SPOTLIGHT feature

Proteomics, Genomics & Microarrays

Avoiding DNA adsorption to tube walls in NGS sample preparation

Emily Flowers, Hanna Oldfield, Gerrit Gutzke, Steve Knight*, Azenta Life Sciences

Polypropylene (PP) is the ideal plastic material for (q)PCR tubes as PP is chemically inert, resistant to solvents and is well suited to injection moulding, allowing for (q)PCR tubes to be produced with very thin walls for fast heat transfer and therefore optimum (q)PCR results. Despite the very hydrophobic nature of PP, DNA has been shown to bind to PP tubes, especially in high ionic conditions [1]. Different PP polymers can be used for producing PCR consumables, each of which have different characteristics [3], and several manufacturers have introduced low binding products to their ranges to reduce DNA binding [2]. A particularly important characteristic of PP polymers is the surface charge, which influences binding of DNA in varying amounts.

Binding of DNA to PP is typically not an issue for (q)PCR as during amplification, any DNA stuck to the walls of the tube is released during the denaturation step, maintaining accessibility of the DNA for the reaction. This is, however, becoming more of an issue due to the progressing miniaturisation of reaction volumes and the development of new technologies such as Next Generation Sequencing (NGS). These techniques often use very small amounts of DNA and have several incubation and transfer steps, therefore they require ultralow DNA binding consumables to ensure specificity and consistency of the reaction.

Methods

Transfer steps:

10-fold serial dilutions of digested plasmid ACTA1 (1167bp) from 0.01ng/µl to 10ng/µl were incubated in 96 well PCR plates made from one of the standard in-house PP polymers (Azenta), low binding PP polymer (Azenta) and low binding PP polymer from competitor E. ACTA1 fragments were incubated for 30minutes at room temperature before transferring to the next well for a total of 8, 30 minute incubations (240 minutes total). 2µl samples from each concentration both before and after incubation were run in qPCR (SYBR Fast qPCR kit, KAPA) and Ct values compared.

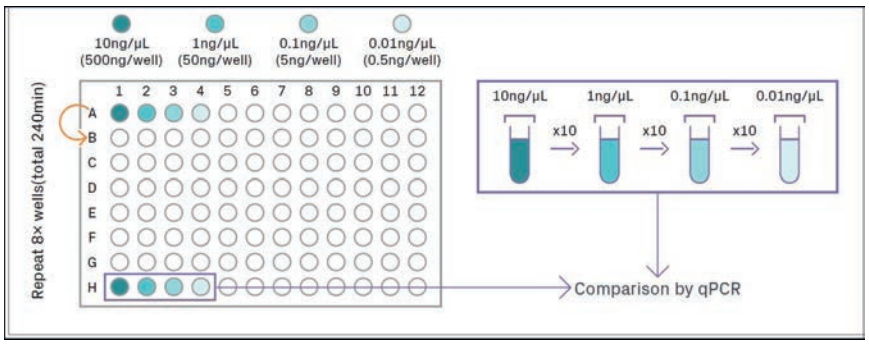

Figure 1: Schematic of DNA template incubation steps.

Incubation temperature:

The above experiment was then carried out using mouse genomic DNA (Clontech) on the PCR plate made from low binding PP polymer (Azenta). DNA was incubated at a broad range of different temperatures to simulate the expected loss at different stages of the reaction cycle. Temperatures tested were 4°C for sample storage, 37°C for enzymatic reaction and 65°C for enzyme denaturation. Samples pre and post-incubation were then run in qPCR (SYBR Fast qPCR kit, KAPA) and the change in Ct values of the samples assessed.

Results

Transfer steps:

On comparison with low binding products from other vendors, serial incubation of a 1.1kb linear DNA fragment showed no significant loss of DNA in Azenta low binding plates, while some loss was seen from low starting concentrations in plates made of alternative materials or competitor low binding branded plates, resulting in significantly higher Ct values (up to 3 cycles).

