CleanNGS: Purification for Next Generation Sequencing Library Preparations

Abstract

With constant evolving methods for Next Generation Sequencing (NGS), there is an increased need for high quality library purification of nucleic acids as well as library fragment size selection methods. Throughout the NGS applications were adopted in both life science research as well as diagnostics, the latest trend that the input amounts of starting materials are decreasing. NGS library preparation today allow starting with as little as picograms of sample input material. Therefore it is crucial to have access to a purification technology providing high recovery as well as high purity and accurate size selection abilities. To meet this demand, CleanNA[1)] offers CleanNGS[1)], designed for accurate size selection and delivering both highest purity and recovery in NGS library preparation processes. This application note shows the quality of the library after purification with CleanNGS magnetic beads. In conclusion this experiment showed that CleanNGS can be used to create libraries with a high quality and purity to meet all the NGS demands.

Introduction

With emerging NGS technologies, there is an increased need for NGS library purification methods providing accurate results starting from low input amounts of DNA and/or RNA. This application note will demonstrate the abilities of CleanNGS to provide both a high recovery as well as a high DNA and/or RNA purity. To demonstrate the purity of CleanNGS purified DNA, several qPCR experiments have been performed using sheared human genomic DNA.

Materials & Methods

Equipment

- Bio-Rad CFX96 Touch™ Real-Time PCR detection system
- Covaris® S2 Focused-ultrasonicator
- Clean Magnet Plate 96-well RN50[1),2)]

Chemicals

- Bioline, Human genomic DNA[2)]
- CleanNGS (P/N CNGS-0050)[1),2)]
- Nuclease Free Water
- PCR primers, RPL13a-F and RPL13a-R
- SYBR qPCR master mix

Labware

- 15 mL Greiner tubes
- PCR plates

Experimental design

Human genomic DNA (Bioline) was sheared to 150, 200, 400 and 1000 bp fragments using the Covaris S2. Fragments have been pooled and 10 µL of 1.5 – 1.8 ng/µL sheared genomic DNA has been purified in a series of 6 using 1.8x ratio (18 µL) of the appropriate beads (3x CleanNGS; 3x Competitor A). Nuclease free water was used as a NTC. After binding the beads were washed twice using 80% ethanol and dried at RT for 5 minutes. An on-bead qPCR was performed using the Bio-Rad CFX Touch after adding 20 µL of SYBR master mix to the bead pellet containing the purified genomic DNA.

qPCR was performed using RPL13A-F and RPL13A-R primers.

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Results

The average Ct value for CleanNGS is 19.20 and for competitor A 21.15. Ct values for each sample are displayed in table 1. Corresponding amplification plots are shown in figure 1.

Sample ID	CleanNGS	Competitor A	NTC
1	18.00	21.23	29.47
2	19.56	21.39	28.70
3	20.06	20.82	28.62
Average	**19.20**	**21.15**	**28.93**

Table 1. Ct values on-bead qPCR comparing CleanNGS to competitor A.

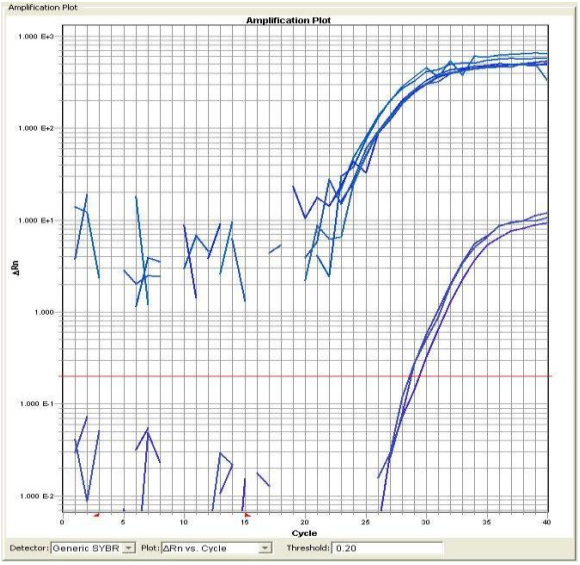

Figure 1. qPCR amplification plot on bead qPCR.

Conclusion & Discussion

The qPCR results showed that CleanNGS provides a solution without inhibition for NGS applications.
With the experiment we demonstrated the efficiency of an on-bead qPCR for both CleanNGS as well as competitor A. By performing an on-bead qPCR, we demonstrate both the DNA and/or RNA purity, concentration as well as the CleanNGS beads do not cause any qPCR inhibition versus competitor A.

This comparison between CleanNGS and competitor A, showed that the average Ct value of the qPCR performed with CleanNGS is 2 Ct lower.
This shows that the CleanNGS purified product on average performs better than competitor A when taking into account the combination of both purity as well as recovery (final yield). Since CleanNGS can be used both manually as well as automated it can be adopted in any NGS laboratory independent of sample throughput. To enable a broader usage of CleanNGS within NGS, but also in RNA applications such as MicroArrays, CleanNGS will be produced RNase free from the start of 2017. 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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