Chromatrap

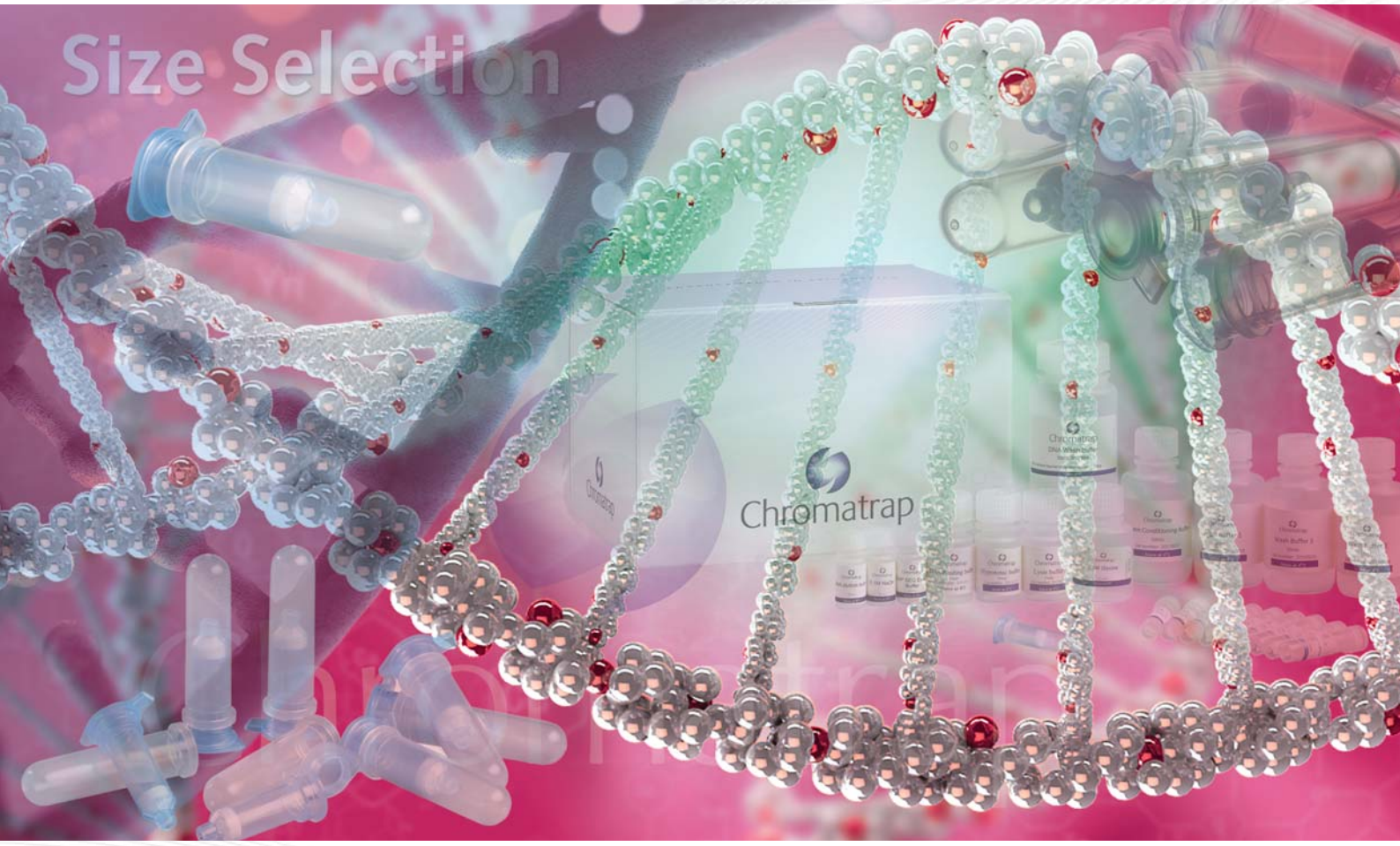
V 1.0

Chromatrap® Size Selection

Chromatrap® spin columns for the efficient purification and size selection of DNA from enzymatic reactions and library preparations

