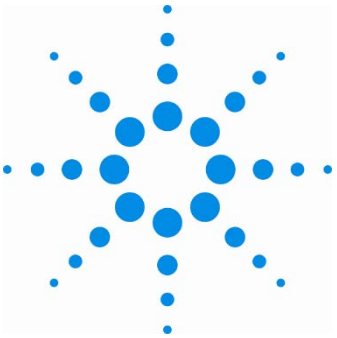

RecoverEase DNA Isolation Kit

Instruction Manual

Catalog #720202 (30 preparations) and #720203 (15 preparations)

Revision D0

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

720202-12

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RecoverEase DNA Isolation Kit

Contents

RecoverEase DNA Isolation Kit

MATERIALS PROVIDED

Material provided	Quantity	
	Catalog #720202[a]	Catalog #720203[b]
Lysis buffer[c]	2 × 150 ml	150 ml
Proteinase K solution[c,d] (2.0 mg/ml)	2 × 1.5 ml	1.5 ml
Digestion buffer[c]	2 × 1.5 ml	1.5 ml
RNace-It ribonuclease cocktail[e]	2 × 50 µl	50 µl
Cell strainers, sterile, yellow (100-µm mesh size)	30	15
Dialysis tubes and Dialysis caps[f]	30	15
Floats	30	15
Applicators, sterile	30	15

[a] This kit contains sufficient reagents and materials for 30 DNA isolations.

[b] This kit contains sufficient reagents and materials for 15 DNA isolations.

[c] See *Preparation of Media and Reagents*.

[d] Immediately on receipt, thaw and dispense the proteinase K solution into usable aliquots to avoid multiple freeze-thaw cycles. Store the aliquots at –20°C.

[e] Available separately (Agilent Catalog #400720).

[f] A patented product registered to G-Biosciences/ Geno Technology, Inc.

STORAGE CONDITIONS

Proteinase K Solution: –20°C

Note *Immediately on receipt of the RecoverEase DNA isolation kit, thaw and dispense the proteinase K solution into usable aliquots to avoid multiple freeze–thaw cycles. Each DNA preparation requires 70–100 µl/isolation.*

RNace-It Ribonuclease Cocktail: –20°C
Lysis Buffer: 4°C
Digestion Buffer: 4°C
Dialysis Tubes and Caps: Room temperature protected from light
All Other Components: Room temperature

ADDITIONAL MATERIALS REQUIRED

Wheaton Dounce tissue grinder with two pestles, clean (7-ml)
- A loose-fitting pestle (pestle B) for tissue disaggregation and complete homogenization
- A tighter-fitting pestle (pestle A) for release of the cell nuclei

Scissors or razor blades, clean
Forceps, clean
Conical tubes, sterile (50-ml) (e.g., BD Falcon #2098 polypropylene tubes or equivalent)
Wide-bore pipet tips
TE buffer, sterile (see *Preparation of Media and Reagents*)
Dialysis reservoir, clean and sterile

Revision D0

INTRODUCTION

The RecoverEase DNA isolation kit quickly isolates high-molecular-weight genomic DNA from a variety of tissues without organic solvent extractions or ethanol precipitation. The procedure involves physical disaggregation of the tissue, coarse filtration, and isolation of the cell nuclei via short centrifugation, followed by incubation with a highly active protease solution containing proteinase K to predigest the cellular proteins. Digestion continues as the DNA preparation is transferred to a convenient, free-floating dialysis tube that allows overnight dialysis of the digested peptides across a semipermeable membrane. After dialysis, the fully hydrated, purified high-molecular-weight DNA is simply collected from the dialysis tube. The resulting DNA, which consistently measures from 100 to 500 kb in length, is ready for immediate use in experimental applications including Southern blot analysis, the polymerase chain reaction (PCR), analysis by pulsed-field electrophoresis, or the recovery of lambda shuttle vectors from transgenic animals.

The benefits of the RecoverEase DNA isolation kit include the early removal of nucleases in the cell cytosol, elimination of toxic organic solvents, such as phenol and chloroform, and the reduction of shearing forces associated with extractions and excessive handling of the DNA. In addition, the DNA is fully hydrated and ready for immediate use after dialysis.

Certain tissues, like bladder and skin, are not amenable to homogenization or nuclei isolation and are thus not recommended starting materials for genomic DNA isolation using the RecoverEase DNA isolation kit.

PREPARING THE TISSUE SAMPLES

1. Perform tissue extraction as quickly as possible.

2. Either immediately use the fresh tissue sample (proceed to *Isolating Genomic DNA from a Variety of Cellular Tissues*) or wrap the excised tissue sample completely in aluminum foil and flash freeze the sample in liquid nitrogen.

