TECHNICAL NOTE

Increased coverage of the *TERT* promoter using TruSight™ Oncology 500 High-Throughput

A simple protocol modification improves performance in GC-rich regions

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Introduction

Telomere length has known associations with numerous human diseases, including cancer. The enzyme telomerase reverse transcriptase (TERT) plays a key role in maintaining telomeres. Mutations within the *TERT* promoter have been found in over 50 cancer types.[1] In particular, two hotspot point mutations within the *TERT* promoter have been found in 71% of melanomas[2,3] and 83% of glioblastomas,[4] making accurate sequencing of this region of the gene critical for prognostic applications. However, the *TERT* promoter region is GC-rich, consisting of > 80% GC bases,[5] complicating DNA amplification and sequencing.

In this technical note, we describe a modification to the enriched library amplification (EL-PCR) step within the TruSight Oncology 500 High-Throughput protocol that optimizes amplification and subsequent sequencing of GC-rich regions, such as the *TERT* promoter, without compromising performance of non-GC–rich portions of the genome. Similar results were demonstrated with TruSight Oncology 500 (data not shown).

Methods

Modified EL-PCR protocol

During library preparation, probes containing PCR amplification sequences and sequencing adapters bind to specific regions of the DNA. These pieces of DNA are amplified by PCR and purified before loading on to a flow cell that binds the fragments using the ligated adapters. As previously noted, GC-rich regions can be problematic for PCR amplification, resulting in lower yields and lower coverage of these regions during next-generation sequencing (NGS). To overcome the GC-rich challenges experienced in NGS, Illumina scientists increased cycle times during the final amplification (EL-PCR) of libraries in the TruSight Oncology 500 High-Throughput protocol (Table 1).

Experimental conditions

Testing of the changes to cycle times was performed using formalin-fixed paraffin-embedded (FFPE) DNA samples positive for bladder or thyroid cancer and a SeraCare cell line control sample. Libraries were generated following the TruSight Oncology 500 High-Throughput Reference Guide[6] using the standard or modified EL-PCR protocol. The

Table 1: Comparison of the standard and modified EL-PCR protocols

Step	Temperature (°C)	Standard duration	Modified duration	No. of cycles
Denaturation	98°C	30 sec	30 sec	1
Cycling	98°C	10 sec	**30 sec**	18
	60°C	30 sec	30 sec	
	72°C	30 sec	**60 sec**	
Extension	72°C	5 min	5 min	1
Hold	10°C	hold	hold	–

modified EL-PCR protocol extended the amplification time from 41 to 57 minutes. Prepared libraries were sequenced on the NovaSeq™ 6000 Sequencing System using the S2 flow cell and a 2 × 101 bp read length. Analysis was performed using DRAGEN™ TruSight Oncology 500 Analysis Software v2.1.0.

Results

Coverage improvement of the GC-rich *TERT* promoter region, and most high-GC regions, was observed for libraries prepared using the modified EL-PCR protocol (Figure 1 and Figure 2). At the same time, coverage of non-GC–rich regions remained high and did not appear impacted by changes to the EL-PCR protocol (Figure 3). Results were further confirmed with additional studies on samples positive for bladder, bone, breast, kidney, lung, skin, or uterine cancer.[7] It is important to note that the quality of the original sample will impact results; higher quality samples, those with a lower ΔCq, will show better coverage compared to lower quality samples.

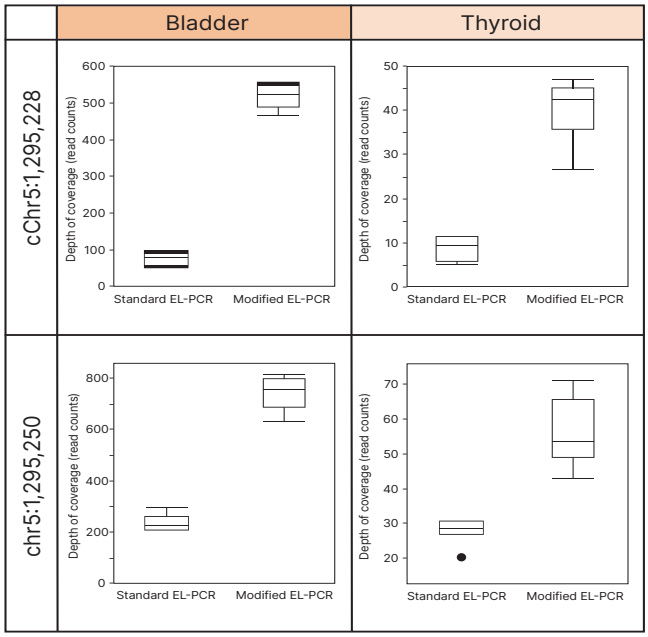

Figure 1: Improved coverage of the TERT promoter using the modified EL-PCR protocol—Analysis shown for two recurrent hotspot mutation positions in the *TERT* promoter, chr5:1,295,228 and chr5:1,295,250 in bladder (n = 8) and thyroid (n = 6) cancer samples. When mutated, these sites show a cytidine-to-thymidine transition.[3]

