The importance of automated NGS library quality control when identifying rare mutations

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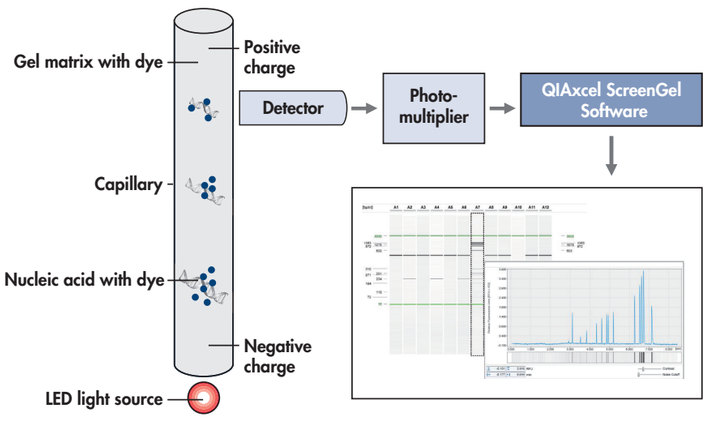
Introduction

We developed a novel method for the reliable and efficient identification of rare mutations in genetically complex diseases (1). It is based on target gene enrichment technology combined with next generation sequencing (NGS). Good library quality is crucial for the success of this method.

Library preparation is a complex, multistep process. It is important to assess the sample quality after several of the steps to ensure that the final library is of the appropriate quality, with suitable DNA size and concentration.

For our library quality control method, we chose the QIAxcel® Advanced capillary electrophoresis system because it uses ready-to-run gel cartridges, and has very short, fully automated runs that are suitable for high-throughput analyses.

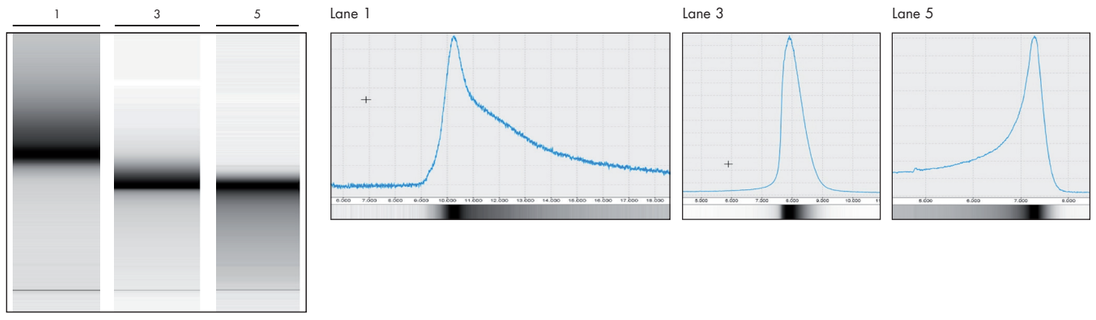


The principle of QIAxcel capillary electrophoresis sample separation.

Genomic DNA quality control

Effective genomic DNA purification techniques are crucial for success in downstream procedures, but optimized quality control of purified gDNA is equally important. It helps to avoid time and money being wasted, particularly in the case of costly procedures, such as NGS, where gDNA is fragmented and used for library preparation.

Our gDNA quality control was automated by using the QIAxcel Advanced System. The results are displayed as electropherograms. The shapes of the peaks reveal the quality of the DNA.



Results of gel electrophoresis of genomic DNA. Lane 1: Very good quality gDNA. Lane 3: Partially degraded DNA. Lane 5: Highly degraded DNA.

Identifying rare mutations in Diamond-Blackfan anemia

Diamond-Blackfan anemia (DBA) is a rare congenital stem cell disorder associated with monoallelic inactivating mutations in the ribosomal protein (RP) genes. It leads to bone marrow failure syndrome by causing defects in erythroid progenitor and precursor cell development.

Target gene enrichment combined with high-throughput sequencing is a robust methodology for the genetic detection of DBA. It shows considerable improvement over existing methods. Using this method, we identified and validated known and novel inactivating mutations in 88% of individuals with DBA (results not shown).

We purified DNA that was to be used for the NGS library preparation from peripheral blood leukocytes using the QIAamp® DNA Mini Kit on the QIAcube®.

The NGS libraries were prepared using the Agilent® SureSelectXP protocol. Library quality control was performed after DNA shearing, after adapter library ligation, and after DNA capture, each time with the QIAxcel DNA Screening Kit on the QIAxcel Advanced System using the AM320 method. Sample results are shown in Panel 4.

