

Automated Genomic DNA Extraction from Buccal Cells on the Tecan Freedom® EVO Workstation

- Rapid purification of genomic DNA in a normalized or high yielding format
- No centrifugation or vacuum filtration
- Automated solutions for different throughputs available

Nucleic Acid Sample Preparation

Genomic DNA from buccal cells with ChargeSwitch® Technology

Driven by the need for increased sample throughput for genome sequencing projects and diagnostic applications, Tecan has developed a range of automated solutions for nucleic acid purification kits which allows fully automated processing on the tecan robots, with a long walk-away time.

Isolation of high-quality DNA is a prerequisite for success in any molecular biology assay. In the coupling of Tecan robotic platforms with ChargeSwitch® Technology, researchers can confidently leave their robotic systems and concentrate their efforts elsewhere. ChargeSwitch® Technology delivers high-yield and high-purity nucleic acid in a rapid and cost effective manner.

Low-throughput



Medium-throughput



High-throughput



Ultra high-throughput



Figure 1–Tecan automated platforms

CHARGE SWITCH TECHNOLOGY

ChargeSwitch® Technology, is a revolutionary approach to nucleic acid purification.

Scientists can now purify DNA in a **100% aqueous environment** without using ionic chaotropes, such as guanidinium isothiocyanate, or organic reagents, such as ethanol, phenol, chloroform or IPA.

These reagents, used in most nucleic acid purification technologies are hazardous and can cause problems for liquid handling systems. Additionally, these chemicals can potentially break through to the final elution, interfering with, and sometimes causing failure of downstream, enzyme-dependant processes.

ChargeSwitch® Technology is a unique chemistry that acts as a pH-dependant ionic switch (Figure 2). When “switched on” at a lower pH, it becomes positively charged and binds DNA. When “switched off” by raising pH, the charge is neutralized allowing purified DNA to be released.

GENOMIC DNA ISOLATION FROM BUCCAL CELLS

Extracting genomic DNA from buccal swabs or pelleted mouthwashes is extremely useful as a quick, non-invasive technique for collection and isolation of DNA.

DNA extracted by this method is used in many applications including genotyping, detection of disease markers and for comparison to crime scene samples.

The quantity of cells, and therefore DNA, obtained from a buccal swab or pelleted mouthwash

can often be very low. Techniques used for purification of DNA from buccal cells must therefore be sensitive, reliable and 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 genomic DNA from buccal cells, all of which use either:

- Harsh ionic chaotropes
- Hazardous organic solvents
- Expensive and undesirable alcohols

All of these reagents can break through during the purification process and can have a detrimental effect on the efficiency of enzymes such as Taq polymerase. This can result in reaction failure.

ChargeSwitch® Technology avoids the use of any chaotropes, organic solvents or alcohols giving you confidence and peace of mind in a highly sensitive reaction.

AUTOMATION OF PROTOCOL

Automated protocols for genomic DNA isolation from buccal cells generally require either a centrifugation or vacuum step in the protocol that can cause a bottleneck in the process.

With 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.

Normalization of Purified Genomic DNA
Variations in template quantity may have an effect on the final STR profile. Too much

template may lead to overamplification and saturation. Too little template may result in partial profiles or failures.

Through limitation of binding capacity, ChargeSwitch® Technology can provide a normalized yield of purified genomic DNA in the range of 1-3 ng/μl. At this concentration, the need for quantification is removed. Additionally users can readily modify the amount of beads added in order to adjust the normalization

CHARGE SWITCH® PROTOCOL (FIGURE 3)

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.

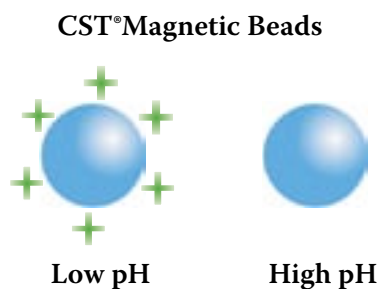


Figure 2—ChargeSwitch® Magnetic Beads

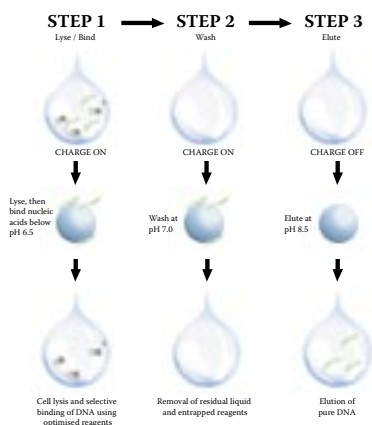


Figure 3—ChargeSwitch® protocol

RESULTS

Data interpretation The purification of genomic DNA from buccal cells using ChargeSwitch® on Tecan workstations is a reliable walk-away protocol with many advantages. The protocol avoids the use of chaotropic salts, organic solvents and alcohols. The ChargeSwitch® Genomic DNA Purification protocol (Buccal Cells, Normalized Yield) can remove the need for quantitation prior to STR analysis, saving time and increasing throughput (Figures 4 and 5). Fully automated on the Tecan Freedom EVO workstation, the protocol offers a walk-away capability with no risk of cross contamination (Figure 6). 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 (Figure 7). The protocol is fully automatable on Tecan workstations.

