The basic guide for magnetic DNA purification

by Alanna Klose

Fluorescent tag

Magnetic particle

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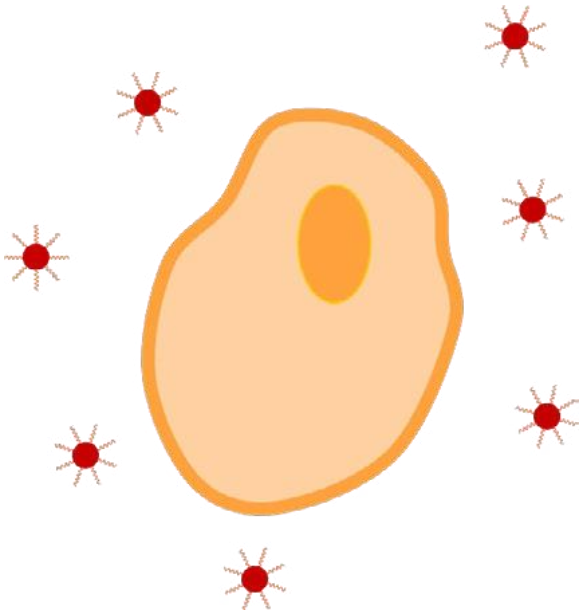
SUMARY

Introduction



Nucleic acid separation can be fickle. DNA is fragile, and RNA even more so. Many commercial kits are designed to streamline the process, but they may not result in high yield or high purity DNA or RNA every time. Every laboratory is different; working habits vary, and experimental goals are not identical. It is tempting to rely on a single kit for routine isolation of genetic material because it is familiar, your lab may have published previous work with an established protocol, or you might not have the luxury or freedom to try something new. However, if you are experiencing consistently low RNA or DNA yield or purity then you may be able to justify taking time to gain a deeper understanding of the process, familiarizing yourself with the tools available, and possibly reworking your strategy.

Even if your current isolation strategy is performing adequately it may be worthwhile to investigate recent developments in the field. New technology such as magnetic DNA purification could potentially increase the specificity of extraction, decrease isolation time, increase throughput, and even automate the process. Isolation and purification of DNA and RNA is a fundamental procedure in the modern laboratory, and the high cost of sequencing technology means it is essential to consistently extract pure, high-quality genetic material every time.

Additionally, the explosion of nanotechnology in the past decade has created an incubator for the creation of new biomedical diagnostic devices. New methods to isolate nucleic acid have improved our ability to quickly diagnose disease. Sample collection and diagnosis can occur at the point of care without being transferred to a laboratory. By eliminating the need for traditional culture methods the time from sample collection to diagnosis is a fraction of what it used to be. Such point-of-care rapid diagnostic systems are in demand, and the market has room for creative solutions and growth.

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Chapter 1. Nucleic Acid Isolation

Our understanding of genetic material has substantially increased since Friederich Miescher first extracted DNA in 1869. He discovered that a material exists within cells that precipitates out of acidic solution and dissolves into alkaline solution. He called it nuclein because it seemed to be located within the nucleus. It took until 1953 for the structure of DNA to be elucidated. It was during this time that procedures to isolate DNA began to emerge. Later, during the 1960's and 70's scientists were furiously untangling the cellular environment, and the discovery of RNA with its various forms and functions further refined DNA purification procedures. It was no longer enough to simply separate DNA from protein and salt impurities; it became necessary to remove contaminating RNA as well. Concurrently, scientists became interested in purifying messenger RNA (mRNA). Soon it became essential to purify not only DNA (genomic or plasmid), but also RNA in its various forms.

As our understanding of nucleic acid chemistry deepened, more researchers saw the benefit of isolating and studying genetic material, and new tools such as solid-phase centrifugation columns hit the market. These columns took advantage of selective binding of negatively charged nucleic acids mediated by salt concentration and solution pH. Recently, superparamagnetic nanoparticles have been introduced as solid-phase support systems for the purification of nucleic acids by magnetic separation.

There are three general steps to nucleic acid isolation

1. Cell lysis
2. Removal of contaminating proteins, nucleic acids, and salts and deactivating DNAases or RNAases.
3. Recovery of DNA or RNA

Step 1: cell lysis

The first step in nucleic acid isolation is breaking apart the cell wall and/or membrane to release the genetic material. This is accomplished with the use of lysis buffer, rotor homogenizer, bead mill, freeze thaw cycles, or sonication. Lysis buffer contains a detergent to help break down cell membranes, and an enzyme such as protease K for digesting protein components. The homogenizer and bead mill provide rough mechanical shearing to disrupt the tissue and cells. Cell lysis produces a solution in which the cellular contents are no longer neatly compartmentalized. Therefore, DNAases and RNAases threaten to enzymatically destroy the genetic material. EDTA

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Early nucleic acid purification methods relied on density gradient separation. Later strategies such as guanidinium thiocyanate-phenol-chloroform extraction took advantage of the variable solubility of cellular components in organic or aqueous solvent.

can be used to deactivate DNAases by chelating divalent ions that are necessary for enzymatic activity, and RNAases are permanently destroyed by beta-mercaptoethanol. Additionally, to decrease the chance of contamination with environmental RNAases or DNAases, it is important to use DEPC-treated water and buffers, to wear gloves, and to maintain a clean workspace.

