SpinSmart DNA Extraction From Agarose Gels Protocol

Denville Scientific

Abstract

The SpinSmart PCR purification and gel extraction technologies utilize a lysis buffer containing chaotropic salts that allow DNA to bind to a silica membrane. Binding buffer PCR 1 is added to a PCR reaction or agarose gel slice; the mixture is subsequently loaded directly onto SpinSmart PCR Columns. Salts, enzymes, and other soluble components are washed away with ethanolic PCR 2 Wash buffer. Purified DNA is eluted using PCR 3 Elution buffer (5 mM Tris/HCl, pH 8.5).

Citation: Denville Scientific SpinSmart DNA Extraction From Agarose Gels Protocol. **protocols.io** dx.doi.org/10.17504/protocols.io.gqfbvtn
Published: 15 Dec 2016

Guidelines

Components

SpinSmart PCR Purification and Gel Extraction Kit Contents

Catalog Number	50 preps CM-510-50	250 preps CM-510-250
PCR 1 Binding Buffer	2 x 25 ml	2 x 120 ml
PCR 2 Wash Buffer (Concentrate)*	2 x 6 ml	40 ml
PCR 3 Elution Buffer (5 mM Tris/HCl, pH 8.5)	15 ml	50 ml
SpinSmart PCR Columns (yellow ring)	50	250
Collection Tubes (2ml)	50	250
User Manual	1	1

Equipment and reagents to be supplied by user

Consumables

96 - 100% ethanol

1.5 ml microcentrifuge tubes

Disposable pipette tips

Equipment

Manual pipettors

Centrifuge for microcentrifuge tubes

Heating block

Vortex mixer

Personal protection equipment (lab coat, gloves, goggles)

The SpinSmart PCR Purification and Gel Purification Procedures

The SpinSmart PCR purification and gel extraction technologies utilize a lysis buffer containing chaotropic salts that allow DNA to bind to a silica membrane. Binding buffer PCR 1 is added to a PCR reaction or agarose gel slice; the mixture is subsequently loaded directly onto **SpinSmart PCR Columns**. Salts, enzymes, and other soluble components are washed away with ethanolic PCR 2 Wash buffer. Purified DNA is eluted using PCR 3 Elution buffer (5 mM Tris/HCl, pH 8.5).

SpinSmart PCR Kit Specifications

SpinSmart PCR kits are designed for DNA purification from TAE/TBE agarose gels and for the direct purification of PCR* products.

SpinSmart PCR buffers are formulated to completely remove primers from PCR* reactions. Small double-stranded DNA fragments still remain bound and are purified with high efficiency.

SpinSmart PCR kits will effectively purify DNA fragments from detergent-rich PCR* reaction buffers.

DNA absorption to the membrane is pH-dependent. TAE standard gels or reaction mixtures with pH 6-8 should be used for best results.

Both standard and low melting agarose gels can be used.

SpinSmart PCR purified DNA fragments are ready to use in downstream applications like automated fluorescent DNA sequencing, PCR (PCR is patented by Roche Diagnostics), ligation reactions, or other types of enzymatic manipulation.

SpinSmart PCR Parameters	
DNA fragments from agarose gels	60 bp – 10 kbp
Elution volume	15-50 µl
Binding capacity	15 µg
Time/prep	10 min for 6 preps
Removal of small DNA fragments and **primer-dimers**	see pages 6-7

Removal of small DNA fragments and primer-dimers

Spin Smart PCR is specially formulated to remove unused, single stranded primers while effectively purifying PCR products down to 60 bp. In some cases, a PCR reaction may yield unwanted small double stranded fragments, such as primer-dimers or small PCR products resulting from unspecific annealing. SpinSmart PCR offers a simple method to remove these products that can interfere with your downstream sequencing or cloning applications.

By simply diluting PCR 1 Binding Buffer with sterile water, you can decrease the ability of small DNA fragments to bind to the membrane without compromising larger fragment recovery. A simple

dilution series should be tested, ranging from 1:1 – 1:9 (PCR 1:H2O) in order to determine the appropriate cutoff range for your reaction. As you approach the 1:9 dilution, the larger fragment recovery will sequentially decrease as well.

Rule of Thumb: The smaller the fragment you wish to exclude, the less you will need to dilute the PCR 1 Binding Buffer.

Elution procedures

DNA should be eluted using the PCR 3 Elution Buffer. If necessary, sterile water or other low salt elution buffers may be used, however the pH must be in the range of 7.0 – 8.5 for optimal recovery.

Typical recovery of 70-95% can be obtained with DNA fragments between 50bp -10kbp with an elution volume of 15 µl. For larger amounts of DNA (5-15 µg of DNA; from PCR* reactions > 100 µl or gel slices > 200 mg), elution with at least 50 µl of PCR 3 Elution Buffer is recommended.

Pre-warmed PCR 3 Elution Buffer can improve the yields of larger fragments (> 5-10 kbp). Add pre-warmed PCR 3 Elution Buffer (70°C) to the membrane, and incubate for 1-2 minutes, then centrifuge as directed in the standard protocol.

DNA Recovery with SpinSmart PCR		
Fragment length	**Elution volume**	**Recovery**
65 bp	15 µl	85%
	25 µl	90%
	50 µl	95%
	100 µl	95%
400 bp	15 µl	85%
	25 µl	95%
	50 µl	100%
	100 µl	100%
700 bp	15 µl	85%
	25 µl	90%
	50 µl	95%
	100 ul	95%
1500 bp	15 µl	85%
	25 µl	85%
	50 µl	90%
	100 µl	95%

Storage and preparation of solutions

PCR 1 Wash Buffer contains chaotropic salt. Wear gloves and goggles!

