA to tubes and wait for 30 min on ice.
4. Heat shock 42oC for 45 sec.
5. Incubate on ice for 2 min
6. Add in LB medium 500 ul to each tube
7. Shaker in 37oC for 20 min
8. 50 ul plate on agar plates

Detailed procedure (Later afternoon, duration ~ 2 hours)

1. Get the ice bucket and keep DH5a (-80C) chemical competent cells in cie for 5-10 min for them to thaw.
2. Turn on the heat shock machine to low setting at 42C, add water to the suface to make a water bath for even heat-up. Put the LB plate at 37C incubator to warm 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 45 sec at 42C. If the thermometer readsa higher temperature, add some water to cool it down first.
5. Cool down in ice for 2 min.
6. Add DH5a to LB media, incubate in shaker at 37C for 1 hour.
7. Add 50-100 ul DNA mix to the plate.
8. Soak spreader in ethanol, 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 overnight (16-18 hours).

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1. Pick 1 colony, put in 5 ml LB with 5 uL amp with fire (11 am)
2. 37C 8hrs shake
3. Check if tube become coudy (shake see if things are floating inside)
4. If yes, put 0.5 ml of the culture into 250 ml LB with 250 ul amp with fire. If not wait longer.
5. 37C 12-16 hours shake.

Amp. 1:1000 dilution
Kanamycin 1:500 dilution when preparing LB.