GC biotech

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 as well as library fragment size selection methods. Throughout the NGS applications adopted in both life science research as well as diagnostics, the latest trend is input amounts of starting materials are decreasing. NGS library preparation today allow starting with as little as picograms of sample input material. This makes it 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. The conclusion of this experiment is 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, high recovery and accurate size selection abilities. To demonstrate the purity of CleanNGS purified DNA, a qPCR experiment has been performed using sheared human genomic DNA.

Materials & Methods

Equipment

- Bio-Rad CFX96 Touch™ Real-Time PCR detection system
- Covaris® S2 Focused-ultrasonicator
- Permagen™ 96-well Magnetic Separation Plate (P/N S480)[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.

Results

A series of 6 samples containing of sheared human genomic DNA have been purified and used for qPCR. 3 samples were purified using CleanNGS and 3 samples using competitor A.

qPCR was performed using RPL31a-F and RPL13a-R primers. 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..

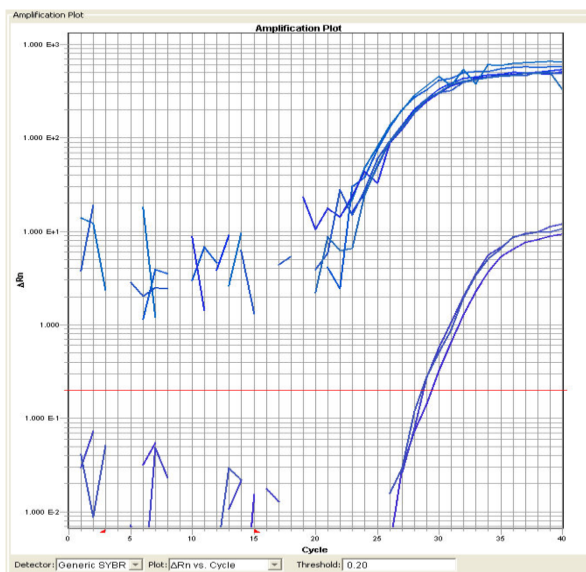

Figure 1. qPCR amplification plot on bead qPCR.

Conclusion & Discussion

The qPCR results show 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 competitor A. We have chosen for an on-bead qPCR test to eliminate any differences between the two kits tested which might be caused by elution efficiency.

This comparison between CleanNGS and competitor A, shows the average Ct value of the qPCR performed with CleanNGS is 2 Ct lower. This lower Ct value does not mean the CleanNGS purified template contains fewer inhibitors compared to the template purified using competitor A.

In previous experiments, we found the recovery of input template (DNA or RNA) to be approximately 5% higher with CleanNGS when comparing to competitor A. As found in any literature written about qPCR, a higher concentration of input material will also result in a lower Ct value. The qPCR in this experiment does show, the CleanNGS purified product does not cause inhibition when the purified template is used in qPCR. This shows 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 off 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

Trademarks

Bio-Rad is a registered trademark of Bio-Rad Laboratories, Inc.

Bioline is a registered trademark of Meridian Life Science, Inc.

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