All data presented have been produced with a Tecan Freedom instrument running with Gemini software. The optimized software scripts can be easily used on the Freedom EVO robotic workstation the newest robotic platform from Tecan.

Obtaining automated ChargeSwitch® protocols
To find out more information about ChargeSwitch® Technology and its use in genomic DNA extraction from buccal cells, please go to www.invitrogen.com

ChargeSwitch® protocols are also available for:

- Automated plasmid micropreps with no centrifugation or vacuum filtration
- Automated PCR clean-up with adjustable size exclusion
- Automated genomic DNA purification from blood with no centrifugation or vacuum filtration

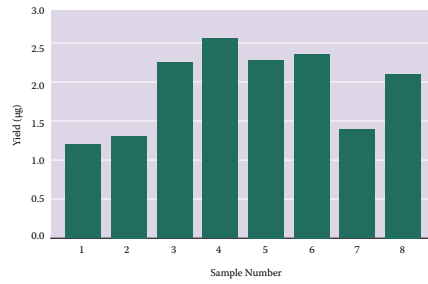


Figure 4—High yield data Yield of genomic DNA purified from 8 samples using the ChargeSwitch® Genomic DNA Purification protocol (Buccal Cells, High Yield) on the Tecan robotic workstation.. The variation in concentration is due to the amount of epithelial cells collected in the sampling process.

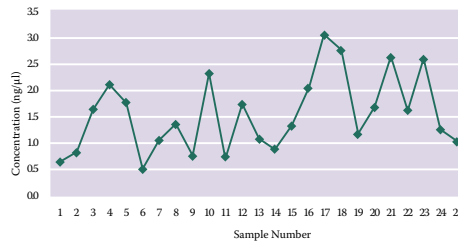


Figure 5—Normalised yield data Shows the concentration of genomic DNA purified from 25 samples using the ChargeSwitch® Genomic DNA Purification protocol (Buccal Cells, Normalized Yield) automated on the Tecan robotic workstation. With the normalized protocol concentrations are kept to below 3 ng/µl.

6.01	0.00	4.55	0.00	11.66	0.00	3.63	0.00
0.00	3.02	0.00	6.53	0.00	6.73	0.00	3.72
5.22	0.00	6.30	0.00	6.82	0.00	3.58	0.00
0.00	4.13	0.00	12.30	0.00	5.20	0.00	5.31
3.20	0.00	7.48	0.00	2.87	0.00	5.38	0.00
0.00	2.20	0.00	8.82	0.00	4.34	0.00	5.97
1.90	0.00	3.30	0.00	4.26	0.00	10.95	0.00
0.00	7.79	0.00	2.86	0.00	14.82	0.00	12.38
1.96	0.00	3.40	0.00	7.86	0.00	7.48	0.00

Figure 6—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 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.

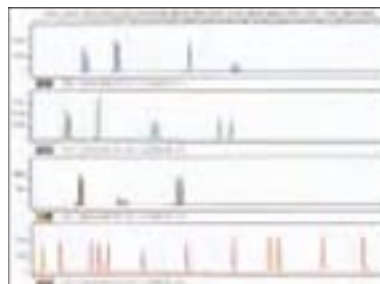


Figure 7—STR profile analysis STR profile generated from genomic DNA purified from buccal cells using the CST® Genomic DNA Purification protocol (Buccal Cells, Normalized Yield). One microliter of the purified material was added to an STR PCR reaction without quantification. The normalized protocol provides DNA up to a concentration of 3 ng/µl, an ideal concentration for STR analysis.

ORDERING INFORMATION

Product	Quantity	Cat. no.
ChargeSwitch® gDNA Buccal Cell Kit	960 preps	CS11021-10
ChargeSwitch® gDNA Normalized Buccal Cell Kit	960 preps	CS11020-10
ChargeSwitch® NoSpin Plasmid Micro Kit	96 preps	CS10201
ChargeSwitch® NoSpin Plasmid Micro Kit	960 preps	CS10201-10
ChargeSwitch® PCR Clean-Up Kit	960 preps	CS12000-10
ChargeSwitch® gDNA 10-20 µl Blood Kit	960 preps	CS11010-10
ChargeSwitch® Forensic DNA Purification Kit	960 preps	CS11200-10
ChargeSwitch® gDNA Plant Kit	960 preps	CS18000-10
96-well Magnetic Separator	96-well	CS15096
Quant-iT™ PicoGreen® dsDNA Assay Kit	200–2000 assays	P7589
Quant-iT™ PicoGreen® dsDNA Reagent	200–2000 assays	P7581

For more information visit www.invitrogen.com/naprep

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