



High Throughput Sequencing Using the Genetic Analysis System

Introduction

Since the publication of DNA's double helical structure, electrophoresis has been the standard tool used for the analysis of nucleic acids. Capillary electrophoresis (CE) technology extends the power of electrophoresis by using a format that incorporates direct detection, increased sensitivity and automation.

The Genetic Analysis System presents these unique advantages over other available systems:

- * capillary-based on-column detection allows for ease of lane tracking and maximal sensitivity
- * non-scanning detector offers a highly stable platform
- * sample integrity maintained through direct sample injection, the use of separate buffer wells for each sample, and providing individual buffer trays for each plate
- * high throughput with full automation and walk-away operation
- * user-friendly operating and analytical software

Continuing the efforts to increase the throughput while reducing the cost of current sequencing technology, the Genetic Analysis System is offered in a 24, 96 and 192 capillary format. In addition, individual sample pricing is further reduced using cost effective preparative methods which provide clean template for sequencing reactions and for later electrophoresis. Since the resulting PCR product post clean-up is ultrapure, the amount of the sequencing reaction can also be considerably reduced without loss of signal during data acquisition.

Using the Genetic Analysis System along with the prescribed sample preparation protocols, the researcher can rapidly produce high quality sequence at a low cost per sample. The accompanying software allows adaptable data analysis with the option to manually scrutinize data or to assign parameters for automatic analysis.

Sample Preparation- PCR Purification

Materials:

AMPure Binding Solution, Agencourt #000131(60ml) or #000132 (450ml)
SPRIPlate 384 Magnet, Agencourt #000222; 96 Magnet, Agencourt #000220
TE, Quality Biological #351-010-130
Thermo-fast 384 well plate, Abgene #AB-0820; 96 well skirted plate, Abgene #AB-0800

- 1.) Thoroughly mix AMPure Binding Solution by swirling and inverting until it appears homogenous.
- 2.) Add 18 ul of Binding Solution to 10 ul PCR reaction and mix by pipetting up and down 10 times.
- 3.) Incubate the PCR product with the Binding Solution for 5 minutes at room temperature.
- 4.) Place the plate onto the SPRIPlate 384 or 96 and allow beads to bind to the magnet for 10-20 minutes or until the solution appears clear.
- 5.) Aspirate 20 ul (384 format) or 23 ul (96 format) of solution from each well of the plate using a multichannel pipette and discard. A 384 well plate may be inverted on a paper towel and spun at 20 g for 15 seconds to remove any remaining waste.
- 6.) Dispense 40 ul (384 format) or 200 ul (96 format) of 70% ethanol to each well of the plate and allow to stand for 30 seconds.
- 7.) Remove ethanol by gently flicking the plate over a sink and blotting paper towels, making sure the plate remains on the magnet at all times to prevent loss of DNA.
- 8.) Repeat ethanol wash and removal.
- 9.) Remove ethanol residue using a multichannel pipette and discard, or a 384 well plate may be inverted on a paper towel and spun at 20 g for 15 seconds to remove any remaining waste.
- 10.) Keeping the plate on the magnet, allow plate to dry at room temperature for 30 minutes.
- 11.) Once the sample is dry, add 30 ul of TE to each well.
- 12.) Remove the magnet and spin plate at 20g for 20 seconds.
- 13.) Wait 10 minutes before proceeding to next step to allow beads to hydrate.
- 14.) Mix samples by pipetting up and down 10 times.
- 15.) Spin down at 20 g for 20 seconds.
- 16.) Place plate on magnet and allow solution to separate for 10-20 minutes or until the solution clears.
- 17.) Transfer 20 ul of purified product to new 384 or 96 well plate.
- 18.) Use purified product directly in subsequent sequencing steps.

