

Additional Information

Standard PCR-ST Protocol

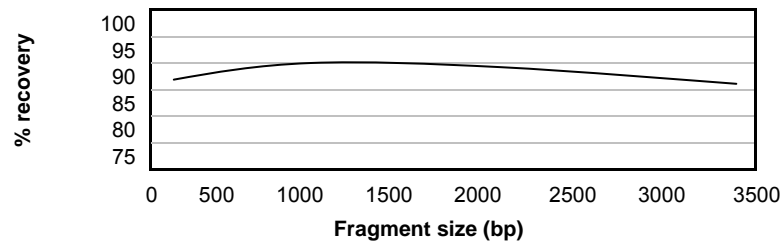


Table 1: Average recovery for DNA fragments of various lengths.

Modified PCR-ST Protocol for smaller fragments

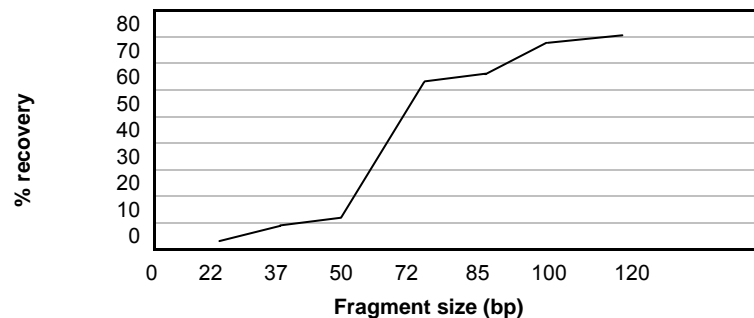


Table 2: Average recovery for small DNA fragments using modified protocol.

Genaxxon bioscience PSI Clone PCR Purification Kit

Product	Cat#	Package size
PSI Clone PCR Purification Kit	S5303.0075	75 preps
PSI Clone 96 high throughput PCR purification kit	S5303.2196	1 x 96 well plate
Big BAC DNA Isolation Kit	S5302.0550	5 x 250 ml culture volume
96 well plate BAC DNA Isolation Kit	S5302.0196	1 x 96 well plate
96 well plate BAC DNA Isolation Kit	S5302.0496	4 x 96 well plate

Purification protocol using the PSI Clone Big BAC Kit

Introduction:

The PSI CLONE PCR ST purification kit is designed to provide the researcher with a rapid method for processing PCR reaction products in a convenient single tube format for centrifugal application. The protocol is fast and results in high yield, high purity DNA which is suitable for use in molecular biology procedures.

DNA fragments (ranging from 100 bp to 10 kb) from PCR reactions bind to the membrane in the spin tube supplied with the kit. Subsequent wash steps remove residual primers, nucleotides, enzymes, and salts. Following wash steps, DNA is eluted in a low salt buffer in high yield (table 1) and is suitable for further molecular operations without additional processing.

Residual primers, nucleotides, enzymes and salts in PCR amplification reaction products may interfere with molecular operations (e.g. cloning, sequencing etc.). The PSI Clone PCR ST kit efficiently removes these residual reactants using a convenient single tube format. The purified amplicons are ready for direct sequencing or subcloning.

The PSI Clone Mini BAC DNA Kit utilizes a simple spin column protocol free of organics to yield high purity BAC DNA.

Material required but not supplied:

- 95% to 100% ethanol
- centrifuge for microtubes

Kit components and storage requirements

PCR Spin Tubes	75 tubes	Supplied ready to use
Binding buffer	12 ml	This solution is subject to crystallisation at temperatures below 22°C. If crystals form, warm the buffer to 50°C to dissolve, prior to use. Store above 22°C. Protect from light. Expiration date as stated on label.
Wash buffer *	12 ml	Provided as concentrate, the Wash Buffer requires dilution with ethanol to a final ethanol concentration of 75-80% * Store at RT. Expiration date as stated on label.
Elution buffer	10 ml	Use as provided. Store at RT. Expiration date as stated on label.
Wash tubes	75 tubes	Provided for use with binding and wash steps.
Sample collection tubes	75 tubes	Provided for use with final elution step.

* add 48 mL ethanol (95-100%) to the Wash Buffer concentrate prior to use.

Related Products

Product	Cat#	Pack size
ExtraClean DNA Kit (standard soil)	S5360.0050	50 Purifications
ExtraClean DNA Kit (humic acids)	S5361.0050	50 Purifications
Justspin Gel Extraction columns	S5337.0050	50 Extractions

Protocol

Processing of amplification products from PCR reactions using centrifugation.

Add 48 mL ethanol (95 – 100%) to the Wash Buffer concentrate (#2) prior to use.

- Add 1 volume of PCR binding buffer ^{1,2} to 1 volume of PCR reaction ^{1,2} product and mix thoroughly (vortex).
- Put a single PCR column in a wash tube (2 mL) and transfer the DNA mixture to the PCR column (filter) and centrifuge at 8,000 x g for 30-60 seconds. Discard the flow thru..
- Add 400 µL PCR wash buffer (diluted with ethanol) to the PCR column and centrifuge a 8,000 x g for 30-60 seconds. Discard the flow thru.
- Repeat step 3 once more. Ensure that the membrane is spun dry.
 - (optional) re-spin (as in step 4 above) the PCR column empty to remove residual wash buffer if membrane has not been spun dry.
- Add 50-65 µL PCR elution buffer and incubate 2-5 minutes to allow buffer to soak into the membrane..
- Place the PCR column in a DNase free (autoclaved) collection tube and centrifuge at 8,000 x g for 30-60 seconds to collect the eluant. Discard the PCR column and save the flow thru (eluant).

Notes:

- Follow this protocol for amplicons in the range of 150 bp to 10 kb.
- For fragments smaller than 150 bp and larger than 70 bp, revise step 1 of the protocol. Use 5 volumes of PCR binding buffer to 1 volume of PCR reaction product (table 2). Additional binding buffer will be required and is available as Catalogue number PC-503 (60 ml bottle of binding buffer).

Trouble shooting

Failure to follow the protocol at the wash step may result in low recovery of fragments

- Check that ethanol was added to the Wash Buffer (#2)
- Thoroughly dry the spin tubes before adding the Elution Buffer