CleanNGS: Size selection and purification for Next Generation Sequencing Library Preparations

Abstract

Single Cell Sequencing is an emerging technique in the field of Next Generation Sequencing (NGS). There is a big need for high quality library preparation and size selection methods, when starting with the RNA isolated from a single cell. Sample purification is an important part of the library preparation. This experiment describes the use of CleanNGS[1)] magnetic beads provided by CleanNA[1)] to perform these purifications and size selection in the library preparation for Single Cell Sequencing. The conclusions from this experiment showed the prepared libraries have a high purity and high quality and meet all the demands for size selection.

Introduction

With emerging NGS technologies, for example like Single Cell Sequencing, there is an increased need for NGS library purification methods providing accurate results starting from low input amounts of DNA and/or RNA. In this application note we describe the abilities of CleanNGS to provide, high recovery and accurate size selection abilities. A Single Cell library series have been prepared to demonstrate the CleanNGS purification, recovery and size selection abilities.

Materials & Methods

Equipment

- Agilent, BioAnalyzer™ 2100
- DeNovix®, DS-11 FX
- Clean Magnet Plate 96-Well RN50 (P/N CMAG-RN50) [1),2)]

Chemicals

- CleanNGS (P/N CNGS-0050)[1),2)]
- DeNovix Broad Range Assay
- Ethanol absolute
- Nuclease free water
- Single Cell Library preparation enzymes and buffers

Labware

- 15 mL Greiner tubes
- PCR plates

Experimental design

For Single Cell Sequencing library preparation, we used a series of 4 Single Cell libraries were prepared in duplicate, using CleanNGS in one and competitor A in the other duplicate series for the cleanups and size selection.
The procedure for library preparation is as below described:

- FACS sorting, to obtain 1 single cell per well
- Single Cell lysis and annealing of the RT-primer
- Reverse transcription
- Pre-amplification
- Clean-up using CleanNGS
- Tagmentation
- Tagmentation enzyme inactivation
- Amplification PCR
- Clean-up using CleanNGS
- Library check via Agilent BioAnalyzer and DeNovix DS-11 FX

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Results

Yield and size of the samples after library preparation was compared to competitor A. Library fragment size was compared using Agilent Technologies BioAnalyzer, while final library concentration and yield were determined via fluorescence measurement using a DeNovix DS-11 FX and the DeNovix Broad Range Assay. Average library size and the concentration of each library can be found in table 1.

Sample ID	CleanNGS		Competitor A	
	Avg. size (bp)	Conc. (ng/µL)	Avg. size (bp)	Conc. (ng/µL)
1	696	26.7	691	25.9
2	580	27.1	587	25.3
3	569	27.6	567	25.4
4	607	28.3	603	27.1
Average	**613**	**27.4**	**612**	**25.9**

Table 1. Comparison of average library size and concentration between CleanNGS and competitor A.

The corresponding BioAnalyzer data can be found in figures 1 till 5, showing the electropherograms from samples processed with CleanNGS and competitor A.

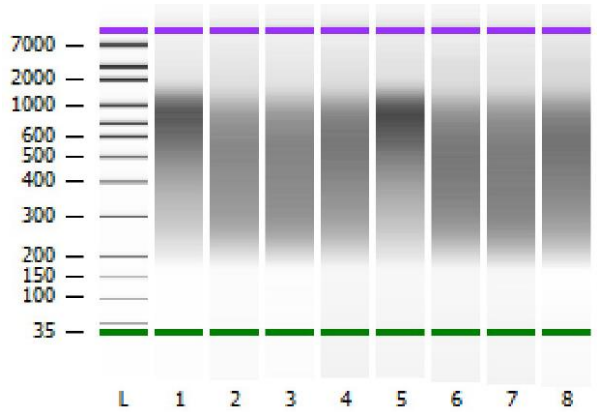

Figure 1. BioAnalyzer overview of the libraries. L=ladder, samples 1-4=competitor A, samples 5–8=CleanNGS.

