$CsCl_2$ Maxi DNA Prep

Experimental Design Considerations

- Yield depends on bacterial strain (DH5 typically gives high yields while BL21 gives poor yields)
- Yield also depends on backbone of plasmid with old pBR322 plasmids and GEX plasmids giving low yields
- Better yields are obtained if cells are allowed to grow at saturation for several hours. Therefore, cells should be cultured for at least the minimum incubation times listed

Protocol

-Inoculate 5ml media (+100ug/ml Amp) in Morning
-Inoculate 250ml 2XYT (100ug/ml Amp) with 5ml culture in afternoon.
-Incubate O/N (at least 16hrs).
-Spin 5 minutes at 5K in 500ml centrifuge bottles
-Suspend pellet in 6ml Soln. I and transfer to 50ml conical tube (polypropylene).
-Add 12 ml Soln. II (0.2N NaOH, 1% SDS), shake vigorously.
-Add 9 ml Soln. III, rock back and forth GENTLY until you see white fluffy precipitate.
-Spin 5 min. on high (ca. 3K) in clinical centrifuge.
-Transfer aqueous to new 50 ml tube (don't transfer white wafer on top).
-Add 10 ml Phenol/CHCl3, and shake vigerously
- Spin at high speed for 5 minutes
- Transfer top phase to new 50 ml tube.
-Add 100% EtOH up to 50ml.
-Let sit ca. 20-40 min.
-Spin 20 min. clinical centrifuge (high speed).
-Pour off EtOH, wipe out excess EtOH with kimwipe.
-Add 4ml TE, disrupt pellet so that it floats on surface - it will then dissolve on it's own.
-Add 4.7g CsCl2.
-Add 200ul 10mg/ml Ethidium Bromide.
-Transfer to vTI80 opti-seal centrifuge tubes.
-Spin 4hrs, 75k or O/N at 65k.
-Take out DNA with syringe (21-gauge needle), take off needle before transferring to 15ml polystyrene conical tube.
-Extract several times with 1ml of H_2O saturated n-butanol until clear [if there is difficulty extracting EtBr, it probably means that the EtBr is old].
-Add TE to bring up volume to 3ml.
-Add 300ul 3M NaOAc, +8ml EtOH, let sit at RT 20 min (if you have low yield, keep at –20C overnight)
-Spin in clinical centrifuge 20min. on high.
-Wash with cold 70% EtOH.
-Let dry [we usually wrap two kimwipes around the eraser end of a pencil to dry the inside of tube (2X)]
-Resuspend in TE (typically 500-1000ul)

Buffers

SOLUTION I	1L
50mM glucose	9g
25mMTris (8.0)	25ml 1M
10mM EDTA	20ml 0.5M

SOLUTION III	1 liter
5M KOAc	295g KOAc
Glacial Acetic Acid	115ml Acetic Acid

2xYT	1L
tryptone	16g
yeast extract	10g
NaCl	5g

TE
10mM Tris (pH 7.6)
1mM EDTA (pH 8.0)