# How to isolate maxi-EBV DNA from 6x 400 ml *E. coli* bugs

One of the bigger problems is to obtain a descent amount of high quality plasmid DNA from E. coli clones that carry single copy plasmids such as maxi- or mini-EBV plasmids. I have found that one can get along with a medium scale preparation in combination with two rounds of ethidium bromide/CsCl gradients. This modification of the Maniatis standard protocol delivers between 10 to 100 µg of maxi-EBV DNA. This is how it works*:

**Grow bugs:**

Start six 400 ml overnight cultures in six two-liter-flasks at the appropriate temperature and with the recommended antibiotic concentration [for F-factor plasmids in DH10B use normal LB medium (Maniatis recipe), 30µg/ml chloramphenicol at 37°C in the air shaker]. It is advisable to have a small scale (5ml) overnight culture ready to inoculate the six large flasks. This 5 ml culture should be started from a single colony in order to rely on a single, genetically stable and well-defined clone. A persistent problem are DNA preparations that contain more than one plasmid or even defective ones!

**Harvest bugs:**

Spin each of the 400 ml culture in one 500 ml screw-capped centrifuge bottle at 5000 rpm at 20°C for 10 min. Decant the supernatant carefully to obtain an almost liquid-free pellet.

Resuspend each of the six pellets in 8 ml of solution I (Maniatis recipe, see below for exact composition), after complete resuspension, transfer the content of the six bottles into six 50 ml flip-cap tubes (Kendro/Sorvall or equivalent ones). Put on ice. Add 0.5 ml of a freshly prepared lyzozyme solution (10 mg/ml in water) into the resuspended bugs, swirl, and let the whole shebang sit on ice for five to ten minutes. Sometimes it gets viscous, sometimes not; any phenotype does not seem to be meaningful at all.

**Lysis:**

Add 16 ml of the freshly prepared solution II (Maniatis recipe: 0.2 M NAOH, 1% SDS, see below for exact preparation) to each flip-cap tube and mix immediately by vigorous shaking for 10 sec. Do this one tube after another. [DO NOT add solution II to all tubes and THEN start mixing. This will lead to poor yield and massive contamination with bacterial DNA.] Set on ice for (not more than) 10 min, DO NOT shake or vortex the tube anymore.

Add 12 ml of solution III (Maniatis recipe) to each flip-cap tube and invert the tubes gently ten to twenty times first. After the solutions are somewhat mixed, start shaking the tube liberally to mix the content thoroughly and bring the pH down again. Set on wet ice

for at least 30 min or over night; spin at maximum speed at 20,000 rpm, 4°C, for 30 min at least.

**Precipitation:**

After centrifugation, combine the six supernatants in one 500 ml screw-capped centrifuge bottle, measure the volume (by weighing for example), add 0.7 volumes [NOT MORE!] of 2-propanol (iso-propanol); let stand at room temperature for 10 min. Typically, one gets between 210 to 220 ml of supernatant. Add 150 ml of 2-propanol, not more than 0.7 of the initial volume.

Spin at 9000 to 10,000 rpm, at 20°C, for 30 min to collect the precipitate; decant supernatant. Add 250 ml of 80% ethanol/$H_2O$ (vol/vol), shake until the pellet is loose, spin again at maximum speed at 20°C for 30 min, decant the supernatant (CAREFUL, pellet is now substantially smaller and does not adhere well to the wall of the bottle), remove traces of liquid.

Add 5 ml of special TE buffer (50 mM Tris, pH 8.0 and 20 mM EDTA) to the 500 ml bottle, add 100 µl of proteinase K solution (10 mg/ml in $H_2O$, freshly made or kept frozen), let rock slowly (!) on a platform shaker or tumbler at room temperature to help dissolve the pellets which will take 10 to 30 min usually. Do not vortex. The pellet will become translucent, break up in pieces, and then will dissolve gradually. Watch out carefully for pieces of not yet dissolved, almost clear particles! Add 100 µl of RNase A solution (1mg/ml in $H_2O$, boiled for 10 min before use, kept frozen), let go at room temperature for another10 min. Collect the dissolved solution in a 50 ml Falcon tube.

