DNA Purification for Case Transgene Pronuclear Injection

Updated 2/26/08 RM

Materials:

- Stratagene StrataPrep™ DNA Gel Extraction Kit (Catalog #400766 or #400768) http://www.stratagene.com/products/displayProduct.aspx?pid=448
- 0.22um Millipore Ultrafreee® -MC Sterile GV Durapore® (1.5mL yellow dot tubes with filter cups) (Catalog #UFC30GV0S) **(4 labeled: A1, A2, B1, B2)** http://www.millipore.com/catalogue/itemdetail.do?id=UFC30GV0S
- Plasmid DNA
- Appropriate Enzymes & Buffers
- DNA Purification Work Sheet
- UV illuminator
- Gel Photograph Equipment
- Agarose
- Microwave
- Ethidium Bromide
- Razor Blades (clean)
- DNA Molecular Weight Ladders (e.g. Gibco 1kb, lambda HindIII)
- Water bath at 50°C
- Microcentrifuge
- UV spectrophotometer with Cuvettes
- Micropipettor and Sterile Tips
- $T_{10}E_{0.1}$, pH8.5 buffer
- dH_20
- 3x SDS sample buffer
- 1x TAE buffer
- 1.5mL sterile microcentrifuge tubes **(3 x A, 3 x B, 1 x Blank)**
- 2.0mL sterile microcentrifuge tubes **(4 labeled: A1, A2, B1, B2)**
- 2.0mL microspin tubes (from StrataPrep kit) with filter cups **(2 labeled: A, B)**

DNA Digestion & Gel Electrophoresis:

1. After receiving the plasmid DNA, label the ordering number on the tube & store at -20°C in Molecular Biology freezer box.
2. Order restriction enzymes if necessary.
3. Verify the restriction enzymes, digestion solution and digestion temperature to release the insert from the construct. Use recipe provided by investigator if available.
4. Start **DNA Purification Work Sheet** (attached).
5. Check DNA concentration using UV spec. Compare to investigator concentration (use ours).
6. Set up the digestion reactions and incubate at appropriate temperature (usually 37°C) for 3 hours to overnight.
    a. Usually digest 20-40 ug DNA/reaction (minimum of 10ug/reaction)

b. Set up 2 identical digests in parallel, e.g. 30ug plasmid DNA x 2 (**tube A + tube B**)= 60 ug total
c. Generally increase enzyme units slightly above predicted activity
d. Total reaction volume >10x total enzyme concentration (to dilute glycerol)

7. Set up ~1% agarose gel (2.5g of Agarose in 250ml 1xTAE). Microwave ~2.5'to dissolve. Cool ~10' before adding ~20ul of 10mg/mL ethidium bromide to the agarose solution. Pour and set the gel using a comb with taped wells. (2 wells hold ~ 100uL).

Suggested Gel Lane Schematic

- Lane 1 = **Molecular Weight Marker**
- Lane 2 = Blank
- Lane 3 = **Plasmid DNA (Uncut)**
- Lane 4 = Blank
- Lane 5 = Blank
- Lane 6/7/8 (taped) = **Tube A:Digested DNA**
- Lane 9 = Blank
- Lane 10 = Blank
- Lane 11 = Blank
- Lane 12/13/14 (taped) = **Tube B: Digested DNA**

8. Add appropriate volume 3x SDS Sample buffer & water to the samples. Load samples. Leave space between sample lanes so as to facilitate subsequent cutting of the target band out of gel.
9. Run electrophoresis at ~200V for 1-5 hours.
10. Take a picture of the gel. (Limit the use of UV light to protect the DNA integrity.)

DNA Purification:

1. Turn on 50°C water bath.
2. Using a clean razor blade, identify the target bands (transgene insert from samples A & B) and cut them out of the gel.
3. Cut each gel slice into 2 pieces (**A1 & A2 + B1 & B2**). Place 1 slice each inside 4 x 2.0mL microcentrifuge tubes labeled A1, A2, B1, B2. **[2 digestion tubes will be distributed into 4 purification tubes.]**

Starting Modified StrataPrep DNA Gel Extraction procedure

[StrataPrep kit: 80% recovery for inserts 250bp-9kb/50% recovery for 9kb-23kb (max)]

1. Add 750uL (increased compared to StrataPrep protocol) of DNA extraction buffer to the gel slice in the tube. Heat at 50°C for at least 10 min with occasional mixing (often takes up to 1.5 hours to completely dissolve). Be sure that the gel is completely dissolved before continuing the next step. (This is critical; solid gel will plug up the filters and yield will be very low. Gel is difficult to see. Look at tube up close while tapping and turning to confirm dissolved.)
2. Label 2 of the **Microspin Cups** (2mL microcentrifuge tubes + spin cup filters) provided by the StrataPrep kit. (**Labels: A, B**)

