TECHNICAL NOTE

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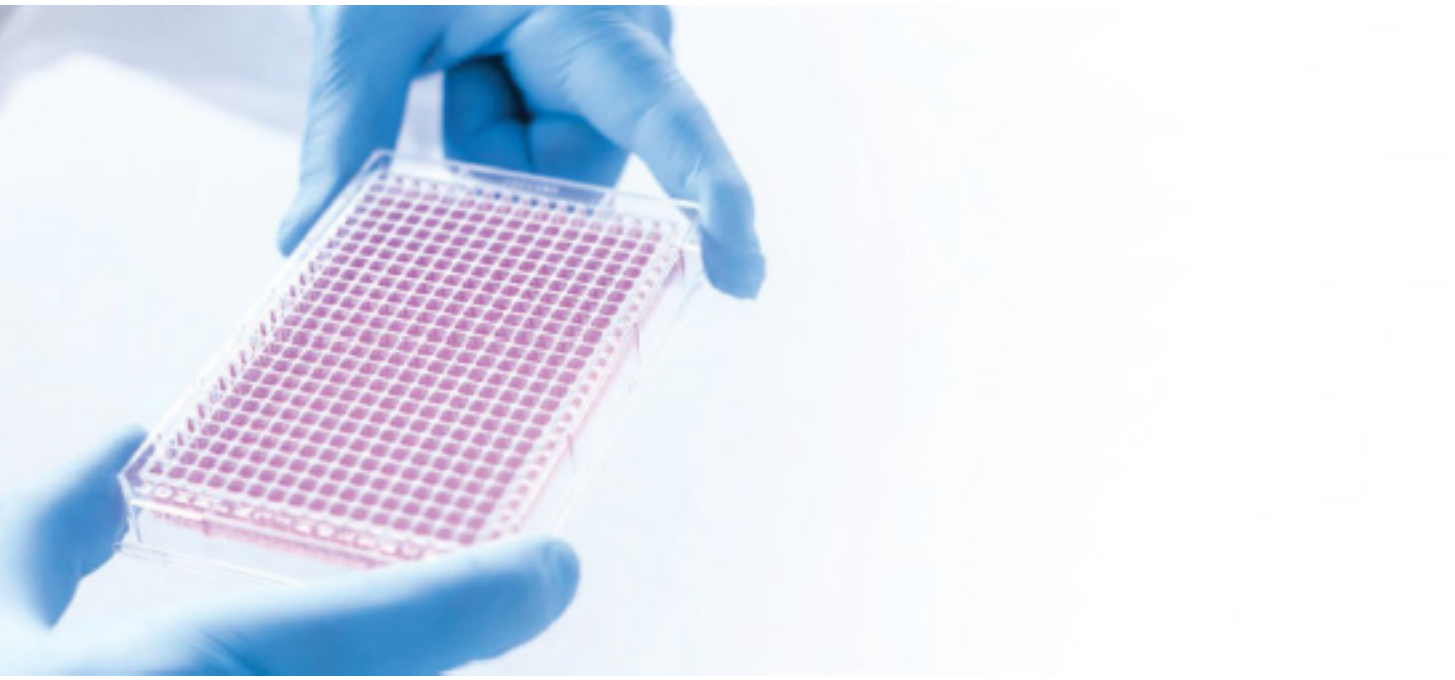
The C.WASH™ Enables Automated and Tip-free Bead-based DNA Purification with Uniform Results and No Cross-Contamination

Thomas Clapes, PhD, Julia Stifel, PhD

CYTENA, Freiburg, Germany



Introduction

The C.WASH™ is a plate washer and liquid dispenser designed specifically for cellular assays and bead-based DNA purification. It also simplifies and accelerates next-generation sequencing (NGS) library prep workflows. For all applications, the C.WASH ensures that no well-to-well cross-contamination occurs during liquid removal or reagent dispensing.

In this experiment, we evaluated the likelihood of sample cross-contamination when using the C.WASH for automated bead-based DNA purification. The instrument quickly and gently removes liquids from polymerase chain reaction (PCR) plates using centrifugal force, while magnetic plate carriers maintain the magnetic beads at the bottom of the plates.

The noncontact dispenser quickly and uniformly adds reagents, such as ethanol, during the washing steps. Noncontact washing saves several hundred (96-well plate) to over a thousand (384-well plate) pipette tips per plate. We took advantage of the tandemly repeated sequences that comprise a significant percentage of total genomic DNA in mammals to demonstrate this. Major satellite repeats (MSRs) consist of repeated sequences of 234 base pair (bp) monomers that are enriched in pericentromeric regions and implicated in heterochromatin formation. Their abundance in the genome as well as their relatively small size make MSRs the perfect indicators for detecting cross-contamination during quantitative polymerase chain reaction (qPCR). We demonstrate that C.WASH bead cleanup does not lead to cross-contamination of the DNA samples from adjacent wells or other samples.

