Application Note

The MD Anderson SPRI® Experience: Using Agencourt® CleanSEQ® and Agencourt AMPure® in HLA Sequencing-Based Typing

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Introduction

The human leukocyte antigen (HLA) system is comprised of a group of genes essential to immune function. This group of genes encodes cell-surface antigen-presenting proteins on the outside of a cell, which identify the cell as a self cell or non-self cell. The immune system seeks out non-self cells for destruction. Matching HLA types reduce the risk of transplant rejection or an adverse immune response. Solid organ transplantation and allogeneic stem cell transplantation represent a common treatment for end-stage organ failure and several hematological and non-hematological malignancies. Matching the patient with an unrelated donor for HLA molecules significantly decreases the probability of graft rejection, exposure to any graft diseases, and transplant-related mortality. HLA type screening methods are categorized as low, medium, and high resolution, where sequencing-based typing (SBT) provides the highest resolution and is considered the gold standard of HLA typing.

The major advantages of HLA sequencing-based typing are:

1 - Able to cover HLA complexity and polymorphism

2 - Capable of detecting new, undefined alleles

3 - Easily automated for high throughput and data consistency

HLA-SBT is the method of choice at the University of Texas M.D. Anderson Cancer Research Center for screening and matching organ and tissue donors with recipients at the center.

Objectives for Streamlining HLA-SBT Workflow Using Agencourt Chemistry

With the increasing cost of BigDye[1] Terminator and the interference it presents during sequencing, the M.D. Anderson Cancer Center was seeking solutions for hastening their research and reducing the operational costs of the HLA-SBT pipeline. Incorporated BigDye Terminator decreases flexibility, can limit research to particular clean up protocols, and increases unexpected nucleotide base calls, which can be interpreted as a heterozygous base call. Un-incorporated BigDye Terminator increases testing flexibility by expanding the clean up options and decreasing the possibility of unexpected nucleotide base calls. Achieving a BigDye ratio under 1:4 in an appropriate elution buffer would result in high quality reads, high signal to noise ratios between 20-2000, low background noise, and a good 'G' signal. M.D. Anderson had three major goals for streamlining the HLA-SBT pipeline:

1 - Increase sensitivity

2 - Decrease BigDye dilution

3 - Achieve ease of use via automation

By using Agencourt chemistry, all the objectives were met.

Agencourt chemistry is based on SPRI (Solid Phase Reversible Immobilization) paramagnetic bead-based technology and is a patented purification technology developed at MIT's Whitehead Institute (Hawkins, et. al., Nucleic Acids Res. 1995 (23): 4742-4743). It is a simple and highly efficient means of magnetic-based nucleic acid isolation used extensively in the Human Genome Project. The purification process consists of immobilizing nucleic acids onto paramagnetic microparticles under specific buffer conditions. Flexibility is added to the system through modification of the binding buffer, which alters the type and size of immobilized nucleic acids. Sample contaminants are easily removed without the need for the centrifugation or filtration, thus creating an automation-friendly format.

Increasing Sensitivity

Sample Format

- 96 unpurified PCR2 products (~20 l)
  - 48 PCR from A Locus
  - 48 PCR from DR Locus

- HLA primers
  - A Locus: A2F, A2R, A3F, A3R
  - DR Locus: DR2F, DR2R

Methods: Post-PCR Clean Up Using the Agencourt AMPure System

Purified 15 µl of 20 µl of PCR product with 5 µl remaining in original plate for control and run on an agarose gel. Samples were purified on a Beckman Coulter Biomek® NXᴾ 96 Multichannel using 27 µl of Agencourt AMPure reagent for 15 µl PCR. A standard method protocol was used (10 tip mixes, 5 minute binding, 5 minute separation, 2 x 70% ethanol washes). Samples were dried for 5 minutes, eluted with 40 µl DiH2O, and 35 µl were transferred away from the beads. Equivalent volumes of unpurified and purified were run on a 2% Agarose gel to assess recovery.

Results

As shown in Figure 1, increased sensitivity was accomplished by capturing differences in chemistries, which contain differing prime ends. By using an effecient PCR clean up method, base calls will open up closest to the prime ends. This is the most important step prior to sequencing. In addition, using a better clean up method before sequencing will allow for more base calls without missing either prime end. Greater than 90% recovery was easily achieved by using the Agencourt AMPure system.

