

Fosmid/Cosmid subcloning
(Jessica Mark-Welch, Ashita Dhillon)

Start with 10 ug highly purified supercoiled DNA in 150 ul

Incubate DNA 37°C for 30 min, vortexing every 10 min
Centrifuge RT max speed 20 minutes

Shear using HydroShear 25 cycles, speed 7 [or nebulize]

Run on 1% NuSieve GTG gel, 1X TAE, with ethidium bromide, in cold room

Cut out band using transilluminator (2-3 kbp)

Add equal volume of TE

Spin 30 min RT, top speed in microfuge
Collect supernatant

Ppt. with 0.1 vol 3 M NaOAc, 2 vol EtOH
Centrifuge 4°C 20-30 min
Wash pellet with 70% ethanol
Dry 2-3 min in SpeedVac

Resuspend in 100 ul 10 mM Tris pH 8
Check concentration using spectrophotometer or gel

T4 Pol repair

1X T4 buffer, NEB
50 ug/ml BSA
100 uM each dNTP
1 U T4 DNA Pol
1 ug DNA
water to 50 ul total
Incubate 12°C for 20 min

Add 5.5 ul 100 mM EDTA (to 10 mM)

heat inactivate 75°C 10 min
EtOH ppt as before

Phosphatase reaction

Resuspend in 50 ul SA buffer, 1X
Add 2 ul SAP (Shrimp alkaline phosphatase) at 1 U /ul
Incubate 37°C 1 hr
heat inactivate 65°C 15 min minimum

EtOH pt as before

A-tailing reaction:

Resuspend in 19.5 ul water plus 2.5 ul Qiagen 10X PCR buffer
transfer to PCR tube
Add 2.5 ul dNTPs at 2.5 mM each
0.5 ul Qiagen Taq at 5 U/ul
Total 25 ul
Incubate at 72°C for 30 min

Phenol-chloroform extract, EtOH ppt, resuspend in 4 ul water

Proceed to TOPO-TA cloning

25-30 min ligation step
Use 2 ul to transform
(may need to transform all 6 ul for adequate coverage)