USER GUIDE

invitrogen™
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Easy-DNA™ Kit

For genomic DNA isolation

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For Research Use Only. Not for use in diagnostic procedures.

life technologies™

Contents

Important Information

Shipping/Storage

The Easy-DNA™ Kit is shipped at room temperature and should be stored at room temperature. For long-term storage (>6 months), store the mussel glycogen, RNase, and Protein Degrader at –20°C.

Kit Contents

The following components are included in the Easy-DNA™ Kit. Store each component at room temperature.

Item	Volume	Composition
Solution A	55 mL	Lysis Solution
Solution B	25 mL	Precipitation Solution
TE Buffer	100 mL	10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0
Mussel Glycogen	750 µL	2 mg/mL in sterile water
RNase	750 µL	2 mg/mL in sterile water
Protein Degrader	750 µL	5 mg/mL in sterile water

Precipitates

The following table describes what to do if precipitates form in solutions in this kit.

If there is a precipitate in....	Then....
Solution A	Warm the solution to 45°C and swirl to dissolve precipitate. Be sure to cool to room temperature before use.
Solution B	Stir the solution before use and let the precipitate settle to the bottom. When pipetting be sure not to draw up precipitate.
The Protein Degrader solution after thawing (only if stored at –20°C)	Warm the solution at 37°C for 5 minutes.

Introduction

Overview

Easy-DNA™ Kit

The Easy-DNA™ Kit is a simple, quick, and inexpensive method for the isolation of DNA from a variety of sources. DNA from the sources listed has been successfully isolated and used to produce PCR products or for Southern blot experiments.

- Fresh, dried, frozen, or heparinized blood
- Tissue culture cells, both suspended and trypsinized
- Mammalian tissue
- E. coli
- Yeast cells
- Plant leaves
- Hair follicles
- Mouse tails
- Baculovirus (viral particles)

Experimental Overview

Cells are lysed by the addition of Solution A and subsequent incubation at 65°C. Proteins and lipids are precipitated and extracted by the addition of Solution B and chloroform. The solution is then centrifuged to separate the solution into two phases with a solid interface separating the two phases. The DNA is in the upper, clear aqueous phase, the proteins and lipids are in the solid interface, and the chloroform forms the lower phase. The DNA is then removed, precipitated with ethanol, and resuspended in TE buffer. Purified DNA may be used for PCR or restriction digestion, or may be stored at 4°C.

Expected Yields

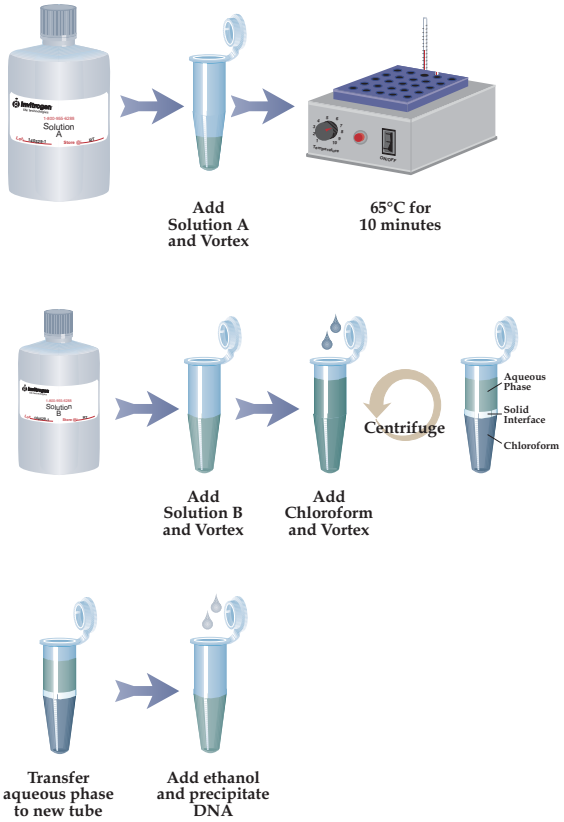
The following table gives the approximate yield of DNA from a variety of samples.