Steps 2 and 3: Removal of contaminates and recovery of DNA and RNA

Guanidinium thiocyanate-phenol-chloroform extraction

This early purification technique takes advantage of the variable solubility of cellular components in organic or aqueous solvent, and sensitivity to salt concentration. The addition of phenol and chloroform to cell lysate causes the solution to fall out into a hydrophobic phase and a hydrophilic phase. Nucleic acids will remain in the hydrophilic phase while proteins will be in the hydrophobic phase. Guanidinium thiocyanate is a chaotropic agent that disrupts hydrogen-bonding. When guanidinium thiocyanate is used in a phenol-chloroform extraction it helps separate RNA and DNA into two different aqueous layers. After centrifugation the RNA will be dissolved in the top aqueous layer, with DNA below it, and the proteins will be in the organic hydrophobic layer at the bottom. Guanidinium thiocyanate also denatures proteins, including RNAases.

Ethanol precipitation and solid-phase column support systems

Nucleic acids can also be collected through ethanol precipitation methods. This technique requires solid phase support systems. The first support system developed for this purpose was a column. The column is static and the solution is poured through it. Under appropriate salt concentrations and pH the nucleic acids bind to the column while other contaminants flow through. This procedure relies on multiple washing and centrifugation steps to remove contaminants before finally eluting and capturing the DNA or RNA.

Magnetic nucleic acid purification

More recently, superparamagnetic particles and magnetic separation have been used to capture nucleic acids. The benefit of this method is that the particles are free to move around within the solution, which improves nucleic acid adsorption and capture efficiency. The superparamagnetic nature of the particles allows them to be manipulated by an external magnet and retained in place while the contaminating proteins and salts are washed away. These systems can rely on salt concentration and ethanol precipitation or they can use more sophisticated chemistry for the reversible-binding of DNA or RNA in a specific or nonspecific manner.

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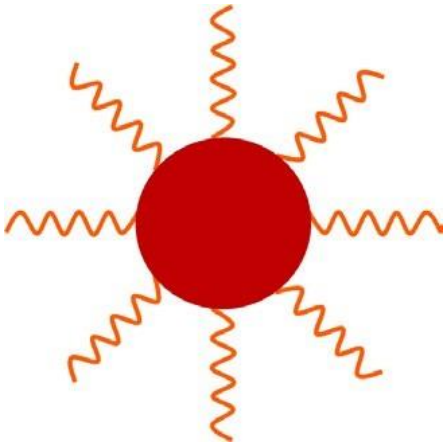
Chapter 2. Solid phase support Systems



The traditional solid-phase support system is a static column. These columns are made of silica matrices or anion-exchange resins. They are porous and allow the solution to flow through them. These non-magnetic solid-phase columns require centrifugation to force the solution through. More recently, magnetic particles are being used as mobile solid-phase support systems for capture and purification of DNA and RNA. These magnetic particles are added to solution and are free to move around during the DNA or RNA adsorption period. They are then retrieved by magnetic separation. No centrifugation is needed for magnetic-particle nucleic acid purification.

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Traditional silica-based columns

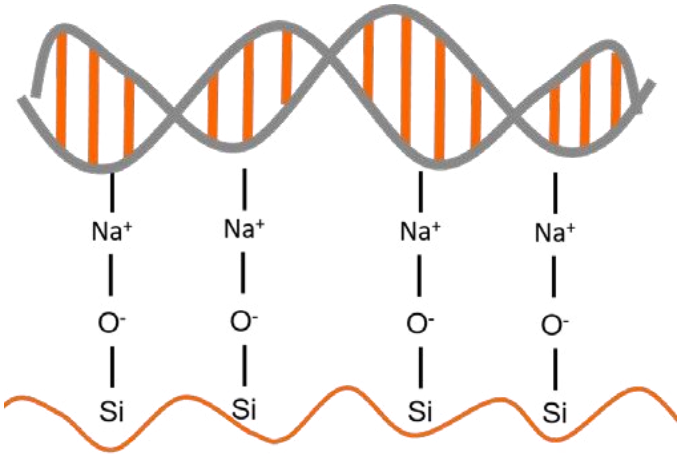
DNA or RNA adsorbs specifically to silica matrices in a high-salt buffer at optimal pH. These matrices are made of silica nano- or micro-particles packed into a resin. The pores between the particles allow the solution to flow through the column. Proteins, carbohydrates, and other contaminants are washed out through the column. The purified DNA or RNA is then collected in a final elution step with a low-salt buffer.

Anion-exhange resin columns

These columns are made of a silica nanoparticle resin that has a large pore size and a dense hydrophilic coating of positively charged DEAE groups. Plasmid DNA binds to the DEAE groups in a medium salt buffer while impurities are washed away. Finally, DNA is eluted in a high-salt buffer. Other anion-exchange resins such as cellulose, dextran, or agarose have been used to make separation columns, but they work in a narrower salt concentration range. The salt concentration and pH can be modified to selectively bind RNA to the column while washing away DNA and other contaminants. Commercial kits are optimized to isolate RNA or DNA, and often have options of differentiating between plasmid and genomic DNA, or isolating specific types of RNA.

Magnetic Particles

The magnetic particles consist of a superparamagnetic core, protected by a coating. The coating prevents irreversible aggregation of the magnetic particles and allows functionalization by the attachment of ligands for adsorption of DNA or RNA. The magnetic particles have a higher surface area than packed columns, which gives them a higher capacity to adsorb nucleic acids, and their ability to be added to solution improves adsorption efficiency. The solution containing DNA or RNA must flow through the column, which limits the time for DNA or RNA to adsorb, whereas magnetic particles can be incubated in the solution for as long as necessary to achieve optimal adsorption.



