

Application Note

Techniques and Technical Insight to Optimize Use of Whatman Products

CloneSaver Cards are ideal for rapid clone screening through PCR and transformation, and for the long-term, stable storage of clones.

CloneSaver™ Cards employ a filter-based method for archiving and purifying clones that condenses the functions of -80°C freezers and purification kits down to the size of an index card. CloneSaver uses patented Whatman FTA® technology to provide years of ambient clone archiving, protect against and recover from phage infection, and deliver analysis-quality DNA. Clones that are stored and purified using CloneSaver are suitable for many common downstream applications including PCR and transformation (electroporation and heat-shock).

CloneSaver Cards, consisting of a chemically impregnated cellulose matrix, lyse cells on contact and sequester the DNA regardless of glycerol content or growth media. Once separate from the lysed cell and bound to the CloneSaver fibers, the DNA is ready for room-temperature storage in a lab drawer, card catalog or lab notebook. CloneSaver is also suitable for accepting and archiving purified DNA at room temperature. When the sample is required for analysis—whether it is 12, 24 or 48 months later—a small disk is removed from the CloneSaver Card and quickly rinsed. The purified plasmid that remains on the disk can be used directly in analysis or eluted into a small volume and aliquoted into several different reactions. Where traditional methodologies require multiple steps, including at least one overnight incubation, CloneSaver takes clones from “picking list” to analysis in just one hour.

General Protocol

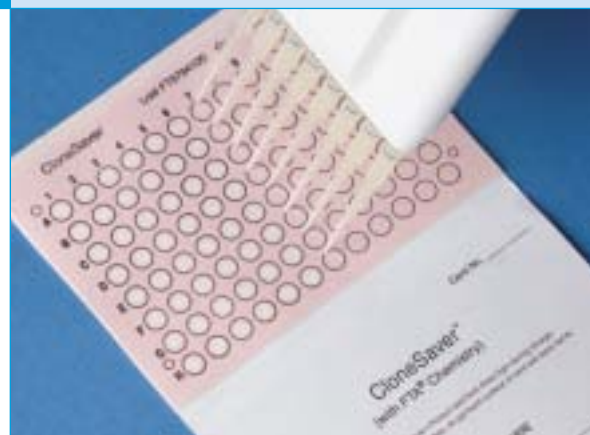
CloneSaver provides a single archive and purification protocol regardless of the downstream application.

- 1) Apply up to 7 μL of culture, glycerol stock or purified plasmid/BAC to a CloneSaver circle. Pink coloration will turn white where sample is applied.
- 2) Allow to dry and store in a dry environment, desiccated if using glycerol or if long-term storage is desired.
- 3) When sample is needed, remove a 2.0 mm disk from white sample area and place in desired vessel (PCR tube, microfuge tube or any vessel with 250 μL capacity).
- 4) Briefly rinse disk with 200 μL of TE^{-1*} by aspirating up and down four times and discarding rinse.
- 5) Repeat step 4 once for a total of two rinses. Be sure to remove any standing rinse around disk.

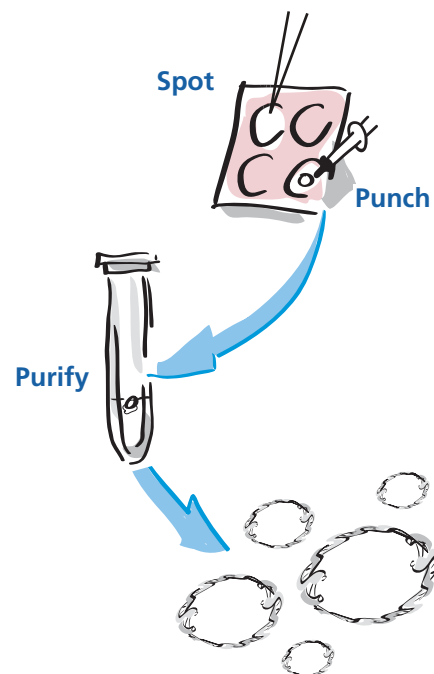
At this point, the plasmid/BAC DNA is clean of any cell debris that may have been present and the disk is clean of any residual FTA chemical. The clone of interest is ready to be analyzed or used to re-transform a new culture. To use the purified plasmid DNA for PCR or transformation, follow the ensuing steps on the subsequent pages.

