TATAA Alu Repeat qPCR Assays

A Tool for Contamination Control and Quality Assessment in Single Cell and Cell-Free DNA Analysis

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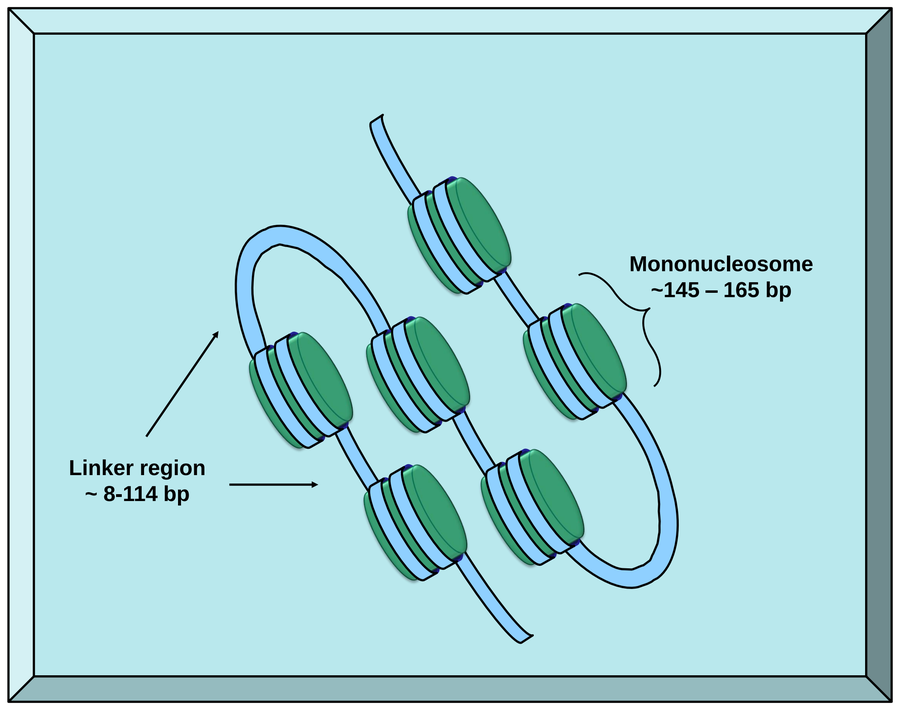

Laboratory reagents including PCR master mixes, primers and probes, are commonly contaminated with residual human genomic DNA (hgDNA) from the manufacturing process. For conventional measurements, the contamination is usually negligible, but poses a significant, if not detrimental, problem in nucleic acid analysis applications where low-abundant targets are studied, such as single cell and rare mutation analysis. To test for hgDNA contamination in reagents TATAA Biocenter has developed ultra-sensitive qPCR assays targeting human specific Alu repeats. Alu repeats are short sequences of about 300 bp dispersed across our genome, summing up to more than one million copies. This corresponds to more than 10% of our hereditary material, rendering Alu repeats the most abundant sequence of the human genome. Therefore, the TATAA assays reliably detect even minuscules amounts of human hgDNA contamination. The ALU quality control panel was in part developed for the CANCER-ID consortium, developing standard operating procedures for liquid biopsy analyses, and as quality control tool for ring trials run in collaboration with the SPIDIA consortium standardizing the preanalytical workflow.

Contamination control of laboratory reagents and material

The TATAA Alu assays are designed to target multiple copies to generate reliable results also for very low amount of human gDNA. The shortest assay, ALU-60, generates the greatest number of amplicons, and is well suited for contamination control.

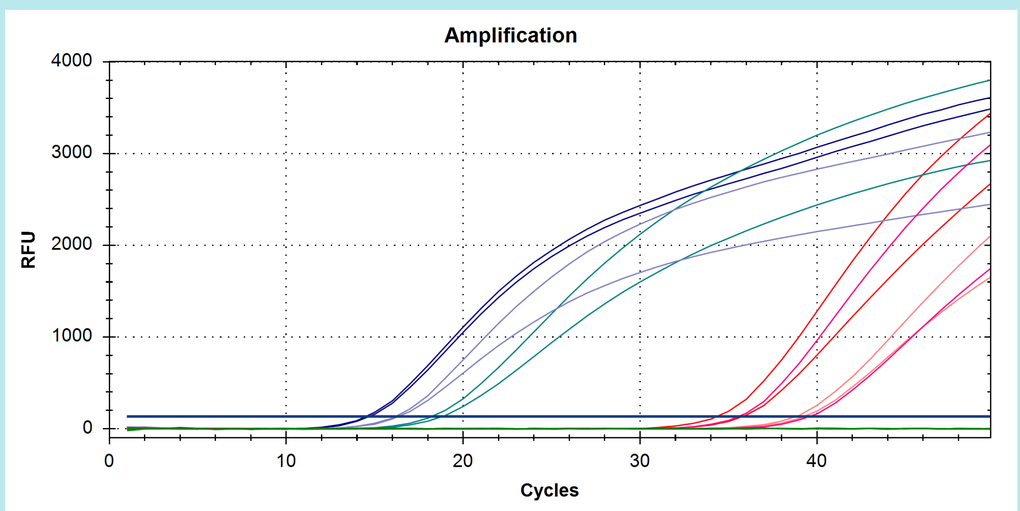

Fig 1. Mastermixes from three different suppliers were tested for human gDNA contamination using TATAA ALU-60 assay. Blue curves are positive controls (4 ng gDNA/reaction), red curves are NTCs (all with significant contamination, generating C_q:s of 32-40), and the green curves (not amplified) are samples treated with PCR Decontamination kit™ from ArcticZymes.

