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Application Note

Optimized Washing of Disposable Tips after Transfer of DNA Samples

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Abstract

The automation of liquid handling tasks in molecular biology allows the parallel processing of large sample numbers. The reproducibility of data and the possibility to exclude the confusion of samples are excellent criteria for automation. Washing of tips instead of tip changing is a well-established procedure in high throughput screening to reduce costs. Washing of tips in automated molecular biology applications is specifically challenging because single contaminating nucleic acid molecules may disturb the results. In this study an optimized tip wash routine was developed which removes all DNA reliably; no DNA contamination was detectable by PCR.

Introduction

Automated washing of disposable tips without risk of cross contamination reduces the costs for liquid handling applications in drug research and life science (1a-c). The efficiency of the tip wash procedure influences the success of the experiments directly. The high efficiency of CyBio´s active Tip Wash Station also for molecular biology applications was already proven for a siRNA transfection example. It could be demonstrated that one wash cycle with the active shallow well Tip Wash Station 96 was sufficient for the removal of any remnants of a siRNA transfection complex thus allowing tip washing as a suitable alternative to tip changing in siRNA transfection experiments (2).

For pipetting instruments, which offer the possibility to reuse tips, a tip wash station and its proper performance are crucial. This performance is determined by different factors: the optimal settings of instrumental parameters (like piston speeds, wash volumes, immersion depths of tips), the process itself (how many wash cycles, temperature influences, reaction times) and the types of liquids involved (e.g. water, organic solvents, detergents or chemicals). In this application note the tip wash procedure was optimized for the automated handling of DNA samples.

Figure 1: *This figure shows the CyBi®-Well 96/250 µl equipped with an active deep well Tip Wash Station 96, which was used to optimize the tip washing after DNA sample transfer.*

Materials

Devices

- » CyBi®-Well 96/250 µl with 4-place linear carriage and stacker
- » PCR Cycler TGradient (Biometra)
- » UV documentation (Biostep) with camera (Olympus)
- » Electrophoresis (Biometra)

Accessories

- » Active deep well Tip Wash Station 96 with source and drain pump (CyBio #OL3397-25-280)
- » Heating adapter with temperature control (CyBio #OL3396-259-24, OL3396-266-24)

Consumables

- » CyBi-TipTray 96/250 DW, PCR certified (CyBio #OL3800-25-759-P)
- » 1.5 ml tube (Eppendorf #0030 123.328)
- » 200 µl PCR tube (Eppendorf #0030 124.332)
- » 96-well plate (Greiner #651201)
- » Reservoir (Nunc #370905)

Reagents

- » Human DNA (Jena Bioscience #PCR-354)
- » Custom primer set LINE-1 (Invitrogen #35645101)
- » Taq master/ high yield (Jena Bioscience #PCR-101)
- » Molecular biology grade water (AppliChem #A7398)
- » UltraPure™10x TBE buffer (Invitrogen #15581-044)
- » Agarose (Starlab #N3101-0100)
- » Ethidiumbromid (Sigma #E-1510)
- » Gene Ruler 50 bp DNA Ladder (Fermentas #SM0373)
- » 10x Blue Juice™Gel Loading buffer (Invitrogen #10816-015)
- » Hydrogen Peroxide (H_2O_2) (Aldrich #34988-7)
- » Sodium Hypochlorite (commercial bleach, 2,8g NaOCl / 100g)
- » DNA ExitusPlus IF (AppliChem #A7409)

Methods

10 µl human DNA were aspirated and dispensed back into the same well of a 96-well micro plate to simulate a DNA transfer by using the CyBi®-Well 96/250 µl pipetting robot. Subsequently, the tips were cleaned with 30 µl of the tested wash solution. The different wash solutions (25% H_2O_2, 10% commercial bleach and (heated) DNA Exitus) were pipetted within a reservoir. After cleaning with chemicals the tips were rinsed 5 times with 50 µl water by using the active deep well Tip Wash Station 96. Finally, they were rinsed 3 times with 20 µl PCR master mix. The final pump speeds were 200 rpm for the drain pump and 120 rpm for the source pump of the active deep well Tip Wash Station 96. The aspirate and dispense steps were performed with a piston speed of 80 µl/s.

