Introduction to RNA Extraction

BMES Cell Team

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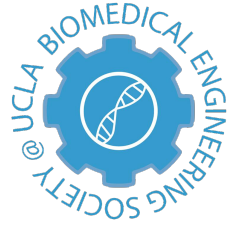


Background

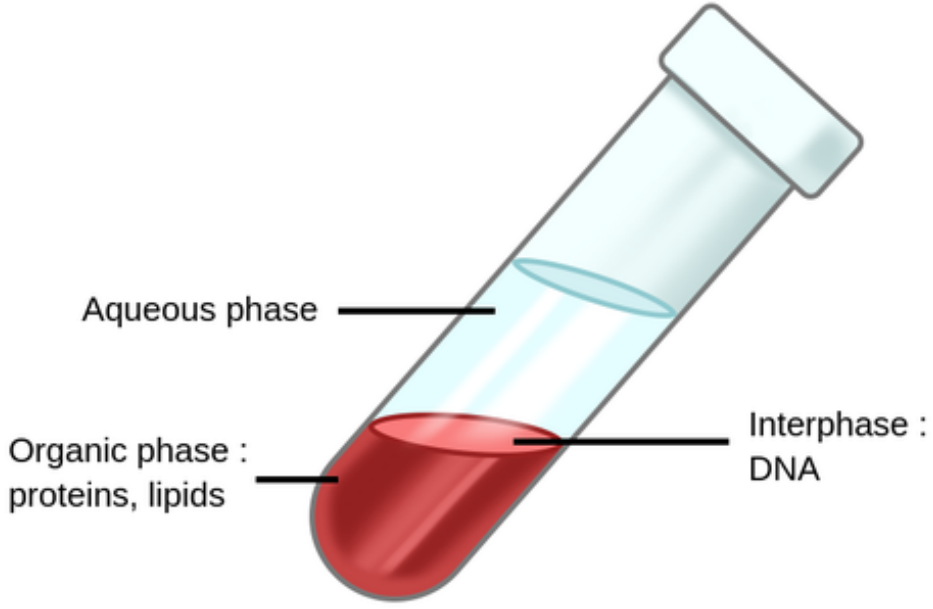
Gene Expression

- In your Labster Simulation, you will be using pigs as a model animal to perform mRNA extraction
- Recall that mRNA is the product of transcription; it contains the nucleic acids that code for a protein
- In the case of protein-coding genes, the abundance of mRNA reflects the level of gene expression

mRNA Extraction

- A common method used to isolate RNA is guanidinium thiocyanate-phenol-chloroform extraction

- This solvent is made up of protein denaturing molecules, and organic phase, and an aqueous phase



mRNA Extraction

The main steps of RNA isolation are as follows:

1. Cell lysis and disruption of cellular structures
2. Separation of the RNA from cell debris
3. Purification of the RNA from the DNA and proteins
4. Precipitation of RNA
5. Wash and resuspension of the RNA

- After centrifugation, RNA ends up in the aqueous phase and is precipitated by adding isopropanol

mRNA Purification with Magnetic Beads

- To purify mRNA, we want to bioconjugate magnetic beads to mRNA
- To do this, we must attach the magnetic beads with oligodT (a chain of Thymines), which will bind to a section of mRNA called the polyA tail
  → Recall that A pairs with T and C pairs with G
- When placed in a magnetic rack, the beads that are conjugated to the mRNA will be separated from the solution
- We can then pipette the solution containing unwanted materials and wash the beads with 70-80% ethanol
- After several rounds of washing, we can elute (separate) the mRNA molecules from the magnetic beads and transfer them to a new tube

mRNA Purification with Magnetic Beads