

REMI

1. Count the cells. Cells must be in the logarithmic phase of growth. Density should be between 1 and 4×10^6 cells/ml at the time of harvest.
2. Incubate cells on ice for 15 minutes. Put cuvettes and glass test tubes on ice.
3. Centrifuge cells for 5 minutes in a clinical centrifuge at 2000 RPM, 4°C.
4. Discard supernatant. Aspirate as much medium as possible.
5. Disturb the pellet and re-suspend in ice cold electroporation buffer (10 mM sodium phosphate buffer pH 6.1, 50 mM sucrose) to a calculated density of 10^7 cells/ml.
6. Transfer 0.8 ml cells into a cold glass tube, add linear DNA (20-40 μg) and restriction enzyme (100-200 units). Mix.
7. Transfer the mixture to a 0.4 cm gap cuvette. Electroporate at 3 μF , 1 Kv (2.5Kv/cm). Expect a time constant of 0.5-1.1 msec. Two pulses may increase the efficiency.
8. Immediately add cells to growth medium and divide into 4 petri dishes.
9. Grow cells for 24 h, add 5 $\mu\text{g/ml}$ blasticidin S. Colonies should appear after 5-6 days.
10. Controls: a. no DNA, b. no drug selection.