3. Transfer 750uL from tube A1 to microspin cup A. Similarly transfer 750uL from tube B1 to microspin cup B. Avoid damaging the fiber matrix of the spin cup. Snap the cap on.
4. Spin tubes A & B in a microcentrifuge at speed 7 for 1 min, room temperature. (The binding capacity of the spin cup is 10 ug.)
5. Open the caps, remove and retain the microspin cup, and discard the liquid. Replace the cup in the same 2-ml receptacle tube.
6. Continue to transfer (750uL aliquots) and spin the remaining DNA from tubes A1 & A2 into microspin tube A and B1 & B2 into microspin tube B. **[4 DNA purification tubes are reduced into 2 microspin tubes.]**
7. Open the caps, remove and retain the microspin cups, and discard the liquid. Replace the cups in the same 2-ml microspin tubes.
8. To each spin cup, add 750uL of 1x wash buffer. (Make sure this is 1x, not the 2x provided in the kit. Follow kit instructions upon receipt to make 1x and label appropriately.) Snap the cap on. Spin the tube at speed 7 for 1 min.
9. Open the caps, remove and retain the cups, and discard the wash buffer.
10. Place the cups back in the 2mL microspin tubes, and snap the caps on. Spin the tube at speed 7 for 1 min. On removal from the microcentrifuge, make sure that all of the wash buffer is removed from the microspin cups.
11. Transfer the microspin cups to **fresh 1.5mL microcentrifuge tubes (labeled A & B)** and discard the 2mL microspin tubes.
12. To each spin cup, add 50uL of elution buffer $T_{10}E_{0.1}$, pH8.5 (7.8, 8.2, and 8.5 tested, preliminary data) directly onto the top of the fiber matrix of the microspin cup.
13. Incubate the tube at room temperature for 5 min (the time is important).
14. Snap the caps on. Spin at speed 7 for 1 min.
15. Open the lids of the 1.5-ml tubes and discard the microspin cups. **[Save the liquid in the tube, not the cup!]**

[Millipore 0.22um Filter Purifactions]

1. Label 4 x **0.22um Millipore Ultrafree 1.5mL tubes (cap has yellow dot)** with filter cups: **A1, A2, B1, B2**
2. Transfer the contents of the tube A into tube A1 & tube B into tube B1. Spin at speed 7 for 1 min at room temperature.
3. Transfer the contents of the tube A1 into tube A2 & tube B1 into tube B2. Spin at speed 7 for 1 min at room temperature.
4. Label A2 & B2 with Transgene Order Number, mark "purified", UV concentration, PI name, date, and initials. This is the purified DNA stock.

DNA Quantification:

1. Label 3 x 1.5mL tubes **(blank, A, B).**
2. Blank tube = 100uL dH_20
3. A & B each = 2uL purified DNA + 98uL dH_20 (1:50 dilution)
4. Prepare and mix dilutions.

5. Read and record absorbance at 260/280 and 230. Calculate DNA concentration and record yield on DNA Purification Work Sheet.
6. Expected Yield: ~30% normal (~9kb), ~15% big (~20kb)
7. Normal concentration range: 50-300ng/uL

Transgenic Buffer $T_{10}E_{0.1}$, pH 8.5 **500 ml**

Tris	0.605 g
500 mM EDTA	0.1 ml
Millipore water	450 ml

-Adjust pH to 8.5.
-Bring volume to 500 ml with Millipore water.
-Aliquot to 5 x 100-ml bottles.
-Autoclave at Liquid cycle for 20 min.

Prepared on (DATE)	Volume

DNA Purification Work Sheet

Date _____
Initials _____
Order # _____
P.I. _____
Contact _____
Total Size _____
Transgene Size _____

DNA Concentration (plasmid)

theirs: _____
ours: _____

Digestion Reaction

uL DNA = ugDNA
uL H20
uL 10xBuffer=
uL enzyme #1 =
uL enzyme #2 =
uL 100x BSA
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total volume reaction
temperature reaction
time reaction

Gel

% Agarose _____
Volts _____
Start Time
End Time
MW marker

Photos

Label Lanes, Include uncut & MW
Attach: Gel Photo Before Purification: Label Lanes
Attach QC: Gel Photo After Purification:
Comments?

Quantification

		sample 1	sample 2
UV Spec Reading Concentration			
Gel Estimated Concentration			
Yield	start amount (ug)		
	end amount (ug)		
	% recovery		

Label Tube(s) order#, concentration, date, initials, "purified"

StrataPrep® DNA Gel Extraction Kit

INSTRUCTION MANUAL

Catalog #400766 and #400768
Revision #094002c

For In Vitro Use Only

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Stratagene. Stratagene shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

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	0800 917 3282	0800 917 3283	0800 917 3281

All Other Countries

Please contact your local distributor. A complete list of distributors is available at *www.stratagene.com*.

