Protocols for Transformation of bacteria via TSS Method

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Adapted for cultures in Falcon (15 mL) Tubes

1. **Make a 1mL bacteria pre-culture the day before, until saturation, in a 15mL Falcon Tube**
2. **Next morning, dilute this culture 1/100 in a new 15mL Falcon Tube**
3. **Incubate at 37°C in a shaker with the lids of the tubes loose for oxygen penetration into the culture**
4. **Wait around 2-3 hours until OD600 reaches ≈ 0.4-0.5**
5. **Prepare 0.5mL eppendorfs (in case heat shock is done with PCR machine) or 1.5mL eppendorfs (if heat shock is done using a 42°C water bath)**
   - *Put the eppendorfs on ICE*
   - *For each transformation:*
     - Pipette 50µL of cell culture per eppendorf
     - Pipette 50µL of TSS 2x per eppendorf; Mix WELL but GENTLY
6. **Incubate on ice for 45 minutes**
7. **Add DNA into each eppendorf according to each transformation (in case of a biobrick, add 1-2µL of the red DNA solution)**
8. **Incubate on ice for 10 minutes**
9. **Heat shock at 42°C by using the PCR machine or water bath for 90 seconds**
10. **Incubate on ice for 10 minutes**
11. **Add 900µL of LB (0.2% glucose is optional) for each eppendorf (if using 0.5mL tubes then transfer solution to new COLD 1.5mL tubes)**
12. **Incubate at 37°C for 1-2 hours**
13. **Plate on LB-Agar supplemented with the appropriate antibiotic. Wait until the next day to harvest colonies.**