APPLICATION NOTE

High quality DNA isolation suitable for ultra rapid whole genome sequencing.

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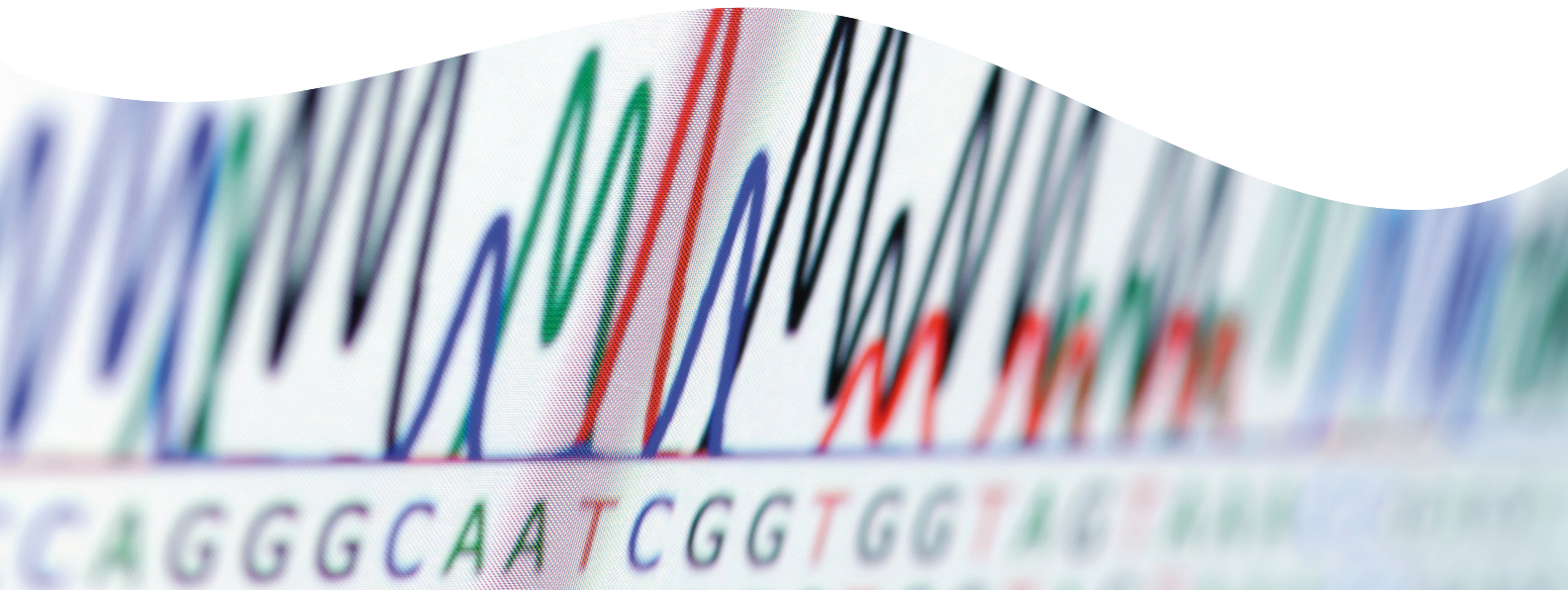
Introduction

Whole Genome Sequencing (WGS) necessitates high quality nucleic acids as well as rapid sequencing and pipelining for most effective implementation. In a pilot study we have previously demonstrated the utility of 50 hours rapid WGS. Herein, we present how fast DNA isolation and modifications to an Illumina® HiSeq® 2500 instrument and optimization of basecalling and variant detection using Isaac software v1 decrease sample preparation and sequencing to less than 35 hours. In order to achieve a minimum run time of 20 hours for a 40x genome (~120GB), cluster generation is completed in 1.5 hours, with an average cycle time of 6.5 minutes. Runs were 2x100 paired end, using v1 chemistry. Importantly the decrease in time did not affect quality scores, with ≥ 90% of data above Q30. Alignment and variant detection are done from BCL files using Isaac software v1 on a single compute node. Variant annotation is completed using custom software, RUNES (Rapid Understanding of Nucleotide variant Effect Software). The total bioinformatics pipeline takes less than 3 hours for completion. Taken together, the modifications made to sequencing and bioinformatics analysis have resulted in an approximately 40% decrease in the amount of time to complete a WGS.



