

PLASMID ISOLATIONS (MINIPREPS)

INTRODUCTION:

After bacteria have been transformed either with plasmid vector DNA or with plasmids containing inserts, the plasmid (or recombinant) DNA needs to be isolated from the bacterial chromosome. There are two reasons for this. First, the bacterial chromosome is very large and would cause a high background on a gel, and would thus cause confusing restriction patterns. The second reason is that often highly purified plasmid DNA is required (e.g., for use as a probe).

To obtain highly purified plasmid DNA, there are two basic methods to use. First is to use column chromatography, many commercial columns are available (HPLC can also be used). The second is to use ethidium bromide in cesium chloride gradients. Ethidium bromide can fit between the stacked bases in DNA, this is termed intercalation. As it does this it forces the bases apart, causing the DNA to unwind and lengthen. However, less of the dye can intercalate into supercoiled DNA than in linear DNA since it is in a covalently closed circle. This is because the ethidium cannot easily force the DNA bases apart since there is a physical restraint. In other words, the covalent closure of the plasmid does not allow unwinding or lengthening of this DNA. When ethidium bromide intercalates into the DNA the buoyant density of the DNA is decreased in the gradient. Since supercoiled DNA (plasmid) takes up less ethidium bromide, it is buoyed up less than the linear DNA in the gradient, thus causing an effective separation of the two forms of DNA in the gradient.

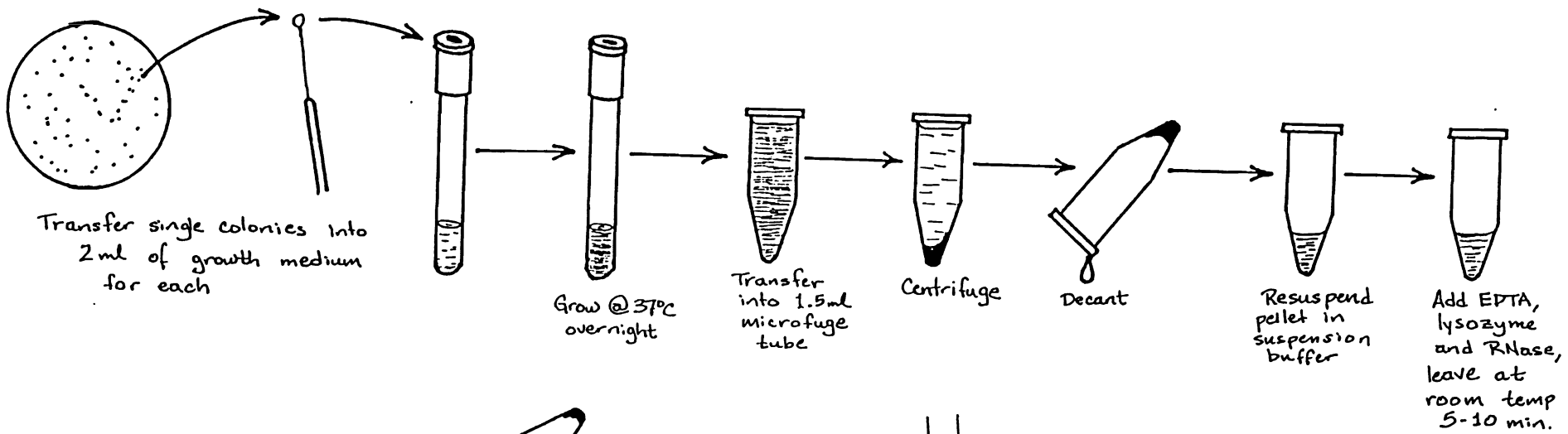
For initial characterization of the plasmids and for other uses when the plasmid DNA may contain some bacterial chromosomal DNA, the plasmids can be isolated using one of the several minipreparation methods. Most methods rely on pretreatment of the bacteria with lysozyme to digest the cell wall, then a lysis step using either detergent, alkaline conditions or boiling, or a combination of these. After the bacterial cells have been lysed, the plasmid is separated from the chromosomal DNA usually by differential precipitation of the DNAs. All rely on the different characteristics of the high molecular weight chromosomal DNA and the low molecular weight plasmid DNA. Higher molecular weight DNA is less soluble than low molecular weight DNA. Because of this, the chromosomal DNA can first be precipitated and discarded, then the conditions in the supernatant can be changed so that the plasmid DNA can be precipitated. In the alkaline precipitation method, the differences in linear DNA (chromosomal) and supercoiled DNA (plasmid) is exploited by denaturation of the linear (chromosomal) DNA, then precipitation to separate the two types of DNA.

The minipreparation (miniprep) method we will use employs lysozyme treatment followed by a detergent lysis and differential precipitation of the plasmid DNA from the chromosomal DNA.