Sequencing was performed on an Illumina® MiSeq® using 150 bp paired-end reads, and multiplexed into two runs of 10 samples each.

Library quality control for NGS

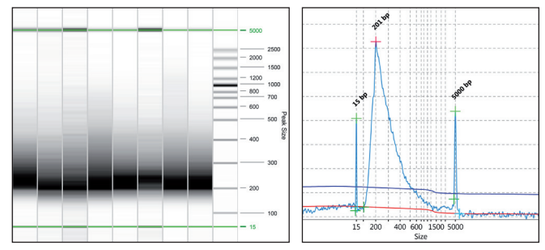
Each analysis was performed with the QIAxcel DNA Screening Kit on the QIAxcel Advanced System.

The AM320 method was used in combination with the 15 bp/5 kb alignment marker and a 100 bp – 2.5 kb DNA size marker.

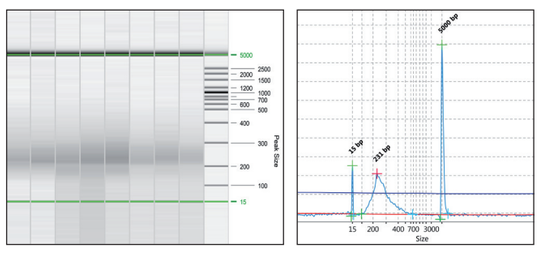
The analysis protocol parameters were: baseline filter 160 sec, minimum distance 5 sec, and threshold 10 S/N.

The run, reference marker table (RMT), and analysis parameters were defined as a standard protocol that was saved with ScreenGel® software and used for all of the runs.

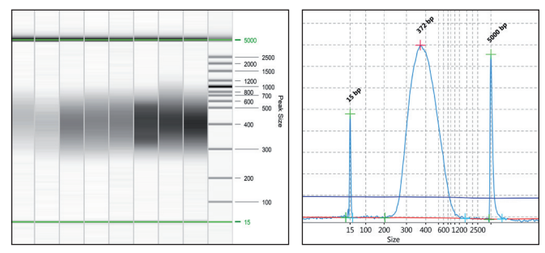
Sheared DNA (Covaris® e220 sonication platform)



Amplified adapter ligated libraries



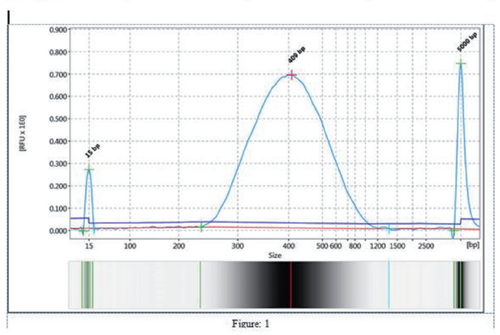
Amplified capture DNA



Comprehensive data analysis and reporting

The QIAxcel Advanced System allows complete analysis of 12 samples in 8 minutes including reporting. A 96-well plate can be run in 1 hour without the need for operator intervention. The QIAxcel ScreenGel software presents the results as a gel-like image, electropherogram, and result table with the requested parameters.

Sample Header			
Experiment Name:	C120322A66_2012-06-14_1 6-30-19	Plate ID:	C120322A66_2012-06-14_1 6-30-19
Position:	D8	Run Number:	1
Run Entry Number:	1		



Fitrure: 1

Result Table							
#	Size [bp]	Conc. [ng/µl]	Mol. [nmol/l]	Normalized Area	NA [%]	Height	Height [%]
1	15	n/a	n/a	0.0040342	n/a	0.2624	n/a
2	409	35.08	129.95	0.0972084	100.00	0.6837	100.00
3	5000	n/a	n/a	0.0082538	n/a	0.7407	n/a
Total		35.08					

Run			
Run Date:	6/14/2012 5:30:20 PM	Instrument ID:	7854

QIAxcel ScreenGel report for an individual sample.

Conclusions

- Target gene enrichment followed by multiplexing and high-throughput sequencing is a powerful approach for finding rare mutations in large sets of genes.
- The advantages over conventional genetic screening using Sanger sequencing on a per-exon/per-gene basis are that it allows for rapid, accurate and cost-effective screening of genes and identification of mutations.
- The method might prove useful when screening for rare mutations in genetically complex diseases.
- DNA quality was monitored throughout the protocol using the QIAxcel Advanced System. This reliable, fast, and cost-effective library quality control ensured the high quality of the final sequencing data.

References

1. Gerrard, G., et al. (2013) Target enrichment and high-throughput sequencing of 80 ribosomal protein genes to identify mutations associated with Diamond-Blackfan anaemia. Brit. J. Haematology **162(4)**, 530.

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