Instructions for use

FTA® Clonesaver Cards
From Qiagen

Description:

CloneSaver Cards are designed for the long-term, room temperature storage of plasmid and BAC DNA clones from bacterial cultures, resuspended colonies or glycerol stocks. DNA stored on CloneSaver Cards is ready for immediate bacterial transformation and PCR amplification or may be archived for later use. CloneSaver Cards utilize patented FTA technology that lyses cells and protects the released DNA. Samples applied to the Card as directed will not contaminate adjacent samples. The specimen area is formatted in a 96-well configuration and its pink colour changes to white upon application of samples enabling one to easily identify sample location.

Precautions:

Handling: Always wear gloves to avoid contamination of FTA CloneSaver Cards. Follow universal precautions when handling biological specimens.

Storage: Store unused cards in original packaging in a cool, dry clean environment. After applying samples, allow CloneSaver Cards to dry, then store at room temperature in a dry environment.

INSTRUCTIONS:

Application of Bacterial Samples:

1. Unfold the CloneSaver Card to expose the pink matrix card (96 well format).
2. Pipette 5 µl of an overnight bacterial culture, e.g., OD_{600}~1.8 in LB, into the centre of one of the 96 printed circles. Bacterial colonies can be applied by first resuspending a single colony in 5 µl of sterile growth media or sterile TE buffer with reduced EDTA (10 mM Tris, 0.1 mM EDTA, pH 8.0) and then spotting the sample. When the sample is applied, the pink colour of the spot will turn white. To prevent cross contamination, apply no more than 5 µl of sample to each circle.
   Bacterial glycerol stocks can also be applied to CloneSaver Cards by simply diluting a small amount in 5 µl growth medium or reduced TE buffer.
3. After applying sample, label the corresponding area of the CloneSaver ID Chart.
4. Let samples air-dry for 1 hour prior to storage or processing. Heat-assisted drying is not recommended. Avoid contact with potential contaminants while drying.
5. Once completely dry; fold the CloneSaver Card so that the pink matrix card is turned down facing the Quick Directions. The cover can be closed over the turned down card.

Storage of Plasmid or BAC DNA on CloneSaver Cards:

Plasmid or BAC DNA on CloneSaver Cards should be stored at room temperature in a cool, dry place (avoid light and excessive humidity). The recommended storage procedure is to place the CloneSaver Card in a Re-sealable Multi-Barrier Pouch (Art. No. HP12.1), if preferred with a Storage Desiccant Packet.

Preparation of CloneSaver Punch for Transformation or PCR:

1. Remove a 2.0-mm punch from the target sample using a Harris Micro-Punch™ (Art. No. HP16.1) or a similar device according to its instructions.
   Note: Since Bacterial clones contain high numbers of DNA copies, it is important to ensure that no residual material is carried from one punch to the next. To clean the punch after each sample, one can either take two punches in a blank area of the CloneSaver Card (or clean filter paper) or wash the punch tip with alcohol between samples.
2. Transfer the CloneSaver punch to a microcentrifuge tube suitable for transformation or PCR.
3. Wash the punch by adding 200 µl of sterile TE buffer with reduced EDTA to the tube and gently pipetting the buffer up and down twice. Completely remove and discard the TE buffer.
   Note: To minimize plasmid loss, do not let the punch remain in TE buffer longer than necessary for the washing step.
4. Repeat step 3 and then remove all traces of TE buffer.
5. The washed punch can now be used for transformation or PCR as described below. If the CloneSaver punch needs to be transferred to a different tube use a sterile pipette tip.

Transformation Using a Washed CloneSaver Punch:

For plasmid DNA, a washed CloneSaver punch can be used to transform either electrocompetent cells or chemically competent cells. For BAC DNA, transformation using a punch with electrocompetent cells is recommended.

1. For the heat shock method of transformation, carefully pipette chemically competent cells directly to the tube containing the washed CloneSaver punch. Perform the transformation according to standard protocols.
2. For transformation by electroporation, place the tube containing the washed CloneSaver punch on ice. Add electrocompetent cells directly to the tube and incubate, on ice, for 10 minutes. Transfer the cells and punch to an electroporation cuvette. Perform transformation according to standard electroporation protocols.
   Note: Electroporation can also be performed using DNA eluted from the punch as described below.

Transformation Using Plasmid DNA Eluted from a CloneSaver Punch:

Sufficient plasmid DNA can be eluted off the CloneSaver punch for transformation by electroporation. This procedure should not be used with BACs stored on CloneSaver Cards or for transformation using chemically competent cells.
Preparation of Eluted DNA from a CloneSaver Punch

1. Prepare a washed punch as described in 'Preparation of CloneSaver Punch for Transformation or PCR', Steps 1-4.
2. Add 5 µl sterile TE buffer with reduced EDTA to the punch in the wash tube. Incubate for 10 minutes at room temperature.
3. Transfer 2 µl of the eluted DNA to a tube containing electrocompetent cells.
4. Perform the transformation according to standard electroporation protocols.
   Note: Transformation efficiency will vary with copy number, plasmid size, concentration of cells spotted,

and efficiency of the competent cells used. Transformation of high efficiency competent cells (with high copy number plasmids up to 10 kb in size) will produce up to 10.000 colonies when 100 µl of a 1 ml transformation is spread on selective agar plates.

PCR Using Either a Washed CloneSaver Punch or DNA Eluted from a CloneSaver Punch:

Sample Preparation – Washed Punch
Prepare a washed punch as described in 'Preparation of CloneSaver Punch for Transformation or PCR', steps 1-4. Add PCR reaction mixture (25 µl) directly to the punch.

Sample Preparation – Eluted DNA
Prepare eluted DNA as described in 'Preparation of Eluted DNA from a CloneSaver Punch'. When DNA is eluted from a CloneSaver Card as described, 1 µl of eluted DNA can be used in a 25 µl PCR reaction.

PCR Procedure
Follow standard PCR protocols. No alterations in the reaction mix or cycling conditions are required.

PERFORMANCE:

CloneSaver Cards are tested to assure successful recovery of plasmid DNA for transformation and PCR amplification. Genomic DNA stored on FTA Cards has been shown to be stable for over 10 years. It is recommended that bacterial samples be applied to properly stored CloneSaver Cards within 3 years of manufacture date

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

For additional protocol information, please contact us under info@carlroth.de.

FTA® CloneSaver Cards	HP11.1
FTA® Pouch CloneSaver	HP12.1

Other FTA Products:

FTA® Cards MINI		CL90.1
FTA® Cards MINI indicated		CL91.1
FTA® Pouch MINI		CL92.1
FTA® Cards CLASSIC		CL93.1
FTA® Cards CLASSIC indicated		CL94.1
FTA® Pouch MAXI		CL95.1
FTA® Purification Reagent		CL99.1
FTA® Foam Tipped Applicator		HP14.1
Harris Uni-Core Punches 1 mm	25 piec.	6729.1
Harris Uni-Core Punches 1.2 mm	4 piec.*	HP15.1
	25 piec.	HP15.2
Harris Uni-Core Punches 2 mm	4 piec.*	HP16.1
	25 piec.	HP16.2
Harris Uni-Core Punches 3 mm	4 piec.*	6798.1
	25 piec.	6798.2
Harris Uni-Core Punches 6 mm	4 piec.*	6799.1
	25 piec.	6799.2

*incl. 2 cutting mats

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