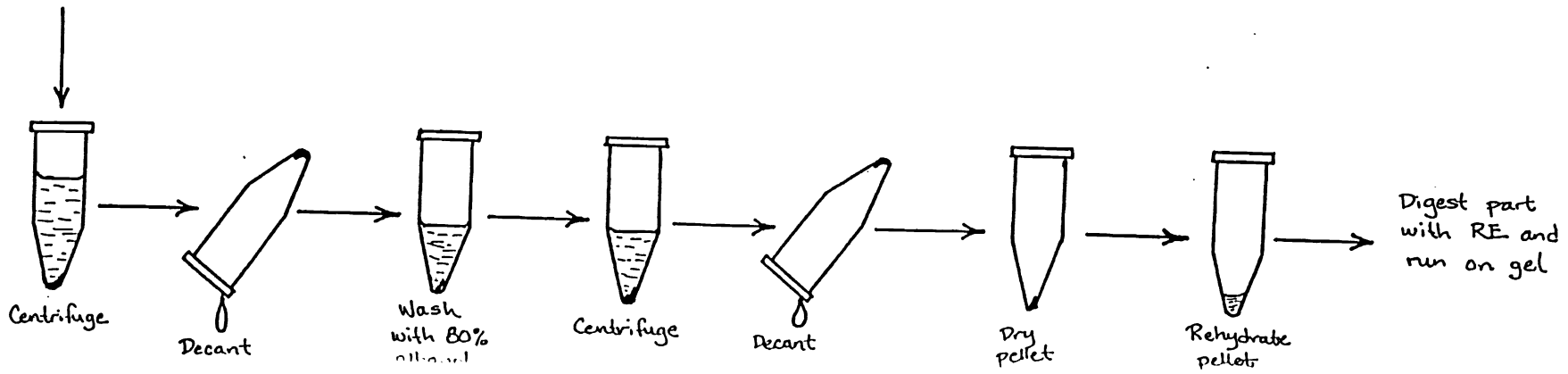
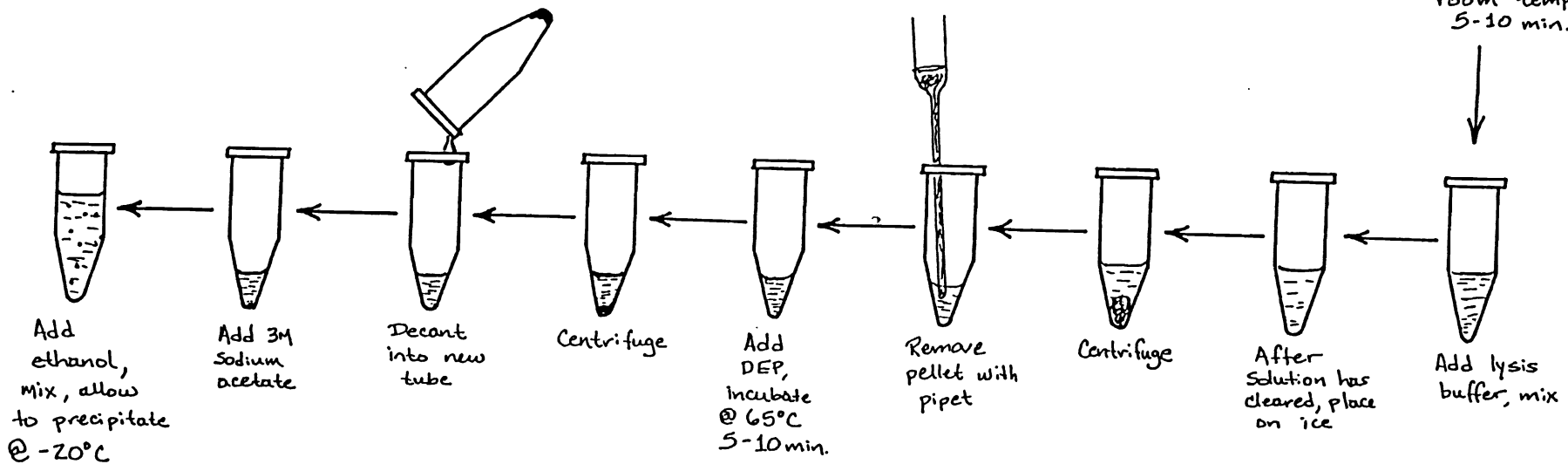
STEPS IN THE PROCEDURE:

1. Grow bacteria in 2 ml of LBA overnight at 37 °C with shaking (for aeration).

2. Transfer 1.5 ml of the culture into a 1.5 ml microfuge tube.
3. Centrifuge for 1 minute. Decant off the supernatant.
4. Add 200 μ l of the suspension buffer. Resuspend the pellet by vortexing.
5. Add 8 μ l of the 0.5M EDTA solution.
6. Add 20 μ l of the 1% lysozyme solution (freshly prepared) and 5 μ l of the RNase solution.
7. Allow to incubate at room temperature for 5-10 minutes.
8. Add 200 μ l of the lysis buffer. Mix thoroughly.
9. Allow to incubate at room temperature until the solution appears clear (indicating that the bacteria have lysed).
10. Put on ice for 5 minutes or longer.
11. Centrifuge for 15 minutes in the cold.
12. Remove the pellet with a disposable Pasteur pipet and discard it. This is the chromosomal DNA. You may lose a portion of the supernatant at this point, but this is better than leaving more of the bacterial chromosome in the supernatant.
13. Add 5 μ l of diethylpyrocarbonate (DEP) to the supernatant. Vortex to mix thoroughly. [Use DEP in the hood.]
14. Incubate at 65 °C for 5-10 minutes.
15. Centrifuge for 1-3 minutes.
16. Decant the supernatant into a new microfuge tube.
17. Add 50 μ l of the 3M sodium acetate solution, and mix thoroughly.
18. Add 1 ml of cold 95% ethanol and mix.
19. Allow to precipitate at -20 °C for 20-30 minutes.
20. Centrifuge for 5 minutes in the cold.
21. Decant off the supernatant and wash the pellet with 500 μ l of 80% ethanol.



120



22. Centrifuge for 1-2 minutes, decant off the ethanol and dry the pellet.
23. Rehydrate the pellet in 50 μ l of 0.1X TE
24. Digest 2 μ l of this solution with restriction enzyme(s) and run on agarose gels to characterize the plasmid DNAs.

SOLUTIONS:

Suspension Buffer

50 mM Tris (pH 8.0)
1 mM EDTA
25% sucrose

RNase solution

1 mg/ml RNase A
100 U/ml RNase T₁

Lysis Buffer

50 mM Tris (pH 8.0)
125 mM EDTA
1% Triton X-100

3M Sodium Acetate