For verification of effects on down stream applications like PCR, tips were rinsed after cleaning directly with master mix without rinsing with water. For positive control the tips were rinsed with PCR master mix after DNA transfer without any wash procedure. The different wash conditions are summarized in Table 1.

For cleaning with heated DNA Exitus (60°C) a reservoir was placed on the heating adapter. We used heated solution because of the recommendation of the manufacturer that this will increase the activity of DNA Exitus and shorten the incubation time.

The PCR master mix for each sample contained 13 µl water, 4 µl Taq Master Mix, 1 µl forward and 1 µl reverse primer, 1 µl DNA (positive control) or water (negative control or for washing). The PCR conditions were 30 sec 94 °C, 30 sec 60 °C, 30 sec 72 °C for 30 cycles.

Detection of DNA contamination was performed by using a 2% agarose gel stained with ethidiumbromide. The electrophoresis conditions were 1 ½ hour, 100 mV and 67 mA. A 50 bp DNA ladder was used to quantify the PCR products.

The primer set detects a 293 bp fragment of the LINE-1 gene. LINE-1 is a multicopy gene and is well established to detect very small amounts of DNA contamination (3).

Table 1: *Tip wash methods*

Wash conditions	Wash performance
No tip washing (positive control)	Aspirate and dispense 10 µl DNA, rinse 3 times with 20 µl master mix
DNA-free water	Aspirate and dispense 10 µl DNA, rinse 1 up to 5 min with 30 µl water, rinse 3 times with 20 µl master mix
25% H_2O_2	Aspirate and dispense 10 µl DNA, rinse 1 up to 5 min with 30 µl H_2O_2, rinse 5 times with 50 µl water, rinse 3 times with 20 µl master mix
10% commercial bleach	Aspirate and dispense 10 µl DNA, rinse 1 up to 5 min with 30 µl bleach, rinse 5 times with 50 µl water, rinse 3 times with 20 µl master mix
Heated DNA Exitus (60°C)	Aspirate and dispense 10 µl DNA , rinse 1 up to 5 min with 30 µl DNA Exitus, rinse 5 times with 50 µl water, rinse 3 times with 20 µl master mix

Results

The PCR results show that cleaning of tips with DNA-free water is not sufficient enough. For all experimental conditions we observed significant bands indicating remaining DNA contamination. The increase of wash cycles from 20 (1min) to 100 (5 min) did not improve the results (data not shown).

Therefore we tested H_2O_2 and commercial bleach. For H_2O_2 the DNA contamination was highly reduced in comparison to the positive control, but small bands still were detectable (Fig 2). The amount of DNA equates the critical value of $\geq$ 1 pg DNA (less than 1 cell).
With commercial bleach already after 1 min of cleaning no DNA was detectable (Fig 2).

Cleaning of tips with heated DNA Exitus also removes DNA completely; we could not detect any DNA contamination whereas the positive control shows distinct DNA bands (Fig 2). The ~300 bp fragment equates to the LINE-1 PCR product of the LINE-1 gene (3).

Furthermore, we tested the effects of the used chemicals on the PCR reaction. For H_2O_2 and bleach we could not detect a significant PCR inhibition.
In contrast, following the cleaning of tips with DNA Exitus without rinsing them with water the PCR reaction was inhibited completely (data not shown). Obviously residues of DNA Exitus still react with DNA during PCR. DNA Exitus is especially reactive at temperatures over 50°C.