Column-based purification systems are widespread, and form the basis for many commercial DNA or RNA purification kits. However, these columns require centrifuges, buffers, solvents, and multiple washing steps. Column purification is only useful for non-specific binding of all DNA or RNA in a sample. Magnetic particles can be used for specific nucleic acid capture. Magnetic DNA/RNA purification doesn't require a centrifuge and can be completed within one tube. This means that magnetic particle nucleic acid purification can be fully automated. Additionally, the ability to functionalize magnetic nanoparticles opens the door to capturing specific DNA or RNA sequences. Reversible binding of magnetic particles also introduces a potential for reuse, which can decrease waste and expenses.

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Chapter 3. Magnetic nucleic acid purification

Magnetic DNA or RNA purification relies on the superparamagnetic property of micro- or nano-scale particles. These particles are most often made of iron oxide, with magnetite (Fe3O4) more commonly used than maghemite (Fe2O3). Superparamagnetic particles are not innately magnetic, but they become magnetized when influenced by a magnetic field. So, if the magnetic field is zero, then the particles are not magnetic at all, but when a magnetic field is applied the particles become magnetized.

Magnetization of superparamagnetic particles

The magnetic property of a material is governed by the alignment state of its atomic dipoles. Magnetization (m) is defined as the extent to which a material becomes magnetized when placed within a magnetic field, and is a measure of the net magnetic dipole moment per unit volume. The magnetization is directly related to the applied magnetic field.

Magnetic susceptibility (χ) is a dimensionless quantity that indicates the degree of magnetization of a material in response to a magnetic field. Magnetic susceptibility is equal to the ratio of magnetic dipole moment to magnetic field.

m = magnetization (magnetic dipole moment per unit volume)
B = applied magnetic field
κ = magnetic susceptibility

$\kappa = m/B$

Non-magnetic materials, like water, are diamagnetic and experience a small negative response to the magnetic field. In the case of diamagnetic materials, the susceptibility is negative and has a value around -10e-5. Paramagnetic materials, like oxygen molecules, experience a slightly positive response to the magnetic field. In the case of paramagnetic materials, the magnetic susceptibility is around 10e-4 or 10e-5. In both cases, the magnetic dipoles attempt to align with the external magnetic field, and fight against thermal agitation. Diamagnetic and paramagnetic dipoles don't interact with each other, and their susceptibility at room temperature is very small.

The situation is very different for magnetic materials, which are technically defined as ferro and ferrimagnetic materials. In magnetic materials, each dipole interacts with its nearest neighbors. This inter-dipole interaction creates a high magnetic response throughout the material, with susceptibilities that can reach values higher than 10e3. The drawback is that the materials show magnetic hysteresis: after applying a magnetic field, the material will have 'memory', meaning that when the applied field returns to zero the material remains at a certain magnetization. This is a very useful property for developing permanent magnets, but it is a problem for many Life Science applications. Ferromagnetic beads will remain magnetized after the removal of an applied magnetic field, and the beads will form irreversible clumps or aggregates.

The ideal material for biological applications would have a high magnetic susceptibility, but no magnetic 'memory'. They would combine a high response to applied magnetic field with a non-magnetic behavior once the magnetic field is removed. Advances in nanotechnology have provided a way to obtain these superparamagnetic properties by reducing the size of the ferro- or ferrimagnetic particles to few nanometers (below the so-called superparamagnetic diameter). Then, this magnetic 'pigment' can be encapsulated in non-magnetic matrices such as silica, polyvinyl alcohol (PVA), dextran, agarose, sepharose, and polystyrene, which can be biofunctionalized and used for life science applications.

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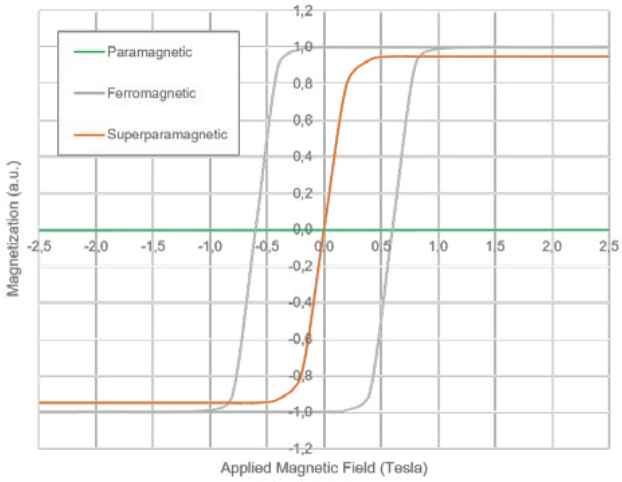
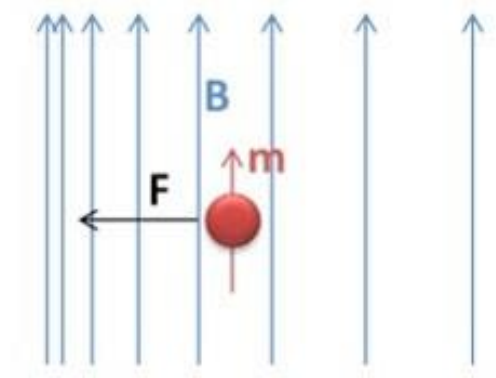


Superparamagnetic particles move in the direction of a magnetic field gradient

A block-shaped permanent magnet produces a magnetic field that decays with distance. A perfectly homogenous magnetic field would induce a rotation on the superparamagnetic beads, but not a displacement. A magnetized superparamagnetic bead will experience a magnetic force if it is exposed to a magnetic field that is not homogenous through space.

$$\mathbf{F} = \nabla(\mathbf{m} \cdot \mathbf{B})$$



If the superparamagnetic particles are saturated, meaning that the magnetization (magnetic moment) is constant, then the magnetic force will be directly proportional to the magnetic field gradient. Below the saturation point the magnetic moment will vary with the magnetic field. If the superparamagnetic particles are not saturated, the magnetization is not constant, and the magnetic force will be lower and dependent upon the square of the applied field. For this reason, modern magnetic bead separation systems are engineered to ensure that superparamagnetic particles are saturated and exposed to a constant magnetic field gradient.