Sample	Yield of DNA
1 µL of blood	5–10 ng
10 µL of blood	20–30 ng
10^7 Sf9 cells	~170–180 µg
10^9 *E. coli* cells	~30–40 µg
50 mg leaf tissue	~4 µg
50 mg of human liver tissue	~150 µg
87 mg of human brain tissue	~170 µg
500 mg of human brain tissue	~700 µg
500 mg of rat heart tissue	~100 µg
2 mL of blood	~40 µg
4×10^9 *Pichia pastoris* cells	~850 µg
Mouse tail (1 cm)	125 µg
1 hair follicle	5–10 ng
750 µL of a baculovirus-infected cell culture	N.D.*

*Not determined, but the DNA yields a discrete PCR product using primers specific for the polyhedrin gene.

Flow Chart

The following figure describes the experimental process.



Materials Supplied by User

The following materials need to be made or supplied by the user. Some reagents apply only to specific protocols, so review the protocol in question before you decide which reagents to prepare.

Reagents

- Chloroform and Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) (Cat. no. 15593-031)
- 80% ethanol and 100% ethanol
- Isopropanol
- YPD medium (see **Recipes**, page 14)
- SCED solution (see **Recipes**, page 14)
- 20% polyethylene glycol in 1 M NaCl (see **Recipes**, page 14)
- Phosphate Buffered Saline (PBS; Cat. no. 10010-023)
- Zymolyase
- 1 M Sorbitol, 100 mM EDTA, 14 mM β-mercaptoethanol

Equipment

- Sterile 1.5-mL microcentrifuge tubes
- Shaking water bath
- Water bath or heat block
- Centrifuge, low-speed
- Microcentrifuge
- Vortex
- Ice bucket with ice
- Sterile 15-mL snap-cap polypropylene tubes (Falcon 2059 or equivalent)

Methods

General Information

General Handling Techniques

When handling DNA, make sure you use sterile solutions. The solutions provided in the kit are sterile and care must be taken to ensure that no DNases are introduced. Make sure all equipment that comes in contact with DNA is sterile. This includes pipette tips, microcentrifuge tubes, 15-mL snap-cap polypropylene tubes, and pipettes. Be sure pipettor barrels are clean and treated with ethanol.

Repeat pipettors can be used for multiple samples because the exact volumes of solutions A, B, chloroform, and ethanol are not critical.

Before Starting

You should have a heat block or water bath set to 65°C, an ice bucket with ice, and have access to either a 4°C microcentrifuge or a 4°C centrifuge depending on the application.

Selection of Protocols

There are eight different protocols depending on the type of sample you have. Below is a table to help you find the right method to use with your sample.

Sample	Protocol Number	Page
Fresh, frozen, or dried blood (1 µL–100 µL) 1–5 hair follicle(s)	1	4
30-minute protocol for 350 µL blood	2	5
Suspension or trypsinized cells (10^3-10^7 cells) *E. coli* cells (0.5–1.0 mL overnight culture, ~10^9 cell/mL) Mammalian tissues (3.5 mg–100 mg) Fresh plant leaves (50 mg)	3	6
Mammalian tissues (100 mg–1 g) Tissue culture cells (10^7–10^8 cells) Fresh blood samples (100 µL–2 mL)	4	7
Yeast cells, small scale isolation (1.5 mL stationary culture)	5	8
Yeast cells, large scale isolation (10 mL stationary culture)	6	9
Baculovirus (750 µL from occlusion-negative infections)	7	10
Mouse tail (1 cm)	8	11

Note

If your sample is not represented above, see the section **Guidelines for Protocol Development**, page 12.

Protocol #1 – Small Blood Samples and Hair Follicles

Before Starting

Place 1 µL to 100 µL of fresh, frozen, or dried blood or 1–5 hair follicle(s) in microcentrifuge tubes. Blood samples should be mixed to form a homogeneous solution.

- Chill 100% and 80% ethanol in a –20°C freezer.
- Thaw mussel glycogen (if stored at –20°C) and keep on ice.
- Equilibrate heat block or water bath to 65°C.

Isolation of DNA

1. Add 50 µL Solution A to blood or hair follicle(s) and vortex in 1 second intervals until evenly dispersed.
2. Incubate at 65°C for 10 minutes.
3. Add 20 µL Solution B, and vortex vigorously until the precipitate moves freely in the tube and the sample is uniformly viscous (10 seconds–1 minute).
4. Add 70 µL chloroform and vortex until viscosity decreases and the mixture is homogeneous (10 seconds–1 minute).
5. Centrifuge at maximum speed in a microcentrifuge for 10 minutes at 4°C to separate the phases and form the interface. Transfer the upper aqueous phase to a fresh microcentrifuge tube. Proceed to **DNA Precipitation**.

