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Plasmid DNA extraction

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ABOUT THE STUDY

A plasmid could even be a little, extrachromosomal DNA molecule within a cell that's physically separated from chromosomal DNA and can replicate independently. They're most typically found as small circular, double-stranded DNA molecules in bacteria; however, plasmids are sometimes present in archaea and eukaryotic organisms. In nature, plasmids often carry genes that benefit the survival of the organism and confer selective advantage like antibiotic resistance. While chromosomes are large and contain all the essential genetic information for living under normal conditions, plasmids are usually very small and contain only additional genes which can be useful in certain situations or conditions. Artificial plasmids are widely used as vectors in molecular cloning, serving to drive the replication of recombinant polymer sequences within host organisms. Within the laboratory, plasmids could even be introduced into a cell via transformation.

HOW TO EXTRACT PLASMID DNA

Cultivate bacterial samples

First, the bacterial cells must cultivate in varying amounts of growth medium. Typically, you'll incorporate an antibiotic into the expansion media, and also the plasmid DNA will pass resistance to the antibiotic to the bacteria. You'll be ready to remove cells from the expansion medium and discard the supernatant through centrifugation. At the best possible of this step, the cells will become cell pellets.

Resuspend the pelleted cells in solution

Next, you want to resuspend the cells in an isotonic solution that contains Tris, EDTA (to destabilize the cell membrane and stop plasmid damage), glucose (to prevent the cells from bursting), and RNase A (to degrade cellular RNA during cell lysis).

Lyse the cells

You will then add an alkaline solution containing hydroxide and sodium dodecyl sulfate (SDS) to facilitate cell lysis and also the denaturation of both genomic and plasmid DNA along with all the proteins within the answer. The highly alkaline solution consisting of NaOH and SDS breaks down the cell membranes and converts the double-stranded DNAs (dsDNA) to single-stranded DNAs (ssDNA).

Neutralize the solution with potassium acetate

A potassium acetate solution neutralizes the sample and separates the plasmid DNA from the genomic DNA (gDNA). The smaller plasmid DNA renatures easily, while the larger, more complicated genomic DNA precipitates out of the solution.

Upon centrifugation, genomic DNA and precipitated proteins form a pellet while plasmid DNA remains soluble. The remaining plasmid DNA within the supernatant is precipitated with ethanol or purified using spin filter technology or a phenol-chloroform mixture.

Precipitate plasmid DNA with ethanol precipitation

Finally, you wish to isolate the plasmid DNA through a process called ethanol precipitation. Once precipitated, you would like to rinse the precipitate (the plasmid DNA) in ice-cold 70% ethanol and let it dry for about 10 minutes therefore the alcohol can evaporate. You'd favor to also resuspend the DNA pellet during an answer containing Tris, EDTA, and RNAses to clean all of the remaining RNAs within the answer.