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Automation of the NGSgo Library Preparation for 96 and 192 Samples on the Biomek i7 Workstation

Introduction

Next-generation sequencing (NGS) is becoming the standard for HLA typing. However, NGS library preparation is labor intensive and interspersed by incubations. To decrease hands-on time and reduce the risk of human error, the GenDx NGSgo library preparation was automated from start to finish. Here we describe the results of the automation of the NGSgo library preparation for Illumina on a Beckman Coulter Biomek i7 Hybrid workstation (Figure 1A) for 1 or 2 plates with 96 samples each. The workflow is fully automated, using amplicon (pools) as input and producing one tube of finalized library per plate.

Materials & Methods

The Biomek layout included an integrated Thermo ATC thermal cycler, peltier devices for cooling and an orbital shaker. Pipetting was performed by a 1200 µl 96-multichannel head and Span-8 pod. The method was designed to enable execution of complete and partial workflows (Figure 1B). The full walk-away solution accommodated overnight runs and allowed the operator to step away from the machine. The two-plate workflow process had been optimized to perform parallel operations, maximizing efficiency (Figure 1C). Reagent tubes could be placed directly from the GenDx NGSgo kit into the tube block on the peltier (Figure 2).

In this study a full plate with 93 samples (5 loci) and three negative controls was processed both in manually and in automated workflows for one as well as two plates. Subsequently, the pooled libraries were sequenced on a MiSeq system and the resulting data was analyzed with NGSengine.

Results

The Biomek i7 workflow yielded functional libraries with an even read distribution over the 93 samples and very little well-to-well contamination (negative controls show < 1% of the average number of reads found in a positive well, Figure 3A). A high percentage of the reads generated could be mapped to the appropriate HLA loci for both the manual and automated libraries (Figure 3B). Insert sizes between the manual and automated libraries were also comparable, though variation between insert sizes was smaller with the automated workflow (Figure 4).

The full plate was processed 3 times by the i7. For a total of 278 samples, sufficient sequence reads were generated and correct HLA genotypes called. For 1 sample, read numbers were too low for typing and this sample was considered a dropout, probably due to a bad tip. Typing concordance between the automated and manual library preparation was > 99%. Quality metrics (mappability, noise, allele balance) were in a similar range between automated and manual library preparations. Figure 5 shows an example of typing results as well as coverage and base variation for HLA-A in 1 sample processed by Biomek i7.

A

B

C

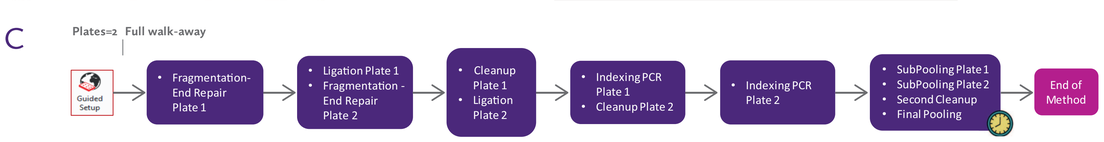


Figure 1. (A) Biomek i7 Hybrid Genomics Workstation (B) Method Options Selector (C) NGSgo full walk-away workflow for 2 plates.

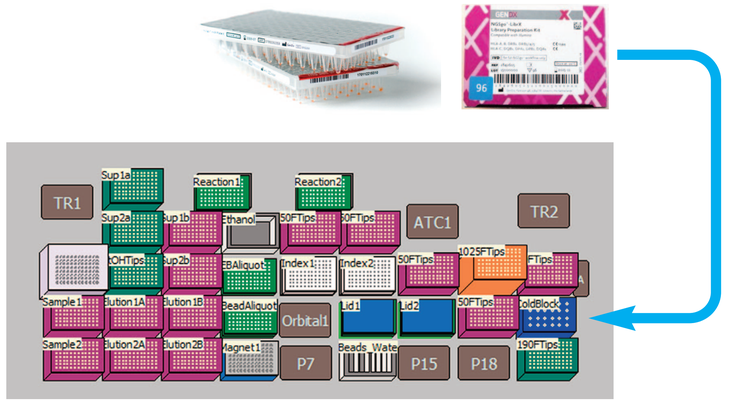
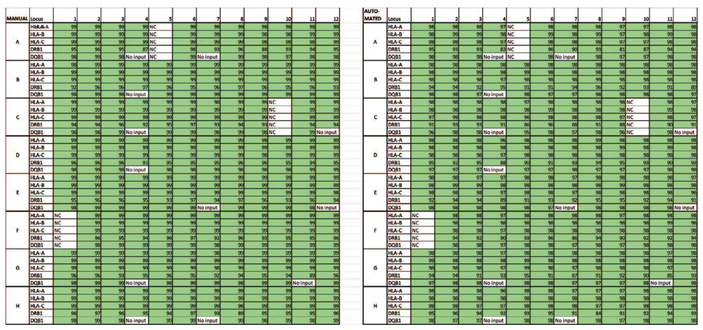