Reversible and irreversible aggregation

Superparamagnetic particles have a high magnetic susceptibility, and once magnetized they can interact with each other to form reversible chain-like aggregates. These chain-like aggregates improve separation time because they move faster than individual particles. Once the magnetic field is removed the particles lose the magnetic moment and the chains are dissolved by thermal fluctuations.

A second kind of aggregation can happen at the retention area. Once the magnetic beads are attracted to the final position, it is necessary to be retained with a force high enough to keep them there when the supernatant is aspirated. However, if the force is excessive, the magnetic beads would be pressed against each other, remaining irreversibly aggregated, or clumped, This will limit the exposed surface area and change the behavior (aggregates would acts as 'bigger' beads), introducing both variability and inconsistency in the process. Irreversible aggregation is a typical problem in classical magnetic separators (magnetic force decreasing sharply with distance). In these devices, increasing the force to capture the farthest beads implies a large multiplier for the retention force, and a leads to a big increase in the likelihood of irreversible aggregation problems. Modern magnetic bead separation systems avoid this problem by generating a constant force high enough to keep the magnetic beads retained when the supernatant is aspirated, but no higher. As the force is kept constant over all the working volume, the farthest beads experience a much higher force than on the classical separators, but are retained much more gently, without risk of irreversible aggregation.

Using superparamagnetic particles for nucleic acid purification

The first step in a nucleic acid purification procedure using magnetic particles is to choose a coating and functionalization strategy. While bare iron oxide particles are able to bind DNA or RNA, they are also prone to irreversible clumping. Coatings are applied to prevent unwanted clumping and also to improve adsorption of nucleic acids to the surface of the magnetic particles. Functionalization by the chemical attachment of molecular moieties makes iron oxide particles able to better adsorb nucleic acids, and is addressed in chapter 4. Control over surface functionalization also allows for specific capture of DNA or RNA, and is discussed in chapter 5.

The next step is to incubate the magnetic particles with the lysed cell solution. The nucleic acids will bind to the magnetic particles, while contaminants such as proteins and polysaccharides will not. The magnetic particle/nucleic acid conjugates are magnetized by an external magnetic field and collected at the sides of the container. The contaminating cell lysate is washed out and replaced with a clean buffer solution. The applied magnetic field is removed and the conjugates are recovered. The extracted DNA or RNA can be released from the magnetic particles by applying an appropriate buffer according to the particle's surface chemistry.

The entire purification process can take place in a single container. No column or centrifugation is needed. The purity of the extracted genetic material is dependent on the binding efficiency and the separation efficiency. The binding efficiency is a function of the type of magnetic particle coating and chemistry used, and the separation efficiency is a function of the separation system. Methods to improve separation efficiency by using advanced magnetic bead separation systems are discussed in chapter 6.

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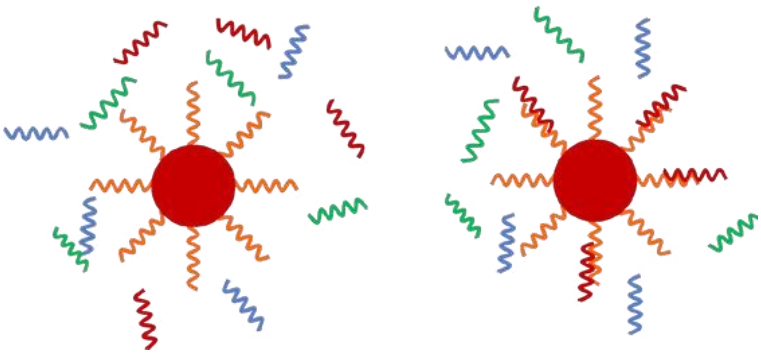
Chapter 4. Magnetic particle coating and surface chemistry for DNA purification



The coating and surface chemistry of magnetic beads governs the binding efficiency of target DNA or RNA. Superparamagnetic beads for life science applications come in two general forms: core-shell type and embedded type. The core-shell synthesis method produces beads composed of a single superparamagnetic core with a polymer or silica surface coating. One example is a bead composed of a magnetite core surrounded with a dextran shell. Other beads of the core-shell type are composed of a polystyrene or polyvinyl alcohol (PVA) core surrounded by superparamagnetic particles and protected by a surface coating. Sometimes these core-shell beads can have multiple layers of superparamagnetic particles alternating with encapsulation material. An alternative method, called embedding, produces superparamagnetic beads composed of a monodisperse matrix such as polystyrene, agarose or sepharose impregnated with multiple 10 nm iron-oxide nanoparticles (magnetic pigment). These beads are typically hundreds of nanometers in diameter and are sealed with a material that prevents loss of the magnetic pigment.

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Regardless of bead type, synthesis method, or core composition, the surfaces of the superparamagnetic beads are chemically modified to allow adsorption of nucleic acids to their surfaces. Any nucleic acid adsorption chemistry is ideally reversible to allow removal of magnetic particles following the extraction procedure. This is necessary because the presence of magnetic particles in the nucleic acid isolate could interfere with downstream qPCR or sequencing methods.