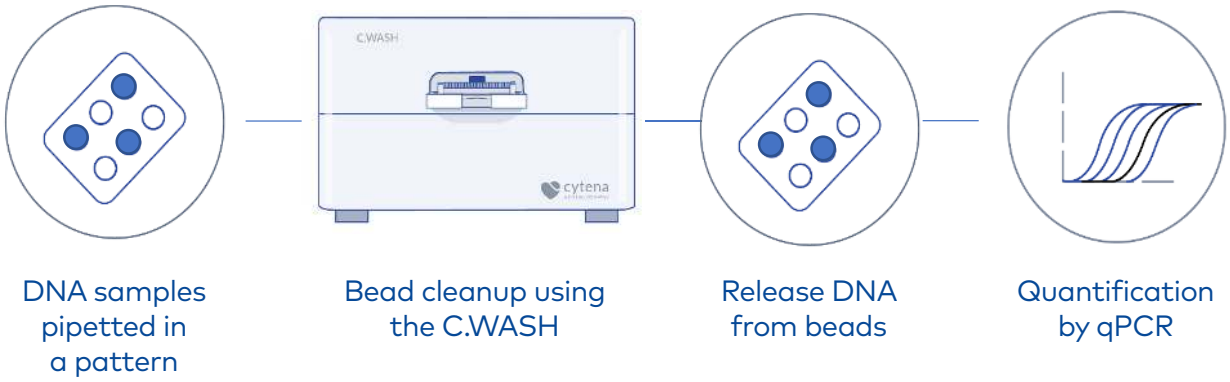


Figure 1. *C.WASH automated DNA purification workflow in a 384-well plate.*

Materials and methods

DNA bead-based cleanup procedure

The bead-based DNA purification protocol used was adapted from the protocol suggested by the manufacturer of the SPRI beads. An unpurified whole-cell lysate of mouse embryonic fibroblasts (MEFs) was used as a DNA template. Five µL of cell lysate was mixed with 10 µL of AMPure XP beads (Beckman Coulter, A63881) in select wells of a 384-well plate (4titude, 4ti-0384) as positive controls.

Wells from the two adjacent columns were filled with 5 µL of UltraPure distilled water (Invitrogen, 10977-035) and mixed with 10 µL of AMPure XP beads as negative controls (Figure 2A). The samples were incubated at room temperature for 8 minutes. Samples were placed on the C.WASH magnetic carrier (CYTENA, 11406) for 5 minutes, until the liquid was clear.