*TE⁻¹ (10 mM Tris, 0.1 mM EDTA, pH 8.0) reduces the common EDTA inhibitory effect of straight TE (10 mM Tris, 1 mM EDTA)

Whatman CloneSaver Protocols for PCR and Transformation.



Three easy steps to clone recovery



Whatman®

PCR

CloneSaver is the ideal medium for performing rapid clone screens via PCR. Plasmid DNA is extracted and protected as soon as the culture or glycerol stock is applied to the card. After rinsing, the CloneSaver disk—containing double stranded plasmid DNA—can be added directly to the amplification master mix. Alternatively, the DNA can be eluted from the disk and a fraction of the eluate can be used for PCR. The option of amplifying directly from the punch or from the eluate is useful when working with different types of sample. High copy-number plasmid, applied to CloneSaver in an overnight culture, would contain too much DNA to use the entire punch for PCR. The plasmid would require elution and a fraction used as template in the master mix. Conversely, a BAC or low copy plasmid applied from a glycerol stock would require the entire rinsed punch be used for amplification.

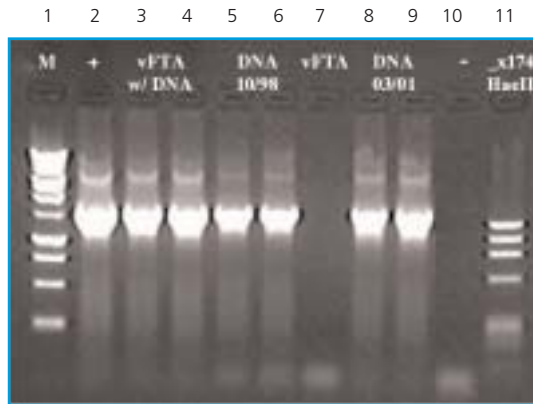
Protocol for PCR Analysis

Follow steps 1–5 of General Protocol and continue as follows:

- 6a) If using the punch: Once all standing liquid is removed, add master mix directly to the rinsed punch.
- 6b) If eluting: Once all standing liquid is removed, add 5 μL of TE^{-1} or dH_2O to the punch and incubate at room temperature for 5 minutes (water is suitable only if the eluate is not to be stored). Add 1 μL of eluate to 25 μL reaction.

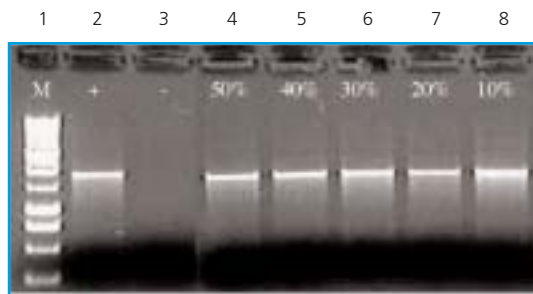
Results

Figure 1. PCR of pMAB32 / JM109 clones applied to CloneSaver over a 30-month period.



- Lane 1: DNA Marker (1 kb. Ladder)
 Lane 2: + Control reaction (using plasmid DNA from a miniprep)
 Lanes 3&4: Control DNA with virgin (unspotted) CloneSaver punch in reaction
 Lanes 5&6: CloneSaver punches spotted in Oct, 1998
 Lane 7: Punch control (negative control with virgin punch in the reaction)
 Lanes 8&9: CloneSaver punches spotted in March, 2001
 Lane 10: - control (negative control PCR with no template DNA in reaction)
 Lane 11: DNA Marker (ϕX174 DNA cut with *Hae* III). Primers were specific for the plasmid pMAB32.
 Forward primer: 5' GAATAAGTGCACATCATCATC 3'
 Reverse primer: 5' GTAATTTCTGGCAAGGTAGAC 3'

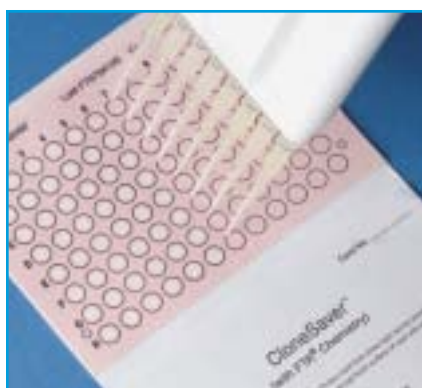
Figure 2. PCR of pGEM-luc/JM109 clones in different amounts of glycerol.



