GENDX

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personalizing diagnostics

Validation of the automated NGSgo Library Preparation Workflow for HLA Typing on the Biomek 4000

Introduction

Next-Generation Sequencing has become a frequently used method for high-resolution HLA typing. The GenDx NGSgo workflow was specifically designed to enable robust implementation of this application. Automation of this workflow on liquid handling workstations reduces the risk of human errors and significantly decreases the time spent performing the workflow. Here we describe automation of the most laborious part of the NGSgo workflow, the library preparation, on a Beckman Coulter Biomek 4000 workstation (Figure 1).

Figure 1. Beckman Coulter BioMek 4000 system with enclosure.

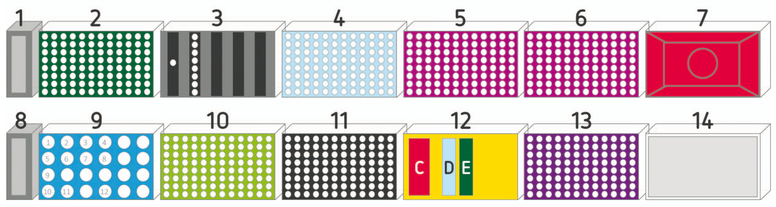
Materials & Methods

A fully hands-off library preparation method was designed to process 4 to 24 samples with any number of HLA loci. Utilization of the Method Options Selector (MOS) coupled to the Guided Labware Setup (GLS) in the Biomek software enables reliable and versatile modular setup. This results in easy-to-use independent protocols for amplification, amplicon pooling and library construction that fully complement each other. The Biomek workstation was configured as shown in Figure 2. On this system a duplicate NGSgo workflow was performed on 15 DNA samples. HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 were included for each sample. After amplification and amplicon quantification, products were equimolarly pooled by the Biomek. The resulting amplicon pools were then processed on the same workstation in a fully automated library preparation. Resulting libraries were checked by gel electrophoresis and qPCR library quantification. Sequencing was performed on an Illumina MiSeq instrument and resulting fastq data was analyzed using NGSengine HLA typing software.

Results

Size-corrected qPCR quantification of the generated libraries showed 12.8nM and 18.6nM libraries (4nM is required for sequencing). Gel electrophoresis showed the expected smears between 400 and 2000bp with the highest intensity between 500 and 1000bp. MiSeq run parameters were indicative of a high-quality sequencing run (Figure 3a). Average sample mappability in NGSengine was found to be 89.1% for Run 1 and 89.7% for Run 2 (Figure 3b). Of the 180 HLA genes typed, three did not meet quality standards. Upon further investigation all three were amplification failures and were consequently excluded from further analysis. All other loci passed all quality standards (Figure 4a). The delta values between highest noise and lowest heterozygous were consistently over 40% for HLA-A, -B, -C, -DQB1 and -DPB1 (Figure 5a) and over 30% for DRB1. Read depth of the sequenced amplicons was predominantly uniform (Figure 5b). The resulting HLA genotypes for these 87 loci were a 100% replicate match between the two runs performed (Figure 4b).

Figure 2. Biomek NGSgo deck layout as set up during library preparation.



#		
1	Liquid waste disposal	Used pipette tips will be placed back
2	Cold well-plate block	
3	Pipette heads	P200L; Single-tip pipette 5-200 µl, MP200; Eight-tip Pipette tool 5-200 µl
4	P250 tips	Ready to go box of pre-sterile tips with barrier (Beckman)
5	P50 tips	
6	P50 tips	
7	TRobot 96, Thermocycler	Thermocycler for; Fragmentation and End-Repair reaction, Adapter Ligation, Indexing PCR
8	Gripper	Head to move plates, plate lids and tubes
9	Cold tube block	Storage of all needed NGSgo-LibrX and NGSgo-IndX reagents (excl. index plate) 1 Fragmentation buffer, 2 End prep buffer, 3 Fragmentation enzyme, 4 End-prep enzyme, 5 Ligation mix, 6 Ligation enhancer, 7 Adapter, 8 Water, 9 HiFi Mix, 10 Fragmentation Master Mix Target, 11 Ligation Master Mix Target, 12 Pool Target
10	PCR plates	
11	Magnet plate	DNA clean-up and size selection
12	Reagent reservoirs	C EtOH, D SPRI Beads, E Elution buffer 80% ethanol, SPRI beads and elution buffer for DNA cleanup and size selection. (this step is performed 2x)
13	NGSgo-IndX plate	Pre-aliquoted indices, resuspension is included in the automation
14	Plate lid for thermocycler	

