

## Preparation of total DNA from living cells

As a genetic engineer, you will need to prepare at least two kinds of DNA for setting up a cloning experiment. First, total cell DNA is required as a source of material from which genes to be cloned are obtained. The genomic DNA preparations might be DNA from bacterial cells, plant cells, or animal cells.

The second type of DNA required for gene cloning experiment is pure plasmid DNA. The isolation steps of both total and plasmid DNA preparations share the same basic principles except critical difference that the plasmid DNA **must** be separated from the main chromosomal DNA existing in the cell.

There are many different methods available for isolating genomic DNA. Choosing the most appropriate method for a specific application needs to take the following issues in consideration. **No single method addresses all these issues.**

- **Source:** What organism/tissue that the DNA will come from?

Some organisms present special difficulties for DNA isolation. Plants cells, for example, are considerably more difficult than animal cells, because of their cell walls, and require special attention.

- **Yield:** How much DNA do you need?

If the source is limited, you will need to use a method that is very efficient at producing a high yield. Fortunately, *E. coli* is easy to grow, and PCR is effective with very small amounts of DNA sample, so this will not be a major issue for us.

- **Purity:** What level of contaminants (RNA, protein, etc.) can be tolerated?

The purification method must eliminate any contaminants that would interfere with subsequent steps. This depends, of course, on what you plan to do with the DNA once you have isolated it. PCR will tolerate a reasonable degree of contamination as long as the contaminants do not inhibit the thermostable DNA polymerase or degrade DNA. We also need to remove proteins from the DNA so that it is a good template for replication.

- **Integrity:** How large are the DNA fragments in your genomic DNA preparation?

High molecular DNA is highly fragile. It is easily cut into smaller pieces by hydrodynamic shearing forces and by DNases.

Hydrodynamic shear is minimized by avoiding vigorous vortexing and pipetting of DNA solutions. A simple precaution is to use micropipette tips with orifices larger than usual ("wide bore tips).

- **Economy:** How much time and money are needed?

Many biotech companies sell kits with all the reagents necessary for genomic preps. You need to look carefully at the cost of these kits relative to the labor that they save.

- **Safety:** How many hazardous materials do you use in the DNA preparation?

A phenol/chloroform reagent widely used in DNA purification is tremendously hazardous. In fact, phenol/chloroform is probably the most hazardous reagent used regularly in molecular biology labs. Phenol is a very strong acid that causes severe burns. Chloroform is a carcinogen.

- **Lysis:**

Cell walls and membranes must be broken to release the DNA and other intracellular components. This is usually accomplished with an appropriate combination of enzymes to digest the cell wall (usually lysozyme) and detergents to disrupt membranes. We use the ionic detergent Sodium Dodecyl Sulfate (SDS) at 80°C to lyse *E. coli*.

- **Removal of protein, carbohydrate, RNA, etc.**

RNA is usually degraded by the addition of RNase. The resulting oligoribonucleotides are separated from the high molecular weight (HMW) DNA by exploiting their differential solubilities in non-polar solvents (usually alcohol/water).

Proteins are subjected to chemical denaturation and/or enzymatic degradation. The most common technique of protein removal involves denaturation and extraction into an organic phase consisting of phenol and chloroform.

Another widely used purification technique is to band the DNA in a CsCl density gradient using ultracentrifugation.

The main basic steps for total DNA from a culture of bacterial cells include:

1. A culture of bacteria is grown and then **harvested**.
2. The cells are broken open to release their contents.
3. This **cell extract** is treated to remove all components except the DNA.
4. The resulting DNA solution is concentrated.

### Procedure

1. Add 1.0 ml of an overnight culture to a 1.5ml microcentrifuge tube.

2. Centrifuge at 15,000 g (or max. speed) for 2 minutes to pellet the cells.

Place your tubes opposite each other to balance to rotor. Do not initiate a spin cycle until the rotor is fully loaded; this minimizes the total number of runs required. A cell pellet should be visible at the bottom of the tube.

