Quality Control of Single Cell DNA Samples with the Agilent D5000 ScreenTape Assays for the Agilent 2200 TapeStation System

Application Note

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Introduction

Next Generation Sequencing (NGS) applications are numerous, allowing advances in many fields of molecular biology including genomics and transcriptomics. With adequate read depth, NGS can be used to detect relatively low abundance mRNAs (4–8 copies/cell). It can also be used to detect rare but important genetic sequence characteristics, such as deletions, insertions, or single nucleotide polymorphisms that are studied in the cancer and pharmacology fields. With current methodologies, both the genome and the whole cell transcript contents can be measured and characterized from a single cell. These analyses require amplification of single cell starting material for detection, the most common amplification method being polymerase chain reaction or PCR. One of the limitations of PCR lies in the available polymerases that can be used with this technique. Through molecular evolution, many different polymerases have been developed with different processivity and fidelity characteristics. However, no polymerase is error free, and when studying low-abundance targets, and even lower abundance polymorphic variations in these targets, enzyme fidelity is important. One way to limit the number of errors introduced by these polymerases is to reduce the number of cycles of amplification. This may be difficult when attempting to amplify low-abundance targets that require a large number of cycles for detection. If the number of PCR cycles is limited, then the detection of this lower amount of amplified material requires greater sensitivity of detection. In this Application Note, we describe the use of the Agilent D5000 and Agilent High Sensitivity D5000 ScreenTape assays for quality control screening of limited-cycle nested PCR amplification of multiple low-abundance genomic targets from single cells for polymorphic analysis by NGS.

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Materials and methods

Materials

Platinum Taq DNA Polymerase (Life Technologies, 10966026), Bio-Rad Mini-Sub cell GT electrophoresis system (Bio-Rad 170-4466), Fotodyne FOTO/Analyst Investigator/FX, Agilent 2200 TapeStation (G2964AA), Agilent D5000 ScreenTape assay (p/n 5067-5588, 5067-5589), Agilent High Sensitivity D5000 ScreenTape assay (p/n 5067-5592, 5067-5593), Covaris S220 Focused-ultrasonicator, DNA nano library construction kit (Illumina FC-121-4001, FC-121-4002)

Methods

Target material is mechanically isolated from single cells. Cells are lysed to release the template DNA (Figure 1, Block 1). Targets are amplified by limited cycles of PCR before nested PCR to further amplify each target (Figure 1, Blocks 2 and 3). Cycle numbers are limited to reduce error rates and maintain sequence fidelity. Nested PCR of each of the targets are then pooled, sheared, and input into the Illumina DNA nano library construction kit to produce libraries that are then sequenced by NGS to identify polymorphisms in the original target sequences (Figure 1, Blocks 4, 5, and 6). The D5000 ScreenTape assay for the Agilent 2200 TapeStation system is used to QC samples after nested PCR amplification (Figure 1, Block 3). After shearing of pooled PCR products (Figure 1, Block 5), and after final library construction (Figure 1, Block 6), samples were analyzed with the High Sensitivity D5000 ScreenTape Assay.

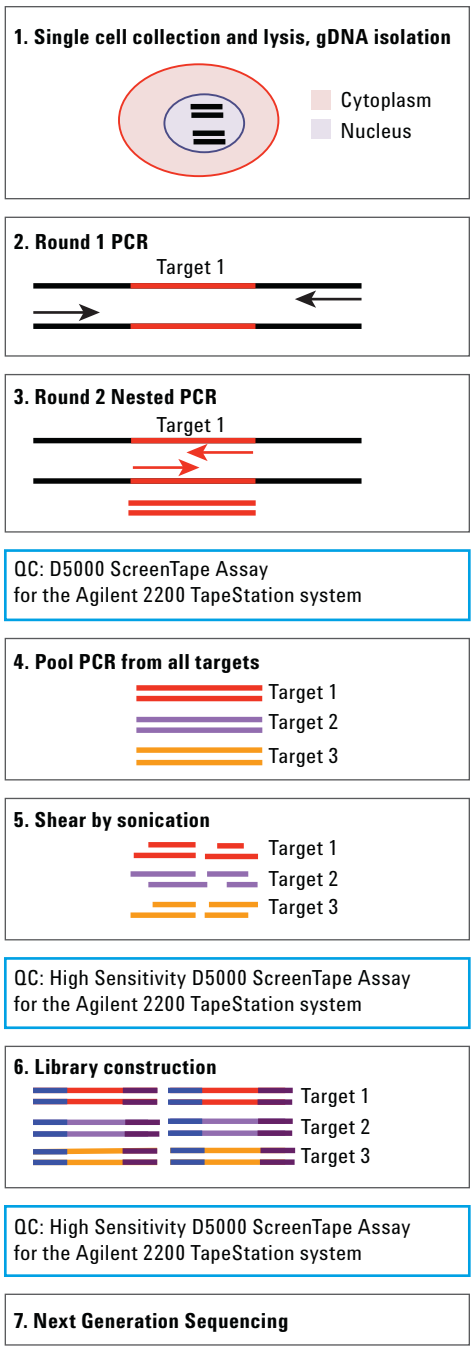

Figure 1. Workflow of PCR amplification of targets and library construction.
Block 1) Single cell, red = cytoplasm, purple = nucleus.
Block 2) Black arrows = primers, red = nested PCR target within first target.
Block 3) Red arrows = primers.
Block 6) Blue ends = Illumina Universal Adapter, purple ends = Illumina Indexed Adapter.
Sampling points in this workflow for QC on the Agilent D5000 ScreenTape and Agilent High Sensitivity D5000 Assay are shown in the blue boxes.

