

Sanger Sequencing Methods and Data Delivery

PCR clean up

PCR clean up is performed using Exo-SAP according to manufacturer instructions.

Sequencing Reaction

Sequencing buffer and a 1:4 dilution of BigDye 3.1 are added and thermocycling performed according to ABI recommendations.

Sequencing Reaction Clean up

Removal of unincorporated sequencing reagents is performed using CleanSeq magnetic beads according to manufacturer instructions. (Agencourt)

Quality Control

To ensure the proper performance of reagents and equipment, 2 control samples are included on every plate sequenced by the Facility. Control #1 is a PCR amplified fragment of 700 bp. Control #2 (plasmid pGEM-3Zf(+)) sequenced with the _21M13 forward primer.) is approximately 1000 bases. If the controls provide quality sequence of >650 bp for #1 and >850 bp for #2 with 95% accuracy and good resolution, technical error on the part of Core Laboratory staff is ruled out and troubleshooting investigation of failed sequencing reactions can begin with examination of sample preparation and primer design.

Data Delivery

Compressed instrument output files are posted in .zip format to our secure website for retrieval by registered users of the investigator's account. After zip files are extracted using winzip or pkzip, PC users can view data using any of several free software packages such as Sequence Scanner (ABI) or Chromas. ABI no longer supports MAC format. 4Peaks (Nucleobytes) is a freeware program available for sequence analysis on MAC. Sequencher (GeneCodes Corp) can be purchased in a MAC compatible format (also available for PC).