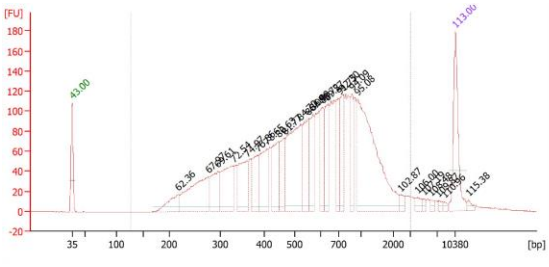

Figure 2. Library 1, competitor A.

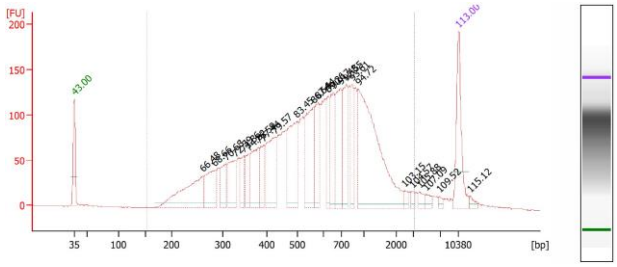

Figure 3. Library 1, CleanNGS

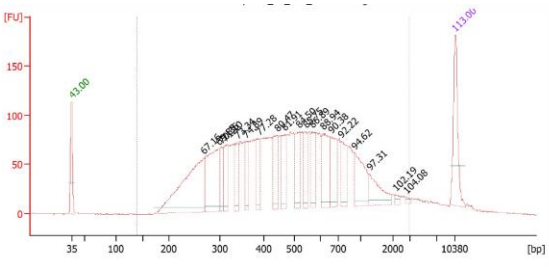

Figure 4. Library 2, competitor A.

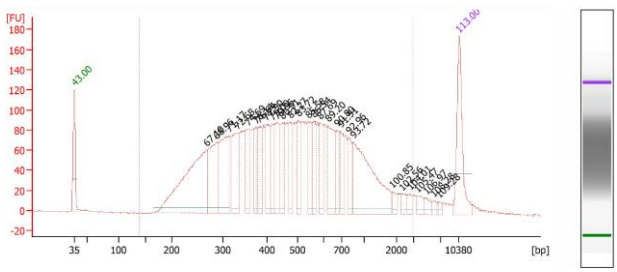

Figure 5. Library 2, CleanNGS.

Conclusion & Discussion

The preparation of the Single Cell Sequencing library showed CleanNGS provides a solution for any NGS application. CleanNGS provides a high recovery and accurate size selection within the library prep processes. The size selection compared to competitor A, shows an average difference of 1 bp, which is considered to be the same. Especially taking into account the Agilent Bioanalyzer has a sizing resolution of ≥ 5 bp. Being well within this range with the size selection comparison shows both kits perform identical.

The recovery compared to competitor A as shown in table 1, has improved by an average of 5% on the samples used within this experiment. By improving the buffer composition of CleanNGS it binds DNA/RNA more effective compared to competitor A. The higher recovery makes CleanNGS a more powerful asset to be used in applications with even the lowest DNA/RNA inputs at the start of the experiment, by providing the best recovery of purified DNA/RNA.

Due to the protocol design, CleanNGS can be used both manually as well as automated. CleanNGS can be adopted in any NGS laboratory independent off sample throughput. Since the CleanNGS protocol is equal to competitor A, CleanNGS can be used as a direct replacement in already established laboratory processes, but providing an increasing recovery.

To enable a broader usage of CleanNGS within NGS, but also in RNA applications such as MicroArrays, CleanNGS is produced RNase free. This will enhance performance of in vitro RNA applications with the goal of providing the best possible recovery within any in vitro RNA application.

References

1. http://www.cleanna.com
2. http://www.gcbiotech.com
3. http://www.bioline.com

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