StrataPrep® DNA Gel Extraction Kit

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StrataPrep® DNA Gel Extraction Kit

MATERIALS PROVIDED

Materials provided	Quantity	
	Catalog #400766[a]	Catalog #400768[b]
DNA extraction buffer	20 ml	100 ml
Wash buffer (2×)	25 ml	125 ml
Microspin cups[c]	50	250
Receptacle tubes (2 ml)	50	250

[a] Contains enough reagents for 50 gel extractions.
[b] Contains enough reagents for 250 gel extractions.
[c] The capacity of the microspin cup is ~0.8 ml.

Caution *The chaotropic salt in the DNA extraction buffer is an irritant.*

STORAGE CONDITIONS

All Components: Room temperature

ADDITIONAL MATERIALS REQUIRED

Elution buffer (see *Preparation of Reagents*)
Ethanol (100%)
Microcentrifuge
Microcentrifuge tubes

Revision #094002c

INTRODUCTION

The StrataPrep® DNA gel extraction kit is a rapid method for extracting gel-fractionated DNA from agarose gels. The method employs a microspin cup that contains a silica-based fiber matrix. In the presence of a chaotropic salt, the agarose is dissolved and the DNA binds to the fiber matrix.[1] Following gel fractionation of the DNA, the desired fragment is cut from the gel, placed in a microcentrifuge tube, combined with the DNA extraction buffer, incubated at 50°C, and transferred to a microspin cup that is seated inside a receptacle tube. The DNA binds to the fiber matrix in the microspin cup. The contaminants are then washed from the microspin cup with a wash buffer. The purified DNA is eluted from the fiber matrix with a low-ionic-strength buffer and captured in a microcentrifuge tube. Double-stranded DNA ≥100 bp is retained. This simple method of DNA extraction eliminates the need for manipulation of resins, toxic phenol–chloroform extractions, and time-consuming ethanol precipitations. The result is purified DNA that is ready for restriction digestion, ligation, and probe labeling.

GEL EXTRACTION PROTOCOL

Note *The following protocol is for the recovery of DNA from a conventional 1% agarose gel (TAE or TBE). If the gel concentration is ≥2%, use twice the volume of DNA extraction buffer for the volume of gel described in the following procedure.*

1. Add 300 µl of DNA extraction buffer for each 100 µl of gel volume [a gel slice with dimensions of 0.8 cm × 0.3 cm × 0.5cm = 0.12 cm^3, ~120 µl (by volume) or ~120 mg (by weight)] to a 1.5-ml microcentrifuge tube.

2. Heat the mixture at 50°C for at least 10 minutes with occasional mixing. Be sure that the gel is completely dissolved before continuing to the next step.

3. Transfer the mixture to a microspin cup that is seated in a 2-ml receptacle tube (exercise caution to avoid damaging the fiber matrix). Snap the cap of the 2-ml receptacle tube onto the top of the microspin cup.

4. Spin the tube in a microcentrifuge at maximum speed for 30 seconds.

   Note *The DNA is retained in the fiber matrix of the microspin cup. The binding capacity of the microspin cup is ~10 µg.*

5. Prepare the 1× wash buffer by adding an equal volume of 100% ethanol to the container of 2× wash buffer: 25 ml of 100% ethanol for catalog #400766 **or** 125 ml of 100% ethanol for catalog #400768. After adding the ethanol, mark the label on the container as suggested: *[✓] 1× (Ethanol Added)*. Store the 1× wash buffer at room temperature.

6. Open the cap of the 2-ml receptacle tube, remove and retain the microspin cup, and discard the liquid. Replace the microspin cup in the 2-ml receptacle tube.

7. Add 750 µl of 1× wash buffer to the microspin cup. Snap the cap of the receptacle tube onto the top of the microspin cup.

8. Spin the tube in a microcentrifuge at maximum speed for 30 seconds.

9. Open the cap of the 2-ml receptacle tube, remove and retain the microspin cup, and discard the wash buffer.

10. Place the microspin cup back in the 2-ml receptacle tube and snap the cap of the receptacle tube onto the microspin cup.

11. Spin the tube in a microcentrifuge at maximum speed for 30 seconds. On removal from the microcentrifuge, make sure that all of the wash buffer is removed from the microspin.