DNA Precipitation

1. Add 445 µL TE buffer and 5 µL mussel glycogen to DNA solution. Add 1 mL of 100% ethanol (–20°C) and mix by inversion.
2. Incubate tube on ice for 30 minutes.
3. Centrifuge at maximum speed in a microcentrifuge for 10–15 minutes at 4°C. Remove ethanol very carefully with a drawn-out Pasteur pipette.
4. Add 500 µL of 80% ethanol (–20°C) and mix by inverting the tube 3–5 times.
5. Centrifuge at maximum speed in a microcentrifuge for 3–5 minutes at 4°C. Remove the 80% ethanol with a drawn-out Pasteur pipette.
6. Centrifuge at maximum speed in a microcentrifuge for 2–3 minutes at 4°C. Remove residual ethanol with a pipettor. Let air dry for 5 minutes.
7. Resuspend the pellet in 10 µL TE buffer. DNA is ready for further experiments. Store at 4°C. See page 5 for expected yields.

Note

No RNase is needed for this preparation as these are small samples of terminally differentiated cells. There is very little RNA transcription occurring.

Protocol #2 – 30 Minute DNA Extraction from Blood Samples

Introduction

The Easy-DNA™ Kit allows you to quickly and easily isolate high molecular weight genomic DNA from a variety of sample types and sizes. The following procedure allows you to isolate genomic DNA from blood samples in less than 30 minutes. The isolated DNA is ready for PCR or RFLP analysis.

Before Starting

- You will need 2.0-mL microcentrifuge tubes for this protocol
- Equilibrate heat block or water bath to 65°C

Isolation of DNA

1. Pipette 350 µL of blood into each microcentrifuge tube. Blood samples should be mixed to form a homogenous solution.
2. Add 500 µL of Solution A to the tube. Mix by inversion several times.
3. Incubate at 65°C for 6 minutes.
4. Remove sample from heat block or water bath and mix by inversion.
5. Add 900 µL of chloroform and vortex vigorously. Be sure the sample is mixed completely. The mixing is complete when the liquid portion of the sample flows freely and the hemoglobin looks like small chocolate-covered particles.
6. Add 200 µL of Solution B and vortex briefly until the sample is uniformly viscous.
7. Centrifuge at maximum speed in a microcentrifuge for 10 minutes at room temperature.
8. Pipette the clear, aqueous phase into a new 1.5-mL microcentrifuge tube.

Precipitation of Genomic DNA

1. Add 1 mL of room temperature 100% ethanol and mix by inversion until a precipitate forms. A precipitate is usually seen within 30–60 seconds. If a precipitate is not seen, allow the tubes to incubate at room temperature for 10 minutes.
2. Centrifuge at maximum speed in a microcentrifuge for 5 minutes at room temperature.
3. Decant the supernatant and add 1 mL of room temperature 70% ethanol.
4. Centrifuge at maximum speed in a microcentrifuge for 2 minutes at room temperature.
5. Decant the supernatant. Centrifuge at maximum speed in a microcentrifuge for 1 minute at room temperature.
6. Remove residual ethanol with a pipette and invert tubes to dry.
7. Add 100–150 µL of autoclaved nuclease-free water to each tube.
8. Incubate at 65°C for 5 minutes.
9. Run genomic DNA on an agarose gel to check for size and yield. Bufferless, precast agarose E-Gel® Go! 1%Starter paks (Cat. no. G4401ST) are available from Life Technologies for fast and easy electrophoresis. Visit our website (**www.lifetechnologies.com**) or call Technical Support (see page 15) for more information. Store purified DNA at 4°C.

Protocol #3 – Small Amounts of Cells, Tissues, or Plant Leaves

Samples

- Suspension or trypsinized cells (10^3–10^7 cells)
- *E. coli* cells (0.5–1.0 mL of an overnight culture, ~1×10^9 cells/mL)
- Mammalian tissues (3.5 mg to 100 mg)
- Fresh plant leaves (50 mg)

Preparation

Cells must be pelleted and the medium decanted. **Resuspend cell pellet in 200 µL 1X PBS** (Cat. no. 10010-023)**.** This will eliminate the formation of a salt pellet when precipitating DNA.

