BiOptic Inc.

NGS APPLICATION NOTE

Introduction

The Next Generation Sequencing (NGS) is a widely used technology in many fields, including genomics, epigenetics, transcriptome, etc. NGS is not only used in basic research but also applied for clinical diagnostics such as cancer screening and drug development.

Currently many commercial NGS platforms have been developed which are based on different principles (e.g., Roche 454FLX, Illumina Genome Sequencer, Applied Biosystems/ ABI SOLiD Sequencer). No matter what kind of platforms, the library preparation is the most critical step in NGS workflow. The major idea of sequencing library preparation is producing adaptor-included DNA fragments with specific size ranges. Therefore, monitoring sample size and quality from sample preparation to sequencing library validation becomes quite important.

Here, we use Qsep100 to test minimal detectable concentration of NGS library sample. The data demonstrates that the specified detection limit of 50 pg library sample can easily be reached by Qsep100 instrument.

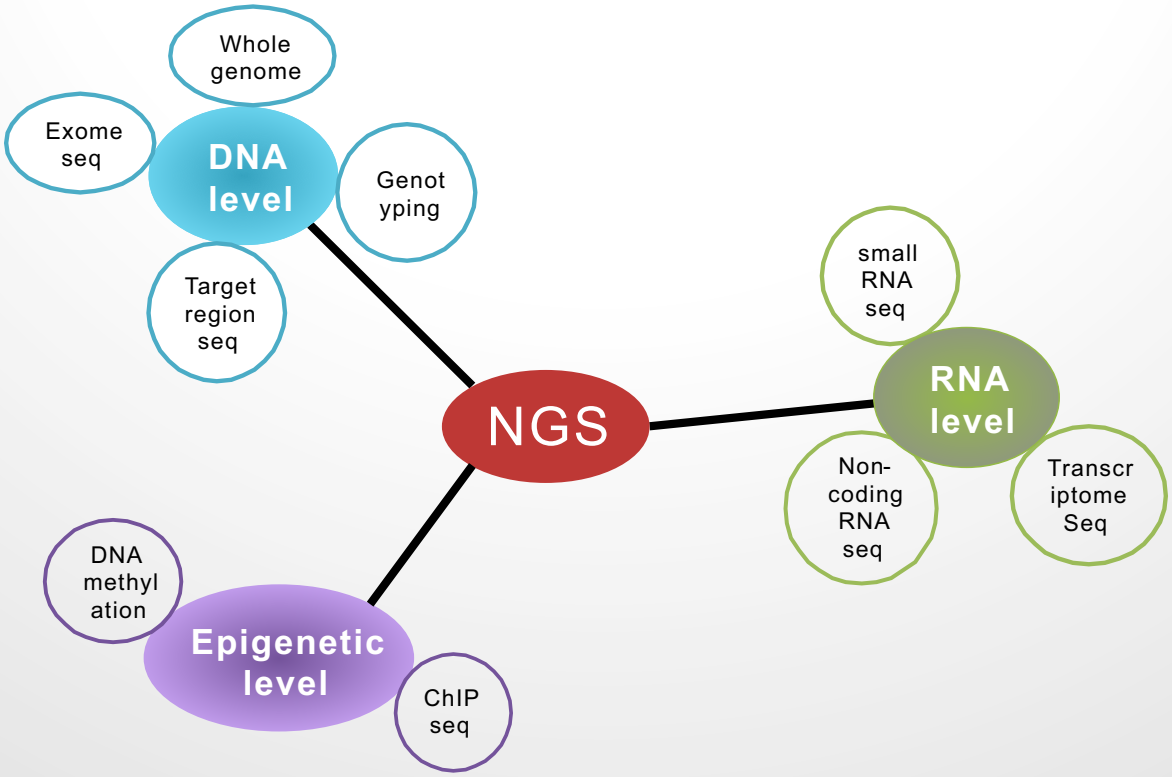


Figure1. Applications of NGS
Different level of upstream samples can be analyzed at NGS platform to meet the needs of applications