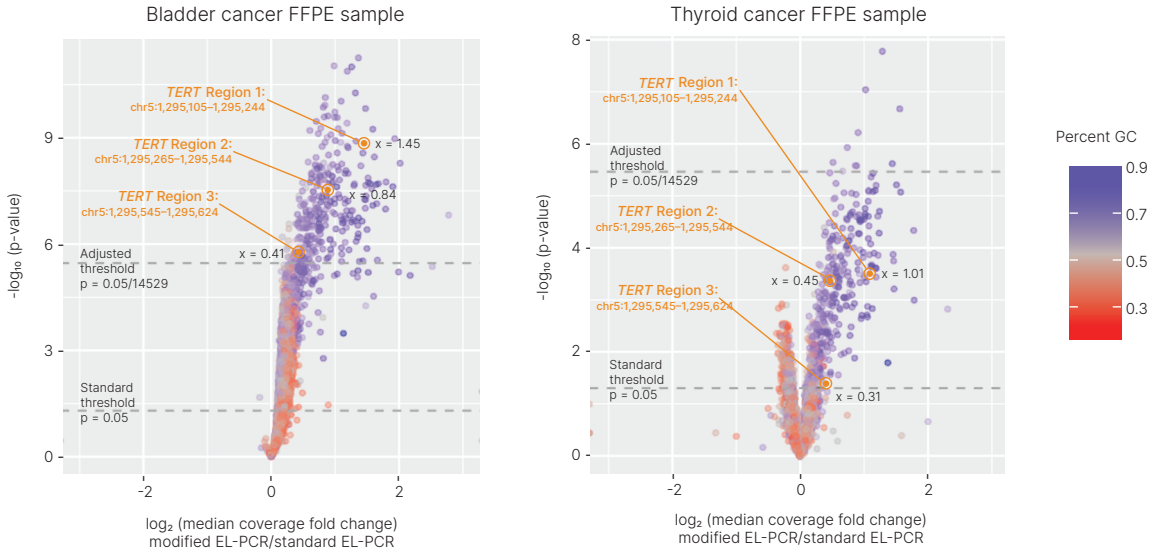

Figure 2: The modified EL-PCR protocol increases coverage of most high-GC regions—TruSight Oncology 500 High-Throughput with the modified EL-PCR protocol was used to analyze bladder and thyroid cancer FFPE samples. FASTQ data was downsampled to 100M read pairs for analysis by volcano plot. The graphs show statistical analysis vs fold change in coverage. Standard ($p < 0.05$) and adjusted ($p < 0.05/14529$) threshold p-values are displayed. X indicates the fold change between the modified and standard EL-PCR protocols for the particular probe region.

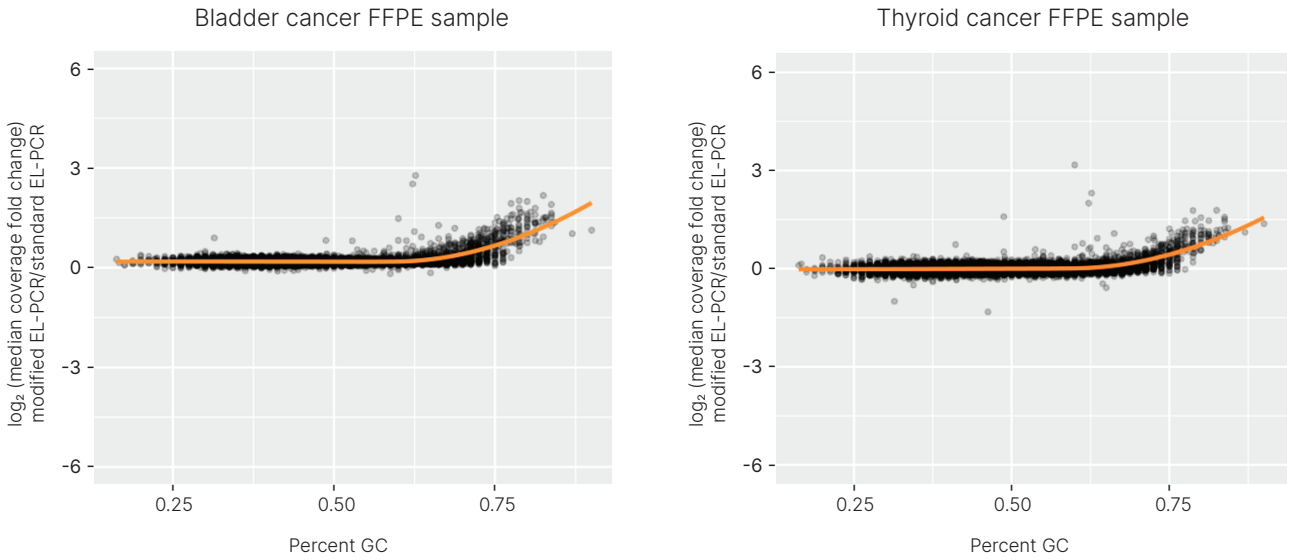

Figure 3: High performance continued to be observed for non-GC–rich regions—While the modified EL-PCR protocol improves coverage of GC-rich regions, it does not impact the overall assay performance. Data points corresponding to regions > 70% GC trend upwards, while those < 70% GC remain constant, indicating the observed increase in coverage is not pulling reads significantly from other regions.

Summary

GC-rich portions of the genome, such as the *TERT* promoter, can pose challenges for NGS. Increasing cycle times during the EL-PCR step in the TruSight Oncology 500 High-Throughput library preparation protocol can increase coverage of these potentially problematic regions while maintaining the high performance observed across non-GC–rich regions.

Questions

Contact techsupport@illumina.com

References

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