Updated 2015.07.28

iGEM Registry: Transformation Protocol

Estimated bench time: 1 hour
Estimated total time: 3 hours (plus 14-18 hour incubation)

Transformations are essential to using the DNA Distribution Kits. However, they can also be one of the more fickle laboratory techniques.

At iGEM HQ, we run test transformations of the DNA Distribution Kit with the following protocol. We have found that it is the best protocol to use with the DNA Distribution Kit and ensures high efficiency transformations.

- At iGEM HQ, we make our own stocks of NEB 10b competent cells. Competent cells purchased from vendors will have better efficiency.
- Make sure to test the competency of your cells with the provided Competent Cell Test Kit.
- **Read through the entire protocol before starting!**

Materials

Resuspended DNA	Resuspend DNA Distribution Kit well(s) with 10µl dH20. Pipet up and down several times, let sit for a few minutes. Resuspension will be red from cresol red dye.
10pg/ul Control DNA	**1µl for control transformation.** pSB1C3 w/ BBa_J04450, RFP on high-copy chloramphenicol resistant plasmid. Located in the Competent Cell Test Kit.
Competent Cells	**50µl per transformation.** iGEM HQ stores competent cells in aliquots of 260µl (5rxns total) at -80°C.
2ml Microtubes	**One tube per transformation.** Label tubes with part name or well location before starting.
Floating Foam Tube Rack	Place 2ml tubes in floating tube rack for better support when working on ice and for the heat shock in the water bath.
Ice & ice bucket	Fill bucket with ice, and pre-chill 2ml tubes (5min). Thaw competent cell stock on ice (10-15min).
Lab Timer	
42°C water bath	Set water bath to 42°C before starting.
SOC Media	**200µl per transformation.** SOC Media is better than LB Media for higher transformation efficiency. SOC Media should not contain antibiotics, and can be easily contaminated.
37°C incubator	Preferably with a rotor/shaker for 2ml tubes. Incubate petri plates overnight (non-agitated).
Petri plates w/ LB agar and antibiotic	**2 plates per transformation:** for 20µl and 200µl platings. Make sure to use appropriate antibiotic. Label with part name or well location before starting.
Sterile spreader or glass beads	Used to spread transformation across petri plates. Be sure to use sterile technique in between platings.
Pipettes and Tips	10µl, 20µl, 200µl tips and pipettes recommended

Setup:

When transforming competent cells, both timing and temperature are very important. Use a lab timer, follow the incubation temperatures closely, and keep materials on ice when required.

Resuspend DNA in selected wells in the Distribution Kit. Label 2ml tubes with part name or well location. Fill lab ice bucket with ice, and pre-chill 2ml tubes (one tube for each transformation, including your control) in a floating foam tube rack.

1. **Thaw competent cells on ice**
   This may take 10-15min for a 260µl stock. Dispose of unused competent cells. Do not refreeze unused thawed cells, as it will drastically reduce transformation efficiency.
2. **Pipette 50µl of competent cells into 2ml tube**
   50µl in a 2ml tube per transformation. Tubes should be labeled, pre-chilled, and in a floating tube rack for support. Keep all tubes on ice. **Don't forget a 2ml tube for your control.**
3. **Pipette 1µl of resuspended DNA into 2ml tube**
   Pipette from well into appropriately labeled tube. Gently pipette up and down a few times. Keep all tubes on ice.
4. **Pipette 1µl of control DNA into 2ml tube**
   Pipette 1µl of 10pg/µl control into your control transformation. Gently pipette up and down a few times. Keep all tubes on ice.
5. **Close 2ml tubes, incubate on ice for 30min**
   Tubes may be gently agitated/flicked to mix solution, but return to ice immediately.
6. **Heat shock tubes at 42°C for 1 min**
   2ml tubes should be in a floating foam tube rack. Place in water bath to ensure the bottoms of the tubes are submerged. Timing is critical.
7. **Incubate on ice for 5min**
   Return transformation tubes to ice bucket.
8. **Pipette 200µl SOC media to each transformation**
   SOC should be stored at 4°C, but can be warmed to room temperature before use. Check for contamination.
9. **Incubate at 37°C for 2 hours, shaker or rotor recommended**
10. **Pipette each transformation on two petri plates for a 20µl and 200µl plating**
    Pipette 20µl and 200µl of the transformation onto appropriately labeled plates. Spread with sterilized spreader or glass beads immediately. This helps ensure that you will be able to pick out a single colony.
11. **Incubate transformations overnight (14-18hr) at 37°C**
    Incubate the plates upside down (agar side facing up). If incubated for too long, colonies may overgrow and the antibiotics may start to break down; un-transformed cells will begin to grow.
12. **Pick single colonies**
    Pick single colonies from transformations: do a colony PCR to verify part size, make glycerol stocks, grow up cell cultures and miniprep.
13. **Count colonies for control transformation**
    Count colonies on the 20µl control plate and calculate your competent cell efficiency. Competent cells should have an efficiency of 1.5×10^8 to 6×10^8 cfu/µg DNA.