Protocol

Protocol for mini-prep with OMEGA Mini-prep Kit

Pick a single colony from a selective plate and inoculate a culture of 4ml LB medium containing the appropriate antibiotic. Incubate for 10-12 h at 37℃ with vigorous shaking

Pellet 1.5 ml bacteria by centrifugation at 12000rpm for 1min at room temperature

Resuspend the bacteria pellet by adding 250ul of solution I/RNase A and vortexing

Add 250 ul of solution II and gently mix by inverting and rotating the tube several times to obtain a clear lysate

Add 350 ul solution III and mix immediately by inverting the tube several time until a flocculent white precipitate

Centrifuge at 12000rpm for 5-10 min at room temperature

Add the cleared supernatant by CAREFULLY aspirating it into a clean HiBind Miniprep Column I assembled in a provided 2 ml collection tube. Centrifuge at 12000rpm for 1 min at room temperature

Discard flow-through liquid and re-use the 2ml collection tube. Add 500ul of Buffer HB to wash the HiBind Miniprep Column I. Centrifuge at 12000rpm for 1 min at room temperature

Discard flow-through liquid and re-use the 2ml collection tube. Add 700ul of DNA Wash Buffer diluted with absolute ethanol to wash the HiBind Miniprep Column I. Centrifuge at 12000rpm for 1 min at room temperature

Repeat wash step with another 700 ul of DNA wash buffer.

Centrifuge the empty column for 2min at 1200rpm.

Place the column into a clean 1.5 ml microcentrifuge tube. Add 30 ul to 50 ul of Elution Buffer. Centrifuge at 12000rpm for 2min at room temperature

Reference: E.Z.N.A. Plasmid Mini Kit I

Protocol for double digestion (20ul)

Pipette the following into a 200ul tube

	Part A	Part B	Linearized plasmid backbone
DNA	10ul	10ul	10ul
dH2O	6ul	6ul	6ul
CutSmart Buffer	3ul	3ul	2.5ul
Enzyme 1	0.5ul EcoRI-HF	0.5ul XbaI	0.5ul EcoRI-HF
Enzyme 2	0.5ul SpeI	0.5ul PstI	0.5ul PstI
Enzyme 3			0.5ul DpnI

Digest 37C/30 min, heat kill 80C/20 min

Notes:

CutSmart Buffer: New Buffer(For more information: www.NebcutSmart.com)

According to personal experience, linearized plasmid backbone 5ul enough.

Protocol for ligation(15ul)

Pipette the following into a 200ul tube

Add 2ul of digested plasmid backbone

DNA part A:3.5ul

DNA part B:3.5ul
dH2O: up to 15ul
10x ligation buffer: 1uL
T4 DNA Ligase: 0.5 ul
Ligate 16C/30 min, heat kill 80C/20 min
Notes:
Use the mixture for transformation

Protocol for transformation

1. Get the competent cell from -70 ℃ and wait for its fusion
2. Add 10 ul ligation mixture into tube(including 50ul competent cell)
3. Pipette up and down several times
4. Ice : 30 min
5. 42C Water : 1min
6. Ice : 5min
7. LB media :250ul
8. 37C incubator : 2h
9. Plate the culture (50ul-100ul)on LB plate containing corresponding antibiotics

Notes:
Competent cell: TOP 10 from TIANGEN BIOTEACH

Protocol for PCR (20 ul)
Pipette the following into a 200ul tube
DNA: 1 ul
Forward primer: 1ul
Reverse primer: 1ul

Super mix: 12ul
dH2O: 5 ul
Reaction condition:

1	94C	10min
2	94C	1min
3	65C	40s
4	72C	1min 30s
5	72C	10min

Cycle: 2to 4 for 30-35
Notes:
Reaction condition only for MerR PCR
Forward MerR sequence (5'to3'):
GTTTCTTCGAATTCGCGGCCGCTTCTAGAGGGAGTCAAACGATATGGAAAA
Reverse MerR sequence (5'to3'):
CTACACCGCGTCCGCACTTACTAGTAGCGGCCGCTGCAGGAAGAAAC