Reversible nucleic acid adsorption systems

a. silica

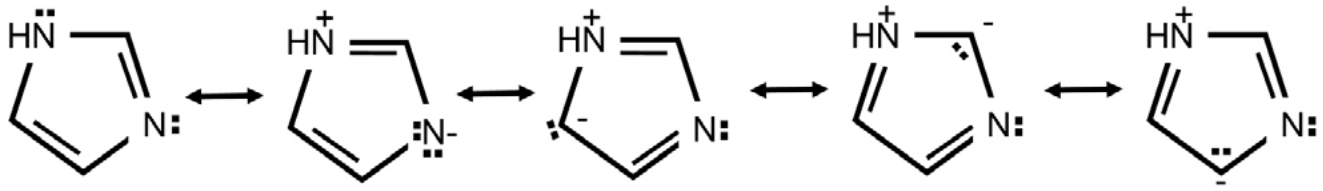
Silica-coated magnetic particles have a reversible binding affinity that is dependent on salt concentration. The DNA binds under high-salt conditions, and is released under low-salt conditions. The mechanics of DNA adsorption to silica is not immediately obvious. From a purely electrostatic viewpoint, DNA should be repelled from silica because they both have negative surface charge. The DNA and the silica each have a Debye double layer. The Debye double layer represents the distance that it takes for the electrostatic potential to decay. It is a function of ionic concentration: the higher the salt concentration, the thinner the double-layer. The idea is that DNA and silica can move closer together in high salt solutions and attractive Van der Waals forces overcome repulsive electrostatic forces. Also, lowering the pH decreases the negative charge density on the surface of the silica, which may reduce the repulsive force between DNA and silica. Following magnetic separation the nucleic acids are released under low ionic conditions.

b. carboxylated surface

Most paramagnetic beads can be functionalized with a surface coating of carboxyl groups. DNA reversibly binds to the surface of these beads in the presence or absence of polyethylene glycol (PEG) in varying salt concentrations. In a similar mechanism to that of silica coatings, the concentration of PEG and salt is very important to the success of this strategy; the electrostatic potential must be overcome to allow attractive Van der Waals forces to prevail.

c. amine and imidazole moieties

Amine groups and imidazole molecules are sensitive to changes in pH. They have a surface charge of zero in neutral solution, but are positive in acidic solution and negative in basic solution. The negatively charged nucleic acid adsorbs to the positively charged amine or imidazole moiety in acidic solution. After magnetic separation the DNA or RNA can be recovered by increasing the pH to make the solution more basic. Desorption of DNA from the imidazole group is most efficient at 80°C, which indicates that other binding strategies are involved other than only electrostatic attraction and repulsion. Amine modification is typically used in conjunction with silica coatings. One magnetic particle commonly used for nucleic acid purification is an amino-modified silica-coated magnetic nanoparticle (ASMNP). Recent research explained in more detail in chapter 7 suggests that the imidazole modification may perform better at reversible adsorption of DNA than ASMNPs.



imidazole

Overall, the reversible DNA adsorption chemistry on the surface of superparamagnetic beads is simple and highly reliant on environmental conditions. The silica, -COOH and -NH2 functional group chemistry is well-understood and easily manipulated by ionic strength, pH, and temperature. This allows for a high degree of control over DNA adsorption and desorption from the bead surface.

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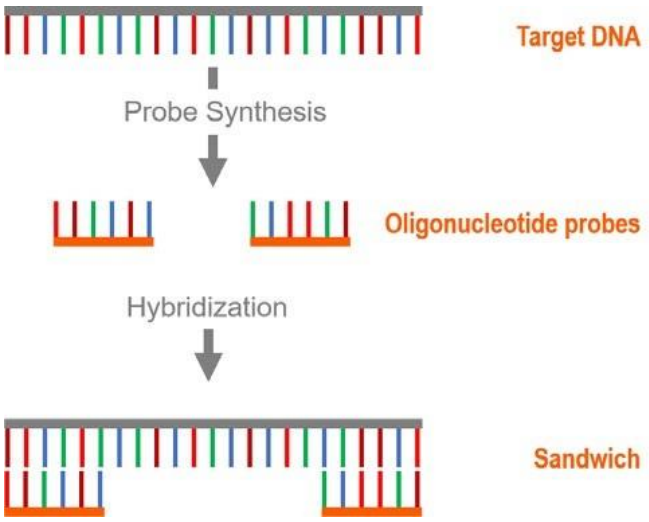
Chapter 5. Specific versus non-specific capture

The reversible-binding systems mentioned in chapter 4 are examples of non-specific capture methods. They capture total DNA and RNA in a sample because they simply rely on the affinity of nucleic acids to the magnetic particle coating or functional moiety. Non-specific capture methods bind all single-strand (ssDNA) or RNA regardless of sequence. A more specific capture system is able to target specific sequences of ssDNA or RNA. This type of specific capture is most applicable to diagnostic systems as an assay for a specific pathogen. Examples in chapter 7 include systems to diagnose methicillin resistant staphyloccocus aureaus (MRSA), specific plant viruses, and infection with malaria-causing bacteria. These strategies could be applied to detect viral, bacterial, or fungal pathogens in a variety of clinical samples.