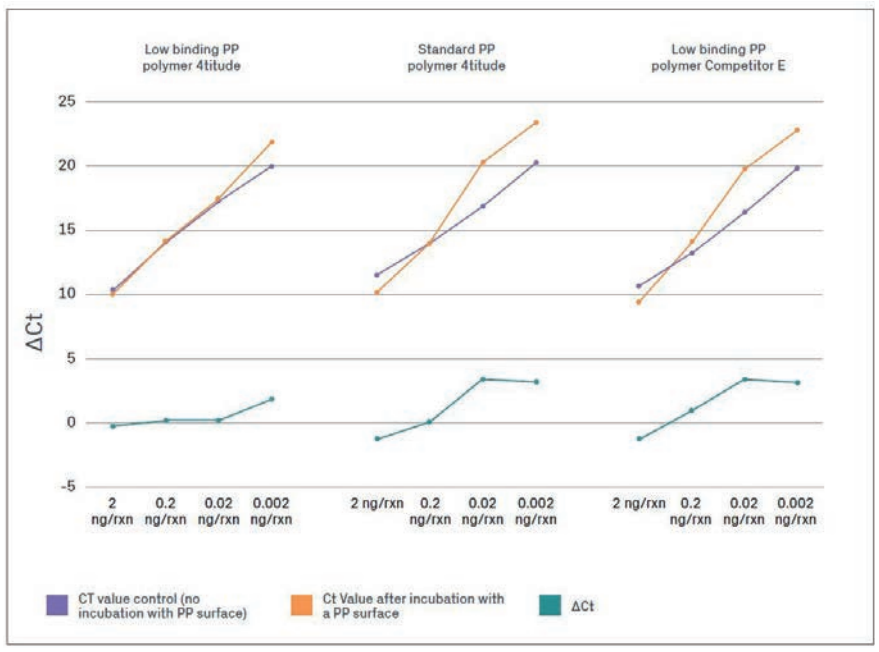

Figure 2: Difference in Ct value seen after incubation of a 1.1kb linear DNA fragment at 37°C with PP tubes made from different polymers. Data created by BioMimOx (UK).

Incubation temperature:

Incubation temperature has no significant impact on DNA binding. Repeating the above described experiments with Azenta Life Sciences low binding PP polymer and mouse genomic DNA at 4°C, 37°C and 65°C showed no significant loss of DNA.

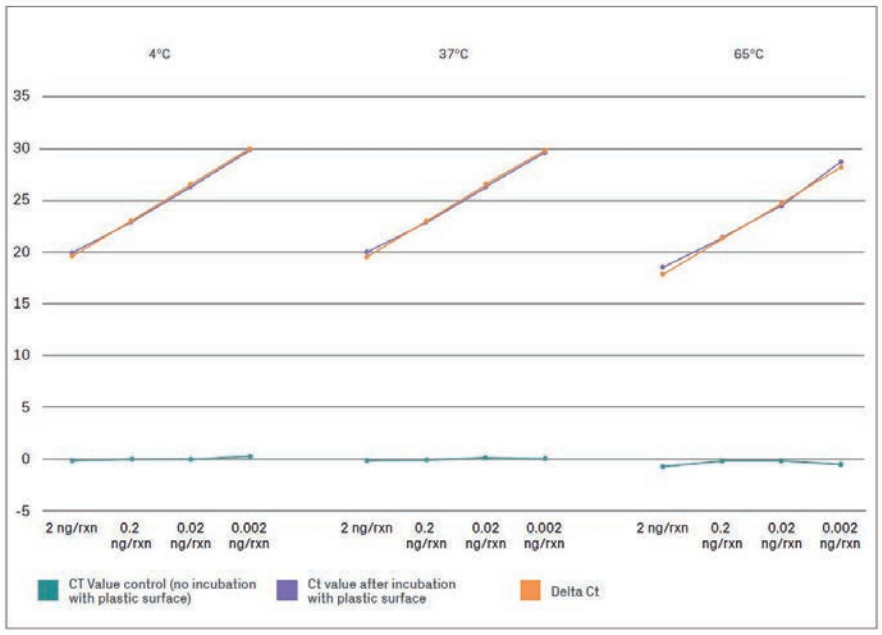

Figure 3: Difference in Ct value seen after incubation of mouse genomic DNA with Azenta low bind PP polymer at 4°C, 37°C and 65°C. Data created by Nippon Genetics Co. Ltd. (Japan)

Conclusion

On comparison with low binding products from other vendors, serial incubation of a 1.1kb linear DNA fragment showed no significant loss of DNA in Azenta Life Sciences low binding plates, while some loss was seen from low starting concentrations in plates made of alternative materials or competitor low binding branded plates, resulting in significantly higher Ct values (up to 3 cycles). Additionally, incubation of mouse genomic DNA over a broad temperature range of 4°C, 37°C and 65°C had no significant impact on DNA binding suggesting no loss of template during storage and PCR. Azenta Life Sciences low binding plates offer ideal properties for highly sensitive assays where low DNA input is vital and therefore any loss during transfer steps would have a significant impact on the success of the assay. Low binding plastics offer advantages not only for sensitive techniques such as NGS but also for the storage of samples and use in the forensic market. Low binding products from Azenta Life Sciences offer reduced nucleic acid binding without the use of surface coatings that can introduce a source of leachables. This is achieved by carefully selecting PP polymers with low-binding characteristics. Azenta is however working in partnership with Furtwangen University (Rottweil, Germany) and Plasma Electronics (Neuenburg, Germany) to investigate novel, chemical resistant coatings for plastic polymers to improve functional binding properties, without affecting samples.

References

1. Gaillard, C. and Strauss, F. (1998) Technical Tips Online Vol 3, 63-65.

2. Belotserkovskii, B. and Johnston, B. (1997) Analytical Chemistry 251, 251-262.

3. Shimizu, E et al. (2010) Shokuhin Eiseigaku Zasshi 51, 43-47.

Further information:

Steve Knight is Marketing Manager of the Consumables & Instruments Group at Azenta Life Sciences and may be contacted on steve.knight@azenta.com