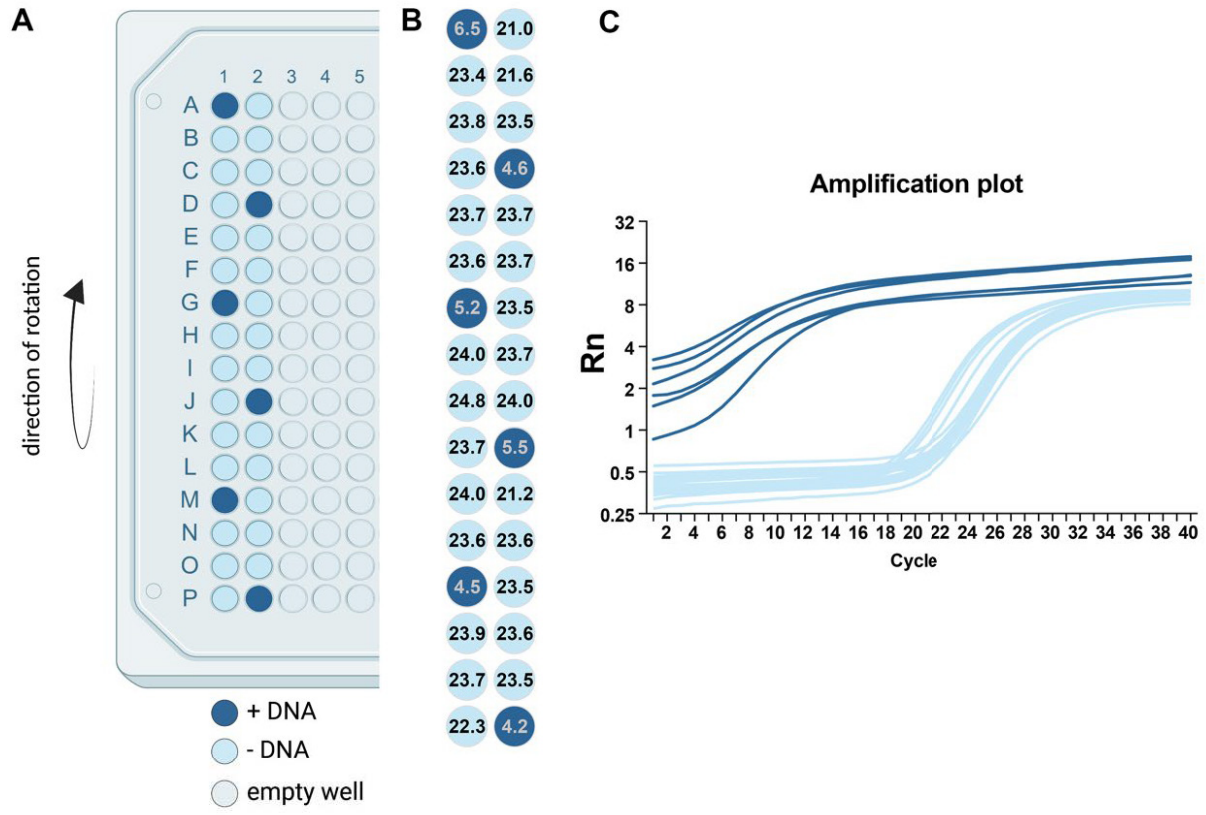


Figure 2. *The C.WASH DNA purification workflow is devoid of cross-contamination. A) Layout of the pipetting scheme in a 384-well plate. The first two columns were filled with either a mix of beads and cell lysate (+DNA) or a mix of beads with water (-DNA). The direction of rotation of the C.WASH is indicated. B) Cycle threshold values obtained for each well after qPCR amplification. C) Amplification plot representing the Rn value (normalized reporter value) of each sample over the cycle of amplification.*

The entire plate on the magnetic carrier was then processed by the C.WASH using the following workflow:

1. Supernatant removal (centrifugal force: 10g)
2. 70% EtOH dispensing (20 µL), wait 30 seconds
3. 70% EtOH removal (centrifugal force: 10g)
4. Repeat ethanol wash (steps 2 and 3)
5. Place sample at room temperature for 2 minutes
6. Add 20 µL of UltraPure distilled water and incubate for 2 minutes to rehydrate

The supernatant of each well was used as a DNA template for qPCR amplification.

qPCR amplification

The qPCR amplification was performed using SYBR Select Master Mix (Applied Biosystems, 4472908) and a StepOnePlus Instrument (Applied Biosystems), according to the manufacturer's recommendations. The PCR reaction (total volume of 10 µL) consisted of SYBR Selected Master Mix (2x), forward and reverse primers (0.1 µM final) and 1 µL of DNA template. The default thermal fast cycling conditions (Primer melting temperature ≥60°C) were used, and the software (StepOne v2.1) provided was employed to manually set the baseline and threshold for the amplification curves.

Results and discussion

The C.WASH is compatible with high-throughput sequencing protocols

To assess the performance of the C.WASH for high-throughput 384-format bead-based cleanup, we purified DNA from a raw cell lysate of MEFs. After bead cleanup, DNA fragments are expected to span between 100 and 10,000 bp, with the peak around 2,000 bp. Since the C.WASH removes liquids in a single spin, it was essential to ensure that no free-floating DNA contaminated other wells during the workflow. Figure 2 shows the raw cycle threshold values for each well (B), in addition to the amplification plots (C). The baseline and cycle threshold values were adjusted manually according to the manufacturer's instructions.

The high frequency of MSRs in the genomic DNA makes them very sensitive methods for assessing cross-contamination from neighboring wells. Indeed, the amplification of MSRs in the positive samples started very early on (cycle threshold values averaged 5.07 ± 0.83). The specific product showed an average melting temperature of 78.62°C ± 0.08 (data not shown). On the other hand, the cycle threshold values of all neighboring wells was on average 23.39 ± 0.88, in a similar order as the no-template controls (23.58 ± 0.83, not shown). This corresponds to typical primer-dimer formation (primer-dimer in contrast to true amplicons has a characteristically lower melting temperature; for this experiment, the average melting temperature was 74.95°C ± 0.1).

These results demonstrate that even in 384-well plates, the C.WASH is compatible with NGS library preparation as it prevents sample-to-sample cross-contamination, with the benefit of saving time. The use of the C.WASH for bead-based cleanup in 384-well plates saves up to 1,152 pipette tips, or 12 boxes of tips.

Conclusion

- The C.WASH reduces the time and cost required to perform bead-based cleanup, when compared to conventional manual methods.
- The C.WASH is compatible with high-throughput 384-well bead-based cleanup.
- The C.WASH prevents any sample-to-sample cross-contamination.

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CYTENA spun off from the University of Freiburg, Germany, in 2014 with its patented single-cell dispensing technology. Today, as part of BICO, the world's leading bioconvergence company, CYTENA continues building on that groundbreaking technology to develop high-precision instruments for isolating, dispensing, imaging and handling biological cells. Its award-winning devices are manufactured in Germany and used at prestigious academic and pharmaceutical labs around the world to automate workflows in numerous application areas, including stable cell line development, single-cell omics, high-throughput screening and drug discovery. CYTENA's breakthrough innovations for the lab combine advanced automation, state-of-the-art software engineering and the latest insights in cell biology to maximize efficiencies in the life sciences and create the future of health. Learn more at cytena.com.

info@cytena.com | +49 761 21632 000 | www.cytena.com