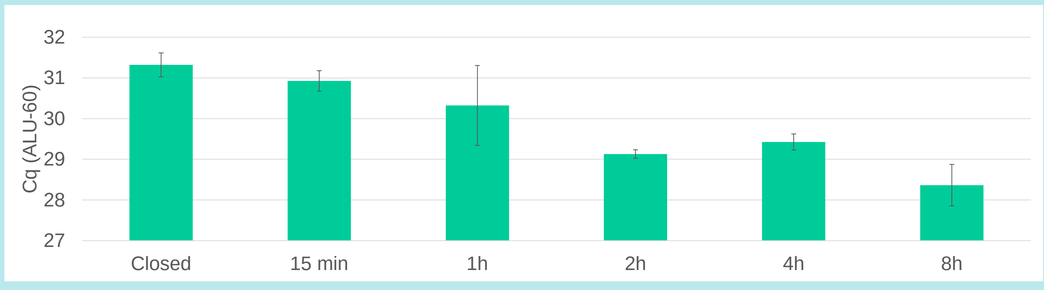

Fig 2. Contamination control of eppendorf tube used for mastermix preparation. The tubes were left open to the atmosphere for various amount of time (0, 15 min, 1h, 2h, 4h and 8h), and contamination was measured with TATAA ALU-60 assay.

Assessment of DNA integrity in liquid biopsy samples.

Alu assays are available that produce different amplicon lengths (60, 135 and 187 bp), which makes it possible to assess the length distribution and integrity of any hgDNA present. Cell free DNA (cfDNA) originates mainly from apoptosis and is predominantly of mononucleosomal length (145-165 bp). By analysing a liquid biopsy sample with TATAA ALU-60 and TATAA ALU-187 assays, the fraction of mononucleosomal cfDNA in the sample can be assessed.

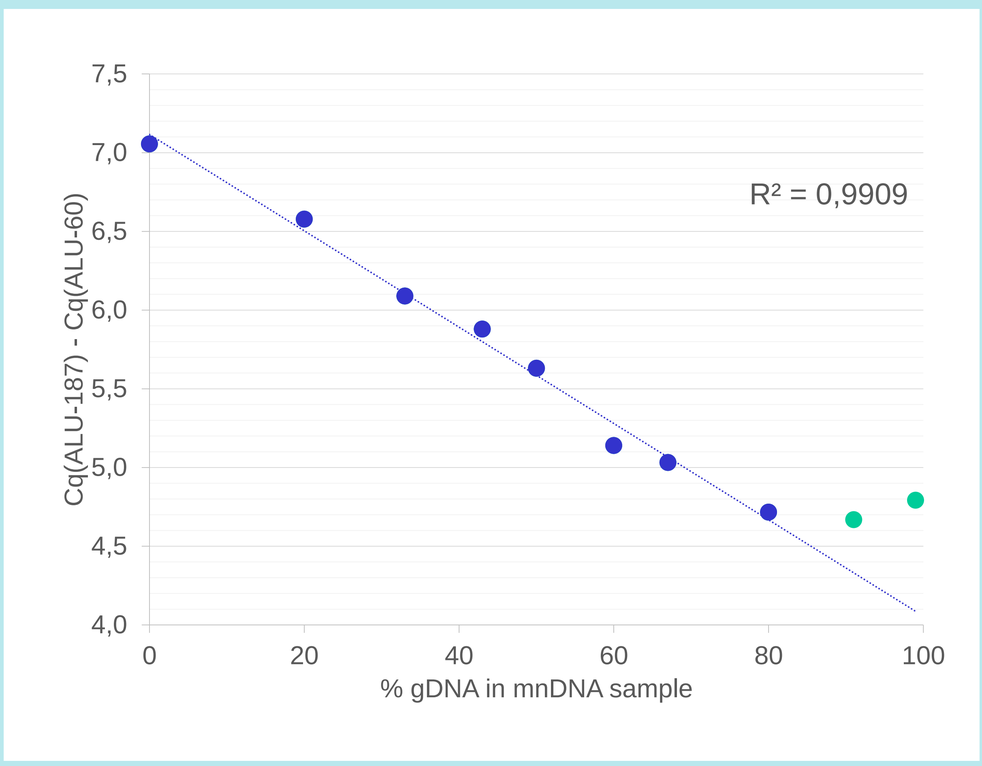

Fig 3. Longer genomic DNA (gDNA) fragments in a mononucleosomal DNA (mnDNA) sample decreases the ΔC_q of ALU-187 and ALU-60 assays. mnDNA prepared from cancer cell lines H441 and H1536 (0,2 ng/reaction, 2:1 ratio) were spiked with High Molecular Weight (HMW) genomic DNA (0-20 ng/reaction) and the samples were analyzed in quadruplicates. The trendline is based on the blue sample points.

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