3. Transfer the supernatant back into the culture tube it came from and discard this culture tube as biohazard waste. Carefully remove as much of the supernatant as you can without disturbing the cell pellet. The pellet may be on the side of the tube, not squarely on the bottom.

4. Resuspend the cell pellet in 600 $\mu$ l of Lysis Solution (LS) which contains SDS and Tris-HCl or EDTA. Gently pipet until the cells are thoroughly resuspended and no cell clumps remain. LS contains the anionic detergent sodium dodecyl sulphate (SDS) to disrupt membranes and denature proteins.

5. Incubate at 80°C for 5 minutes to completely lyse the cells. The samples should now look clear.

6. Cool the tube contents to room temperature.

Do not rely on temperature equilibration with ambient air. Place the tube in a room temperature water bath for several minutes.

7. Add 3 $\mu$ l of RNase solution to the cell lysate. Invert the tube 2–5 times to mix.

8. Incubate at 37°C for 30 minutes to digest RNA. Cool the sample to room temperature.

This step is intended to degrade RNA into small fragments or individual ribonucleotides.

9. Add 200  $\mu$ l of Protein Precipitation Solution (PPS, provided commercially) to the RNase-treated cell lysate. Vortex vigorously at high speed for 20 seconds.

10. Incubate the sample in ice/water slurry for 5 minutes. The sample now has significant whitish insoluble material.

11. Centrifuge at 15,000 g (or max. speed) for 3 minutes. There should be a large pellet of whitish material; protein, on the bottom and sides of the tube.

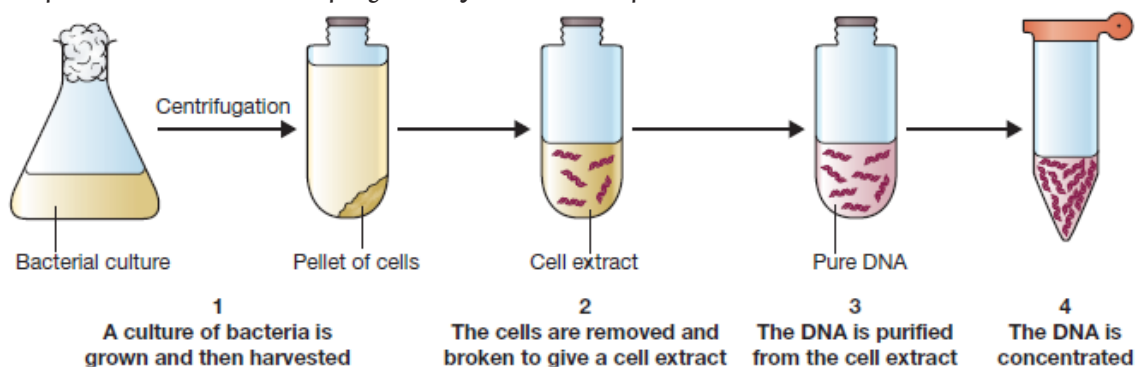
12. Transfer the supernatant ( $\leq$ 800  $\mu$ l) containing the DNA to a clean 1.5ml microcentrifuge tube containing 600 $\mu$ l of room temperature isopropanol (IPA).

Be sure that you don't suck up and transfer any of the precipitate. Use P1000 set to 750  $\mu$ l.

13. Mix the DNA solution with the IPA by gently inverting the tube at least 15 times. The DNA is usually (barely) visible as a small floc of whitish material.