   Note *The liquid nitrogen quickly stabilizes the tissue sample and greatly reduces* in situ *DNA degradation.*

3. Store the frozen tissue sample at –80°C until ready for further use.

ISOLATING GENOMIC DNA FROM VARIOUS TISSUE SAMPLES

Liver, Kidney, Lung, Heart, or Spleen Tissue Samples

1. Chill a clean 7-ml Wheaton Dounce tissue grinder and the lysis buffer on ice. **Do not use a mechanical homogenizer because the associated shearing degrades the genomic DNA within the tissue sample.**

2. Add 5 ml of ice-cold lysis buffer to the tissue grinder.

3. Transfer the appropriate amount of the excised tissue sample (see Table I) to the tissue grinder containing the ice-cold lysis buffer. **Do not use too much tissue**.

TABLE I

Guidelines for the Required Amount of Tissue

Type of tissue	Starting mass (mg)	Average final yield (μg)
Liver	50–80	175
Kidney	~100 (approximately one-half of a whole mouse kidney)	200
Lung	~175 (approximately a whole mouse lung)	125
Heart	~185 (approximately a whole mouse heart)	75
Spleen	~40 (approximately one-half of a whole mouse spleen)	400

4. Disaggregate the tissue sample with pestle B for 3–10 strokes until the sample appears completely homogenized.

   Note *Highly vascularized tissues (e.g., from heart or lung samples) typically require more than 10 strokes to fully disaggregate the tissue samples. Do not proceed to the next step until complete homogenization of the tissue sample is achieved.*

5. Release the cell nuclei within the homogenate using pestle A for 8 strokes. **Avoid twisting pestle A while lowering and raising the pestle into the mortar of the tissue grinder.**

6. Pour the homogenate through a sterile cell strainer into a sterile 50-ml conical tube.

   Note *Vascularized homogenates (e.g., from heart or lung tissue samples) tend to block the filter. If the cell strainer is clogged, swirl a wide-bore pipet tip over the surface to enable the liquid from the homogenized suspension to pass through the mesh.*

7. Add an additional 3 ml of ice-cold lysis buffer to the tissue grinder and pour the wash through the cell strainer to bring the total volume in the conical tube to 8 ml. Discard the cell strainer and place a cap on the tube.

8. Store the conical tube on ice until ready for further use and repeat steps 1–7 for any remaining tissue samples.

9. Centrifuge the conical tube at 1100 × *g* for 12 minutes at 4°C.

10. Uncap the tube and carefully discard the supernatant to avoid losing the cell nuclei pellet.

11. Carefully invert the uncapped conical tube on a paper towel for ~1 minute to drain any excess liquid from the pellet.

12. Dry any residual droplets from the inside walls of the tube using one of the sterile applicators provided. Avoid touching the actual pellet with the applicator because the pellet will adhere to the tip of the applicator.

13. Warm a 70-µl aliquot of the proteinase K solution in a 50°C water bath in advance (i.e., 2–5 minutes prior to use) to activate the enzyme.

14. Prepare the digestion solution by adding 20 µl of RNace-It ribonuclease cocktail/ml of digestion buffer.

15. Add 70 µl of the prepared digestion solution to the cell nuclei pellet and rock the conical tube gently to dislodge the pellet from the bottom of the tube.

    Note *The cell nuclei pellet should float freely in the digestion solution. If the pellet does not float freely in the solution, gently pull up on one edge of the cell nuclei pellet with a standard pipet tip until the pellet breaks loose from the bottom of the tube.*

16. Place the conical tube in a 50°C water bath and add 70 µl of the warmed proteinase K solution to the free-floating pellet. Swirl the conical tube gently to mix.

17. Recap the tube and incubate in a 50°C water bath for 45 minutes, swirling the tube gently every 10–15 minutes.

18. After the proteinase K digestion step is complete, add 300 µl of TE buffer to the DNA sample and stir the sample using a wide-bore pipet tip.

19. Prior to use, prepare the dialysis cap by placing it upside down in a beaker or other suitable container and add 1–2 ml of dialysis buffer to rinse. Keep the dialysis membrane wet until needed.

20. Transfer the DNA sample from step 18 directly into a dialysis tube.

21. Remove the prepared dialysis cap from the beaker and carefully remove excess dialysis buffer with a pipet tip. Screw the dialysis cap onto the dialysis tube containing the DNA sample until it is finger-tight. Invert the dialysis tube to ensure that the entire sample rests upon the dialysis membrane.

