Mini-Lysates for control restrictions

From Wolfgang Hammerschmidt's lab's website

Background

EBV plasmids are present as single copies in *E. coli*. Because of this, yields of DNA preps are generally very low and the DNA you get is of poor quality (i.e. contaminated by chromosomal DNA, sometimes hard to cleave). Wolfgang's protocol yields enough DNA for one restriction with a quality that allows visualisation of restriction fragments down to approx. 3 kB, which is the size of the "Bam-W repeats". Smaller fragments are hard to see, so restriction enzymes should be chosen appropriately ! Some (including myself) find it hard to produce DNA with a suitable quality with this protocol, so I include the alternate protocol I use and which I found to yield more and better quality DNA.

Wolfgang's Protocol

- streak 1/4 (or1/2) plate per clone and restriction enzyme you want to use, incubate o/n – for recombination grow at 42 ºC
- harvest cells by scraping with toothpick and transfer cells into an E'tube containing 200 µl TE + 2 µl RNase (make mixture) , resuspend by vortex
- add fresh 200 µl lysis solution (0.2 M NaOH / 1 % SDS), mix hard by tapping on the bench and incubate on ice 5 min
- add cold 200 µl solution III (Maniatis) mix gently then thoroughly
- keep on ice for 10 min
- centrifuge for 10 min, full speed at 4ºC
- transfer supernatant into a fresh E'tube and then add 0.4 ml isopropanol, mix
- centrifuge 10 min at 20ºC , full speed
- wash pellet with 0.5 ml 70% ethanol
- spin for 5min full speed, throw away supernant
- remove traces of alcohol by spinning for 30sec and pipet liquid out
- resuspend pellet in 20 µl TE, add restriction buffer and enzyme (50 U)
- restrict for at least 4 h
- use complete restriction for one lane of a 300 ml gel
- run at 60-70V for o/n

Alternate Protocol

- grow 1/4 plate per clone and restriction enzyme you want to use
- transfer cells into an E'tube containing 200 µl TE, resuspend
- add 300 µl lysis solution (0.2 M Na-phosphate buffer, pH 13 / 0.5 % SDS), mix
- add 100 µl 5 M K-acetate:acetic acid = 6:1, mix
- add 2µl RNase (10 mg/ml), mix
- spin down for 30 min, full speed at RT
- transfer supernatant into a fresh E'tube containing 0.4 ml 5 x TE, pH 8.5
- add 0.5 ml isopropanol, mix
- leave at RT for approx. 15 min
- spin down 10 min, full speed
- wash pellet with 0.5 ml 75% ethanol
- air dry pellet
- dissolve pellet in 50 µl TE, use 25 µl per restriction
- restrict for at least 4 h, better o/n
- use complete restriction for one lane of a 300 ml gel

Troubleshooting

1. DNA can not be cleaved
    - increase volume of restriction (up to 200 µl)
    - reprecipitate DNA
    - wash more carefully
2. there is no DNA to be cleaved
    - make sure the clones contain a plasmid: they should form well developed colonies o/n at 37¡C on agar containing the appropriate antibiotic
    - make sure your plates contain enough antibiotic (a strain without plasmid must not grow)
    - use a freshly made lysis solution

- make sure you do not remove the DNA pellet together with the supernatant

3. there are not the expected bands
    - make sure the enzyme(s) have cleaved, e.g. by including the original clone in the prep (which you should do anyway...)
    - if the DNA was cleaved: your clone has been rearranged (this can happen sometimes); sorry to say you will have to start again

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