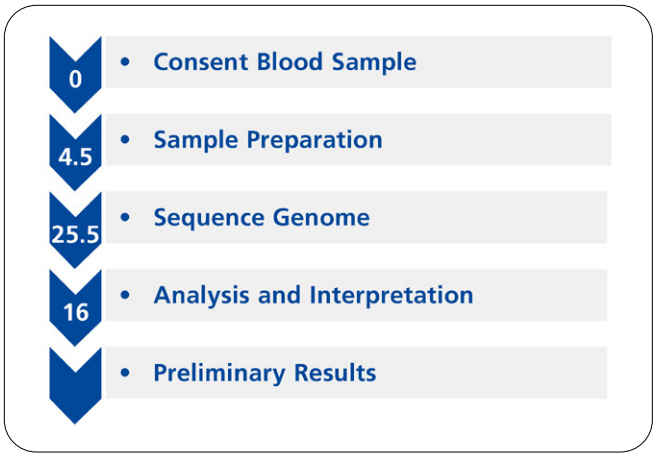


Figure 1: 50 hours protocol.

Methodology

In order to further the decrease time to complete Whole Genome Sequencing, modifications were made to:

- DNA Isolation
- Sample Preparation
- Sequencing
- Alignment and Variant Detection

DNA isolation

DNA isolation was performed using the chemagic™ MSM I instrument (the predecessor to the chemagic 360 instrument.). The instrument is designed to isolate nucleic acids from diverse sample materials in three different configurations: **(LV)** low volume configuration, **(MV)** medium volume configuration, **(HV)** high volume configuration. Associated with the configuration is the sample number that can be maximally extracted per sample set and a sample volume range as indicated in Tab. 1 below.

Table 1: chemagic 360 instrument sample volume configurations.

Volume configuration	Qty. of samples	Sample volumes	chemagic Rod Head
LV	1-96	50 µL - 400 µL	96
MV	1-24	200 µL- 4 mL	24
HV	1-12	1 mL - 18 mL	12

To meet further automation and quality control needs, the systems can be extended with chemagic QA Software and the chemagic Dispenser unit. These come along with pre-installed protocols and allow LIMS-compatible bar code reading/sample tracking as well as automated buffer filling for all volume applications.

The chemagic instruments uses patented magnetic bead based technology for DNA isolation. Rotating needles (rods) and a switchable magnet facilitate an efficient sample mixing and resuspension of DNA bound to magnetic particles. This technology is very beneficial for high sample volumes from 0.2 - 18 mL, resulting in high yields and purities of the isolated DNA within a fast process.

