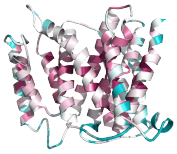
PROVOST & WALLERT RESEARCH

Investigating the Biochemistry & Cellular Physiology of NHE1

EST. 1998

Plasmid DNA Purification

Workflow



Transformation

Cell Strain
DH5α for DNA preparation

Competent cells ON ICE (20-100 µl)

Mix Plasmid DNA (1-5 µl : 1-100 ng)

250 µl SOC/SOB
Shaking 32°C 45 min

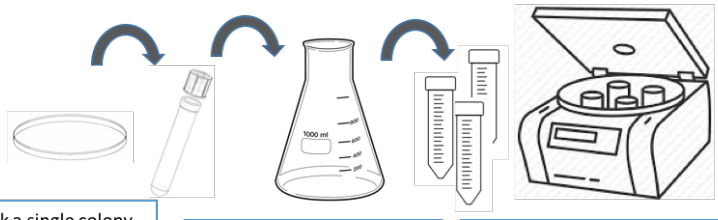
On Ice 20-30 min
Heat Shock 42°C 45 sec

ALTERNATIVE
Isolate streak from glycerol stock

50 µl | *200 µl*

Incubate overnight 32°C
Keep plate with isolated colonies

Inoculation & Expansion

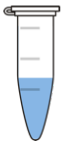


Pick a single colony
Place in loose capped tube over night with shaking

Expand to appropriate volume if needed – never fill flask more than 1.3 full

1000-5000 x g for 15-30 min label and freeze if

Purification



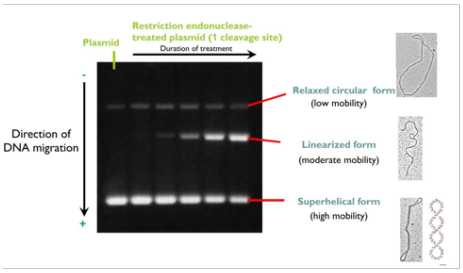
Alkaline Lysis PPT
- Quick and dirty prep for simple use

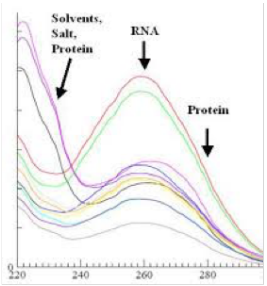
Mini Prep
Spin column format for pure small amounts of DNA

Midi/Maxi Prep
Large vacuum or spin format for pure Plasmid DNA

Follow manufactures instructions. Culture volumes and lysis are the most problematic steps. If plasmid is to be used for mammalian cell transformation – use a endotoxin removal buffer and use sterile tubes with aseptic technique

Analysis & Quantitation





Agarose Gel
ALWAYS check to see the mass is as expected – remember that supercoiled plasmid will run smaller than relaxed or cut DNA. RNA runs at the very bottom of the gel with a thick fuzzy band. Genomic DNA will be at the origin

UV Spectrometric Analysis
Nano Drop, UV Abs, or Take3 Plate:
Determine the OD @ 260 and 280 nm.
dsDNA µg/ml = 50µg/ml x OD 260
260/280 ratio should be between 1.8 & 2.0