Introduction
The post genomic era has seen a rapid growth in the need for high-throughput systems for isolation and purification of excellent quality plasmid DNA from bacterial cultures. There are several alternatives for high-throughput purification of plasmid DNA, such as magnetic beads, 96-well silica plates or individual silica columns connected via vacuum. Our method of choice is magnetic particle, due to the surface area of magnetic particles allowing a higher yield of DNA to be purified in an automated fashion, increasing reproducibility and reducing errors.

Key Words
• Key words – Nucleic acid purification, plasmid, automation, magnetic bead, microbial culture

Goal
Efficient plasmid DNA purification from bacterial cultures is key to many molecular-based experiments. This application note describes an automated process for plasmid DNA purification from bacterial cultures. The method integrates the use of the Thermo Scientific™ KingFisher™ Pure Plasmid Kit and the KingFisher Flex platform for efficient purification of plasmids of various sizes. The system was also used to purify plasmids containing recombinant antibody sequences for sequence identification.

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The KingFisher Pure Plasmid Kit coupled with the KingFisher Flex magnetic particle processor provides an efficient system for high-throughput plasmid DNA purification from bacterial cultures. The KingFisher Flex allows one to use a flexible number of samples with customizable input culture volumes. The system provides a rapid purification process per sample with the ability to conduct 96 parallel extractions in a single run. The kit is also optimized to provide the highest quality and yield without the use of organic solvents.

Here we describe the successful purification and analysis of plasmid DNA of various sizes using the KingFisher Pure Plasmid Kit on the KingFisher Flex instrument, which allows rapid purification of different plasmids with high purity. The method was also adapted to purify antibody sequences containing plasmids for DNA sequencing.
Materials and Methods
The E. coli suspension was cultured overnight and 5 ml of the culture was centrifuged to pellet bacteria. The KingFisher Pure Plasmid Kit was used to isolate plasmid DNA. The bacterial cell pellet was re-suspended in Resuspension Solution containing RNase A and lysed in the Lysis Buffer. After mixing, Neutralization Solution was added and white precipitate containing chromosomal DNA and cell debris was formed. The solution was then centrifuged to remove the precipitate and clear lysate was transferred to Thermo Scientific Microtiter deep well 96 plate. After that, 25 μl of KingFisher Magnetic Beads and 250 μl of isopropanol were added into clear bacterial cell lysate. DNA binding followed by four consecutive washing steps was carried out on a KingFisher Flex automation system. DNA was eluted in 50 μl according to manufacturer’s protocol. The purified plasmid was used for DNA sequencing analysis. DNA sequencing was carried out by a third party service provider using BigDye Terminator v3.1 cycle sequencing kit (Life Technologies). Plasmid DNA isolation with spin columns was performed according to the kit instructions.

Results and Discussion
When conducting high-throughput DNA purification, reproducibility, quality and yield are important factors to ensure good quality sequencing reads. Here we examined the quality of the KingFisher Pure Plasmid Kit in comparison with a commercial spin column kit. Gel electrophoresis analysis shows high quality and yield of two plasmids of varying sizes using the KingFisher kit (Figure 1).

DNA yield and purity were determined using a Thermo Scientific NanoDrop 2000 spectrophotometer. The results show good yields for both plasmids at 20 to 25 μg for KingFisher Pure Plasmid Kit and 5 to 7.5 μg of DNA from the spin columns (Figure 2). The A260/A280 ratios for all samples were approximately 1.9 using both methods.

Figure 2: Comparison of the plasmid purification yield by using a KingFisher Pure Plasmid Kit and a competitor’s spin columns.

Figure 3 shows the plasmid purification of two different antibody clones with the KingFisher Pure Plasmid Kit. The purified plasmids were sequenced and the sequencing reads were accurate and of good quality regardless of the plasmid size.

Conclusions
In this study, we have shown that good yields and purity of plasmid DNA can be obtained using the KingFisher Pure Plasmid Kit in comparison to a conventional spin column. The KingFisher kit is applicable for varying sizes of plasmids with equal efficiency. In conclusion, the KingFisher Pure Plasmid Kit with the KingFisher Flex system enables rapid, high-throughput isolation of high quality plasmid DNA from bacterial cultures.

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