Freeze tissue and plant leaves in liquid nitrogen and pulverize with a mortar and pestle. Place samples in microcentrifuge tubes for processing.

Note: Fresh, minced leaves will yield DNA, but not as much and not as high quality as when the fresh leaves are frozen in liquid nitrogen and pulverized.

Before Starting

- Chill 100% and 80% ethanol in a –20°C freezer.
- Thaw RNase (if stored at –20°C) and keep on ice.
- Equilibrate two heat blocks or water baths, one to 37°C and the other to 65°C.

Isolation of DNA

1. Add 350 µL Solution A to cell suspension, tissue, or plant parts and vortex in 1 second intervals until evenly dispersed.
2. Incubate at 65°C for 10 minutes.
3. Add 150 µL Solution B and vortex vigorously until the precipitate moves freely in the tube, and the sample is uniformly viscous (10 seconds–1 minute).
4. Add 500 µL chloroform and vortex until viscosity decreases and the mixture is homogeneous (10 seconds–1 minute).
5. Centrifuge at maximum speed for 10–20 minutes at 4°C to separate phases. Transfer the upper phase into a fresh microcentrifuge tube. Proceed to **DNA Precipitation**.

DNA Precipitation

1. To the DNA solution, add 1 mL of 100% ethanol (–20°C) and vortex briefly.
2. Incubate tube on ice for 30 minutes.
3. Centrifuge at maximum speed for 10–15 minutes at 4°C. Remove ethanol from the pellet with a drawn-out Pasteur pipette.
4. Add 500 µL of 80% ethanol (–20°C) and mix by inverting the tube 3–5 times.
5. Centrifuge at maximum speed for 3–5 minutes at 4°C. Save the pellet and remove the 80% ethanol with drawn-out Pasteur pipette.
6. Centrifuge at maximum speed for 2–3 minutes at 4°C. Remove residual ethanol with a pipettor. Let air dry 5 minutes.
7. Resuspend the pellet in 100 µL TE buffer. Add 2 µL of a 2 mg/mL RNase to bring the concentration to 40 µg/mL.
8. Incubate at 37°C for 30 minutes. DNA is ready for further experiments. Store at 4°C.

Protocol #4 – Large Samples of Tissue, Cells, or Blood

Samples

- Mammalian tissues (100 mg to 1 g)
- Tissue culture cells (10^7–10^8 cells)
- Blood (100 µL to 2 mL)

Preparation

Pellet cells and decant medium. There is no need to resuspend in 1X PBS.
Freeze mammalian tissues in liquid nitrogen and pulverize.
Place all samples into sterile, snap-cap, 15-mL polypropylene tube.

Before Starting

- Chill 100% and 80% ethanol in a –20°C freezer.
- Thaw RNase (if stored at –20°C) and keep on ice.
- Equilibrate two heat blocks or water baths, one to 37°C and the other to 65°C.

Isolation of DNA

1. Add 3.5 mL Solution A to your cell pellet, tissue, or blood, and vortex in 1 second intervals until evenly dispersed.
2. Incubate at 65°C for 10 minutes.
3. Add 1.5 mL Solution B and vortex vigorously until the precipitate moves freely in the tube, and the sample is uniformly viscous (10 seconds–1 minute).
4. Add 5 mL chloroform and vortex until the viscosity decreases and the mixture is homogeneous (10 seconds–1 minute).
5. Centrifuge at 8000 × *g* for 20 minutes at 4°C to separate the phases and form an interface. Carefully decant the upper, aqueous phase into a fresh 15-mL tube.
6. Add 5 mL phenol:chloroform:isoamyl alcohol to the aqueous phase, vortex, and centrifuge at 8000 × *g* for 5 minutes at 4°C to separate phases.
7. Transfer upper, aqueous phase to a new 15-mL tube. Proceed to **DNA Precipitation**.

