E. coli genome modification protocol

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EXPERIMENT PROTOCOL:

1. Inoculate 4ml of LB with E. coli culture grown overnight and immediately induce with arabinose (10mM final concentration). Grow at 30°C for 4 hours.
2. Centrifuge the cells, then resuspend the bacterial mass in 1 ml ice-cold sterile ddH_2O (for *E. coli*) and mix well until no clumps are visible, keep on ice.
3. Centrifuge each bacterial suspension for 5 min at 5,000 x g in a refrigerated microcentrifuge set to 4 °C, or in a microcentrifuge stored in a cold room set to 4 °C.
4. Discard the supernatant, resuspend the bacterial pellet in the same volume of ice-cold sterile ddH_2O, and repeat the centrifugation step as done before twice more for a total of three washes.
5. Remove supernatant, resuspend, and then loosen the bacterial pellet thoroughly in 40 µl ice cold 2 mM $CaCl_2$ or sterile ddH_2O and keep on ice.
6. Mix 50 µl of cells with 100 ng of pTargetT plasmid and and 400 ng of donor DNA
7. Transfer the electroporation mixture to a 2-mm cuvette and electroporate for 5ms at 2.5 kV, resuspend the mixture immediately afterwards in 1 ml of ice-cold LB medium.
8. Recover cells at 30°C for 1 h before spreading onto LB agar containing chloramphenicol (50 mg/liter) and the respective antibiotic for selection. Incubated overnight at 30°C.
9. Identify transformants by cPCR and sequencing.