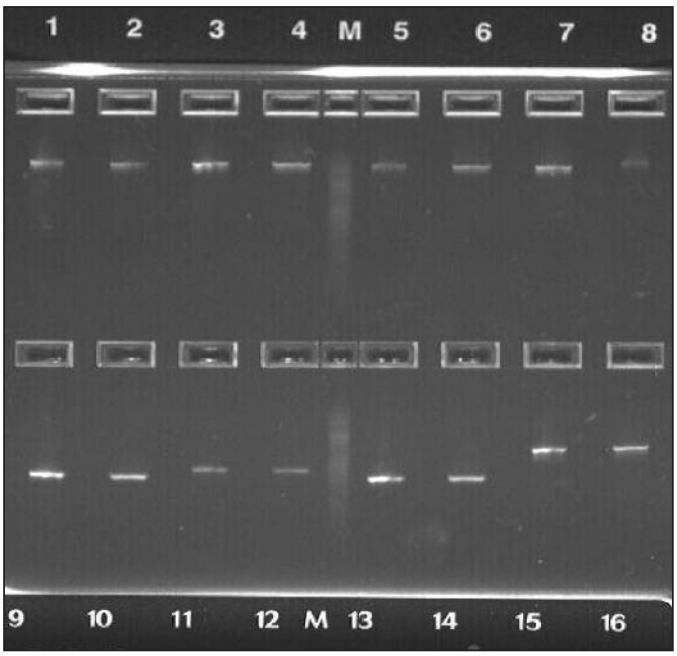

Figure 1. Post PCR clean up: High recovery using Agencourt AMPure. Lane 1: Unp A01 (S4A2), Lane 2: 5.3µl Pur A01, Lane 3: Unp A02 (S4A2), Lane 4: Pur A02, Lane 5: Unp A03 (S4A2), Lane 6: Pur A03, Lane 7: Unp A04 (S4A2), Lane 8: Pur A04, Lane 9: Unp E01 (S4R4), Lane 10: Pur E01, Lane 11: Unp E02 (S4R3), Lane 12: Pur E02, Lane 13: Unp E03 (S4R2), Lane 14: Pur E03, Lane 15: Unp E04 (S4R3), Lane 16: Pur E04.

Decreasing BigDye Dilution and Increasing Cost-Effectiveness

Methods: Pushing BigDye from a 1/4 Dilution to a 1/32 Dilution

Then 10 µl sequencing reactions were purified using Agencourt CleanSEQ chemistry on a Beckman Coulter Biomek NX 96 Multichannel automated laboratory workstation. Standard Agencourt CleanSEQ protocol was used to purify the samples (10 µl Agencourt CleanSEQ reagent, 42 µl of 85% ethanol, 3 minute separation, and two 85% ethanol washes). No drying time was used. Samples were eluted in 45 µl 0.05 mM EDTA and 35 µl were transferred away from beads for detection.

1:32 BigDye Terminator Ratio

0.25 µl BigDye Terminator v1.1

1.88 µl 5X ABI BigDye Buffer

0.87 µl DiH2O

3.00 µl Sequencing Mix

+ 2.00 µl DNA (~17-22 ng)

+ 5.00 µl Primer (Protrans)

10 µl Total Volume

Performed 40 cycles (95ºC for 10 seconds, 50ºC for 5 seconds, and 60ºC for 2 minutes 30 seconds) at 4ºC.

The samples were purified using the Agencourt CleanSEQ system on the Beckman Coulter Biomek NXᴾ workstation. Final elution and transfer occurred offline so that either 0.05 mM EDTA or DiH2O could be added. Samples were run on an ABI 3730 xl.

Results

Cost-effectiveness was achieved by decreasing the BigDye terminator ratio from 1:4 to 1:32. The decrease in dilution factor resulted in reduced operational costs while also maintaining high quality reads, high signal to noise ratios between 20-2000, low background noise (Fig. 2), and a good 'G' signal (Fig. 3). The Agencourt CleanSEQ system allows the use of less BigDye which decreases the chances of getting spurious heterozygous base calls and can also lower intensities on sequences from the ABI sequencer (Fig. 2). New spurious heterozygous peaks could indicate a new allele or erroneous heterozygous base calls.