DNA Precipitation

1. Add 10 mL 100% ethanol (–20°C) to the DNA solution and vortex briefly.
2. Incubate tube on ice for 30 minutes.
3. Centrifuge at 8000 × *g* for 10–15 minutes at 4°C. Keep the pellet and carefully decant ethanol.
4. Add 5 mL 80% ethanol (–20°C) and invert the tube 3–5 times.
5. Centrifuge at 8000 × *g* for 3–5 minutes at 4°C. Carefully decant 80% ethanol.
6. Centrifuge the pellet at 8000 × *g* for 2–3 minutes at 4°C. Remove excess ethanol with a pipettor. Let air dry 5 minutes.
7. Resuspend the pellet in 1 mL TE buffer. Add 20 µL 2 mg/mL RNase to a final concentration of 40 µg/mL.
8. Incubate at 37°C for 30 minutes. DNA is ready for further experiments. Store at 4°C. See page 5 for the expected yield of DNA.

Protocol #5 – Small Scale Isolation of DNA from Yeast Cells

Introduction

The following method was developed at Life Technologies to conveniently isolate DNA from yeast.

Solutions

You will need to prepare the following solutions.

- Minimal Medium (See **Recipes**, page 14)
- TE buffer, pH 7.4 (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, pH 8.0)
- 1 M Sorbitol, 100 mM EDTA, 14 mM β-mercaptoethanol (make fresh)
- Zymolyase, 3 mg/mL stock solution in water (Seikagaku America, Inc.)
- SCED (See **Recipes**, page 14)
- Chloroform and Isopropanol
- 70% or 80% ethanol
- RNase A (not supplied with the kit)

Preparation of Cells

1. Grow the yeast strain at 30°C to an OD_{600} of 5–10 in 2–5 mL of minimal medium.
2. Harvest 1.5 mL of the culture by centrifuging at maximum speed in a microcentrifuge for 1–2 minutes at room temperature.
3. Resuspend cells in 1.5 mL TE and centrifuge as in Step 2.
4. Resuspend cells in 1 mL fresh 1 M Sorbitol, 100 mM EDTA, 14 mM β-mercaptoethanol. Vortex to resuspend.
5. Add 1.5 µL of 3 mg/mL Zymolyase to each tube of cells and incubate at 30°C for 1 hour.
6. Centrifuge at **4000 rpm** in a microcentrifuge for 8 minutes at room temperature.

   Note: Centrifuge with less force as the cells are fragile due to digestion with Zymolyase.
7. **Gently** resuspend cells in 200 µL fresh SCED and incubate at 37°C for 1 hour.

DNA Isolation

1. Add 350 µL Solution A to the cell suspension from Step 7, above and vortex.
2. Incubate at 65°C for 10 minutes.
3. Add 150 µL of Solution B and vortex.
4. Add 600 µL chloroform and vortex.
5. Centrifuge at maximum speed for 20 minutes at room temperature.
6. Transfer the aqueous layer to a fresh tube, add 600 µL isopropanol, and mix by inversion. Incubate at room temperature for 10 minutes.
7. Centrifuge sample at maximum speed for 20 minutes at 4°C.
8. Wash pellet with cold 70% or 80% ethanol, centrifuge at maximum speed for 2 minutes at 4°C, remove ethanol, and air-dry.
9. Resuspend the pellet in 50 µL TE with 50 µg/mL RNase A and incubate overnight at room temperature. Quantitate the amount of DNA. Use 5 µL of this DNA solution in a 50 µL PCR reaction.

Protocol #6 – Large Scale Isolation of DNA from Yeast Cells

Sample Preparation

Prepare YPD medium (see **Recipes**, page 14).
Inoculate 10 mL of YPD medium with a single yeast colony and grow overnight. This will be your source of cells.

Before Starting

- Prepare SCED solution (see **Recipes**, page 14).
- Chill 100% and 80% ethanol in a –20°C freezer.
- Thaw RNase (if stored at –20°C) and keep on ice.
- Equilibrate two heat blocks or water baths, one at 37°C and the other at 65°C.

Washing the Yeast Cells

1. Transfer 10 mL of the overnight culture of yeast cells to a 15-mL snap-cap poly-propylene tube (Falcon 2059 or equivalent). Centrifuge cells at 4000 × *g* for 5–10 minutes at 4°C. Save the pellet and decant the medium.
2. Add 10 mL of sterile, deionized water and tap tube to resuspend cells. Centrifuge cells at 4000 × *g* for 5–10 minutes at 4°C. Keep the pellet and decant the water.
3. Resuspend the pellet in 2 mL SCED and incubate at 37°C for 1 hour.

