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Complex molecular interactions involved in binding of fragmented nucleic acid molecules on magnetic nanoparticles for next generation sequencing applications

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Magnetic fluids or ferrofluids are stable colloidal suspension of magnetic nanoparticles in a carrier liquid that can be aqueous or oil-based. Magnetic nanoparticles have generated considerable research and commercial interest due to their unique properties and wide applicability. One of the key applications for using these nanoparticles is in the field of Genomics, particularly Next Generation Sequencing (NGS).
In the NGS workflow, an important step is selection of fragmented DNA which is then read using a sequencing platform like IlluminaTM or NanoporeTM. The principle behind size selection is that DNA molecules of differing molecular weight can be selectively precipitated using
condensing agents like Polyethylene glycol (PEG) in the presence of a salt like NaCl. The binding of DNA to the beads is dependent on the concentration of the PEG and hence the ratio of the beads to the DNA remains critical. Higher volume of the binding mix helps to
capture lower base pair fragments while lower volume captures higher base pair fragments.
Typically, magnetic nanoparticles without any coating do not offer high DNA recovery rate and therefore to compensate it, pH of the binding system should be maintained at high alkaline conditions. The nanoparticles in such alkaline conditions gains more negative charge on its surface and DNA being negatively charged, with the help of monovalent cation like Na+ serving as a bridge between the DNA and the beads while PEG enables in condensing the DNA from coil to globule shape. Thus, the DNA fragments get selectively precipitated and adsorbed to the surface of the uncoated nanoparticles. A typical profile of size selected DNA
is shown below in Figure 1. We also optimized the volume of the binding buffer required for sequential capture of DNA fragments of varying sizes.
Several sophisticated techniques are involved in this workflow for Biophysical and Biochemical Characterization of uncoated nanoparticles and later to assess the binding chemistry between these nanoparticles and fragmented DNA. Some of these as listed below:
Characterization of magnetic nanoparticles
1. Thermogravimetric analyzer (TGA) and Diffuse Scanning Calorimetry (DSC) (makeMettler-Toledo, model TGA/DSC 1)
2. Fourier transform infrared spectroscopy, FTIR (Thermo Scientific NICOLET FTIR 6700 FTIR)
3. High Resolution Transmission Electron Microscopy (HRTEM) (JEOL JEM 2100 200-
kV transmission electron) microscope (TEM) with a point resolution of 50X to 1.5MX
Assay of fragmentation of DNA and binding of DNA to Magnetic Nanoparticles
1. UV Spectrophotometer (Thermofisher Scientific NanoDrop™ 2000/2000c
Spectrophotometer)
2. Tapestation (Agilent, 4200 TapeStation System)
Sequencing platforms
1. Short read sequencer (Illumina MiSeq)
2. Long read sequencer (Nanopore, Oxford Nanopore)

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