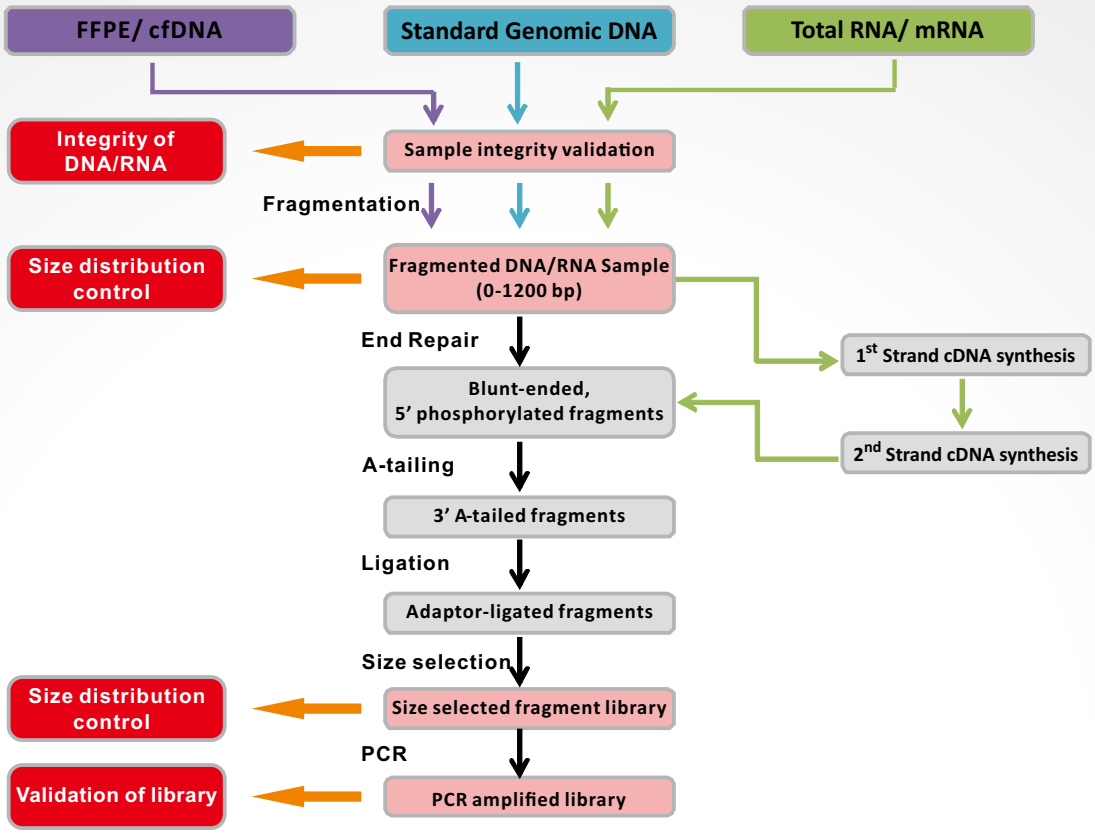


Figure2.
Workflow of Sequencing library construction including many steps of quality control which play important role to make sure of successful analysis. Qsep100 provides an efficient methodology to fully cover the serial QC, which also can match all the NGS instrument workflow.