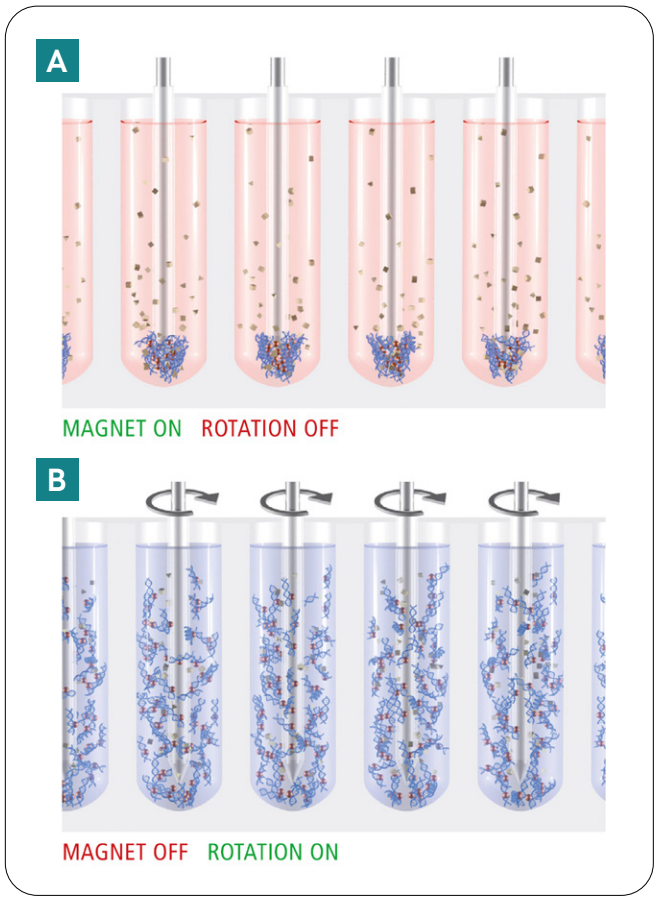


Figure 2: The chemagic™ Separation Technology is based on the use of metal rods that are lowered into a process solution (A). To collect beads from the solution, the rods are magnetized. Pellets form at the tips of the rods, and the rods are withdrawn from the solution with the pelleted beads attached. Resuspension into the next process solution, for example, wash or elution buffer is achieved by switching off the magnetism while rotating the rods (B). This normally difficult step is thus performed quickly and thoroughly, resulting in isolation products with high yields and purities.

Figure 3: chemagic 360 instrument equipped for the isolation of 24 medium volume blkood samples/run.

The configuration of the instrument at Children's Mercy allows for the fast and reliable isolation of 24 medium volume blood samples (200 µL - 4 mL whole blood) in only 1.5 hours. The average yield of DNA is 40 µg/mL.

Hiseq ultra rapid sequencing/bioinformatics analysis

Standard HiSeq 2500 instruments were customized to decrease overall sequencing time. Onboard clustering is utilized and completes in ~1.5 hours. Individual cycle times are ~6.5 minutes. Runs are maintained at 2x100, and standard v1 SBS reagents are used.