Isolation of DNA

1. Add 3.5 mL of Solution A to yeast cells and vortex in 1 second intervals until evenly dispersed.
2. Incubate at 65°C for 10 minutes.
3. Add 1.5 mL of Solution B and vortex vigorously until the precipitate moves freely in the tube, and the sample is uniformly viscous (10 seconds–1 minute).
4. Add 5 mL of chloroform and vortex until the viscosity decreases and the mixture is homogeneous (10 seconds–1 minute).
5. Centrifuge at 1000 × *g* for 20 minutes at 4°C to separate phases and form the interface. Carefully decant the upper, aqueous phase into a fresh 15-mL tube. Proceed to **DNA Precipitation.**

DNA Precipitation

1. Add 10 mL 100% ethanol (–20°C) to the DNA solution, vortex briefly, and incubate the tube on ice for 30 minutes.
2. Centrifuge at 4000 × *g* for 10–15 minutes at 4°C. Decant the ethanol.
3. Add 5 mL 80% ethanol (–20°C) to the pellet and invert the tube several times to wash the pellet. Centrifuge at 4000 × *g* for 3–5 minutes at 4°C.
4. Decant 80% ethanol and centrifuge at 4000 × *g* for 2–3 minutes at 4°C. Remove residual ethanol with a pipettor. Let air dry 5 minutes.
5. Resuspend the pellet in 100 µL TE buffer. Add 2 µL of 2 mg/mL RNase for a final concentration of 40 µg/mL. Incubate at 37°C for 30 minutes.
6. Centrifuge tube at 4000 × *g* for 1 minute at 4°C to remove insoluble particles.
7. Transfer supernatant to a fresh microcentrifuge tube. DNA is ready for further experiments. Store at 4°C. See page 5 for the expected yield of DNA.

Protocol #7 – Isolation of DNA from Baculovirus

Before Starting

- Prepare 20% PEG in 1 M NaCl and chill at 4°C (see **Recipes**, page 14).
- Chill 100% and 70% ethanol in a –20°C freezer.
- Equilibrate a heat block at 65°C.

Sample Preparation

From a 6- or 12-well microtiter plate choose an occlusion-negative well and disrupt the cell monolayer with a sterile pipette tip on a 1-mL pipettor.

Isolation of Viral Particles

1. Transfer 750 µL of an occlusion-negative cell suspension to a microcentrifuge tube. Centrifuge at 1000 × *g* for 3 minutes at room temperature to pellet cells. Transfer the supernatant to a fresh microcentrifuge tube.
2. Add 750 µL cold (4°C) 20% PEG in 1 M NaCl to the tube. Mix three times by inversion, and incubate on ice for 30 minutes.
3. Centrifuge at maximum speed for 10 minutes at 4°C to pellet the viral particles. Keep the pellet and discard the supernatant.
4. Re-centrifuge the tube at maximum speed for 2 minutes at 4°C. Remove residual supernatant with a pipettor.
5. Resuspend viral particles in 100 µL TE buffer or sterile water. Proceed to **Isolation of DNA**.

Isolation of DNA

1. Add 143 µL Solution A to resuspended viral particles and vortex for 1 second to mix.
2. Incubate at 65°C for 6 minutes.
3. Add 58 µL Solution B and vortex vigorously for 5 seconds until mixture is uniform and there is no white plug in the bottom of the tube.
4. Add 258 µL chloroform and vortex until evenly mixed.
5. Centrifuge at maximum speed for 10 minutes at 4°C to separate phases and create the interface. Pipette upper, aqueous phase to fresh microcentrifuge tube and proceed to **DNA Precipitation.**

DNA Precipitation

1. Add 500 µL 100% ethanol (–20°C) to the DNA solution. Invert tube eight times to precipitate DNA.
2. Centrifuge at maximum speed for 5 minutes at 4°C. Keep the pellet and decant the ethanol.
3. Add 500 µL 70% ethanol (–20°C) and centrifuge at maximum speed for 5 minutes at 4°C. Remove 70% ethanol with a drawn-out pipette.
4. Centrifuge at maximum speed for 2–3 minutes at 4°C. Remove residual ethanol with a pipettor and air dry 5 minutes.
5. Resuspend the pellet in 20 µL sterile water. DNA is ready for further experiments. Store at 4°C or –20°C until ready for use.