Measure the final volume (around 5.5 ml), weigh out 6.0 g of solid CsCl (volume of solution equals weight of CsCl plus 10%), add CsCl slowly, warm slightly to help dissolve solid CsCl. Then add 0.5 ml of ethidium bromide stock solution (10mgl/ml) and swirl the tube: a flaky cloudy precipitate will form which normally does not harm in the subsequent ultracentrifuge run. I prefer to spin the Falcon tube at this point and leave the pellet behind.

**Ultracentrifuge runs:**

Fill solution into one 11 ml Kendro Ultra-crimp tube for the Beckman 70.1 TI rotor (or equivalent); use a diaper to avoid a big mess on the bench. Since the volume will no be sufficient to fill the 11 ml tube, add 1.55 g/ml CsCl solution to top off. A CsCl solution with a specific weight of 1.55 g/ml can be obtained by dissolving 48.38 g of CsCl in 51.62 ml of water (obviously this mixture will weigh 100 g but its volume will be about 65 ml, only!)

Seal the tube; don't forget the spacer; spin at 38,000 to 40,000 rpm at 20°C for at least 48 hours. Preferentially I let it go for three days.

Two clearly visible bands should be apparent under 300/354 nm UV light after the run. Cut off the tube with a pair of pliers or make a large hole at the top of the tube. The lower band is collected with a 5 ml syringe and a needle with a large diameter (at least yellow Nr. 1, also called 20G needle), we use needles that are common in veterinarian clinics caring for cattle and horses! The needles have an inner diameter of at least 1 mm!).

Put the content of the syringe into a new 11 ml Ultra-crimp tube for the TI70.1 rotor and fill it with 1.55 g/ml CsCl solution. In case there is little ethidium bromide left, add 50 µl of ethidium bromide stock solution (10mg/ml). Spin in the TI 70.1 rotor at 38,000 to 40,000 rpm at 20°C for at least 48 hours. Two bands should be clearly visible without UV light, both bands can be collected separately since the upper band contains DNA in excellent quality for restriction enzyme mapping and even cell transfections.

Remove ethidium bromide as usual with $H_2O$- and CsCl-saturated isopropanol until no (!) trace of ethidium bromide is left; add 5 ml of $H_2O$ (assuming that your DNA solution has a volume of about 1 to 1.5 ml at this point) and precipitate the DNA with 5ml iso-propanol. Collect the precipitate as usual and wash it in 70% ethanol; resuspend pellet in 200 to 300 µl of TE. At this stage, I prefer Corning Corex tubes (and rubber adapters) since the DNA pellet will tightly adhere to glass but not to plastic. If you must use Falcon tubes be extremely alarmed since the pellet will not stick at all and will be lost easily!

voilà 10 to 100µg of plasmid DNA

**Material needed:**

6 x 8 ml of solution I:
50 mM glucose, 25 mM Tris-HCl pH 8.0, 10 mM EDTA
(from stock solutions of 1 M glucose in $H_2O$; 1M Tris-HCl pH 8.0; 0.5 M EDTA)

6 x 15 ml of solution II:
0.2 N NaOH, 1% SDS (both from stock solutions of 2N and 10%, respectively); make fresh every time, never store concentrated NaOH solution in a glass bottle!

6 x 12 ml of solution III
mix 600 ml of 5 M potassium acetate in $H_2O$ with 115 glacial acetic acid and 285 ml of $H_2O$, keep in the fridge

special TE (50 mM Tris pH 8.0, 20mM EDTA)
CsCl solid

ethidium bromide stock solution (10 mg/ml)
little 1.55 g/ml CsCl solution

six plus one 500 ml volume bottles for Kendro or Beckman high speed centrifuge
2 Kendro Ultra-crimp tubes for TI 70.1 rotor or equivalent

Sugden Lab.

remarks:
* if you are in doubt about the given descriptions ask before you believe your own interpretations!