    Note *If the sample is too viscous, put the dialysis tube into a 50-ml conical tube in an inverted position (i.e., with the dialysis membrane facing down), then centrifuge it for 5 seconds at 500–1000 × g. Do not spin longer than 5 seconds as this may cause the membrane to rupture.*

22. Keeping the dialysis tube in an inverted position, slide the supplied float onto the dialysis tube. Pour at least 500 ml of TE buffer into a dialysis reservoir and float the dialysis tube in the reservoir. To dialyze multiple samples in a single dialysis reservoir, increase the volume of TE buffer proportionally.

23. Ensure that the dialysis membrane is in contact with the dialysis buffer. If there are large air bubbles trapped underneath the dialysis membrane surface, tilt the tube or squirt buffer to remove the air bubble.Gently stir the dialysis buffer. For efficient and complete dialysis, Agilent recommends inverting or gently tapping the dialysis tube 1–2 time during dialysis to mix the sample.

24. Allow the sample to dialyze for at least 5 days (120 hours). During this time, replace the dialysis buffer once a day to maximize the purity of the recovered DNA.

25. After the 5-day dialysis period, remove the dialysis tube from the float. Put the dialysis tube in a 50-ml conical tube in an upright position (i.e., with the dialysis membrane facing up). Immediately spin the conical tube containing the dialysis tube for 5–6 seconds at 500–1000 × *g*. Do not spin longer than 6 seconds as this may cause the membrane to rupture.

26. Discard the dialysis cap and replace with the supplied storage cap, or transfer the DNA sample to an appropriate sterile container using a wide-bore pipet tip.

The recovered genomic DNA is fully hydrated and ready for immediate use. This hydrated genomic DNA preparation may be stored at 4°C for up to 1 month.

Tissue Culture

1. Grow cells to confluence in a T75 flask (~1–5 × 10^7 cells).

   Note *Cells may be washed in Dulbecco's PBS medium, aspirated, flash frozen in liquid nitrogen, and stored in the flask until ready for use. Alternatively, cells may be removed from the flask with a rubber policeman (cell scraper) or trypsin, washed, pelleted, and frozen in liquid nitrogen until ready for use.*

2. Resuspend up to 1 × 10^7 cells from one confluent T75 flask in 5 ml of ice-cold lysis buffer to make lysate for one dialysis cap.

3. Transfer the cells to a Wheaton Dounce tissue grinder and process 5 strokes with pestle A.

4. Pour the homogenate through a sterile cell strainer into a sterile 50-ml conical tube.

   Note *Vascularized homogenates (e.g., from heart or lung tissue samples) tend to block the filter. If the cell strainer is clogged, swirl a wide-bore pipet tip over the surface to enable the liquid from the homogenized suspension to pass through the mesh.*

5. Add an additional 3 ml of ice-cold lysis buffer to the tissue grinder and pour the wash through the cell strainer to bring the total volume in the conical tube to 8 ml. Discard the cell strainer and place a cap on the tube.

6. Store the conical tube on ice until ready for further use and repeat steps 1–5 for any remaining tissue samples.

7. Centrifuge the conical tube at 1100 × *g* for 12 minutes at 4°C.

8. Uncap the tube and carefully discard the supernatant to avoid losing the cell nuclei pellet.

9. Carefully invert the uncapped conical tube on a paper towel for ~1 minute to drain any excess liquid from the pellet.

10. Dry any residual droplets from the inside walls of the tube using one of the sterile applicators provided. Avoid touching the actual pellet with the applicator because the pellet will adhere to the tip of the applicator.

11. Warm a 70-µl aliquot of the proteinase K solution in a 50°C water bath in advance (i.e., 2–5 minutes prior to use) to activate the enzyme.

12. Prepare the digestion solution by adding 20 µl of RNace-It ribonuclease cocktail/ml of digestion buffer.

13. Add 70 µl of the prepared digestion solution to the cell nuclei pellet and rock the conical tube gently to dislodge the pellet from the bottom of the tube.

    Note *The cell nuclei pellet should float freely in the digestion solution. If the pellet does not float freely in the solution, gently pull up on one edge of the cell nuclei pellet with a standard pipet tip until the pellet breaks loose from the bottom of the tube.*

14. Place the conical tube in a 50°C water bath and add 70 µl of the warmed proteinase K solution to the free-floating pellet. Swirl the conical tube gently to mix.

15. Recap the tube and incubate in a 50°C water bath for 45 minutes, swirling the tube gently every 10–15 minutes.

16. After the proteinase K digestion step is complete, add 300 µl of TE buffer to the DNA sample and stir the sample using a wide-bore pipet tip.

17. Prior to use, prepare the dialysis cap by placing it upside down in a beaker or other suitable container and add 1–2 ml of dialysis buffer to rinse. Keep the dialysis membrane wet until needed.