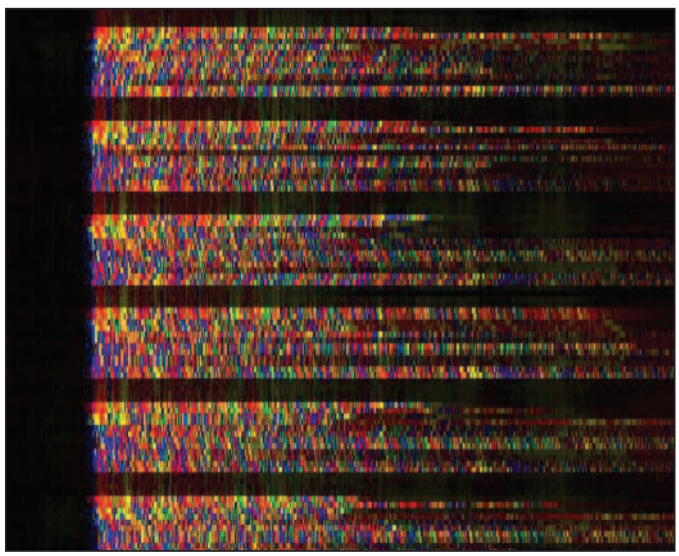

Figure 2. High quality data with Agencourt CleanSEQ: This array image is a representation of array images obtained from ABI 3730 xL.

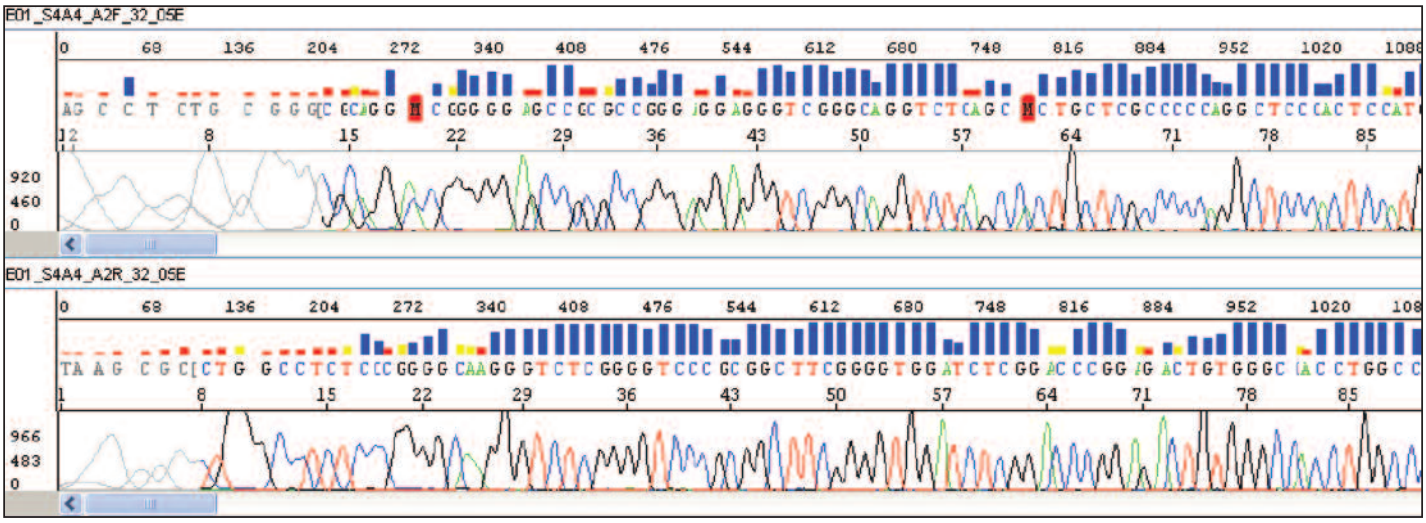

Figure 3. High sensitivity detection with 1/32 BigDye v 1.1 Dilution: 1/32 with 0.05mM EDTA shows clean baseline and good peak resolution.