Protocol #8 – Isolation of DNA from Mouse Tails

Before Starting: Day 1

- Thaw Protein Degrader (if stored at –20°C) and keep on ice. If cloudy, warm at 37°C for 5 minutes until clear.
- Equilibrate a shaking water bath to 60°C.

Isolation of DNA: Day 1

1. To a fresh microcentrifuge tube, mix

TE	320 µL
Solution A	20 µL
Solution B	10 µL
Protein Degrader (5 mg/mL)	5 µL

2. Add 1 cm of freshly cut mouse tail to the microcentrifuge tube and shake (225 rpm) the tube on its side at 60°C overnight (12–20 hours). Be sure to cap the tube tightly.
   Note: After incubation, the mouse tail should be totally digested with only tiny pieces of bone in the bottom of the tube. The solution will be cloudy and may be slightly colored depending on the color of the mouse tail.

Before Starting: Day 2

- Chill 100% and 80% ethanol in a –20°C freezer.
- Thaw RNase (if stored at –20°C) and keep on ice.
- Equilibrate a 37°C heat block or water bath.

Isolation of DNA: Day 2

1. Add 300 µL Solution A and 120 µL Solution B to sample and vortex vigorously until solution is uniformly viscous (10 seconds–1 minute).
2. Add 750 µL chloroform and vortex until the viscosity decreases and the mixture is homogeneous (10 seconds–1 minute).
3. Centrifuge at maximum speed for 10 minutes at 4°C and transfer upper aqueous phase to a fresh microcentrifuge tube.
4. If the upper phase is not clear, a second chloroform extraction is needed. Repeat steps 2 and 3. When upper phase is clear, proceed to **DNA Precipitation.**

DNA Precipitation

1. Add 1.0 mL of 100% ethanol (–20°C) to the now clear upper phase, vortex, and incubate on ice for 30 minutes.
2. Centrifuge at maximum speed for 10–15 minutes at 4°C. Remove ethanol with a drawn-out Pasteur pipette.
3. Add 500 µL 80% ethanol (–20°C) and mix by inverting the tube 3–5 times.
4. Centrifuge at maximum speed for 3–5 minutes at 4°C. Remove 80% ethanol with a drawn-out Pasteur pipette.
5. Centrifuge the tube at maximum speed for 1–3 minutes at 4°C. Remove residual ethanol with a pipettor. Let air dry 5 minutes.
6. Resuspend the pellet in 49 µL TE and add 1 µL 2 mg/mL RNase to a final concentration of 40 µg/mL. Incubate at 37°C for 30 minutes. DNA is ready for use. Store at 4°C.

Guidelines for Protocol Development

Introduction

If none of the protocols given in this manual match the type or size of your sample, then use the following guidelines to develop your own protocol.

Choosing a Starting Protocol

The following table gives suggestions for a protocol based on size and sample type.

If sample size is...	Then, as a guide, use...
Small or valuable (1–100 µL or <1 mg)	Protocol #1
Moderate (3–100 mg tissue, 10^3–10^7 mammalian cells, or 10^9 bacterial cells)	Protocol #3
Large (0.1 g–1 g tissue or 10^7–10^8 mammalian cells)	Protocol #4
Other yeast cells	Protocol #5 or 6
Viral particles	Protocol #7

Do I Need RNase?

The following table describes conditions where you might want to use RNase.

If sample size is...	And consists of...	Then use...
Small	Terminally differentiated cells	No RNase.
Moderate	Viral particles	No RNase.
Moderate or large	Terminally differentiated cells or tissue, bacterial cells, yeast cells, or tissue culture cells	40 µg/mL RNase. Dilute stock solution of RNase with TE buffer if necessary.

Do I Need Protein Degrader?

The following table will help you decide if you need to treat your sample with Protein Degrader. When using the Protein Degrader, remember to add the balance of Solutions A and B after the overnight treatment to properly extract the DNA (see Protocol #8).

