Automated genomic DNA extraction

from buccal cells with Invitrogen's ChargeSwitch® Technology

Extracting genomic DNA (gDNA) from buccal swabs or pelleted mouthwashes is extremely useful as a quick, non-invasive technique for collection and isolation of DNA. This method is used for DNA extraction in many applications, including genotyping, detection of disease markers and for comparison to crime scene samples. In response to the demand for increased sample throughput in these applications, Tecan and Invitrogen have developed a range of ChargeSwitch® Technology-based nucleic acid purification kits that are fully automated using Tecan robots.

The quantity of cells and, therefore, DNA obtained from a buccal swab or pelleted mouthwash can often be very low. As a result, techniques used for purification of DNA from buccal cells must be sensitive and reliable, as well as ensure that the DNA obtained is suitable for relevant downstream applications such as PCR, sequencing and STR analysis. Currently, there are a number of automated techniques for the purification of gDNA from buccal cells, all of which often use harsh ionic chaotropes, such as guanidinium isothiocyanate, hazardous organic reagents such as ethanol, phenol, chloroform or isopropranol (IPA), or expensive and undesirable alcohols. All of these reagents can penetrate during the purification process and this can have a detrimental effect on the efficiency of enzymes such as Taq® polymerase, resulting in reaction failure, as well as potentially causing problems for liquid handling systems, such as salt precipitation in lines. ChargeSwitch® Technology avoids the use of any chaotropes, organic solvents or alcohols and delivers high yield, high purity nucleic acid in a rapid and cost effective manner.

Nucleic acid sample preparation with ChargeSwitch® Technology

ChargeSwitch® Technology is a unique chemistry that acts as a pH-dependent ionic switch, which is "switched on" at a lower pH, becoming positively charged and binding DNA. When "switched off" by raising the pH, the charge is neutralized, allowing purified DNA to be released. The ChargeSwitch® protocol is summarized on the opposite page.

Automating gDNA isolation

Automated purification of gDNA from buccal cells using ChargeSwitch® Technology on Tecan workstations (Figure 1), such as the Genesis™ or Freedom EVO®, is a reliable, walkaway process with many advantages. Automated protocols for isolating gDNA from buccal cells generally require either a centrifugation or vacuum step, which can cause a bottleneck in the process.

Using ChargeSwitch® Technology, coated onto the highest quality magnetic beads available, vacuum and centrifugation steps are no longer necessary, making the technology ideally suited to high throughput environments.

During gDNA isolation, variations in template quantity may have an effect on the final STR profile, where too much template may lead to overamplification and saturation, while too little template may result in partial profiles or failures. Through limitation of binding capacity, ChargeSwitch® Technology can provide a normalized yield of purified gDNA in the range of 1-3 ng/ml. At this concentration, the need for quantification is removed and users can readily modify the amount of beads added in order to adjust the normalization. Furthermore, using the ChargeSwitch® Genomic DNA Purification protocol (ChargeSwitch® gDNA Normalized Buccal Cell Kit, Cat. No.

(a) Low throughput

(b) Medium throughput

(c) High throughput

(d) Ultra high throughput

Figure 1: *Tecan automated platforms*

ChargeSwitch® protocol

Digest

1. Add 1 ml of digestion reagent to a buccal cell swab in a 96-well deep well plate.
2. Incubate at 37-55°C for 20 minutes.

Bind

3. Transfer digest supernatant into a fresh 96-well deep well plate containing purification reagents and ChargeSwitch® beads.
4. Mix and incubate for 5 minutes.

Wash

5. Separate beads on the magnet and discard supernatant.
6. Resuspend ChargeSwitch® beads in 1 ml of wash buffer and wait for a pellet to reform over the magnet.
7. Remove supernatant and repeat wash step.

Elute

8. Remove plate from magnet and resuspend ChargeSwitch® beads in 150 µl of elution buffer.

CS11020) can remove the need for quantitation prior to STR analysis, saving time and increasing throughput (Figure 2).

When fully automated on Tecan workstations, the ChargeSwitch® protocol offers complete walkaway to minimize risk of cross contamination (Figure 3). ChargeSwitch® avoids the use of enzyme inhibitors, increasing the performance of enzymatic reactions. The high yielding kit can provide larger yields for less sensitive downstream reactions, or for when DNA needs to be archived.

For more information about ChargeSwitch® Technology and its use in genomic DNA extraction from buccal cells, please go to www.invitrogen.com/naprep.

ChargeSwitch® protocols are also available for automated plasmid micropreps with no centrifugation or vacuum filtration, automated PCR clean-up with adjustable size exclusion and automated gDNA purification from blood with no centrifugation or vacuum filtration.

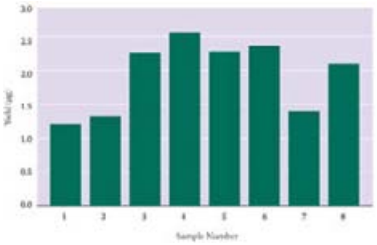

Figure 2: *High yield data*

Yield of gDNA purified from eight samples using the ChargeSwitch® Genomic DNA Purification protocol (ChargeSwitch® gDNA Buccal Cell Kit, Cat No. CS11021) on the Tecan Genesis. The variation in concentration is due to the amount of epithelial cells collected in the sampling process.

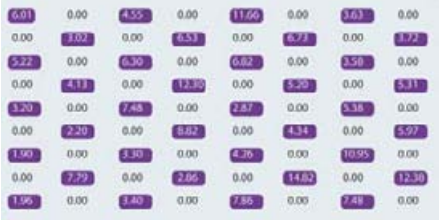

Figure 3: *Low risk of cross contamination*

Single buccal swabs were placed in alternate microtitre plate wells (highlighted in purple) to demonstrate that the adjacent empty wells would not be contaminated by DNA from other samples. DNA was purified using the ChargeSwitch® Genomic DNA Purification protocol (Buccal Cells, High Yield) on the Tecan Genesis robotic workstation. The DNA content of each microtitre plate well was then measured using Quant-it™ PicoGreen® analysis (values are shown in ng/µl). Results indicate that cross contamination of samples does not occur.

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