Analytic steps of Qsep100

Qsep100 set up and operation procedure is listed below:

1. Insert the gel-cartridge and place the samples
2. Select the proper methods (See the table below for details.)
3. Click "Run"
4. Check and analyze results

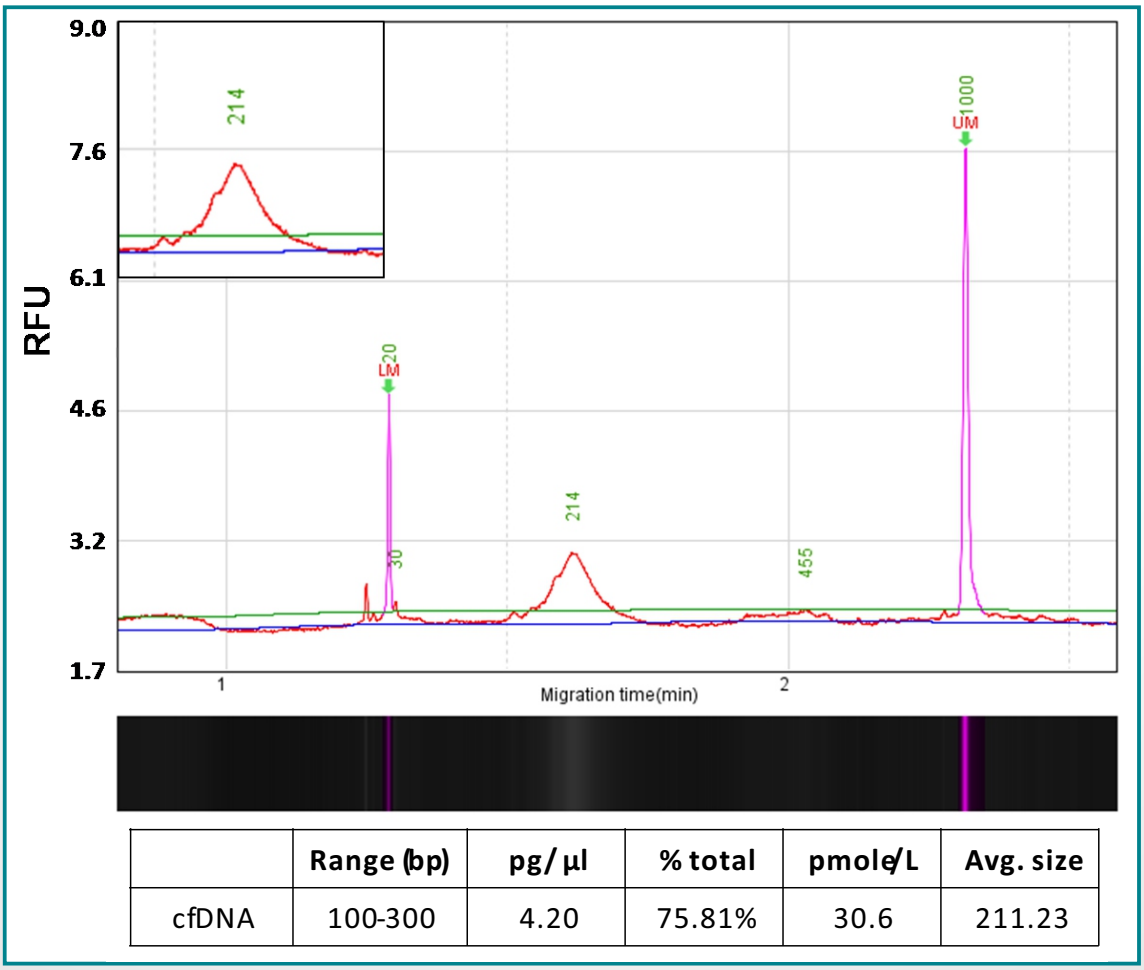
Sample type	Cartridge Kit	Analysis Method
Cell-free DNA	N1 High Sensitivity Cartridge Kit (Cat. C105204)	M8-10-06-300
Genomic DNA	S2 Standard Cartridge Kit (Cat. C105201)	M4-10-08-400
RNA	R1 RNA Cartridge Kit (Cat. C105810)	M4-10-04-600
Fragment DNA	S2 Standard Cartridge Kit (Cat. C105201)	M4-10-08-200
Library DNA	S2 Standard Cartridge Kit (Cat. C105201)	M4-10-08-200

Results

The data displays electropherograms of gDNA, including intact and degraded DNA. The intact gDNA shows a complete, large size peak pattern after the DNA ladder showing up. The three degraded gDNA results gave some small, fragmented peak patterns before the DNA ladder.