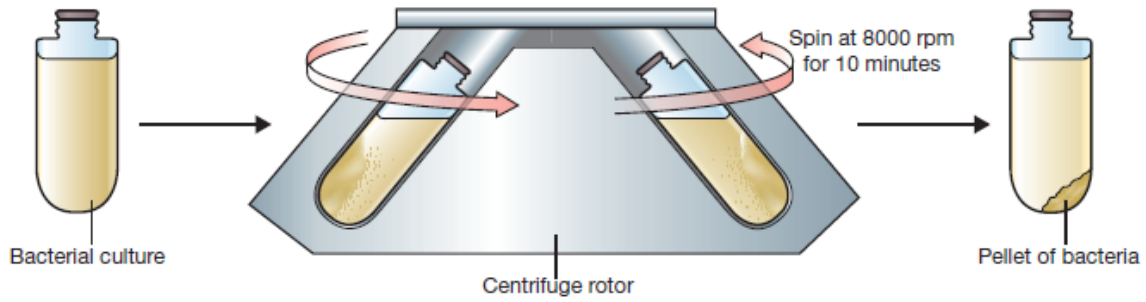
14. Centrifuge at 15,000 g (or max. speed) for 2 minutes.

15. Carefully pour off the supernatant (do not pipette) and invert the tube on clean absorbent paper to drain. The DNA pellet may or may not be visible. Do not allow the DNA pellet to completely dry.
16. Add 600 $\mu$ l of room temperature 70% ethanol and gently invert the tube several times to wash the DNA pellet. Do not resuspend by pipetting.
17. Centrifuge at 15,000 g (or max. speed) for 2 minutes.
18. Carefully pour off the ethanol supernatant (do not pipette) and invert the tube on clean absorbent paper to drain. You want the paper to wick off the ethanol that drains down and collects at the rim of the inverted tube
19. Allow the pellet to air-dry for 10–15 minutes. When all the EtOH is gone there should still be some water left hydrating the DNA.
20. Add 100 $\mu$ l of DNA Rehydration Solution (RH) (TE buffer or sterile DDH<sub>2</sub>O to the tube and rehydrate the DNA by incubating at 65°C for 1 hour. After 30 minutes, flick the bottom of the tube gently to facilitate dissolution and mixing. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C, preferably on a low speed shaker.



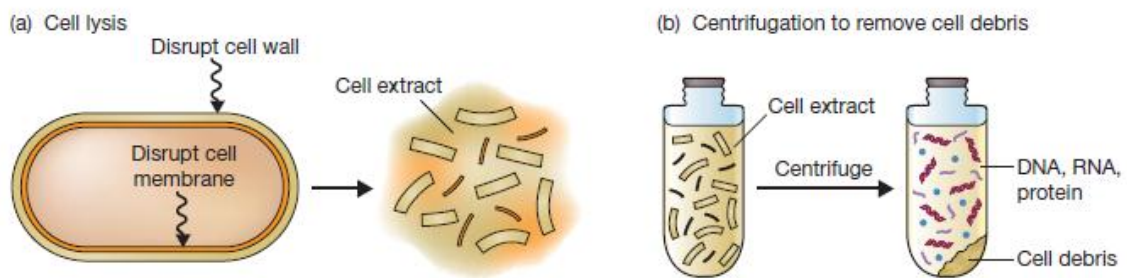
**Figure 3.1**

The basic steps in preparation of total cell DNA from a culture of bacteria.



**Figure 3.3**

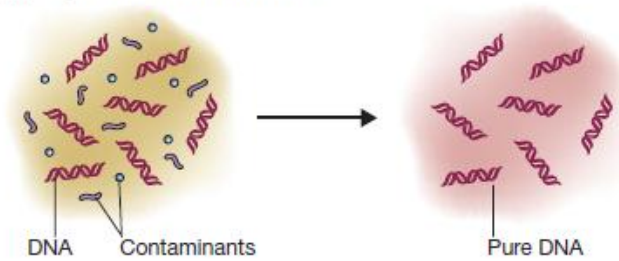
Harvesting bacteria by centrifugation.



**Figure 3.4**

Preparation of a cell extract. (a) Cell lysis. (b) Centrifugation of the cell extract to remove insoluble debris.

(a) Degradation of contaminants



**Figure 3.5**

Two approaches to DNA purification. (a) Treating the mixture with reagents which degrade the contaminants, leaving a pure solution of DNA. (b) Separating the mixture into different fractions, one of which is pure DNA.

(b) Separation of DNA from contaminants