If sample size is...	And consists of...	Then use...
Small	Terminally differentiated cells	No Protein Degrader.
Moderate	tissue with high amounts of protein, i.e. connective tissue	100 µg of Protein Degrader and Protocol #8 to treat your sample.
Large	Tissue with high amounts of protein, i.e. connective tissue	1.0 mg of Protein Degrader and scale-up Protocol #8 to treat your sample.

Do I Need Mussel Glycogen?

If the sample is small (1–100 µL or <1 mg) or if DNA is very dilute, then use mussel glycogen at a final concentration of 20 µg/mL to precipitate DNA.

Troubleshooting

Guidelines

Consult the following table for guidelines to troubleshoot problems which may occur.

If there is...	Then...
No interface	Check and make sure you are using Solutions A and B correctly.
No phases	Check and make sure you added chloroform.
a colored interface	Do not worry. The interface picks up the pigments from your sample.
A low yield or no DNA	• use mussel glycogen at a final concentration of 20 µg/mL to precipitate the DNA, or; • add 100 µg Protein Degrader and follow Protocol #8, or; • freeze sample in liquid nitrogen and pulverize with a mortar and pestle.
Degraded DNA	• add 100 µg Protein Degrader and follow Protocol #8, or; • be sure all solutions are sterile.
RNA in your sample	Add 40 µg/mL RNase and incubate for 30 minutes at 37°C.
A large salt pellet after precipitating your DNA	Re-precipitate the DNA as follows: 1. Resuspend the pellet in 50 µL TE Buffer and add 50 µL 4 M ammonium acetate. 2. Add 200 µL 100% ethanol (–20°C). 3. Centrifuge to pellet DNA and wash the pellet with 80% ethanol. Air dry the pellet for 5 minutes. 4. Resuspend the pellet in the desired volume of TE buffer.

Appendix

Recipes

YPD Medium

Yeast Extract Peptone Dextrose (YPD) Medium (1 liter)

1% Yeast extract

2% Peptone

2% dextrose (D-glucose)

1. Dissolve 10 g of Yeast extract and 20 g Peptone in 960 mL of deionized water.
2. Autoclave at 15 lbs/sq. in. for 20 minutes.
3. Make a 50% dextrose solution by dissolving 50 g of dextrose in 100 mL of deionized water. Filter-sterilize.
4. When autoclaved solution has cooled, add 40 mL of 50% glucose.

Minimal Medium

1.34% Yeast Nitrogen Base

4×10^{-5}% D-biotin

2% dextrose or 1% glycerol

plus any additional nutritional supplements

- Prepare 13.4% YNB (Yeast Nitrogen Base with Ammonium Sulfate, without amino acids), 0.02% D-biotin, 20% dextrose, 10% glycerol, and any other supplements as needed.
- Combine aseptically 800 mL autoclaved water with 100 mL of 13.4% YNB, 2 mL of 0.02% D-biotin, and 100 mL of 20% dextrose or 100 mL of 10% glycerol, plus any other supplements.

SCED Solution

1 M Sorbitol

10 mM Sodium Citrate, pH 5.8

1 mM (ethylenedinitrilo)tetraacetic acid dihydrate (EDTA)

10 mM Dithiothreitol (DTT)

1. For 1 L, dissolve 182.2 g sorbitol, 2.94 g sodium citrate, and 0.372 g EDTA dihydrate in 900 mL deionized water. Adjust the pH to 5.8 with HCl.
2. Bring the volume up to 1 L and autoclave at 15 lbs/sq. in. for 20 minutes. This solution is called SCE and can be stored at room temperature.
3. Make 10 mL of a 1 M DTT solution by dissolving 1.54 g DTT in 10 mL water. Aliquot into 1 mL samples and store frozen at –20°C. DTT can be thawed and refrozen. It is stable for at least 6 months.
4. For 10 mL SCED, thaw and add 100 µL of the 1 M DTT solution to 10 mL of SCE. Use immediately.

20% PEG in 1 M NaCl

20% Polyethylene glycol 8000, 1 M NaCl

1. For 100 mL, mix 20 g PEG 8000 and 5.84 g NaCl in 100 mL water.
2. Autoclave 20 minutes at 15 lbs/sq. in.
3. While the solution is still warm (~55°C), swirl carefully to mix thoroughly.

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