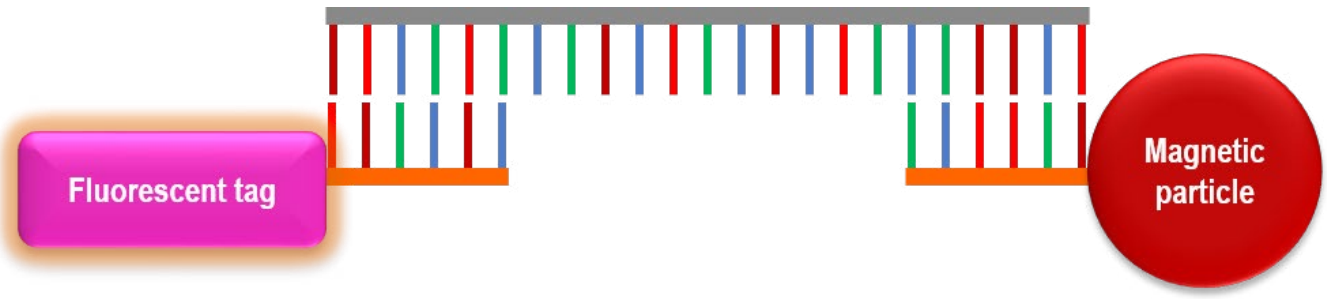
One method for specific capture of single strand nucleic acids involves the attachment of oligonucleotide probes onto the surfaces of magnetic particles. The oligonucleotide probe is fully customizable and is synthesized to be complementary to the target sequence. There are a couple of ways to attach the probes to the surface of the particles. In one system the single-strand oligonucleotides are attached with a non-cleavable triethylene glycol spacer. Another method of capturing specific ssDNA or RNA sequences relies on streptavidin-biotin affinity. Streptavidin and biotin have a very strong specific affinity for each other, and this is a common system for attaching moieties to magnetic particles. The magnetic particles are coated with streptavidin molecules and can bind biotin-tagged single-strand oligonucleotide probes during an incubation period. The biotin tags are added during the initial synthesis of the probes.

Specific nucleic acid capture is highly valuable in situations where the isolation of a specific sequence of DNA or RNA is desired. This type of magnetic particle is very useful in real-time DNA/RNA detection systems if it is combined with a fluorescent or chemiluminescent probe.

Specific nucleic acid capture is highly valuable in situations where the isolation of a specific sequence of DNA or RNA is desired. This type of magnetic particle is very useful in real-time DNA/RNA detection systems if it is combined with a fluorescent or chemiluminescent probe. The simultaneous binding of an oligonucleotide probe and a detection probe to a target ssDNA or RNA strand is called a sandwich hybridization immunoassay.



Sandwich hybridization is a commonly used method in specific ssDNA or RNA capture for diagnostic purposes. Sandwich hybridization involves two oligonucleotide probes that bind to either end of the target ssDNA or RNA. One probe is synthesized complementary to a section of the 5' to 3' end, and the other is complementary to a section of the 3' to 5' end. One probe is conjugated to a magnetic particle. The other probe is conjugated to a diagnostic molecule. When the target ssDNA or RNA is present, the two probes bind to it and form a single complex.



Sandwich hybridization provides a real-time quantitative or qualitative read-out of capture efficiency. The diagnostic molecule depends on whether the read-out is based on a system using fluorescent probes and quantum dots for simple fluorescent signal readout, a more sophisticated fluorescence resonance energy transfer (FRET), molecules for surface-enhanced Raman spectroscopy (SERS), or molecules for a chemiluminescent reaction. A chemiluminescent reaction is a chemical reaction that produces a high-energy intermediate that emits a photon of light as it reacts to its final product. The ability to specifically bind a target sequence of ssDNA or RNA is powerful for identifying the presence or absence of a pathogen. When it is combined with a light-emitting probe this technology becomes extremely valuable as a point-of-care diagnostic tool.

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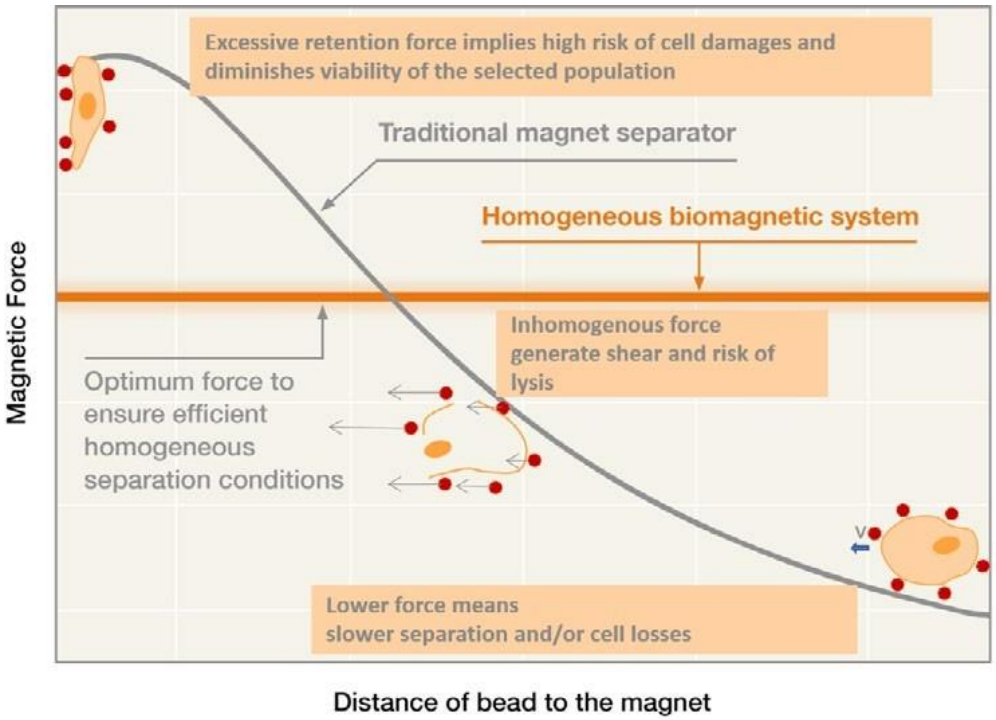
Chapter 6. Advanced Magnetic Bead Separation Systemsfor DNA purification



The traditional magnetic bead separation is a permanent magnet block. The test tube is placed next to the magnet and the magnetic particles in solution move toward the magnet. This system works for very small volumes, but it is not the most efficient method and problems often arise in larger volumes. The downfall of this geometry is that the permanent magnet is only on one side of the tube, which means that the magnetic particles are only drawn to that one side. The magnetic particles close to the mag- net will experience a higher force than the magnetic particles farthest away from the magnet. Magnetic force decreases as distance from the magnet increases, so the particles farthest away might not feel any force if the magnetic strength is not great enough.