18. Transfer the DNA sample from step 16 directly into a dialysis tube.

19. Remove the prepared dialysis cap from the beaker and carefully remove excess dialysis buffer with a pipet tip. Screw the dialysis cap onto the dialysis tube containing the DNA sample until it is finger-tight. Invert the dialysis tube to ensure that the entire sample rests upon the dialysis membrane.

    Note *If the sample is too viscous, put the dialysis tube into a 50-ml conical tube in an inverted position (i.e., with the dialysis membrane facing down), then centrifuge it for 5 seconds at 500–1000 × g. Do not spin longer than 5 seconds as this may cause the membrane to rupture.*

20. Keeping the dialysis tube in an inverted position, slide the supplied float onto the dialysis tube. Pour at least 500 ml of TE buffer into a dialysis reservoir and float the dialysis tube in the reservoir. To dialyze multiple samples in a single dialysis reservoir, increase the volume of TE buffer proportionally.

21. Ensure that the dialysis membrane is in contact with the dialysis buffer. If there are large air bubbles trapped underneath the dialysis membrane surface, tilt the tube or squirt buffer to remove the air bubble.Gently stir the dialysis buffer. For efficient and complete dialysis, Agilent recommends inverting or gently tapping the dialysis tube 1–2 time during dialysis to mix the sample.

22. Allow the sample to dialyze for at least 5 days (120 hours). During this time, replace the dialysis buffer once a day to maximize the purity of the recovered DNA.

23. After the 5-day dialysis period, remove the dialysis tube from the float. Put the dialysis tube in a 50-ml conical tube in an upright position (i.e., with the dialysis membrane facing up). Immediately spin the conical tube containing the dialysis tube for 5–6 seconds at 500–1000 × *g*. Do not spin longer than 6 seconds as this may cause the membrane to rupture.

24. Discard the dialysis cap and replace with the supplied storage cap, or transfer the DNA sample to an appropriate sterile container using a wide-bore pipet tip.

The recovered genomic DNA is fully hydrated and ready for immediate use. This hydrated genomic DNA preparation may be stored at 4°C for up to 1 month.

Brain Tissue Samples

Note *Use ~100 mg of brain tissue (approximately one-quarter of a whole mouse brain).*

1. Chill a clean 7-ml Wheaton Dounce tissue grinder and the lysis buffer on ice. **Do not use a mechanical homogenizer because the associated shearing degrades the genomic DNA within the tissue sample.**

2. Add 5 ml of ice-cold lysis buffer to the tissue grinder.

3. Transfer ~100 mg of the brain tissue sample to the tissue grinder containing the ice-cold lysis buffer. **Do not use too much tissue**.

4. Disaggregate the tissue sample with pestle B for 3–5 strokes only. **Do not use pestle A and do not overhomogenize the tissue sample!**

5. Pour the homogenate through a sterile cell strainer into a sterile 50-ml conical tube.

6. Add an additional 3 ml of ice-cold lysis buffer to the tissue grinder and pour the wash through the cell strainer to bring the total volume in the conical tube to 8 ml. Discard the cell strainer and place a cap on the tube.

7. Store the conical tube on ice until ready for further use and repeat steps 1–6 for any remaining brain tissue samples.

8. Centrifuge the conical tube at 1100 × *g* for 12 minutes at 4°C.

9. Uncap the tube and carefully discard the supernatant to avoid losing the cell nuclei pellet.

10. Carefully invert the uncapped conical tube on a paper towel for ~1 minute to drain any excess liquid from the pellet.

11. Add 10 ml of ice-cold lysis buffer. Swirl the conical tube a few times to remove the nuclei pellet from the bottom of the tube.

12. Centrifuge at 1100 × *g* for 5 minutes at 4°C.

13. Carefully discard the supernatant to avoid losing the cell nuclei pellet.

14. Carefully invert the conical tube on a paper towel for ~1 minute to drain any excess liquid from the pellet.

15. Dry any residual droplets from the inside walls of the tube using one of the sterile applicators provided. Avoid touching the pellet with the applicator because the pellet will adhere to the tip of the applicator.

16. Warm a 70-µl aliquot of the proteinase K solution in a 50°C water bath in advance (i.e., 2–5 minutes prior to use) to activate the enzyme.

17. Prepare the digestion solution by adding 20 µl of RNace-It ribonuclease cocktail/ml of digestion buffer.

18. Add 70 µl of the prepared digestion solution to the cell nuclei pellet and rock the conical tube gently to dislodge the pellet from the bottom of the tube.

