Single-use technology in the production of DNA-free *Taq* DNA polymerase

Kęstutis Bargaila, Vytautas, Budrys, Andrius Krasauskas, Dovilė Lisauskienė, Milda Romeikaitė, Sonata J.-Urbanavičienė, and Juozas Šiurkus

Thermo Fisher Scientific Baltics, V.A. Graičiūno 8, LT-02241 Vilnius, Lithuania

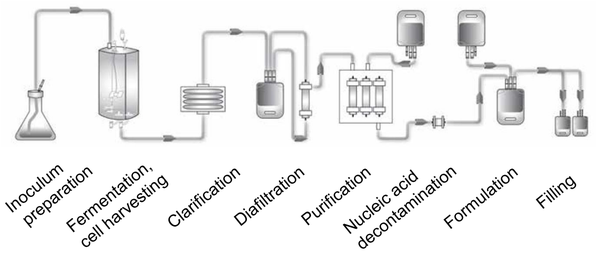
BACKGROUND: Polymerase chain reaction (PCR) technology, used for PCR-based assays, is fast, sensitive, and specific; however, uncontrolled DNA contamination in assays could compromise sensitivity and specificity.

The contaminating DNA can originate from the PCR reagent manufacturing environment, raw materials, human operators, or host cells expressing recombinant proteins. The main source of DNA contamination is the enzyme used for PCR, i.e., *Taq* DNA polymerase. Reported DNA removal methods vary in efficiency and may decrease the sensitivity of the assay.

We introduce Invitrogen™ Platinum™ *Taq* DNA Polymerase, DNA-free – a PCR enzyme with the lowest DNA contamination levels as compared to competing "DNA-free" enzymes. This was achieved through manufacturing innovation, where the enzyme is produced using a closed single-use system (SUS) to minimize the risk of DNA contamination inherent to the conventional manufacturing process.

MATERIALS/METHODS: An SUS was used to manufacture Platinum *Taq* DNA polymerase. Disposable single-use components (e.g., fermenters, bags, filters, and chromatography columns) are connected by sterile single-use tubes for every stage of production. This closed system helps ensure that the entire process is never exposed to the surrounding environment or operator (Fig. 1).

Figure 1. Closed SUS–based manufacturing process.



RESULTS

Unchanged functionality

Platinum *Taq* DNA polymerase manufactured using SUS technology demonstrates the same performance in qPCR as the same enzyme purified using a conventional manufacturing scheme (Fig. 2).

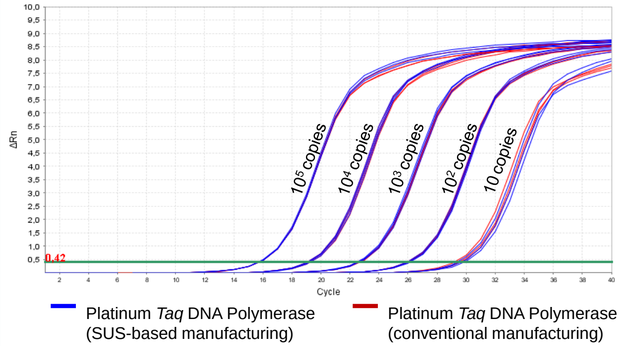


Figure 2. Sensitive, reproducible, and specific qPCR assays. Amplification of varying amounts of bacterial (*E. coli)* DNA using 16S rRNA gene-specific primers.

Uncompromised specificity and sensitivity

For PCR-based assays for pathogen detection, we tested the SUS-manufactured enzyme for absence of signal (false positives) in a no-template control (NTC) in qPCR detecting specific bacteria (*E. coli*). No amplification was observed in all 80 NTC reactions (Fig. 3), demonstrating that use of DNA-free enzyme would not impair analytical specificity of the assay.

The presence of contaminating DNA in an enzyme preparation can lower analytical sensitivity of the assay, since signal from the contaminating DNA can interfere with the detection of low-copy DNA targets (Fig. 4).

The consistent lack of signal in NTC assays increases confidence in the sensitivity of SUS-manufactured polymerase in qPCR for low-copy DNA target detection.

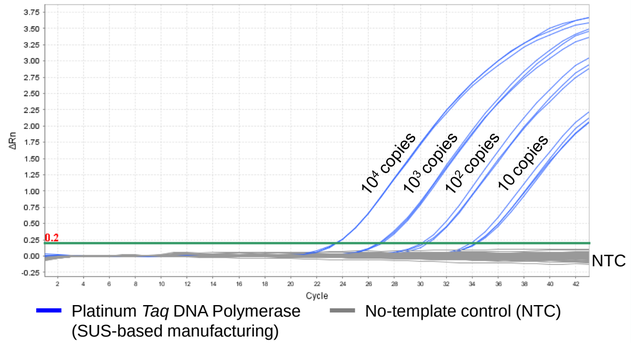


Figure 3. Uncompromised specificity and sensitivity using SUS-manufactured polymerase. qPCR using varying amount of *E. coli* DNA and *E. coli* 23S rRNA gene-specific primers.

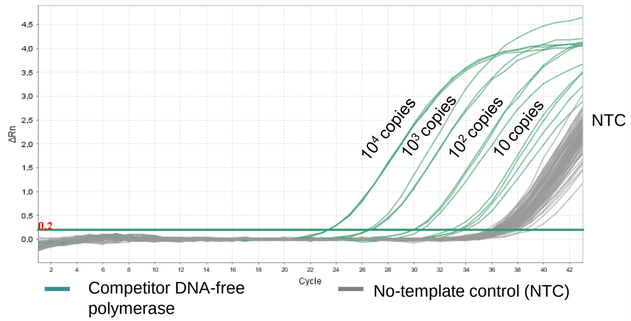


Figure 4. Lower specificity and sensitivity using "DNA-free" polymerase from competitors. qPCR using varying amount of *E. coli* DNA and *E. coli* 23S rRNA gene-specific primers.

Lowest level of contaminating DNA

Platinum *Taq* DNA Polymerase, DNA-free, and "DNA-free" enzymes from other suppliers were tested for DNA contamination. The enzyme manufactured using SUS technology demonstrates the lowest levels of contaminating DNA (Table 1).

Table 1. Detected copies of contaminating genomic DNA in 100 U of *Taq* DNA polymerase.

Enzyme	Bacterial gDNA	Human gDNA	Plasmid DNA
Platinum *Taq*, DNA-free	0.4	0.00	0.4
Enzyme P	19	0.06	400
Enzyme S	13	0.12	11600
Enzyme E	12	0.04	300

Contaminating DNA in enzymes was measured by qPCR using bacterial (16S), human (Alu), or plasmid (ori1) DNA-specific primers after DNA purification from the enzyme.

CONCLUSIONS

The novel SUS-based manufacturing technology, together with tight quality control for DNA presence, enables reliable manufacturing of DNA-free enzyme.

The new DNA-free enzyme enables high analytical sensitivity, specificity, and reproducibility, which are critical for the most demanding PCR-based assay development.

ACKNOWLEDGEMENTS

We would like to thank Eglė Merkienė for her support in development of Platinum *Taq* DNA polymerase, DNA-free.

TRADEMARKS/LICENSING

For Research Use Only. Not for use in diagnostic procedures.