Protocol v1.0

Catalogue no. 500262



ADVANCEMENTS IN EPIGENETICS

SIZE SELECTION

Contents

Introduction

The Chromatrap® Size Selection kit is designed for size selection of DNA fragments from a wide range of sources including PCR mixtures, ChIP samples, restriction enzyme digestions, ligations and NGS sequencing library reactions. Using proprietary filtration media, high quality DNA fragments of desired size can be effectively selected in under 5 minutes. Buffers are optimised to remove any unwanted impurities while providing efficient DNA recovery from samples at the selected size. DNA obtained using the Chromatrap® Size Selection kit is suitable for sensitive reactions such as ligation, PCR, and Next Generation Sequencing (ChIP seq, Medip seq, etc). DNA fragments ≥200 bp, ≥150 bp, ≥100 bp, ≥50 bp can be recovered efficiently and quickly using Chromatrap® DNA size selection columns. The Chromatrap® DNA Size Selection kit is ideal for removing unwanted DNA fragments such as primer dimers formed during NGS library preparation or free nucleotides from PCR reactions.

Kit components and storage

Kit component	Quantity	Storage temperature
Chromatrap® Size Selection columns	50	RT
1.5 ml Collection Tubes	50	RT
SzS Binding Buffer	30 mls	RT
SzS Wash Buffer*	10 mls	RT
SzS Elution Buffer	3 mls	RT

The Chromatrap® Size Selection kit (Cat no. 500262) and its contents can be stored for up to 12 months after the date of receipt without showing a reduction in performance and quality.

Chromatrap® products are intended for research purposes only.

*Preparation of SzS Wash Buffer: Add 40 ml ethanol (96-100%) to the SzS Wash Buffer concentrate and note on the label with the date.

WARNING: *Some of the components of this product are irritants, refer to MSDS sheet for more information and follow the safety guidelines of your research facility.*

Additional materials required

- Ethanol (96-100%)
- Nuclease free water

Protocol

1. Add up to 100 µl of sample DNA to a 1.5 ml microcentrifuge tube. If the sample volume is lower than 100 µl make up to a final volume of 100 µl using nuclease-free water.

2. In a separate 1.5 mL microcentrifuge tube place 500 µl of Chromatrap® SzS Binding Buffer and adjust the amount of 95% ethanol to select for the desired length of DNA fragments (see Table 1 below). Mix well by pipetting the entire volume up and down at least 5 times.

Table 1

DNA fragment size preserved	Volume of 95% Ethanol
≥200 bp	50 µl
≥150 bp	75 µl
≥100 bp	100 µl
≥ 50 bp	250 µl

For example, to remove fragments smaller than 150 bp, add 75 µl 95% ethanol to 500 µl SzS Binding Buffer and mix well

3. Transfer each DNA sample from Step 1 to its corresponding SzS Binding Buffer/ethanol mix from step 2. Mix well by pipetting.

4. Transfer the entire DNA/Binding Buffer/ethanol mixture volume (650 µl-850 µl) to a Chromatrap® DNA Size Selection column and close the cap.

5. Centrifuge 16,000xg for 60 seconds at RT. Discard the flow through and place the spin column back into the same collection tube.

6. Add 700 µl of SzS Wash Buffer to each column and centrifuge 16,000xg for 60 seconds at RT. Discard the flow through and place the spin column back into the same collection tube.

7. Add 250 µl of SzS Wash Buffer to each column and centrifuge 16,000xg for 60 seconds at RT. Discard the flow through and place the spin column back into the same collection tube.

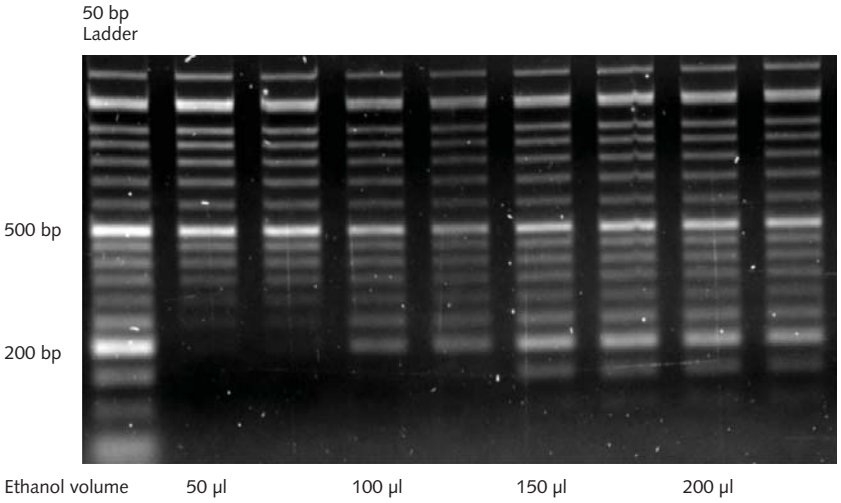
8. Perform a dry spin, 16,000xg for 60 seconds at RT, to remove any residual liquid from the spin column.