One solution is to find a bigger magnet. As the magnetic force decreases very fast with the distances, is not easy (and sometimes impossible) to allow the particle farthest away to feel a sufficient magnetic force. Even so, the particles closest to the magnet will experience an extremely large magnetic force. In cell separation protocols this can be detrimental to the cells because the high magnetic force can destroy the membrane and cause cell death. This is less of a concern in DNA purification because there are no live cells to worry about, but the separation efficiency is still compromised by the irreversible aggregation of magnetic particles closest to the retention area. Additionally, the separation time can be painfully long due to uneven forces throughout the working volume.

Advanced magnetic bead separation systems are engineered to produce a constant magnetic force throughout the working volume. This means that the magnetic force felt by a magnetic particle does not vary greatly with location. Ideally, all particles within the sample will experience a comparable magnetic force. This translates to faster separation times and greater separation efficiency because all of the particles will experience an optimal magnetic force. All particles are captured within a comparable time-

scale because there are no particles left behind, and irreversible aggregation of particles is avoided since it is not necessary to increase retention force to capture the farthest beads. These advanced bio-magnetic separation systems are carefully designed with optimized geometry, magnetic field strength, shape, and gradient. They are also equipped with real-time quantitative software that provides optical monitoring of the separation process. This software provides the additional benefit of standard curves to compare separation efficiency across experiments, which is critical when optimizing a new separation protocol. Also, this added feature takes the gues-swork out of the separation process. It is easy to see when the separation is complete, and a typical separation requires only a few short minutes.

These advanced magnetic bead separation systems are carefully designed with optimized geometry, magnetic field strength, shape, and gradient. They are also equipped with real-time quantitative software that provides optical monitoring of the separation process.

Advanced magnetic bead separation systems are particularly beneficial for nucleic acid isolation because of their short separation times and high yield. Short separation times are important when analyzing a large number of samples. Higher yield is very desirable, especially when isolating DNA or RNA from a rare cell type and a minimum concentration of nucleic acid must extracted for use in qPCR or sequencing.

It is important to remember that the magnetic forces play a fundamental role in the magnetic separation process. When faced with difficulties in their separation strategy many people might first think that their choice of magnetic particle, coating, or functionalization chemistry is at fault. However, the root of long separation times and poor yield often lies in a poorly designed separation system. An advanced magnetic separation system can save frustration, time, and money.

Chapter 7. Magnetic DNA purification in recent research

Superparamagnetic particles have been used in recent research for specific and nonspecific capture of DNA and RNA. Discussed here are examples of successful isolation strategies using silica-coated magnetic particles, imidazole moieties, amine surface moieties, oligonucleotide probes, and sandwich hybridization.

Silica-coated magnetic particles (SMPs) have been used to detect plant virus RNA.5 The SMPs adsorbed total nucleic acid from lily and grapevine leaves in the presence of guanidinium thiocyanate. The captured viral RNA was evaluated by qPCR and compared to two traditional extraction methods: a commercial column-based kit and a Trizol extraction. The silica-coated magnetic particle nucleic acid extraction was shown to be comparable to the two traditional methods. The SMP method was able to extract nucleic acid in quantities sufficient to identify the presence of virus RNA in plant samples.

Amino-modified silica-coated magnetic nanoparticles (ASMNPs) can improve DNA adsorption efficiency by introducing an electrostatic attraction between the positively charged amine groups and the negatively charged phosphate groups on the DNA backbone. One study has shown that the conformation of the DNA in solution can affect its adsorption efficiency to the surface of ASMNPs.3 A globular conformation is more efficient than an elongated coil. If the DNA is elongated it is able to wrap around the surface of the particle and block multiple binding sites. This prevents other DNA molecules from binding the same particle. However, if the ionic strength of the solution is increased, the DNA compacts into a globular shape and more DNA molecules can adsorb to a single ASMNP. The adsorption efficiency increased with increasing NaCl concentration to a maximum at 1.0M NaCl, and decreased slightly at higher ionic strength.

Imidazole moieties on the surface of magnetic nanoparticles have been used in conjunction with magnetic separation to capture genomic DNA from a single bacterial cell.1 This procedure requires careful attention to pH and temperature. The DNA adsorbed to the particles in acidic solution, and following magnetic separation it was released into basic solution. The ability to successfully isolate target DNA from a single bacteria cell 8 out of 10 times is very impressive. Many magnetic-particle based systems fail at such low concentrations of DNA. Typical silica-coated and amine modified magnetic particles are very good at isolating DNA from solutions containing 109 bacterial genomes and fail at 1-103 genomes.

