Optimizing Influenza Genome Sequencing with Magnetic Beads for DNA Size Selection

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Objective

- Optimize the viral sequencing process by altering individual steps of the overall procedure to be more efficient and accurate.
- Create a solid, successful protocol for size selection using magnetic beads that can be applied to a wide range of viruses and other samples.

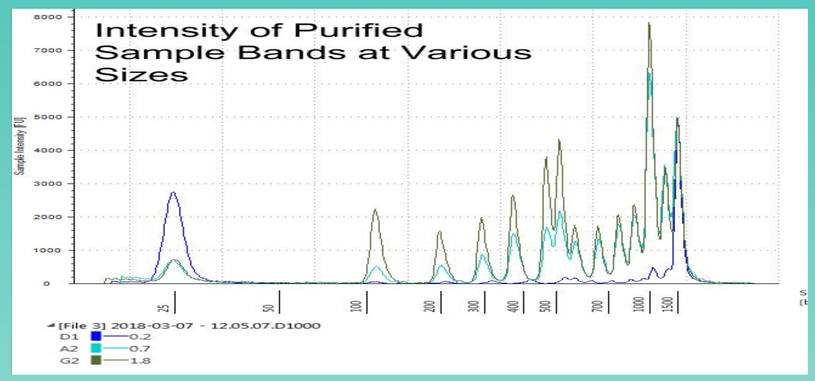
Introduction

Viral sequencing is an important component in the field of vaccine development because it allows researchers to classify clinical samples and determine what strains are prevalent and should potentially be used in the next round of vaccinations. One of the most important parts of viral sequencing is proper sample preparation in order to get accurate reads from the sequencer. In order to get enough genetic material to read, sequencing requires a method called Polymerase chain reaction (PCR), which makes multiple copies of a strand of DNA. Samples must be purified after PCR before any quantification of DNA concentration can be measured. One of the best methods for this PCR cleanup is magnetic bead purification, which uses magnetic beads that have the ability to bind with DNA that can pellet on a magnet to allow for easy DNA isolation and removal of excess materials. When these beads are used correctly, little to no DNA is lost from the sample, making it an ideal method. We have explored a new use for these beads, which is size selection of DNA. By changing the dilution ratio of the beads to DNA in a sample, we have been able to select which sizes of DNA fragments we get after purifications, from all sizes to only longer segments. This can be useful in speeding up sequencing by eliminating external steps to isolate particular sized fragments from viral genomes, such as influenza, which is comprised of 8 different sized segments with unique functions, for sequencing purposes.

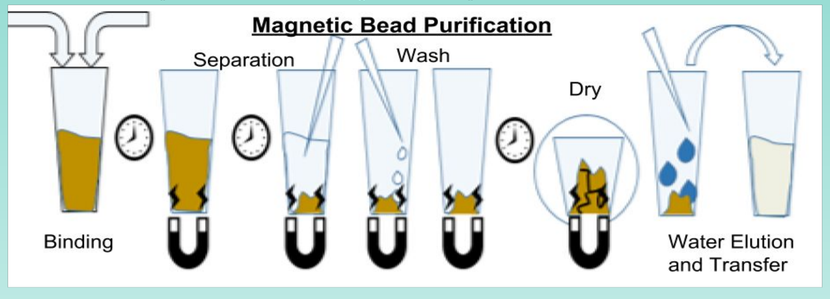
Size Selection Method

The magnetic beads used in this purification method are a ferrofluid, which means they are individually suspended in liquid reagents and will not clump together unless they are put on a magnet. One of these reagents, polyethylene glycol (PEG), is able to push DNA (which is negatively charged) onto the surface of the beads (which are covered with carboxyl molecules) and forces the two to bind. In a typical use of magnetic purification for DNA sample preparation, a 1.8 dilution is used (90ul beads:50ul DNA). Generally, this standard concentration is designed to remove fragments of DNA that are smaller than 100bp. Since viruses and other samples can have various sizes of DNA fragments, for this experiment we used a known 1000 base pair ladder that, when undisturbed, should have bands of DNA fragments at particular sizes, such as 100bp, 200bp, 500bp, etc so we could guarantee that certain sizes were originally in the sample and compare it to the base pair sizes that resulted after the purification. Various dilutions of beads were used to purify the samples, from 0.1 to 1.8, and there was a positive control of unpurified ladder and a negative control of just beads. After purification and quantification to ensure presence of DNA, sample are run on a TapeStation, which separates DNA fragments by size and charge, and electronically displays each base pair size in a graph. Ideally, we are looking for a loss of fragments at lower dilutions, which would mean the 1.8 graph should have many peaks at all base pair lengths, while the 0.1 and 0.2 should only have intense peaks at the start and end of the graph. Most viruses are split into various segments that are different lengths and sizes, so this size selection could be a beneficial method to isolate particular regions of a viral genome that are one size and exclude the other, smaller segments.