9. Transfer the Chromatrap® DNA Size Selection column to a new clean 1.5 mL microcentrifuge tube.

10. To elute the DNA, add 20-50 µl SzS Elution Buffer to the centre of the filter and incubate for at least 1 minute at RT. Centrifuge at full speed for 60 seconds to collect the eluted DNA.

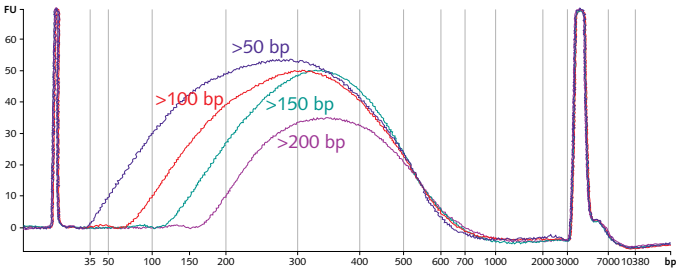
Appendix

Figure 1. Efficient purification of 50-200 bp fragments from a full range of DNA sizes.



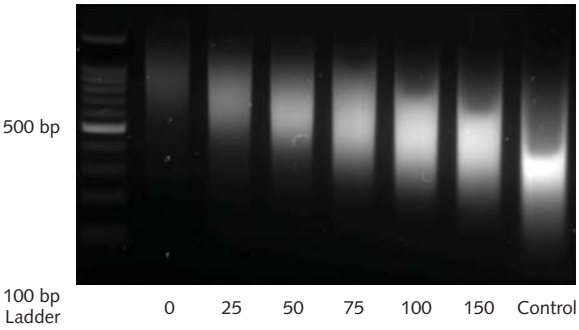
Chromatrap®'s Size-Selection Kit was used to purify DNA excluding fragment sizes from 50-200 bp. Following purification, 10 µl from each 30 µl elution was loaded on a 2% agarose gel. 10 µl of DNA ladder (50-200 bp ladder) was used per sample. Highly efficient DNA recovery within the expected size range is clearly demonstrated.

Figure 2A. Efficient purification and size selection of 50-200 bp sheared salmon sperm DNA analysed by Bioanalyser.



A - Bioanalyser traces of the salmon sperm size selection and purification. A - 1 in 40 dilution was made of each purified sample in nuclease free water and 1 µl of the dilution was analysed using a High Sensitivity DNA chip on an Agilent 2000 bioanalyser.

Figure 2B. Efficient purification and size selection of 50-200 bp sheared salmon sperm DNA.



B - Chromatrap®'s Size Selection kit was used to purify DNA excluding sizes from 50-200 bp. 10 µg of salmon sperm DNA fragmented by sonication (50- 1000 bp) was used per sample. Following purification, 10 µl from each 30 µl elution was loaded on 2% agarose gel.

Troubleshooting Guide and FAQs

1. I have very little DNA recovery following DNA purification, why?

Ensure that the ethanol used is always between 96 and 100%.
Ensure ethanol (96-100%) has been added to the SzS Wash Buffer.
Make sure that the lid to the wash buffer is fastened tightly after use to prevent ethanol evaporation.
Add SzS Elution Buffer directly to the centre of each Chromatrap® Size Selection column to ensure the membrane is completely covered for the best elution efficiency.
Input DNA may be low, check efficiency of reactions being carried out prior to clean up and size selection.

2. How do I choose my cutoff value?

To maximise the removal of undesired bands, always choose a cutoff at least 50 bp above the size you want to remove. Please check Table 1 for required volumes.