    Note *The cell nuclei pellet should float freely in the digestion solution. If the pellet does not float freely in the solution, gently pull up on one edge of the pellet with a standard pipet tip until the pellet breaks loose from the bottom of the tube.*

19. Place the conical tube in a 50°C water bath and add 70 µl of the warmed proteinase K solution to the free-floating pellet. Swirl the conical tube gently to mix.

20. Recap the tube and incubate in a 50°C water bath for 45 minutes, swirling the tube gently every 10–15 minutes.

21. After the proteinase K digestion step is complete, add 300 µl of TE buffer to the DNA sample and stir the sample using a wide-bore pipet tip.

22. Prior to use, prepare the dialysis cap by placing it upside down in a beaker or other suitable container and add 1–2 ml of dialysis buffer to rinse. Keep the dialysis membrane wet until needed.

23. Transfer the DNA sample from step 21 directly into a dialysis tube.

24. Remove the prepared dialysis cap from the beaker and carefully remove excess dialysis buffer with a pipet tip. Screw the dialysis cap onto the dialysis tube containing the DNA sample until it is finger-tight. Invert the dialysis tube to ensure that the entire sample rests upon the dialysis membrane.

    Note *If the sample is too viscous, put the dialysis tube into a 50-ml conical tube in an inverted position (i.e., with the dialysis membrane facing down), then centrifuge it for 5 seconds at 500–1000 × g. Do not spin longer than 5 seconds as this may cause the membrane to rupture.*

25. Keeping the dialysis tube in an inverted position, slide the supplied float onto the dialysis tube. Pour at least 500 ml of TE buffer into a dialysis reservoir and float the dialysis tube in the reservoir. To dialyze multiple samples in a single dialysis reservoir, increase the volume of TE buffer proportionally.

26. Ensure that the dialysis membrane is in contact with the dialysis buffer. If there are large air bubbles trapped underneath the dialysis membrane surface, tilt the tube or squirt buffer to remove the air bubble.Gently stir the dialysis buffer. For efficient and complete dialysis, Agilent recommends inverting or gently tapping the dialysis tube 1–2 time during dialysis to mix the sample.

27. Allow the sample to dialyze for at least 5 days (120 hours). During this time, replace the dialysis buffer once a day to maximize the purity of the recovered DNA.

28. After the 5-day dialysis period, remove the dialysis tube from the float. Put the dialysis tube in a 50-ml conical tube in an upright position (i.e., with the dialysis membrane facing up). Immediately spin the conical tube containing the dialysis tube for 5–6 seconds at 500–1000 × *g*. Do not spin longer than 6 seconds as this may cause the membrane to rupture.

29. Discard the dialysis cap and replace with the supplied storage cap, or transfer the DNA sample to an appropriate sterile container using a wide-bore pipet tip.

The recovered genomic DNA is fully hydrated and ready for immediate use. This hydrated genomic DNA preparation may be stored at 4°C for up to 1 month.

Bone Marrow Tissue Samples

Note *Use two whole mouse femurs and snip off the ends of each femur with scissors.*

Preparing Bone Marrow Tissue Samples from Femurs

Using a 25-gauge, 5/8-inch needle, inject 4 ml of ice-cold lysis buffer lengthwise through one femur to force the bone marrow from the femur into a 50-ml conical tube. Repeat this process with a second femur and combine the bone marrow tissue samples for a total volume of 8 ml.

Isolating Genomic DNA from Bone Marrow Tissue Samples

1. Vortex the bone marrow tissue sample briefly for 3–5 seconds on medium speed and place the conical tube on ice for ~10 minutes, swirling occasionally.

2. Centrifuge at 1100 × *g* for 15 minutes at 4°C.

3. Uncap the tube and carefully discard the supernatant to avoid losing the cell nuclei pellet.

4. Carefully invert the uncapped conical tube on a paper towel for ~1 minute to drain any excess liquid from the pellet.

5. Dry any residual droplets from the inside walls of the tube using one of the sterile applicators provided. Avoid touching the pellet with the applicator as the pellet will adhere to the tip of the applicator.

6. Warm a 70-µl aliquot of the proteinase K solution in a 50°C water bath in advance (i.e., 2–5 minutes prior to use) to activate the enzyme.

7. Prepare the digestion solution by adding 20 µl of RNace-It ribonuclease cocktail/ml of digestion buffer.

8. Add 70 µl of the prepared digestion solution to the cell nuclei pellet and gently rock the conical tube to dislodge the pellet from the bottom of the tube.

   Note *The cell nuclei pellet should float freely in the digestion solution. If the pellet does not float freely in the solution, gently pull up on one edge of the pellet with a standard pipet tip until the pellet breaks loose from the bottom of the tube.*

9. Place the conical tube in a 50°C water bath and add 70 µl of the warmed proteinase K solution to the free-floating pellet. Swirl the conical tube gently to mix.