- Lane 1: 1 kb ladder
 Lane 2: + control using purified plasmid
 Lane 3: — control using water
 Lanes 4–8: Amplified product from samples applied to CloneSaver containing varying concentrations of glycerol. Primers are from cloning sites and result in 1.8 kb product when used with pGEM with luciferase insert.

Transformation

CloneSaver is a highly effective storage device because it not only houses the plasmid in an analysis-ready state, it allows the plasmid to be reconstituted via transformation. CloneSaver releases plasmid that is suitable for either electroporation or heat-shock methods and, similar to PCR, CloneSaver does not require overnight growth steps or plasmid purification kits. Electroporation, because of its superior efficiency, can work with either a small aliquot of eluted plasmid or directly from the rinsed punch. Conversely, and due to its inherent inefficiency, heat-shock transformation works best when all available plasmid DNA is added to the reaction.



Protocols for Transformation

Follow steps 1–5 of General Protocol and continue as follows on each protocol:

Electroporation from Eluate

- 6) Once all standing liquid is removed from the punch after the final rinse, add 5 μL of TE⁻¹ or diH₂O to the punch and incubate at room temperature for 5 minutes (water is suitable only if the eluate is not to be stored).
- 7) Add 2 μL of eluate and 20 μL of electro-competent cells to a chilled microfuge tube.
- 8) Incubate on ice for 10 minutes and proceed as normal.

Electroporation Directly from the Punch

- 6) Once all standing liquid is removed from the punch after the final rinse, add 20 μL of electro-competent cells and perform electroporation as usual.

Heat-shock

- 6) Once all standing liquid is removed from the punch after the final rinse, add 5 μL of TE⁻¹ or diH₂O to the punch and incubate at room temperature for 10 minutes (water is suitable only if the eluate is not to be stored).
- 7) Place tube containing punch and eluate on ice for 5 minutes.
- 8) Add chemically competent cells directly to tube and perform heat shock as usual.

Results

Table 1. Various plasmids spotted to CloneSaver and recovered using transformation

Vector [†]	Size (kb)	Copy Number	cfu/Transformation Electroporation [‡]	cfu/Transformation Heat-Shock [§]
pUC19	2.7	500–700	$3.4 \times 10^4 \pm 2.3 \times 10^4$	1009 ± 275
pEZ11104	2.7	100	$2.1 \times 10^5 \pm 0.8 \times 10^5$	9076 ± 4950
pMAB32	11.2	100	$2.8 \times 10^4 \pm 1.7 \times 10^4$	8090 ± 6500
pSUsrmb2	5.8	6–10	$1.4 \times 10^4 \pm 0.9 \times 10^4$	8645 ± 2497

Table 2. Purified plasmid (pGEM-luc) applied to CloneSaver and recovered using electroporation

	Amount of DNA Applied (ng)	cfu/Transformation
DNA Eluted from the Punch	50	1.54×10^5
	125	2.31×10^5
	250	6.40×10^5
DNA Transformed from the Punch	50	8.7×10^4
	125	7.34×10^5
	250	1.0×10^6

Table 3. Electroporation using BAC clones stored on CloneSaver

	Size (kb)	Cfu/Transformation**
Human clone in pBeloBAC	130	1508 ± 729

[†] All vectors spotted to CloneSaver in JM109 strain of *E. coli*

[‡] The competent cells used in the transformation are ElectroMax™ DH5 α

[§] The competent cells used in the transformation are MaxEfficiency DH5 α

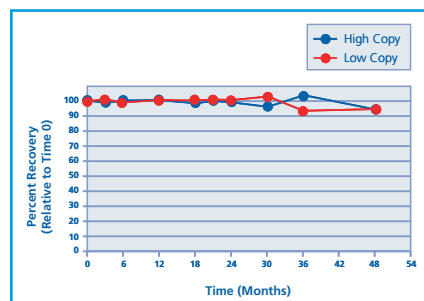
** The competent cells used in the transformation are ElectroMax™ DH10B

Longevity

CloneSaver, like all FTA products, has the unmatched ability to protect nucleic acid samples at room temperature for many years. FTA protects against DNA degradation caused by light and UV exposure, as well as damage caused by microbial growth. Studies have proven the quality and suitability of archived clones for downstream processing after 48 months of storage. Sample colonies were spotted onto CloneSaver 4 years ago and sampled every 6 months. This sampling process involved taking a punch, performing a transformation, and counting the colonies. The counts were plotted relative to day 0. Figure 3 illustrates the capability of CloneSaver to store clones as shown by relative transformation efficiencies. Over time (48 months), both high and low copy number clones maintain the capability of transformation efficiency relative to day one.

