Standard Operation Procedure (S.O.P.) for DNA Purification using Wizard SV Gel and PCR Clean-Up System

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This S.O.P. will direct you in the procedure of DNA purification through the Wizard SV Gel and PCR Clean-Up System (cat. no. A9281). The afore mentioned method provides high yield, ultrapure linear DNA, supercoiled DNA, or single-stranded linear or circular DNA. The purified DNA is useful for transfection, in vitro transcription/translation, enzymatic modifications and digestion, sequencing, cloning, and transformation, among others. This kit allows purification up to 40 µg of DNA fragments (100 bp to 10 kb) from standard or low-melt agarose gels, PCR amplifications, and restriction enzyme digestion.

Different factors could affect DNA purification, including

- DNA size: small and large fragment display reduced yield.
- DNA Strand: single-stranded DNA has lower yield than double-stranded DNA.

Important:

- ***This kit is designed to purify DNA from gels or PCR purifications, but we have successfully used for purification from restriction enzyme digestion.***
- ***This kit is designed to purify DNA from gels in Tris acetate (TAE) or Tris borate (TBE) buffers.***
- ***Expected yield for single-stranded DNA is lower than for double-stranded DNA and also depends on the size of the DNA fragment. See Appendix.***
- ***Check if ethanol (95%) was added to the Membrane Wash Solution (a box in the side label should be checked). If not, add the indicated volume of ethanol before use. See manufacturer manual.***
- ***All components are stored at room temperature.***
- ***Close the buffer bottles immediately after use to avoid acidification by CO_2.***
- ***The Spin Column is made up of two parts: the SV Minicolumn (clear tube with a white silica membrane band) and the Recollection Tube (clear).***
- ***Mineral oil does not interference with the purification.***
- ***For DNA samples that does not contain a single fragment, gel purification of the band of interest is recommended.***
- ***All tips and microtubes must be autoclaved.***
- ***All volumes are given on a per culture tube basis.***

Materials:

- 70% Alcohol
- Waste Container with Detergent (1% Alconox, stored at room temperature)
- Micropipettes and Tips for 100, 200, and 1000 µL
- 1.5 mL Microtubes
- Spin Column (SV Minicolumn + Recollection Tube)
- Membrane Binding Solution (stored at room temperature)
- Membrane Wash Solution (stored at room temperature)
- Nuclease-free Water (stored at room temperature)
- Microcentrifuge for 1.5 mL Microtubes
- Vortex

Procedure:

a) The working area should be sanitized using 70% ethanol prior to placing any materials on the bench. Prepare the waste container with 1% alconox.

b) Organize, assemble, and label the appropriate number of Spin Columns and 1.5 mL microtubes to be used during the purification process. Each DNA sample to be purified must have one Spin Column for purification and one 1.5 mL microtube for the elution step.

c) Briefly centrifuge the tube containing the DNA sample and add an equal volume of Membrane Binding Solution.
   - If not all the DNA sample will be purified, vortex and transfer the required volume of DNA to a new microtube of appropriate size, then add the Membrane Binding Solution.

d) Transfer the mixture to the SV Minicolumn of an assembled Spin Column and incubate for 1 minute at room temperature.
   - ***Remember, the maximum binding capacity of the SV Minicolumn is 40 μg***.
   - The maximal volume capacity of the SV Minicolumn is 2 mL. For volume larger than 700 μL, continue to pass the sample through the SV Minicolumn (***Step e***) until all the mixture has been processed. In case it is necessary, empty the recollection tube.

e) Centrifuge the Spin Column at 16,000 x *g* for 1 minute. Remove the SV Minicolumn from the Spin Column and discard the liquid in the Recollection Tube into the waste container. Return the SV Minicolumn to the Recollection Tube.
   - ***Failure to spin at 16,000 x g can result in reduced yield***.

f) Wash the SV Minicolumn by adding 700 μL of Membrane Wash Solution. Centrifuge the Spin Column for 1 minute at 16,000 x *g* and empty the Recollection Tube as explained above (***step e***).

g) Wash again the SV Column by adding 500 μL of Membrane Wash Solution and centrifuge the Spin Column for 5 minutes at 16,000 x *g*.

h) Empty the Recollection Tube as explained in step e, ***being careful not to wet the bottom of the column with the flowthrough***. Assemble the Spin Column again and centrifuge for 1 minute at 16,000 x *g*.

i) Carefully transfer the SV Minicolumn to the labeled 1.5 mL microtube. Add 50 μL of nuclease-free water directly to the center of the SV Minicolumn. ***Do not touch the membrane***. Incubate at room temperature for 1 minute and centrifuge for 1 minute at 16,000 x *g*.
   - If the DNA needs to be concentrated, it may be eluted in as 15 μL of water without significant reduction in yield. If are using <50 μL of water, verify the membrane is completely covered with water. ***Volumes less than 15 μL are not recommended***.

j) Discard the SV Minicolumn and store the DNA at 4 °C if it will be used in short time (<2 weeks).
   - If long-term storage is required, place the stock at −20 °C (preferably at –80 °C).
   - ***Avoid freeze and thaw cycles because degrades DNA***.

k) Decontaminate all the equipment that was used with 70% ethanol. Carefully discard the alconox solution in the waste container into the proper waste container and discard the materials in a biological waste (trash can with red bag).

l) Be sure all equipment are shut down. Return any material and equipment to its proper place. The pipettes must be returned to their maximal volume.

Appendix:

Percent Recovery Versus Elution Volume for a 700 bp PCR Product

Elution Volume (μL)	% Recovery vs 50 μL H_2O
10	35%
15	98%
25	98%
50	100%
75	100%
100	100%