Figure 4. Analysis quality: A. Analysis quality metrics minimum, average and maximum combined for all loci. B. Results per locus.

A.

Analysis Quality metrics		Min	Average	Max
Locus Mappability (%)	80-100 60-80 0-60	82.0	95.9	98.0
Read length (bp)	>120 100-120 <100	126	135	140
Read depth (median)	>200 100-200 <100	301	2479	10909
Exon mismatches	[Ex] 0 [Ex] ≥ 1	[Ex] 0	[Ex] 0	[Ex] 0
Phasing regions	[R] 1 [R] ≥ 3	1	1.4	3
Insert size (bp)	300 150-300 <150	203	300	407

B.

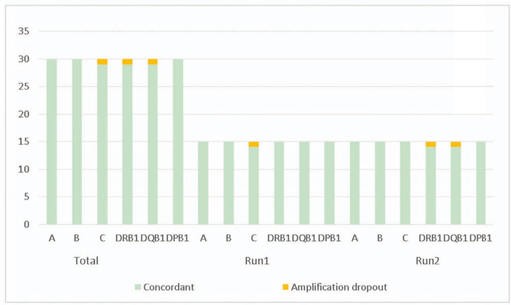


Figure 3. Run quality: A. Quality metrics. B. Mappability per sample in duplicate runs.

A.

Run Quality metrics		
KAPA (nM)	>22.5 4-22.5 0-4	12.8 (run 1) 18.6 (run 2)
Cluster density (K/mm2)	800-1200 	1152
Cluster passing filter (%)	>80 <80 	89,7
Basecalls >Q30 (%)	>80 <80 	89,6
Read identified (%)	97-100 95-97 0-95	96.3

B.

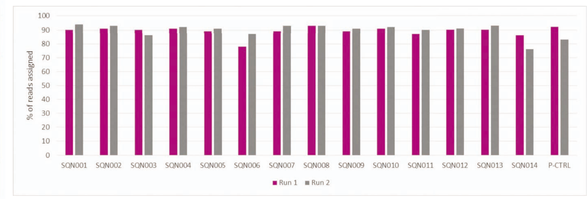
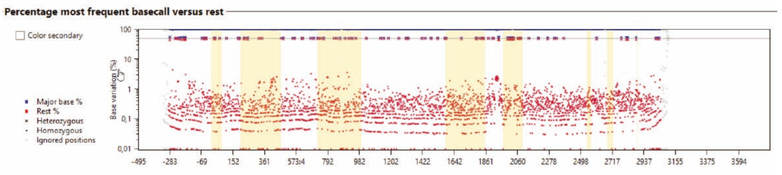
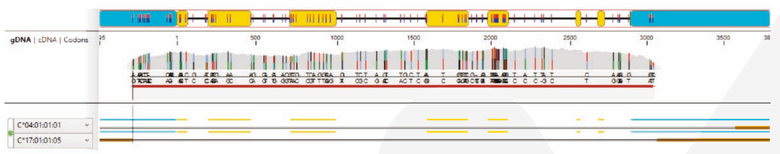


Figure 5. NGSengine plots: A. Clear separation between noise and true heterozygous positions. B. Alignment plot showing uniform read distribution across the amplicon.

A.



B.



Conclusions

- NGSgo library preparation for 4 to 24 samples can be performed on the Biomek 4000 platform, without manual intervention.
- The automated workflow results in high-quality reproducible libraries.
- Performing automated NGSgo library preparation as described here reduces hands-on time from 1.5 hours to 20 minutes as compared to manual execution.

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