Nucleic Acid Isolation Using Paramagnetic Particles
Brent Bishop & Craig Conder
InnovaBio, Utah Valley University

INTRODUCTION

MagneSil paramagnetic particles, created by Promega Corporation offer an alternative method for extraction of both plasmid and genomic DNA from all cell types. The paramagnetic particles are made of magnetite (Fe₃O₄), and covered with a thin layer of silica. DNA has a natural affinity for silica which allows it to easily bind to the surface of the beads. DNA purification starts with lysing the cells, freeing the DNA from inside the cell. A majority of larger proteins and other cellular components are then removed by centrifugation. MagneSil paramagnetic particles are then added and the cell’s DNA binds to the beads. The central magnetite core makes it possible for the particles to readily come out of solution when placed in a magnetic field, separating the DNA from the rest of the cell debris. After the DNA has been captured and separated from the unwanted cell content, the particles are washed with an elution buffer to effectively separate the DNA from the silica beads so it can be collected and analyzed.

PURPOSE

- Prepare necessary buffers for plasmid DNA isolation with MagneSil paramagnetic particles based on published literature.
- Optimize a protocol to purify plasmid DNA using MagneSil particles that can be compared to QiAgen’s mini prep method.
- Optimize the protocol for use with the Magnabot 96 well plate holder and 0.7ml microcentrifuge tubes.
- Compare and contrast QiAgen mini prep buffers and homemade MagneSil buffers.
- Optimize a protocol for isolation of genomic DNA using MagneSil paramagnetic particles with pre-made genomic DNA MagneSil buffers.

MATERIALS AND METHODS


Methods. E. coli DH5α cells transformed with pET32a plasmid were cultured in 1, 2, or 5ml LB broth overnight. Varying volumes of QiAgen mini prep buffers; P1, P2, P3, and N3, and homemade MagneSil resuspension, lysis, and neutralization buffers were added to individual cultures. Different volumes of MagneSil beads were added to collected supernatants and mixed by vortexing. Magnabot 96 plates were used to pellet the beads and the supernatant discarded. Beads were washed twice with 75 – 95% ethanol of varying volumes and then dried for different amounts of time. DNA was eluted from the beads in different volumes of QiAgen’s or homemade MagneSil EB buffers. Eluted DNA was analyzed by spectrophotometry, restriction digest and gel electrophoresis.

Genomic trials were performed with NIH 3T3 mouse cells and human cheek cells from several participants. Collected cells were pelleted and mixed with MagneSil’s Blood Lysis buffer and different volumes of beads. Beads were then treated with a series of salt washes followed by alcohol washes. After drying, samples were heated to 80°C and homemade EB added. DNA was analyzed as mentioned above.

RESULTS

First experiment comparing MagneSil beads to QiAgen’s mini prep spin column for plasmid purification.

RESULTS CONTINUED

MagneSil vs. QiAgen after optimization of MagneSil plasmid purification protocol.

Genomic DNA purification using MagneSil beads.

CONCLUSIONS

MagneSil paramagnetic particles is a viable way to extract plasmid and/or genomic DNA from cells. The protocol has been optimized for use in the InnovaBio lab with lab-made reagents which gives comparable yields to other methods previously used in the lab. Extracted DNA can then be used for additional experiments such as PCR, transformations, or sequencing.

REFERENCES