SpinSmart PCR kit components should be stored at room temperature and are stable for up to one year.

The following should be prepared before starting any SpinSmart PCR purification or gel extraction protocols:

Add the indicated volume of 96-100% ethanol to PCR 2 Wash Buffer Concentrate.

	50 preps	**250 preps**
PCR 2 Wash Buffer (Concentrate)	2 x 6 ml add 24 ml 96-100% EtOH to each bottle	40 ml add 160 ml 96-100% EtOH

Safety instructions – risk and safety phrases

The following components of the SpinSmart PCR kits contain hazardous materials.
Wear gloves and goggles and follow the safety instructions given in this section!

Buffer	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
PCR 1	Guanidine thiocyanate	Xn	Harmful by inhalation, in contact with skin and if swallowed	R 20/21/22	S 13

Risk Phrases

R 20/21/22 Harmful by inhalation, in contact with the skin and if swallowed
Safety Phrases

S 13 Keep away from food, drink and animal feedstuffs

∗ Label not necessary, if quantity below 125 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

Troubleshooting

For troubleshooting see the product manual:

https://www.denvillescientific.com/Files/Files/Denville/ProductDocs/CM-510-50_and_CM-510-250_manual.pdf

Before start

The following should be prepared before starting any SpinSmart PCR purification or gel extraction protocols:

Add the indicated volume of 96-100% ethanol to PCR 2 Wash Buffer Concentrate.

	50 preps	250 preps
PCR 2 Wash Buffer (Concentrate)	2 x 6 ml add 24 ml 96-100% EtOH to each bottle	40 ml add 160 ml 96-100% EtOH

Materials

SpinSmart™ PCR Purification & Gel Extraction Columns Only, with Collection Tubes, 50 per pack CM-500-50 by Denville Scientific Inc.

Protocol

Excise DNA fragment / Solubilize gel slice

Step 1.

Excise the DNA fragment from an agarose gel using a clean scalpel.

Excise DNA fragment / Solubilize gel slice

Step 2.

Excise gel slice containing the fragment carefully to minimize the gel volume. Determine the weight of the gel slice and transfer to a clean tube.

Excise DNA fragment / Solubilize gel slice

Step 3.

For each 100 mg of agarose gel add 200 µl PCR 1 Binding Buffer.

NOTES

Nicole Clouse 11 Dec 2016

For gels containing > 2% agarose, double the volume of PCR 1 Binding Buffer. Max Gel slice size is 400 mg for normal gel (<2%) or 200 mg for high percentage gel (> 2%).

Excise DNA fragment / Solubilize gel slice

Step 4.

Incubate sample at **50°C** until gel slice is fully dissolved (**5-10 min**).

Vortex the sample briefly every 2-3 min during incubation.

DURATION

00:05:00

Bind DNA

Step 5.

Place a SpinSmart PCR Binding Column (yellow ring) into a Collection Tube (2 ml) and load the sample.

Bind DNA

Step 6.

Centrifuge for **1 min** at **11,000** x g.

DURATION

00:01:00

Bind DNA

Step 7.

Discard flow-through and place the SpinSmart PCR Binding Column back into the Collection Tube.

Wash silica membrane

Step 8.

Add **600 µl PCR 2 Wash Buffer**.

Wash silica membrane

Step 9.

Centrifuge for **1 min at 11,000 x g.**

DURATION

00:01:00

Wash silica membrane

Step 10.

Discard flow-through and place the SpinSmart PCR Binding Column back into the Collection Tube.

NOTES

Nicole Clouse 11 Dec 2016

Optional: To prevent salt carryover for sensitive procedures, add an additional 200 µl PCR 2 Wash Buffer and repeat wash steps 8-10.

Dry silica membrane

Step 11.

Centrifuge for 2 min at 11,000 x g to remove PCR 2 Wash Buffer

DURATION

00:02:00

NOTES

Nicole Clouse 11 Dec 2016

Residual ethanol from Wash Buffer PCR 2 can inhibit subsequent reactions and must be removed in this step. Complete EtOH removal can be achieved by incubation of SpinSmart PCR Columns for 2-5 min at 70°C prior to elution.

DNA Elution

Step 12.

Place the SpinSmart PCR Binding Column into a clean 1.5 ml microcentrifuge tube (not provided)

DNA Elution

Step 13.

Add **15 - 50 µl PCR 3 Elution Buffer** and incubate at **room temperature** for **1 min to increase** the yield of eluted DNA.

DURATION

00:01:00

DNA Elution

Step 14.

Centrifuge for **1 min at 11,000 x g**.

DURATION

00:01:00

NOTES

Nicole Clouse 11 Dec 2016

Prewarmed PCR 3 Elution Buffer (70°C) can be used to increase the yield of larger fragments (> 5-10 kbp).

Warnings

PCR 1 Wash Buffer contains chaotropic salt. Wear gloves and goggles!

Safety instructions – risk and safety phrases

The following components of the SpinSmart PCR kits contain hazardous materials.
Wear gloves and goggles and follow the safety instructions given in this section!

Buffer	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
PCR 1	Guanidine thiocyanate	Xn*	Harmful by inhalation, in contact with skin and if swallowed	R 20/21/22	S 13

Risk Phrases

R 20/21/22 Harmful by inhalation, in contact with the skin and if swallowed
Safety Phrases

S 13 Keep away from food, drink and animal feedstuffs

∗ Label not necessary, if quantity below 125 g or ml (according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)