

Automated sequencing clean up: high throughput dye terminator removal using Agencourt® Bioscience's CleanSEQ®

Application

The removal of excess dye terminators and other impurities from completed cycle sequencing reactions is critical for high quality sequence data. Although there are a number of methods available (e.g. Ethanol precipitation, Sephadex® filtration) many of them are laborious and time consuming, which is not ideal for a high throughput facility. Recently the Canadian Centre for DNA Barcoding (CCDB) switched to Agencourt® CleanSEQ®, a magnetic bead based solution which offers fast automated processing, while generating high quality sequence reads.

Method Overview

Agencourt® CleanSEQ® is a magnetic bead-based sequencing purification system incorporating SPRI® technology (Solid Phase Reversible Immobilization). Sequencing products are bound to magnetic beads. These beads are then exposed to a strong magnetic field (via a magnetic ring) while contaminants are washed away. Clean sequencing products are then eluted from the beads and run on the ABI 3730xl DNA Analyzer. The CleanSEQ® system is flexible, simple and can be easily automated using the Biomek FX® liquid handling platform or performed manually for lower throughput laboratories. Other key advantages to the CleanSEQ® system is the ability to reduce the amount of dye terminator (BigDye® v3.1) in the cycle sequencing reaction and it is scalable to the 384 well format. The Canadian Centre for DNA Barcoding now uses a 1/24 dilution of BigDye® v3.1 (from a 1/16 dilution) and efforts are underway to scale to the 384 well format for further reduction of cost and greater throughput.

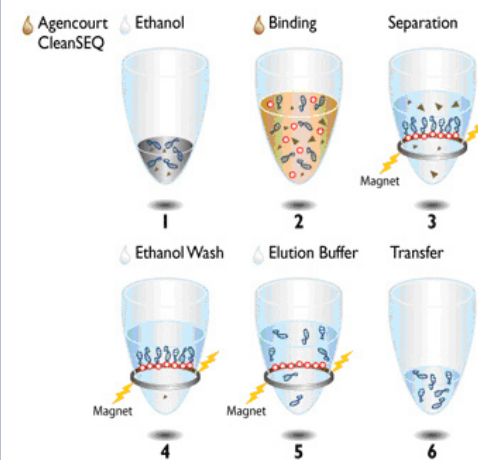
The CleanSEQ® reagent is compatible with all major sequencing platforms and chemistries including Applied Biosystems®, Beckman Coulter and GE Healthcare. Detailed protocols for each platform can be found at <http://www.agencourt.com/>

More Information:

1. Ivanova NV, Grainger C (2007). Dye terminator sequencing of COI for the 3730xl DNA Analyzer (Applied Biosystems®). Published online at <http://www.dnabarcoding.ca/>
2. Agencourt® CleanSEQ® Dye-Terminator Removal Protocol (2006). Published online at http://www.agencourt.com/products/spri_reagents/cleanseq
3. Hajibabaei M, deWaard JR, Ivanova NV et al. (2005). Critical factors for assembling a high volume of DNA barcodes. Philosophical Transactions of the Royal Society: Biological Sciences 360:1959 – 1967.

At a glance

- » Produces sequence data with longer Phred 20 read lengths and higher signal intensities.
- » Allows for greater dilution of dye terminators (BigDye® v3.1)
- » Tested on more than 50 000 samples of various taxonomic groups
- » Fully automated and high throughput; 94 samples complete in 5 mins.
- » Scalable to 384 format for further reduction of cost and higher throughput



Materials:

- a. Agencourt® CleanSEQ® Kit (50 ml). Agencourt part # 000136
- b. Agencourt® SPRIPlate® Magnetic Plate. Agencourt part # 000219