10. Recap the tube and incubate in a 50°C water bath for 45 minutes, swirling the tube gently every 10–15 minutes.

11. After the proteinase K digestion step is complete, add 300 µl of TE buffer to the DNA sample and stir the sample using a wide-bore pipet tip.

12. Prior to use, prepare the dialysis cap by placing it upside down in a beaker or other suitable container and add 1–2 ml of dialysis buffer to rinse. Keep the dialysis membrane wet until needed.

13. Transfer the DNA sample from step 11 directly into a dialysis tube.

14. Remove the prepared dialysis cap from the beaker and carefully remove excess dialysis buffer with a pipet tip. Screw the dialysis cap onto the dialysis tube containing the DNA sample until it is finger-tight. Invert the dialysis tube to ensure that the entire sample rests upon the dialysis membrane.

    Note *If the sample is too viscous, put the dialysis tube into a 50-ml conical tube in an inverted position (i.e., with the dialysis membrane facing down), then centrifuge it for 5 seconds at 500–1000 × g. Do not spin longer than 5 seconds as this may cause the membrane to rupture.*

15. Keeping the dialysis tube in an inverted position, slide the supplied float onto the dialysis tube. Pour at least 500 ml of TE buffer into a dialysis reservoir and float the dialysis tube in the reservoir. To dialyze multiple samples in a single dialysis reservoir, increase the volume of TE buffer proportionally.

16. Ensure that the dialysis membrane is in contact with the dialysis buffer. If there are large air bubbles trapped underneath the dialysis membrane surface, tilt the tube or squirt buffer to remove the air bubble.Gently stir the dialysis buffer. For efficient and complete dialysis, Agilent recommends inverting or gently tapping the dialysis tube 1–2 time during dialysis to mix the sample.

17. Allow the sample to dialyze for at least 5 days (120 hours). During this time, replace the dialysis buffer once a day to maximize the purity of the recovered DNA.

18. After the 5-day dialysis period, remove the dialysis tube from the float. Put the dialysis tube in a 50-ml conical tube in an upright position (i.e., with the dialysis membrane facing up). Immediately spin the conical tube containing the dialysis tube for 5–6 seconds at 500–1000 × *g*. Do not spin longer than 6 seconds as this may cause the membrane to rupture.

19. Discard the dialysis cap and replace with the supplied storage cap, or transfer the DNA sample to an appropriate sterile container using a wide-bore pipet tip.

The recovered genomic DNA is fully hydrated and ready for immediate use. This hydrated genomic DNA preparation may be stored at 4°C for up to 1 month.

Whole Testis Tissue Samples

Note *Use ~50 mg of whole testis tissue (approximately one-half of a whole mouse testis).*

1. Chill a clean 7-ml Wheaton Dounce tissue grinder and the lysis buffer on ice. **Do not use a mechanical homogenizer because the associated shearing degrades the genomic DNA within the tissue sample.**

2. Add 5 ml of ice-cold lysis buffer to the tissue grinder.

3. Transfer ~50 mg of the whole testis tissue sample to the tissue grinder containing the ice-cold lysis buffer. **Do not use too much tissue**.

4. Disaggregate the tissue sample with pestle B for 3–10 strokes until the sample appears completely homogenized.

5. Release the cell nuclei within the homogenate using pestle A for 8 strokes. **Avoid twisting pestle A while lowering and raising the pestle into the mortar of the tissue grinder.**

6. Pour the homogenate through a sterile cell strainer into a sterile 50-ml conical tube.

7. Add an additional 3 ml of ice-cold lysis buffer to the tissue grinder and pour the wash through the cell strainer to bring the total volume in the conical tube to 8 ml. Discard the cell strainer and place a cap on the tube.

8. Store the conical tube on ice until ready for further use and repeat steps 1–7 for any remaining whole testis tissue samples.

9. Centrifuge the conical tube at 1100 × *g* for 12 minutes at 4°C.

10. Uncap the tube and carefully discard the supernatant to avoid losing the cell nuclei pellet.

11. Carefully invert the uncapped conical tube on a paper towel for ~1 minute to drain any excess liquid from the pellet.

12. Dry any residual droplets from the inside walls of the tube using one of the sterile applicators provided. Avoid touching the actual pellet with the applicator because the pellet will adhere to the tip of the applicator.

13. Warm a 100-µl aliquot of the proteinase K solution[§] in a 50°C water bath in advance (i.e., 2–5 minutes prior to use) to activate the enzyme.