Monitoring integrity of total RNA is a typical QC step for downstream experiments, such as RNA-seq. Samples range are from intact (RQN8.9) to degraded (RQN2.3).

In order to obtain high quality sequencing data, the size of sheared DNA must be within a critical size range, which is dependent on NGS platform. In this case, DNA is sheared into small fragments with average size of 895 bp.



	Range (bp)	pg/µl	% total	pmole/L	Avg. size
cfDNA	100-300	4.20	75.81%	30.6	211.23

Figure 3a.
Representative cell-free DNA sample result using the Qsep100 analyzer with N1 high sensitivity cartridge kit. Low amounts cfDNA, yields less than 5 pg/µl , is directly detectable by Qsep100 instrument.

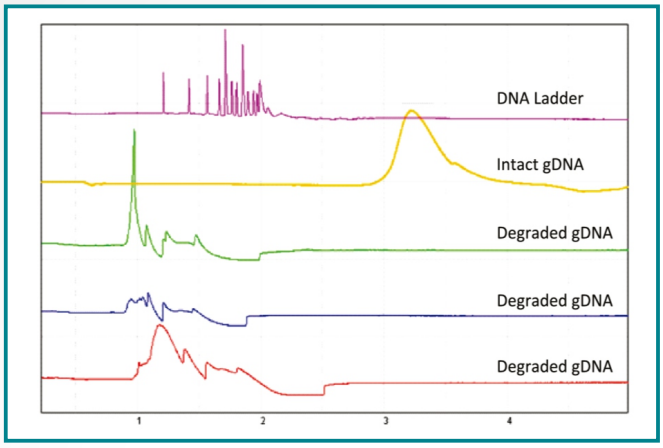


Figure 3b.
Quality analysis of intact and degraded genomic DNA. The data displays electropherograms of gDNA, including intact and degraded DNA. The intact gDNA shows a complete, large size peak pattern after the DNA ladder showing up. The three degraded gDNA results gave some small, fragmented peak patterns before the DNA ladder.

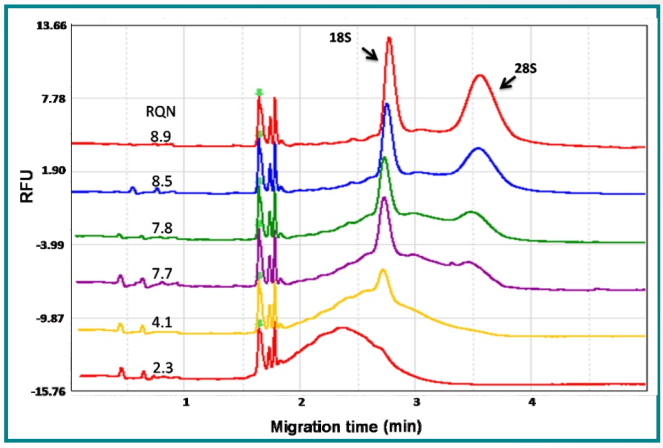
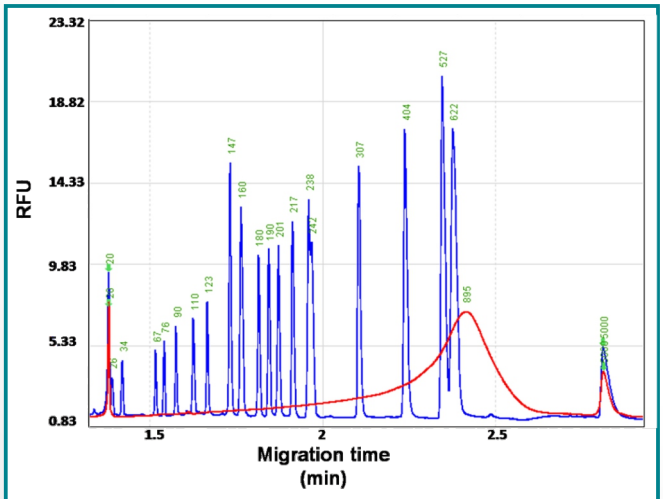