Sample Preparation- Sequencing Reactions

Materials:

PE9700 thermocycler or equivalent
Big Dye V.3 terminator kit
384 well plates, PE Biosystems #4305505; 96 well plate, PGC Scientific #62-6049-03
MicroAmp clear adhesive films, Applied Biosystems #4306311
3M plastic PCR sealer, BioRad #9101707
Half-BD terminator sequencing mix, Sigma #H-1407

- 1.) Thaw appropriate amount of Big Dye (e.g. 1.6mL aliquot for two 384 well plates).
- 2.) Add 1/8 volume of Half-BD terminator sequencing mix to thawed Big Dye (i.e. 200 ul of Half-BD to 1.6mL of Big Dye). This provides extra volume for slight errors in pipette calibration.
- 3.) Add 2 ul of thawed Big Dye (plus Half-BD), 1.5 ul 5 uM primer, and 1.5 ul of purified template for each reaction in the plate.

NOTE: The amount of purified template to be added must be determined empirically, but it is suggested to use 50 ng as a general starting point.

- 4.) Cover plate with MicroAmp adhesive film, seal with 3M plate sealer, and pulse contents to bottom of the wells in centrifuge (1second @ 1000g).
- 5.) Cycle sequence as per Big Dye V.3 protocol using 35 cycles instead of 25 cycles.
- 6.) Store at -20° C or proceed to purification protocol.

Sample Preparation- Sequencing Reactions Purification

Materials:

Sodium acetate, pH 4.6, Applied Biosystems #400320

Non-denatured 100% ethanol

dH₂O

Aluminum foil adhesive tape, Costar #6570

3M Plastic PCR sealer, BioRad #9101707

0.5M EDTA, pH 8.0

HiDi formamide, Applied Biosystems #4311320

Precipitation Mixture:

<u>(volumes in ul)</u>	<u>1X</u>	<u>120X</u>	<u>440X</u>	<u>880X</u>	<u>1760X</u>
3M NaOAc, pH 4.6	0.75	90	330	660	1320
Non-denatured 100% EtOH	17.5	2100	7700	15400	30800
dH ₂ O	1.75	210	770	1540	3080

- 1.) Add 20ul of Precipitation mixture to 5ul of sequencing reactions.
- 2.) Mix by pipetting up and down 5 times.
- 3.) Cover plate with aluminum foil adhesive tape and seal with 3M PCR sealer device.
- 4.) Centrifuge plate for 1 second @ 3000g.
- 5.) Incubate at room temperature for 15 minutes.
- 6.) Centrifuge plate for 30 minutes @ 3000g.
- 7.) Decant the aqueous phase by inverting on a paper towel. Centrifuge the plate inverted on top of paper towel for 60 seconds @ 20g.
- 8.) Add 25 ul of 70% ethanol and centrifuge 5 minutes @ 3000g.
- 9.) Decant the aqueous phase by inverting on a paper towel. Centrifuge the plate inverted on top of paper towel for 60 seconds @ 20g.
- 10.) Speed vacuum plate for 7 minutes @ 35°C, or allow to air dry (approximately 15 minutes).
- 11.) Add 18ul of injection buffer (0.02mM EDTA in HiDi formamide*) and cover with aluminum adhesive tape.
- 12.) Centrifuge 1 second @ 3000g.
- 13.) Resuspend pellets by pipetting up and down 5 times (or allow to resuspend at least 1 hour @ 4°C).
- 14.) Transfer 2.5 ul of sample to a new 384 well plate for electrophoresis.
- 15.) Store samples at -20°C or proceed with electrophoresis.

* Dilute 1ul of 0.5M EDTA, pH 8.0 into 25 ml of HiDi formamide.

CheckMate Genetic Analysis Running Conditions

Wetware

	<u>Bottle 1</u>	<u>Bottle 2</u>	<u>Syringe</u>	<u>Bottle 3</u>	<u>Bottle 4</u>
	192; 96 format	192; 96 format			
<i>Purge</i> (ml/min,min)	30,15; 20,10	0,0; 0,0	12 ml	10,3	N/A
<i>Cap. Flow</i> (ml/min,min)	40,5; 25,5	50,5; 25,5	28ul/cap. *	6,6	2 ml

* Volume of matrix used to fill capillaries.

Voltage

	<u>PreRun</u>	<u>Inject</u>	<u>Current Monitoring</u>	<u>Data Acquire</u>
V (kV)	5	2	7	7
Time	6 min.	60 sec.	15 min.	150 min. (~900 bp read)

Temperature Settings

60C run with a 59C to 60C temperature ramp at injection.