Results

Figure 3. Transformation performance of plasmid stored on CloneSaver over a 48-month period.



High copy plasmid (pMAB32 and pEZ11104) and low copy plasmid (pSUsrcMB2) was applied to CloneSaver and used for electroporation over time. The recovery at each time point is represented as a percent of the initial recovery prior to application to CloneSaver (time 0).

The transformation performance obtained from the 48-month samples demonstrates that CloneSaver protects the DNA during storage and provides an effective transformation method.

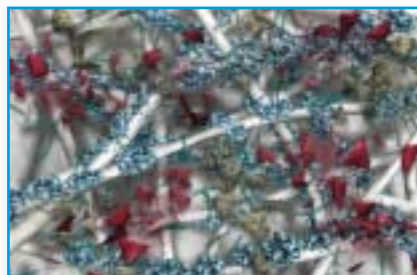
Phage Inactivation

CloneSaver protects against a leading cause of clone loss: phage contamination. CloneSaver not only protects clones against phage contamination, but clones that have already been infected can be applied to CloneSaver and rescued, making them suitable for transformation into a healthy culture. The figures below demonstrate how FTA can rescue infected samples and destroy bacteriophage upon application to a CloneSaver card.



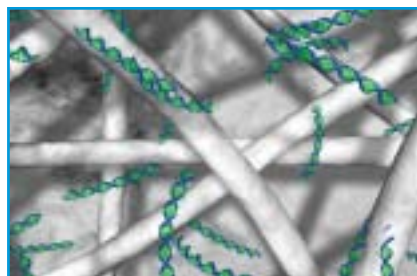
1. FTA matrix prior to sample addition.

The crystalline structures show FTA reagent attached to the paper fibers.



2. FTA after sample application.

FTA is impregnated with chemicals that lyse cell membranes and denature proteins on contact—including bacteriophage. Intact plasmids or BACs are then protected for room temperature archiving.



3. FTA after washing with TE-1 buffer.

After washing with buffer, the phage contamination is gone and the clone DNA is ready for downstream applications.

Whatman Quality

Whatman is a global leader in separations technology and is known in the scientific community for providing innovative Life Science products and solutions. Our instinct for simplification accelerates the rate of discovery, reduces costs and saves time. In order to focus on the unique needs of customers, Whatman is organized into four business development units: LabSciences, BioScience, MedTech-Diagnostics and MedTech-Devices. For more information, visit www.whatman.com.

Whatman, CloneSaver and FTA are registered trademarks of the Whatman Group.

North America Whatman Inc.
200 Park Avenue
Florham Park, NJ 07932 USA
Technical Support: 1-800-922-0361
Customer Service: 1-800-631-7290
E-mail: info@whatman.com

Europe Whatman International Ltd
Springfield Mill, James Whatman Way
Sandling Road, Maidstone
Kent ME14 2LE UK
Tel: + 44 (0)1622 676670
Fax: + 44 (0)1622 691425
E-mail: information@whatman.com

Japan Whatman Japan KK
Daiwa Ueno Building 1F 6-10
Ueno 5-chome, Taito-ku
Tokyo 110-0005, Japan
Tel: +81 (0)3 3832 6707
Fax: +81 (0)3 3832 6457
E-mail: japaninfo@whatman.com

Asia Pacific Whatman Asia Pacific Pte Ltd
171 Chin Swee Road
#08-01 San Centre
Singapore 169877
Tel: +65 6534 0138
Fax: +65 6534 2166
E-mail: wap@whatman.com

Additional information
on CloneSaver and
other FTA products is
available from Whatman.
www.whatman.com
or 1-800-922-0361

Whatman®

Leaders in Separations Technology
www.whatman.com