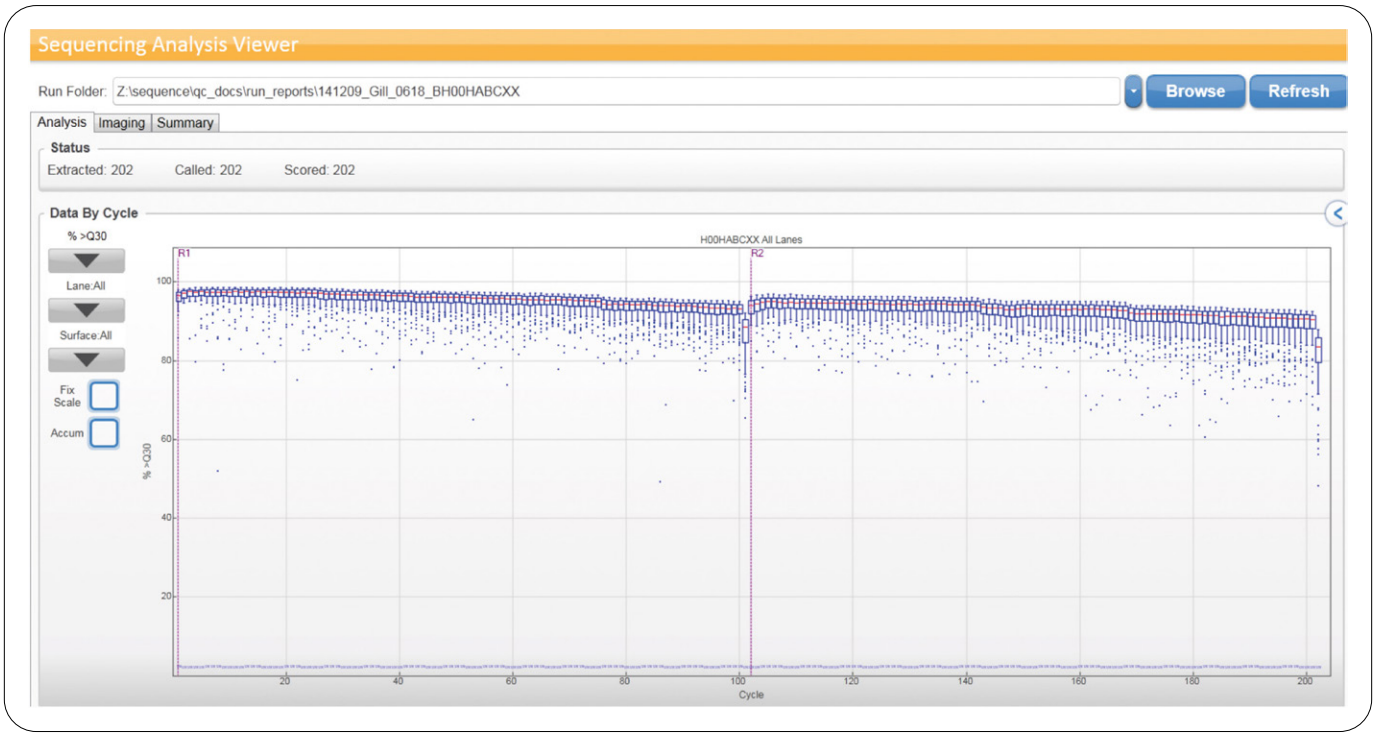


Figure 4: Screenshot, Illumina Sequencing Analysis Viewer.

The average sequencing run time was ~20 hours with bioinformatics analysis done in ~3.5 hours. The average amount of data was 62 GB; including two runs* (see tab. 2, run 617 and 624). with fluidics errors resulting in the loss of data from the entire bottom of the flow cell.

Quality remained high throughout the run. Alignment and variant detection done with Illumina Isaac software v1, variant characterization and functional annotation done with CMH's RUNES software.

Table 2: Example run timings from 14 ultra rapid runs.

Run	Time	GB	% > Q30	Isaac alignment	Variant calling	RUNES	Total bioinformatics
602	19 hr 53 min	50.12	90.0	2 hr 12 min	29 min	30 min	3 hr 11 min
603	19 hr 54 min	58.90	91.3				
604	21 hr 31 min	66.00	90.6	2 hr 30 min	34 min	30 min	3 hr 34 min
605	21 hr 29 min	66.07	91.1				
606	19 hr 43 min	59.94	91.3	2 hr 21 min	31 min	29 min	3 hr 21 min
607	19 hr 45 min	62.75	91.9				
617*	21 hr 8 min	36.57	93.0	2 hr 34 min	29 min	36 min	3 hr 39 min
618	20 hr 52 min	71.31	93.6				
621	23 hr 10 min	77.64	93.1	3 hr 22 min	39 min	41 min	4 hr 42 min
622	23 hr 8 min	78.61	92.6				
623	19 hr 34 min	58.83	95.4	1 hr 57 min	24 min	37 min	2 hr 58 min
624*	19 hr 33 min	36.67	95.2				
633	22 hr 17 min	70.70	90.4	2 hr 57 min	30 min	36 min	4 hr 3 min
634	22 hr 19 min	71.15	90.7				

Ultra rapid protocol

Figure 5: Modified ultra rapid WGS.

Modifications were made to all aspects of WGS. The largest decrease in time was accomplished by implementing Isaac for alignment. Run times were decreased ~5 hours by modifying the chemistries and cycle times of a standard HiSeq 2500 in rapid mode. Further modest time savings in sample preparation were accomplished by using the KapaHyper library prep kit omitting the final PCR amplification step. In total, including DNA isolation WGS may be completed in <35 hours, including analysis.

Sensitivity/Specificity

Sensitivity and Specificity are calculated using NA12878, which has been highly characterized for SNPs and indels. With ~40x coverage from two flowcells in ultra rapid mode configuration, sensitivity and specificity were both >99%.

Table 3: Calculation of sensitivity and specificity.

	True +	False -	False +	True -	Total	Sens.	Spec.
NA12878 - 633 + 634	2702149	7747	3216	479489	3181638	99.71%	99.33%

Conclusion

The chemagic instruments enable fast DNA isolation of high volume blood samples by coincidentally providing high quality DNA suitable for sequencing. Modifications to all aspects of Whole Genome Sequencing have led to a 40% decrease in the time.

- Importantly, ultra rapid run mode does not affect run quality scores or variant detection sensitivity and specificity.
- Further modifications will enable WGS to be completed in <24 hours [1].

Acknowledgements

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References

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