Figure 3c.
RQN (RNA Quality Number) value determines the integrity of RNA. Monitoring integrity oftotal RNA is a typical QC step for downstream experiments, such as RNA-seq. Samples range are from intact (RQN8.9) to degraded (RQN2.3).



	Range (bp)	ng/ µl	% total	nmole/L	Avg. size
Library	100-5000	55.98	99.71%	101.71	846.96
	400-600	7.63	13.62%	23.75	493.97
	600-800	7.16	15.03%	15.53	709.73
	800-1000	9.22	19.37%	15.82	896.50
	1000-1200	5.29	10.40%	7.52	1082.95

Figure 4a.
Quality control of fragmented DNA. In order to obtain high quality sequencing data, the size of sheared DNA must be within a critical size range, which is dependent on NGS platform. In this case, DNA is sheared into small fragments with average size of 895 bp.

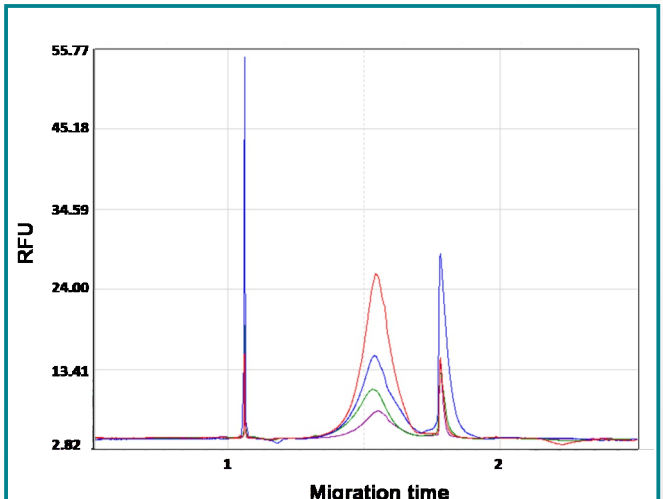


Figure 4b.
High sensitivity result of serially diluted library sample. Average size of DNA library is 515 bp. Library concentrations are from 0.33 ng/µl to 0.07 ng/µl.

Summary

Qsep100system is a high-quality control platform for NGS, which provides the easiest & simplest solution for monitoring each stage of library preparation to ensure success in sequencing. Thefully automated system completely covers the QC protocol from upstream of genomic DNA, total RNA to the final step of DNA library. With ready to use gel cartridge, users can set up the instrument in 1 minute, and get results within3-5 minutes. The data can also be released by batch or individually with fully detailed information.

System Highlights

- Automated Sample Handling: Automated process for 96-well capacity
- Disposable Gel-Cartridge: No need for gel preparation. RFID tagged for up to 100-300 runs
- Fast Analysis: 2-7 minutes per run
- Detection Sensitivity: Detects as low as 0.1 ng/ μl
- Resolution: Up to 1-4 bp DNA fragments (between 100-500bp)
- Data View: Electropherogram and gel-image format
- Software: Digital data for qualitative and quantitative analysis
- Compact Design: Fits on most lab benches
- Competitive Cost for System and Consumable

References

1. Michael A. Quail et al. A large genome centre's improvements to the Illumina sequencing system. Nat Methods 5, 1005-1010 (2008).
2. Michael L. Metxker. Sequencing technologies-the next generation. Nat Rev Genetics 11, 31-46 (2010).
3. Desai AN and Jere A. Next-generation sequencing: ready for the clinics? Clin Genetics 81, 503-510 (2012).

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