Results and Discussion

Because the genomic targets in this study are low abundance, some targets from single cells cannot be seen on an ethidium bromide stained agarose gel (Figure 2A, targets highlighted in red). Increasing the PCR cycle number to allow detection of these products is not recommended since this increases the likelihood of Taq enzyme introduced mutations in the original sequence. Instead, those products that cannot be observed by conventional means are analyzed with the D5000 ScreenTape assay. This assay is sensitive enough to detect low-abundance PCR amplicons at the expected size without further amplification or manipulation of the samples (Figure 2B).

Identification of the correct amplicon can be performed with confidence because of the sizing accuracy of this assay, with measured amplicon sizes significantly correlating with expected amplicon sizes (Figure 2C, Spearman R = 0.9466, $p < 0.0001$). The High Sensitivity D5000 ScreenTape assay can further be used for those targets below the limit of detection of the D5000 ScreenTape assay. For this workflow, the High Sensitivity D5000 ScreenTape assay is particularly useful for quantifying sheared PCR fragments to accurately calculate input amounts and sizes for library construction (Figure 3). This step is very important, because imprecise input amounts can lead to libraries that either under cluster and produce fewer reads or over cluster, which again will reduce read count. Accurate input results in complex DNA libraries averaging 600 bp (Figure 3).

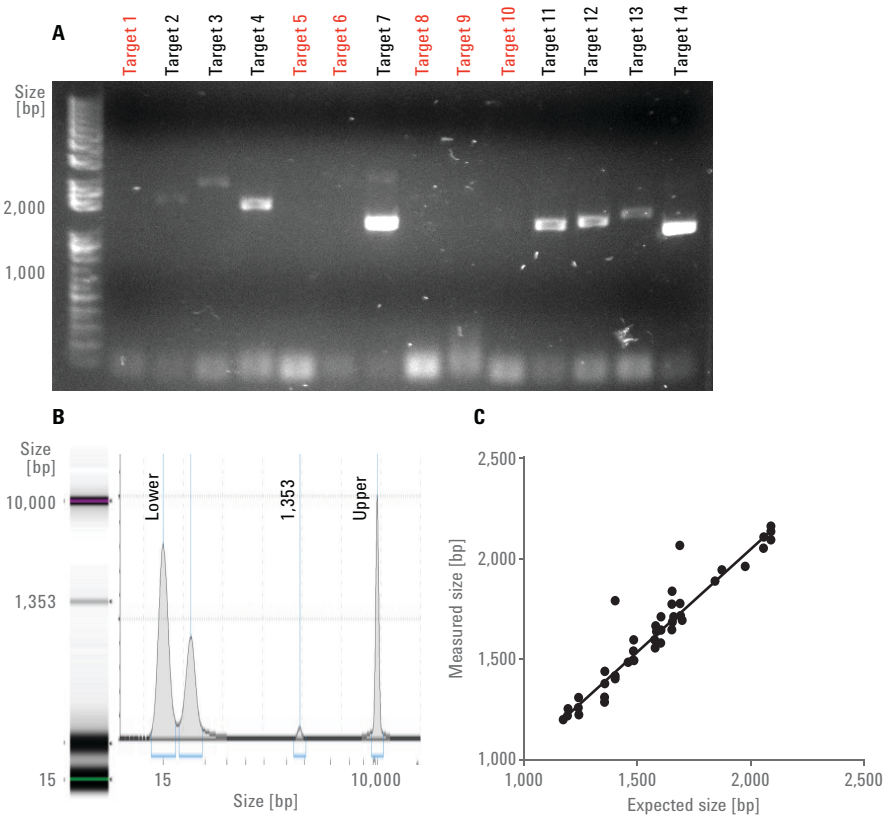

Figure 2. Visualization of nested PCR products. A) Ethidium bromide stained agarose gel of nested PCR of a subset of targets. Targets for this particular sample that were not detectable by this method are highlighted in red. B) Characteristic gel image and electropherogram of one of the red targets undetectable by method A) that is then detected using the D5000 ScreenTape assay. C) Plot of size correlation between expected amplicon size and amplicon size measured with the Agilent D5000 ScreenTape assay.

Figure 3. Characteristic gel image from the Agilent High Sensitivity D5000 ScreenTape assay of sheared amplicons input into library construction and the resulting DNA library.

Conclusion

The Agilent D5000 and Agilent High Sensitivity D5000 ScreenTape assays provide unique sizing accuracy and sensitivity, making the detection of amplicons from low-abundance targets from single cells much easier. This sensitivity omits the need for additional PCR amplification cycles, thus minimizing PCR associated errors that can confound interpretation of Next Generation Sequencing results. Further, the High Sensitivity D5000 ScreenTape assay allows precise quality control of the library construction process. As such, these assays have become integral to this particular set of single cell studies.

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