

Cell transformation protocol (Alex Van Slyke)

Transformation-

Thaw cells from -80C freezer on ice
Warm up agar plates
Aliquot 50uL of bacteria for each sample in microcentrifuge tubes
Add ~0.5-1uL of DNA (1-200ng) to 50uL of bacteria UNLESS LIGATION PRODUCT
Add half or more of the ligation reaction to 50uL of cells if ligation product
Leave cells on ice for 30 min
Heat shock in 42C water bath for 45sec
Put tubes back on ice
Add 500 uL SOC media
Put tubes in shaking incubator at 37C for 45 min
Plate 5-30uL on agar plates UNLESS LIGATION PRODUCT
Plate 100-250uL on agar plates if ligation product
Put plates upside down in incubator at 37C for ~18 hours

<http://www.addgene.org/plasmid-protocols/bacterial-transformation/>

Mix'n'Go (using ampicillin resistant plasmid):

Thaw cells from -80C freezer on ice
Warm up agar plates
Aliquot 50uL of bacteria for each sample in microcentrifuge tubes
Add ~0.5-1uL of DNA to 50uL of bacteria UNLESS LIGATION PRODUCT
Add half or more of the ligation reaction to 50uL of cells if ligation product
Leave cells on ice for 5 minutes.
Add 500uL SOC media
Plate 5-30uL on agar plates UNLESS LIGATION PRODUCT
Plate 100-250uL on agar plates if ligation product
Put plates upside down in incubator at 37C for ~18 hours

https://files.zymoresearch.com/protocols/_t3001_t3002_mix_go_e_coli_transformation_kit_buffer_set.pdf

Day 2:

Pick colonies with pipette tip
Drop whole pipette tip with bacteria into LB media with antibiotic
Can use 35mL of media to run 3 parallel minipreps
OR
1 disposal tube with 8mL to run a single miniprep
Place shaking in incubator at 37C for 17 ± 1 hours
Antibiotic concentrations:

<https://www.addgene.org/mol-bio-reference/#antibiotics>