CsCL gradient DNA ultracentrifugation

NOTE: Long ultracentrifugation step = balance the rotor.

NOTE: This is MaxiPrep DNA: super clean stuff (now called Qiagen grade). This DNA can be used for pretty much anything, including sequencing and the transformation of mammalian cells.

Innoc. 500ml 2xYT and grow overnight.
Centriguge 4000rpm to collect
Resuspend in 5ml Solution I and transfer to 50ml Falcon
Add 10mls Solution II and mix by inversion. Incub 10 mins on ice.
Add 7.5mls Solution III and mix by inversion. Incub 10 mins on ice.
Centrifuge 10 mins 4000rpm 4ºC.

Transfer S/N to fresh 50ml Falcon and extract with 10 mls PCI
Remove S/N and add 15mls IPA. Vortex and centrifuge 10 mins 4000rpm 4ºC.
Discard the S/N and wash the pellet with 10mls 70% EtOH.

Remove all the liquid and resuspend the pellet in 4ml TE pH 8.0.
Add 8.4g CsCl and make solution to 14g with TE pH8.0.
Add 300µl 10mg/ml EtBr. Vortex and centrifuge 10 mins 4000rpm RTº.
Remove the liquid to an ultra tube leaving the crappy protein precipitate.

Use the 16 x 75mm tubes. Overlay with a CsCl solution (ρ = 1.48g/L).
Balance and heat seal the tubes.
Spin in a Beckman 70Ti 55000rpm, >16h, **25ºC**. Do NOT spin at 4oC – the CsCl can ppt and this may unbalance the rotor - nastiness might then ensue.

Extract the LOWER band with an 18G needle. Extract with three changes of 5ml water saturated butan-1-ol. Keep going until the pink color no longer remains. After the final extraction add an equal volume of 1M NH_4Oac and six volumes of **RTº** EtOH (if you cool it the CsCl will precipitate ant the huge pellet you're looking at is not DNA). Mix and centrifuge 3000rpm RTº. Wash the pellet with 70% EtOH. Drain, air-dry and resuspend in TE pH8.0. Estimate the concentration and aliquot as required.