14. Prepare the digestion solution by adding 20 µl of RNace-It ribonuclease cocktail/ml of digestion buffer.

15. Add 100 µl of the prepared digestion solution to the cell nuclei pellet and gently rock the conical tube to dislodge the pellet from the bottom of the tube.

    Note *The cell nuclei pellet should float freely in the digestion solution. If the pellet does not float freely in the solution, gently pull up on one edge of the cell nuclei pellet with a standard pipet tip until the pellet breaks loose from the bottom of the tube.*

16. Place the conical tube in a 50°C water bath and add 100 µl of the warmed proteinase K solution to the free-floating pellet. Swirl the conical tube gently to mix.

17. Recap the tube and incubate in a 50°C water bath for 2½–3 hours, swirling the tube gently every 15–30 minutes.

18. After the proteinase K digestion step is complete, add 300 µl of TE buffer to the DNA sample and stir the sample using a wide-bore pipet tip.

19. Prior to use, prepare the dialysis cap by placing it upside down in a beaker or other suitable container and add 1–2 ml of dialysis buffer to rinse. Keep the dialysis membrane wet until needed.

20. Transfer the DNA sample from step 18 directly into a dialysis tube.

21. Remove the prepared dialysis cap from the beaker and carefully remove excess dialysis buffer with a pipet tip. Screw the dialysis cap onto the dialysis tube containing the DNA sample until it is finger-tight. Invert the dialysis tube to ensure that the entire sample rests upon the dialysis membrane.

    Note *If the sample is too viscous, put the dialysis tube into a 50-ml conical tube in an inverted position (i.e., with the dialysis membrane facing down), then centrifuge it for 5 seconds at 500–1000 × g. Do not spin longer than 5 seconds as this may cause the membrane to rupture.*

22. Keeping the dialysis tube in an inverted position, slide the supplied float onto the dialysis tube. Pour at least 500 ml of TE buffer into a dialysis reservoir and float the dialysis tube in the reservoir. To dialyze multiple samples in a single dialysis reservoir, increase the volume of TE buffer proportionally.

23. Ensure that the dialysis membrane is in contact with the dialysis buffer. If there are large air bubbles trapped underneath the dialysis membrane surface, tilt the tube or squirt buffer to remove the air bubble.Gently stir the dialysis buffer. For efficient and complete dialysis, Agilent recommends inverting or gently tapping the dialysis tube 1–2 time during dialysis to mix the sample.

24. Allow the sample to dialyze for at least 5 days (120 hours). During this time, replace the dialysis buffer once a day to maximize the purity of the recovered DNA.

25. After the 5-day dialysis period, remove the dialysis tube from the float. Put the dialysis tube in a 50-ml conical tube in an upright position (i.e., with the dialysis membrane facing up). Immediately spin the conical tube containing the dialysis tube for 5–6 seconds at 500–1000 × *g*. Do not spin longer than 6 seconds as this may cause the membrane to rupture.

26. Discard the dialysis cap and replace with the supplied storage cap, or transfer the DNA sample to an appropriate sterile container using a wide-bore pipet tip.

The recovered genomic DNA is fully hydrated and ready for immediate use. This hydrated genomic DNA preparation may be stored at 4°C for up to 1 month.

TROUBLESHOOTING

Standard Troubleshooting for Isolating Genomic DNA

Observation	Suggestion(s)
Following dialysis, the recovered genomic DNA appears cloudy	Continue dialysis for an additional 48 hours with gentle agitation, increase the volume of the TE buffer to at least 500 ml for each sample, and/or change the TE buffer once or twice during dialysis
	Decrease the starting amount of the tissue sample to the suggested guidelines
The recovered DNA is viscous and difficult to pipet	To avoid shearing and to facilitate transfer of the genomic DNA for use in further applications, use a wide-bore pipet tip to transfer the DNA into a sterile microcentrifuge tube
	If the genomic DNA does not transfer easily, lower the wide-bore pipet tip to the bottom of the tube and twist the tip against the tube bottom to remove an aliquot of the DNA