DNA from a variety of species has been identified by specific magnetic purification using streptavidin-coated magnetic beads and biotinylated probes.4 Each probe is designed to have a sequence complementary to the target ssDNA with a biotin tag on the 3' end. The probes are incubated with ssDNA to allow hybridization. Then, the streptavidin-coated magnetic beads are treated with a blocking step to prevent nonspecific binding of DNA before being added to the solution containing hybridized probe-target DNA. Streptavidin and biotin have a high affinity, which allows the target DNA to conjugate to the magnetic beads via the biotinylated probes. The target DNA is recovered by magnetic separation followed by increasing the temperature to denature the hydrogen bonds between the target DNA and the oligonucleotide probes. This technique was used to specifically capture DNA from a handful of species including the Indiana bat, trumpeter swan, white-tailed kite, Bliss Rapid snail, and broad-tailed hummingbird. It could easily be adapted for specific capture of viral, fungal, and bacterial pathogens.

A specific DNA capture method has been used to extract Plasmodium faciparum DNA, the bacterium responsible for causing Malaria.2 The method used sandwich hybridization and surface-enhanced Raman spectroscopy (SERS) to provide a real-time readout of DNA capture. This system allowed for rapid diagnosis of Malaria by measuring the presence or absence of Plasmodium faciparum DNA in a serum sample. SERS is a non-fluorescent method of identifying molecules attached to metallic surfaces. Raman scattering is a type of spectroscopy that measures information about the vibrational modes of molecules. A laser shines light onto the molecule, some light passes through, but some light is scattered. The Raman spectra are specific to each molecule. The molecule used in this study was called a nanorattle, and it was attached to a oligonucleotide probe for sandwich hybridization. When the Plasmodium faciparum DNA is present in the serum, a sandwich of magnetic-particle, target DNA, nanorattle is made. This is magnetically separated and measured by SERS. The characteristic signal of the nanorattle will indicate the presence of Malaria in the serum.

Chapter 8. Future of Magnetic DNA purification

The future of magnetic DNA purification is automated in large high-throughput machines, integrated onto a miniature lab-on-a-chip device for point-of-care diagnostics, or a mixture of both. Most importantly, magnetic DNA purification enables cleaner protocols that don't require large chunks of a laboratory technician's time to be spent moving columns into and out of a centrifuge. Magnetic separation and superparamagnetic beads allow the entire purification process to occur in one tube, which opens the door to high-throughput 96-well runs or to compact point-of-care rapid detection systems.

The vision of fully automated magnetic DNA purification systems is already being realized. Automated DNA extraction devices are currently on the market, and more are sure to be modified throughout the next decade. Many of the current machines use 96-well plates, so they offer an ability to analyze many samples at once, introducing a level of high-throughput in DNA purification that column-based methods simply cannot achieve. One downfall of these devices is their size and cost. These machines are not ideal for resource-poor settings or for small laboratories, but they are excellent for quality control companies or large clinical laboratories that must analyze many samples as quickly as possible.

The age of nanotechnology has brought with it the ability to design and manufacture lab-on-a-chip devices. These centimeter-sized chips are patterned with nano- or micro-fluidic channels that direct the laminar flow of liquid solutions. A user needs only to place a drop of the sample solution onto the chip and it will move through a series of coordinated reaction wells. Some of these systems use external magnets to direct the flow of superparamagnetic particles. The chips can also use fluorescent or chemiluminescent reactions to produce qualitative or quantitative readouts. These systems are portable, rapid, and relatively inexpensive so they are ideal for small laboratories or low-resource settings. These devices are also the key to creating point-of-care diagnostics that could potentially eliminate the need to transport clinical samples to and from full laboratories.

An example of magnetic microfluidic system is one developed for the rapid detection of methicillin-resistant Staphylococcus aureus (MRSA).6 Conventional assays for the presence of MRSA rely on time-consuming culture methods. This microfluidic system is much more rapid. It relies on magnetic particles coated with oligonucleotide probes with specific sequences complementary to MRSA DNA. These probe-conjugated magnetic particles and the clinical sample are loaded into the main chamber. Washing buffer and loop-mediated isothermal amplification (LAMP) reagents are placed into separate chambers that are connected to the main chamber by microfluidic channels. The MRSA bacteria in the clinical sample is lysed to release its DNA. The DNA binds to the oligonucleotide probes, which are bound to the magnetic particles. A permanent magnet is placed underneath the main chamber and all contaminating liquid is vacuum-pumped into a waste chamber. The captured DNA is then washed and reacted with the LAMP reagents all via microfluidic channels on the chip. The DNA undergoes isothermal amplification for 40 minutes and the results are read with a spectrophotometer. From clinical sample to diagnostic result this MRSA assay takes less than one hour and all takes place on one compact chip. There is no centrifuge required, no qPCR machine, no harsh chemicals, no tedious washing steps, and no expensive columns. The future of lab-on-a-chip diagnostic assays is magnetic and miniature.

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Conclusion

The extraction and purification of DNA and RNA is fundamental to modern research. Our desire to obtain large yields of high quality DNA has spurred rapid innovation in extraction technology. Magnetic nanoparticles and magnetic separation are at the forefront of this movement. Magnetic particles introduce a level of specificity to DNA extraction that simply cannot be achieved by traditional chemical and column-based methods. Additionally, magnet separation is rapid and suitable for automation and miniaturization. Magnetic separation is allowing DNA and RNA purification to escape the confines of the laboratory and move into the clinic. Lab-on-a-chip devices are revolutionizing the diagnosis of infection and disease.

Designing a magnetic DNA purification protocol can seem overwhelming. It is tempting to fixate on choosing a magnetic particle coating and functionalization strategy. However, it is critical to give equal thought towards choosing a magnetic separation system. A magnetic particle that adsorbs DNA perfectly will be useless without a well-engineered separation system. Advanced magnetic bead separation systems can be used to optimize a separation protocol in order to achieve rapid and consistent purification of DNA and RNA.

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