Bioseparation

محاضرة 5

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DNA Purification Basics

There are five **basic steps** of DNA extraction that are consistent across all the possible DNA purification chemistries:

1) disruption of the cellular structure to **create a lysate**.

2) **separation** of the soluble DNA from cell debris and other insoluble material.

3) **binding** the DNA of interest to a purification matrix.

4) washing proteins and other contaminants away from the matrix and

5) **elution** of the DNA.

1. Creation of Lysate

The first step in any nucleic acid purification reaction is releasing the DNA/RNA into solution. The goal of lysis is to rapidly and completely disrupt cells in a sample to release nucleic acid into the lysate. There are four general techniques for lysing materials: physical methods, enzymatic methods, chemical methods and combinations of the three.

DNA Purification Basics

- 2. Clearing of Lysate
- Depending on the starting material, cellular lysates may need to have cellular debris removed prior to nucleic acid purification to reduce the carryover of unwanted materials (proteins, lipids and saccharides from cellular structures) into the purification reaction, which can clog membranes or interfere with downstream applications. Usually clearing is accomplished by centrifugation, filtration or bead-based methods.

- Centrifugation can require more hands-on time, but it is able to address large amounts of debris. Filtering can be a rapid method, but samples with a large amount of debris can clog the filter. Bead-based clearing, like the method used with Promega particle-based plasmid prep kits, can be used in automated protocols, but can be overwhelmed with biomass.
- Once a cleared lysate is generated, the DNA can then be purified by many different chemistries, such as silica, ion exchange, cellulose or precipitation-based methods.

- **3. Binding to the Purification Matrix**
- Regardless of the method used to create a cleared lysate, the DNA of interest can be isolated using a variety of different methods. Many companies offers genomic DNA isolation systems based on sample lysis by detergents, and purification by binding to matrices (silica, cellulose and ion exchange), which is where interest has primarily been focused in recent years.
- Each of these chemistries can influence the efficiency and purity of the isolation, and each have a characteristic binding capacity. Bind capacity is an indication of how much nucleic acid an isolation chemistry can bind before it reaches the capacity of the system and no longer isolates more of that nucleic acid. We can build design features into these chemistries by manipulating the binding conditions to enrich for different categories of nucleic acid (e.g., chemistries that selectively bind RNA versus DNA or large versus small fragments).

- **Solution-Based Chemistry**
- This type of chemistry does not rely on a binding matrix, but rather on alcohol precipitation. Following the creation of lysate, the cell debris and proteins are precipitated using a high-concentration salt solution. The high concentration of salt causes the proteins to fall out of solution, and then centrifugation separates the soluble nucleic acid from the cell debris and precipitated protein (1).

- The DNA is then precipitated by adding isopropanol to the high-concentration salt solution. This forces the large genomic DNA molecules out of solution, while the smaller RNA fragments remain soluble. The insoluble DNA is then pelleted and separated from salt, isopropanol and RNA fragments via centrifugation.

- Additional washing of the pellet with ethanol removes the remaining salt and enhances evaporation. Lastly, the DNA pellet is resuspended in an aqueous buffer like Tris-EDTA or nuclease-free water and, once dissolved, is ready for use in downstream applications.

- **Silica-Binding Chemistry**
- The technology for these genomic DNA purification systems is based on binding of the DNA to silica under high-salt conditions. The key to isolating any nucleic acid with silica is the presence of a chaotropic salt like guanidine hydrochloride. Chaotropic salts present in high quantities are able to disrupt cells, deactivate nucleases and allow nucleic acid to bind to silica.

- Once the genomic DNA is bound to the silica membrane, the nucleic acid is washed with a salt/ethanol solution. These washes remove contaminating proteins, lipopolysaccharides and small RNAs to increase purity while keeping the DNA bound to the silica membrane column. Once the washes are finished, the genomic DNA is eluted under low-salt conditions using either nuclease-free water or TE buffer.

- Binding to silica is not DNA specific, so if pure DNA is required, there is also the option to add ribonuclease (RNase A) to the elution buffer. RNA may be may be copurified with gDNA, and the addition of RNase to the elution buffer ensures the removal of the vast majority of contaminating RNA.

- **Cellulose-Binding Chemistry**
- More recently, commercialized DNA isolation methods that use a cellulose-based matrix. Nucleic acid binds to cellulose in the presence of high salt and alcohols. Generally, the binding capacity of cellulose-based methods is very high. Conditions can be adjusted to preferentially bind different species and sizes of nucleic acid. As a result of the combination of binding capacity and relatively small elution volume, we can get high concentration eluates for nucleic acids.

- **Ion Exchange Chemistry**
- Ion exchange chemistry is based on the interaction that occurs between positively-charged particles and the negatively-charged phosphates that are present in DNA. The DNA binds under low salt conditions, and contaminating proteins and RNA can then be washed away with higher salt solutions. The DNA is eluted under high salt conditions, and then recovered by ethanol precipitation.

- **4. Washing**
- Wash buffers generally contain alcohols and can be used to remove proteins, salts and other contaminants from the sample or the upstream binding buffers. Alcohols additionally help associate nucleic acid with the matrix.

- **5. Elution**
- DNA is soluble in low-ionic-strength solution such as TE buffer or nuclease-free water. When such an aqueous buffer is applied to a silica membrane, the DNA is released from the silica, and the eluate is collected. The purified, high-quality DNA is then ready to use in a wide variety of demanding downstream applications, such as multiplex PCR, coupled in vitro transcription/translation systems, transfection and sequencing reactions.

Purifying RNA

- Break the cells → solubilize components → inactivate RNAses
  by the addition of guanidinium thiocyanate (very powerful denaturant)

- Extract RNA using phenol/chloroform (at low pH)

- Precipitate the RNA using ethanol/LiCl

- Store RNA:

  in DEPC-treated H_20 (-80°C)