12. Transfer the microspin cup to a fresh 1.5-ml microcentrifuge tube[‖] and discard the 2-ml receptacle tube.

13. Add 50 µl of elution buffer directly onto the top of the fiber matrix at the bottom of the microspin cup.

    Note *For eluting DNA from the microspin cup, use a low-ionic-strength buffer (≤10 mM in concentration, pH 7–9) or sterile deionized water. For most applications, 10 mM Tris base (pH adjusted to 8.5 with HCl) is recommended; however, TE (10 mM Tris HCl, pH 8.0, 1mM EDTA) may be used for applications in which EDTA will not interfere with subsequent reactions.*

14. Incubate the tube at room temperature for 5 minutes.

    Note *Maximum recovery of the DNA from the microspin cup depends on the pH, ionic strength, and volume of the elution buffer added to the microspin cup, the placement of the elution buffer into the microspin cup, and the incubation time. Maximum recovery is obtained if not less than 50 µl of elution buffer is added directly onto the fiber matrix at the bottom of the microspin cup, and the tube is incubated for 5 minutes.*

15. Snap the cap of the 1.5-ml microcentrifuge tube onto the microspin cup and spin the tube in a microcentrifuge at maximum speed for 30 seconds.

16. Open the lid of the 1.5-ml microcentrifuge tube and discard the microspin cup.

    Notes *The purified DNA is in the bottom of the 1.5-ml microcentrifuge tube. Snap the lid of the 1.5-ml microcentrifuge tube closed to store the purified DNA.*

    An 80% recovery is expected from DNA that is 250bp–9kb; a 50% recovery is expected for longer DNA up to 23kb.

[‖] 1.5-ml flat snap cap microcentrifuge tubes from Continental Laboratory Products, Inc. are recommended

TROUBLESHOOTING

Observation	Suggestion
Low recovery of the desired DNA	Gel may not be completely dissolved following addition of the DNA extraction buffer. Verify that the volume of the DNA extraction buffer is correct for the volume of the agarose gel. If the gel concentration is ≥2%, add twice the volume of DNA extraction buffer
	Ensure that the 2× wash buffer is diluted with an equal volume of 100% ethanol so that the DNA is washed and retained on the microspin cup. Prepare 1× wash buffer by adding an equal volume of 100% ethanol to the 2× wash buffer
	Do not use a solution of high ionic strength or low pH as the elution buffer. Instead, use a low-ionic-strength (≤10 mM) buffer, pH 7–9
	Do not dispense the elution buffer down the side of the microspin cup. Add the elution buffer directly onto the fiber matrix of the microspin cup to ensure complete coverage of the membrane
	Incubate the tube for 5 minutes after adding the elution buffer
The DNA floats out of the well of the agarose gel	Make sure that the 1× wash buffer is completely removed from the microspin cup before adding the elution buffer to avoid ethanol contamination

PREPARATION OF REAGENTS

Elution Buffer

10 mM Tris base
Adjust pH to 8.5 with HCl
or
10 mM Tris base
1 mM EDTA
Adjust pH to 8.0 with HCl
or
Sterile ddH_2O

2× Wash Buffer

10 mM Tris-HCl (pH 7.5)
100 mM NaCl
2.5 mM EDTA

REFERENCE

1. Vogelstein, B. and Gillespie, D. (1979) *Proc Natl Acad Sci U S A* 76(2):615-9.

ENDNOTES

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on Stratagene's website at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

StrataPrep® DNA Gel Extraction Kit

Catalog #400766 and #400768

QUICK-REFERENCE PROTOCOL

- Add the appropriate volume of DNA extraction buffer to the gel slice
- Heat at 50°C for at least 10 minutes (until gel is completely dissolved)
- Transfer the DNA-DNA extraction buffer mixture into a microspin cup that is seated in a 2-ml receptacle tube
- Spin the tube in a microcentrifuge for 30 seconds. Open the cap of the 2-ml receptacle tube, remove and retain the microspin cup, and discard the liquid
- Prepare the 1× wash buffer by adding an equal volume of 100% ethanol to the container of 2× wash buffer
- Add 750 µl of 1× wash buffer to the microspin cup
- Spin the tube in the microcentrifuge for 30 seconds
- Open the cap of the 2-ml receptacle tube, remove and retain the microspin cup, and discard the wash buffer
- Place the microspin cup back in the 2-ml receptacle tube and snap the cap of the 2-ml receptacle tube onto the microspin cup
- Spin the tube in a microcentrifuge for 30 seconds
- Transfer the microspin cup to a fresh 1.5-ml microcentrifuge tube and discard the 2-ml receptacle tube
- Add 50 µl of elution buffer directly onto the fiber matrix at the bottom of the microspin cup
- Incubate the tube at room temperature for 5 minutes
- To collect the DNA, spin the tube in a microcentrifuge for 30 seconds
- Open the lid of the 1.5-ml microcentrifuge tube and discard the microspin cup

Note *The DNA is in the bottom of the 1.5-ml microcentrifuge tube.*