Table 1

DNA fragment size preserved	Volume of 95%Ethanol
≥200 bp	50 µl
≥150 bp	75 µl
≥100 bp	100 µl
≥ 50 bp	250 µl

3. My purity ratio is low/below the limits which indicate the sample is not pure, why?

This may be due to residual ethanol from the wash buffer. An additional dry spin (full speed for 60 seconds at RT) should ensure the removal of any residual DNA Wash Buffer from your sample.
Make sure to perform both washes using the SzS Wash Buffer.
If necessary, eluted samples can be heated to 56°C for 10 minutes before loading into the gel to evaporate any residual ethanol.

4. My sample floats out of the well when trying to load a gel what can I do?

This indicates ethanol carryover. The SzS Wash Buffer may not have been completely removed during dry centrifugation step. Try an additional dry spin (full speed for 60 seconds at RT) to ensure the removal of any residual SzS Wash Buffer.
If necessary, eluted samples can be heated to 56°C for 10 minutes before loading into the gel to evaporate any residual ethanol.

5. Can I reduce the elution volume?

This kit has been optimised for an elution volume of 50 µl. However, this may be reduced to a minimum of 20 µl. For NGS library preparation the elution volume is 20 µl.
It is possible to elute in smaller volumes, however this may lead to a reduction in DNA yield.

6. Can I increase the recovery of my DNA eluted?

The yield of DNA may be improved by incubating the SzS Elution Buffer on the columns for 5 minutes before elution by heating the SzS Elution Buffer to 50°C.

7. Can I elute in water?

The yield of DNA may be improved by incubating the SzS Elution Buffer on the columns for an additional 5 minutes during elution or by heating the SzS Elution Buffer to 50°C prior to elution.

Other products available from Chromatrap®

ChIP products

Product	Quantity	Catalogue no.
Chromatrap® ChIP-seq Pro A	24	500189
Chromatrap® ChIP-seq Pro G	24	500190
Chromatrap® HT ChIP-seq Pro A	1 x 96	500214
Chromatrap® HT ChIP-seq Pro G	1 x 96	500215
Chromatrap® Enzymatic ChIP-seq Pro A	24	500191
Chromatrap® Enzymatic ChIP-seq Pro G	24	500192
Chromatrap® HT Enzymatic ChIP-seq Pro A	1 x 96	500216
Chromatrap® HT Enzymatic ChIP-seq Pro G	1 x 96	500217
Chromatrap® ChIP qPCR Pro A	24	500071
Chromatrap® ChIP qPCR Pro G	24	500117
Chromatrap® Premium ChIP qPCR Pro A	24	500115
Chromatrap® Premium ChIP qPCR Pro G	24	500116
Chromatrap® HT ChIP qPCR Pro A	1 x 96	500161
Chromatrap® HT ChIP qPCR Pro G	1 x 96	500163
Chromatrap® HT Enzymatic ChIP qPCR Pro A	1 x 96	500162
Chromatrap® HT Enzymatic ChIP qPCR Pro G	1 x 96	500164
Chromatrap® Enzymatic ChIP qPCR Pro A	24	500166
Chromatrap® Enzymatic ChIP qPCR Pro G	24	500168
Chromatrap® Premium Enzymatic ChIP qPCR Pro A	24	500167
Chromatrap® Premium Enzymatic ChIP qPCR Pro G	24	500169
Chromatrap® FFPE ChIP-seq Pro A	24	500235
Chromatrap® FFPE ChIP-seq Pro G	24	500236
Chromatrap® Native ChIP-seq Pro A	24	500237
Chromatrap® Native ChIP-seq Pro G	24	500238
Chromatrap® Sonication Shearing		500239
Chromatrap® Enzymatic Shearing		500165

DNA products

Product	Quantity	Catalogue no.
Chromatrap® DNA Purification	50	500218
Chromatrap® Gel Purification	50	500219
Chromatrap® HT DNA Purification	2 x 96	500220
Chromatrap® HT DNA Purify and Concentrate	2 x 96	500240
Chromatrap® DNA Extraction	50	500260
Chromatrap® HT DNA Extraction	2 x 96	500261
Chromatrap® Size Selection	50	500262
Chromatrap® HT Size Selection	2 x 96	500263

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