Figure 2. Guided Labware setup. Plates and tubes are transferred directly from the kit to the Biomek.

A

B



manual Biomek i7

Figure 3. (A) Read distribution 93 samples and 3 negative controls (NC). (B) Locus mappability of manual and Biomek i7 runs.

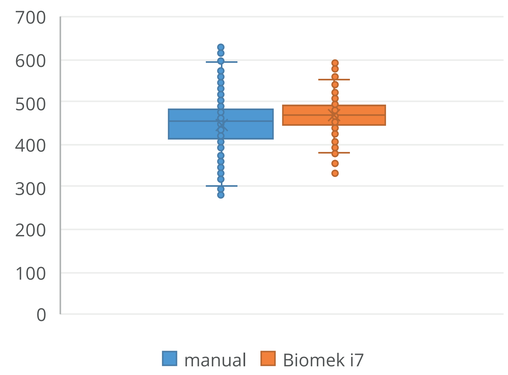
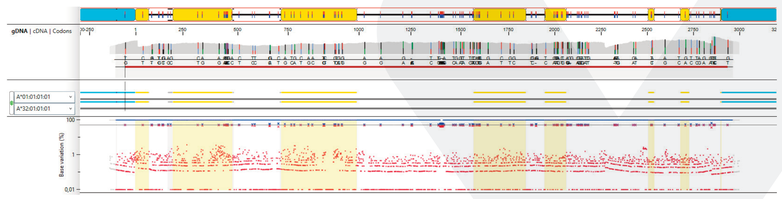


Figure 4. Median insert sizes of manual and Biomek i7 runs.

A



B

Pre-type	Manual run	1 plate automated run	2 plates automated run_plate 1	2 plates automated run_plate 2	Concordance
A*01:01:01:01, A*32:01:01:01	A*01:01:01:01, A*32:01:01:01	A*01:01:01:01, A*32:01:01:01	A*01:01:01:01, A*32:01:01:01	A*01:01:01:01, A*32:01:01:01	TRUE
B*08:01:01:01, B*35:01:01:02	B*08:01:01:01, B*35:01:01:02	B*08:01:01:01, B*35:01:01:02	B*08:01:01:01, B*35:01:01:02	B*08:01:01:01, B*35:01:01:02	TRUE
C*04:01:01:01, C*07:01:01:01	C*04:01:01:01, C*07:01:01:01	C*04:01:01:01, C*07:01:01:01	C*04:01:01:01, C*07:01:01:01	C*04:01:01:01, C*07:01:01:01	TRUE
DRB1*03:01:01:01, DRB1*11:03:01	DRB1*03:01:01:01, DRB1*11:03:01	DRB1*03:01:01:01, DRB1*11:03:01	DRB1*03:01:01:01, DRB1*11:03:01	DRB1*03:01:01:01, DRB1*11:03:01	TRUE
DQB1*02:01:01, DQB1*03:01:01:02	DQB1*02:01:01, DQB1*03:01:01:02	DQB1*02:01:01, DQB1*03:01:01:02	DQB1*02:01:01, DQB1*03:01:01:02	DQB1*02:01:01, DQB1*03:01:01:02	TRUE

Figure 5. (A) Coverage and base variation plots for HLA-A from NGSengine (B) Example of typing concordance between manual and Biomek i7 runs.

Conclusions

Automation of the NGSgo library preparation on the Biomek i7 consistently yields high quality libraries, with a significant reduction of hands-on time.

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