DNA Ladder Graph



Dilutions of 0.2, 0.7 and 1.8 are depicted in this graph. Based on the peak comparison, the short fragments of DNA decreased at the lower concentrations of beads. The 1.8 sample had intense peaks all the way from 100bp to 1000bp. The 0.7 sample had similar sizes represented, but with less intensity in the peaks.This gradual increase in intense peaks indicates a loss of fragments at each particular size.



Magnetic Purification Process

1. A known 1000 base pair ladder is used to model a sample with a range of sizes. (Viruses have a range of DNA fragments sizes.)
2. Magnetic ferrofluid and 50ul of 1000bp ladder are mixed and left for 5 minutes to bind.
3. Various dilutions of beads were used from 0.1 to 1.8
4. A strong magnet pulls magnetic beads with bound DNA into a pellet
5. Remaining liquid supernatant removed
6. Pellet washed with ethanol twice to precipitate DNA bound to beads
7. Ethanol removed and sample left on magnet to dry
8. Water resuspends DNA. Beads separate in the pellet.
9. Results compared to positive control of unpurified ladder and negative control of just beads.
10. DNA Quantified by Qubit, which uses a dye that fluoresces in the presence of DNA to detect the concentration of DNA in the tube
11. DNA sample are run on TapeStation, which separates DNA fragments by size and charge. A more intense peak on the graph means there are more fragments of that size in the sample.

Results and Conclusion

As shown in this graph depicting the intensity of purified DNA ladder sample bands at various dilutions, the short fragments of DNA were decreased with a low dilution of beads in comparison to the higher concentrations of beads. The sample of 1.8 concentration is what the manufacturer of the beads suggests be used, and the peaks starting at 100bp and increasing at all the other sizes is true to the advertised sensitivity of the beads. The medium dilutions had similar sizes represented, but the intensity of their peaks were all significantly lower, indicating a loss in quantity of fragments at each particular size of base pairs. The stark contrast between the highest dilution and the lower dilution of 0.2 was the most promising, with there being almost zero peaks for the lowest dilution except for the larger base pair sizes. This gradual increase shown strongly indicates that this method of size selection using magnetic beads in a typical purification setting was successful. With this new application of magnetic beads to isolate different segments of DNA during the purification process, the next step is substituting the 1000bp DNA ladder that was used as a constant in the experiment with actual viral samples that have been collected. This size selection method is important in optimizing viral sequencing because it essentially takes away a separate step that would need to be done in order to isolate certain parts of the viral genomeand combines it with the purification process that already needs to be done. For example, the influenza genome is comprised of 8 different segments with different functions, so this method could potentially be applied to isolate specific lengths of the genome that contain one of these particular segments and cut out the smaller fragments.

Biotechnology Applications

Viral sequencing is a very useful application of biotechnology because it allows researchers to view the genomic makeup of a virus and classify it against others in order to find potential strain mutations and to make decisions regarding future vaccinations. Many important traits of viruses are found on the proteins that coat their surfaces, such as hemagglutinin and neuraminidase on influenza viruses, so when one mutates, it can completely change traits of the virus such as the degree of infection it can provide or the timeline of infection, so being able to map them out and detect mutations from previous strains via sequencing is beneficial to the world of biotechnology and health care. In combination with the GenBank and bioinformatics, viral sequencing has the capability to link various unknown strains of viruses from direct clinical samples to the other known sequences in the world. Overall, using sequencing as a biotechnology application to improve the vaccine development process has the capability to improve the lives of many and help make vaccines more accessible to the public.

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