	96 MC			96 PS			384 MC			96 MC-384 Quad			384 PS			Span 8		3000
Agencourt CleanSEQ®	A29218			A29192 v2 A42284 v3			A29219			A29221			A29194 v2 A29220 v3			A35560		A35566
	7/hour	10/hour	8/hour	7/hour	10/hour	8/hour	7/hour	10/hour	8/hour	3/hour	4/hour	4/hour	9/hour	10/hour	10/hour	1/hour	1/hour	1/hour
Agencourt AMPure®	A29216			A29201 v2 A35785 v3			A35029			A29217			A29189 v2 A35786 v3			A35561		A35787
	6/hour	8.5/hour	7/hour	5/hour	7/hour	7/hour	6/hour	8/hour	6/hour	3/hour	3/hour	3/hour	4/hour	7/hour	5/hour	1/hour	1/hour	0.75/hour

Table 1. Agencourt software methods and plate throughput for Beckman Coulter Biomek NX^P, FX^P, FX^P dual pod and 3000.

Achieving Ease of Use

Being able to adapt quickly is crucial to basic research, drug discovery, and translational medicine. Agencourt SPRI reagents and Beckman Coulter automation create a complete, fully supported, and scalable solution for nucleic acid extraction. Researchers can process anywhere from a few samples to thousands without additional hardware or personnel.

By incorporating a wide range of features in a small-footprint design, the Biomek family of automated workstations takes care of every aspect of liquid handling and accelerates the path to discovery. As shown in Table 1, automating nucleic acid purification on Beckman Coulter automated workstations improves ease of use and increases throughput (Fig. 4).

The combination of Agencourt chemistry and Beckman Coulter automation helped M.D. Anderson researchers more easily adapt manual methods onto an automated platform (Fig. 4). Ease of use was accomplished by automating sample preparation using the Biomek NX^P workstation for clean up of both PCR and cycling samples. With the addition of Agencourt paramagnetic bead-based reagents, M.D. Anderson was able to process three trays at a time without centrifugation or vacuum filtration. Thus, saving time on sample preparation and reducing the overall HLA-SBT cost per sample.

Figure 4. Image of the M.D. Anderson automated HLA-SBT platform on a Biomek NX^P deck configuration for Agencourt AMPure and Agencourt CleanSEQ chemistries.

Conclusions

The M.D. Anderson Cancer Center objectives for streamlining the HLA-SBT pipeline objectives were all met by utilizing the turnkey solution provided by Beckman Coulter's Agencourt reagents and Biomek automates workstations. Previously used clean up methods involved time-consuming processes, which required centrifugation, vacuum manifold filtration, and thermocyclers. These methods reduced throughput and productivity. Replacing such chemistries with SPRI technology in the HLA-SBT pipeline (Fig. 5) significantly

cut the processing time required by technicians and allowed more walk away time to focus on other skill-requiring tasks such as data analysis. In addition, the walk away solution provided by Agencourt chemistries on the Biomek NX[P] allowed for scaling up the HLS-SBT pipeline throughput while continuing to use the same automation platform.

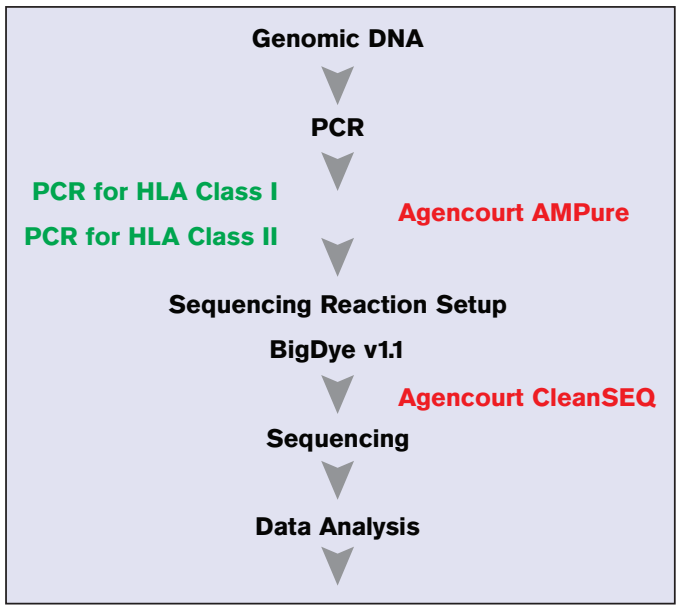

Figure 5. HLA Sequencing Workflow.

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