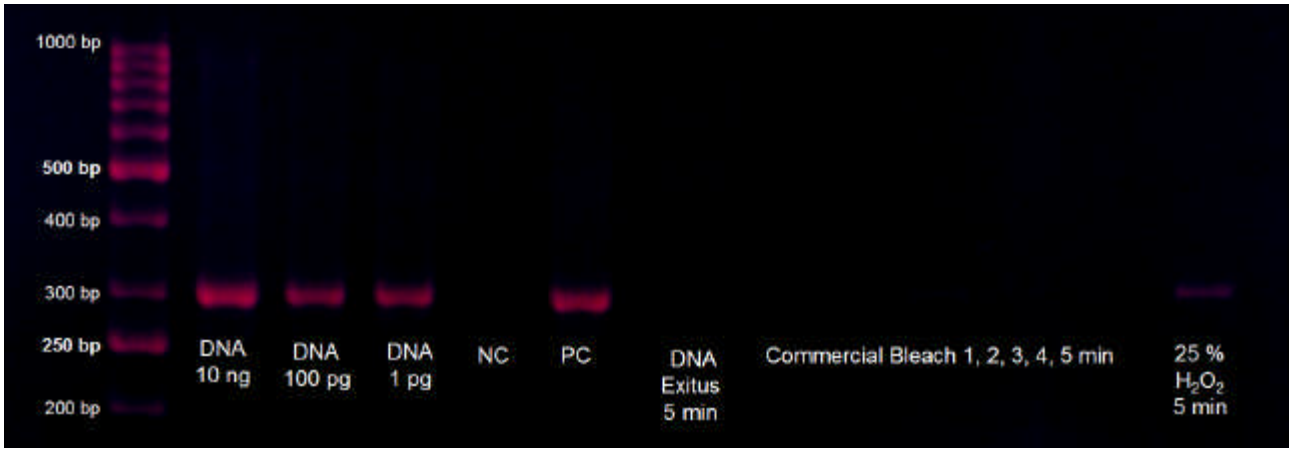

Figure 2: *This figure shows ethidiumbromide stained agarose gel with the 293 bp PCR product of LINE-1 gene. After transfer of DNA (10ng/µl, 10 µl were aspirated and dispensed) the tips underwent different wash procedures and rinsed with PCR master mix (see table 1). Cleaning with H_2O_2 is not sufficient; residues of DNA were detectable. Commercial bleach and heated DNA Exitus remove DNA reliably. DNA Exitus should be heated and applied not less than 5 min.*
NC = negative control, PC = positive control

Discussion

In this study we optimized a tip wash routine for the handling of DNA samples which eliminates DNA cross contamination reliably and allows an automated and reproducible set up at the CyBi®-Well and CyBi®-Well vario.
At first we established a very sensitive PCR test to be able to detect DNA contamination < 1 pg (less than 1 cell).
We used this PCR method to analyze residues of DNA contamination after the cleaning of tips with different chemicals. Rinsing with water only did not remove DNA contaminations. Rinsing with H_2O_2 reduced DNA contamination, but only commercial bleach and DNA Exitus removed DNA completely.

To avoid DNA cross contaminations and effects on down stream applications we recommend the following wash procedure.
First, the tips should be rinsed with commercial bleach for at least 1 minute in a reservoir.
Cleaning with heated DNA Exitus for at least 5 minutes is also possible.
Following this procedure tips should be rinsed with sterile DNA free water to remove all residues of chemicals. For this step we recommend the active deep well Tip Wash Station 96, which ensures the continuous supply of fresh water and allows automation.

Literature

(1a) Heidi Prüfer and Michael Busch
Performance Data demonstrating the Efficiency of the Tip Wash Station of the CyBi®-Well Family. *CyBio AG*
http://www.cybio-ag.com/files.php?dl_mg_id=627&file=dl_mg_1183467328.pdf

(1b) Katrin Undisz, PhD and Heidi Prüfer
Stability of the Precision of Different CyBi®-Well vario Heads after Multiple Tip Washing Routines. *CyBio AG*
http://www.cybio-ag.com/files.php?dl_mg_id=741&file=dl_mg_1222170864.pdf

(1c) Katrin Undisz, PhD
Start-up Routine and Tip Washing Efficiency Data of the Active Deep Well Tip Wash Station 384. *CyBio AG*
http://www.cybio-ag.com/files.php?dl_mg_id=692&file=dl_mg_1198225263.pdf

(2) Mark Hewitson, Hanno Herman, Neil Benn
How to perform high throughput siRNA transfections. *Cenix Bioscience GmbH*
http://www.cybio-ag.com/files.php?dl_mg_id=679&file=dl_mg_1195134764.pdf

(3) Deragon JM, Sinett D, Mitchell G, Potier M, Labuda D
Use of gamma irradiation to eliminate DNA contamination for PCR. *Nucleic Acids Res. 1990 Oct 25; 18(20):6149*
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC332437/?tool=pubmed

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