Additional Troubleshooting for Users of Lambda Shuttle Vector Transgenic Systems

Observation	Suggestion(s)
The recovered DNA yields low packaging efficiencies with Transpack packaging extract (<100,000 pfu/reaction)	Continue dialysis for an additional 48 hours with gentle agitation, increase the volume of the TE buffer to 500 ml for each sample, and/or change the TE buffer once or twice during dialysis
	Decrease the starting amount of the tissue sample to the suggested guidelines outlined in this instruction manual to ensure the digestion is sufficient
	Pipet the first viscous clump **only**; further pipetting primarily yields TE buffer, resulting in the recovered DNA being too dilute
	Use a smaller magnetic stir bar and stir the TE buffer less vigorously
	Concentrate the DNA by precipitating and rehydrating in a smaller volume of TE buffer
The recovered genomic DNA is extremely viscous but yields a concentration of <0.5 mg/ml	Conventional genomic DNA isolation methods that use organic extractions and ethanol precipitation steps typically yield a mixture of high- and low-molecular-weight DNA; therefore, concentrations of ≥0.5 mg/ml are required to obtain high packaging efficiencies. Because the RecoverEase DNA isolation kit yields exclusively high-molecular-weight genomic DNA, less-concentrated DNA (i.e., at concentrations <0.5 mg/ml) is required to obtain *significantly* higher packaging efficiencies than are observed for isolation of genomic DNA via conventional techniques

PREPARATION OF MEDIA AND REAGENTS

Note *All reagents should be prepared with distilled water (dH_2O).*

Digestion Buffer (pH 8.0)

1.75 g of Na_2HPO_4
8.0 g of NaCl
0.2 g of KCl
0.2 g of KH_2PO_4
20 ml of 0.5 M EDTA (pH 8.0)
800 ml of sterile dH_2O
Adjust the pH to 8.0 with 1 N NaOH
Add sterile dH_2O to a final volume of 1 liter
Autoclave for 30 minutes

Lysis Buffer (per Liter)

8.20 g of NaCl
0.22 g of KCl
120 g of sucrose
0.30 g of EDTA
10 ml of Triton® X-100
1.58 g of Tris-HCl (pH 8.3)
800 ml of sterile dH_2O
Adjust the pH to 8.3
Add sterile dH_2O to a final volume of 1 liter
Filter sterilize
Store unopened at room temperature for up to 1 year
Store at 4°C after opening

Proteinase K Solution (per 50 ml)

100 mg of proteinase K
30 ml of sterile dH_2O
10 ml of 10% (w/v) sodium dodecyl sulfate (SDS)
10 ml of 0.5 M EDTA (pH 7.5)

Note *Store this solution in small, usable aliquots in a freezer at –20°C for up to 1 year.* ***Do not refreeze after the initial thawing***

TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA
Autoclave for 30 minutes
Store at room temperature for up to 1 year

REFERENCES

1. Rogers, B. J., Sylvester, V. and Provost, G. S. (1996) *Strategies* 9(3):73-74.
2. Monforte, J. A., Rudd, C. J. and Winegar, R. A. (1995) *Environ Mol Mutagen* 23:46.

ENDNOTES

Triton® is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *www.agilent.com*. MSDS documents are not included with product shipments.

RecoverEase DNA Isolation Kit

Catalog #720202 (30 preparations) and #720203 (15 preparations)

QUICK-REFERENCE PROTOCOLS

Liver, Kidney, Lung, Heart, or Spleen Tissue Samples

- Prepare the tissue sample
- Transfer the appropriate amount of the tissue sample to a chilled Wheaton Dounce tissue grinder containing 5 ml of ice-cold lysis buffer
- Disaggregate with pestle B for 3–10 strokes
- Release the cell nuclei using pestle A for 8 strokes
- Pour the homogenate through a sterile cell strainer into a sterile 50-ml conical tube
- Rinse the tissue grinder with 3 ml of ice-cold lysis buffer and pour the wash through the cell strainer into the 50-ml conical tube for a total volume of 8 ml
- Centrifuge at 1100 × *g* for 12 minutes at 4°C
- Carefully discard the supernatant, invert the conical tube to drain any excess liquid, and dry the tube with one of the sterile applicators provided
- Add 70 µl of prepared digestion solution to the cell nuclei pellet and gently rock the tube to allow the pellet to float freely
- Place the conical tube in a 50°C water bath, add 70 µl of proteinase K solution (prewarmed to 50°C) to the free-floating nuclei pellet, and swirl gently to mix
- Incubate in a 50°C water bath for 45 minutes, swirling gently every 10–15 minutes
- After the proteinase K digestion step is complete, add 300 µl of TE buffer to the DNA sample and stir the sample using a wide-bore pipet tip.
- Prior to use, prepare the dialysis cap by placing it upside down in a beaker or other suitable container and add 1–2 ml of dialysis buffer to rinse. Keep the dialysis membrane wet until needed.
- Transfer the DNA sample directly into a dialysis tube.
- Remove the prepared dialysis cap from the beaker and carefully remove excess dialysis buffer with a pipet tip. Screw the dialysis cap onto the dialysis tube containing the DNA sample until it is finger-tight. Invert the dialysis tube to ensure that the entire sample rests upon the dialysis membrane.

- Keeping the dialysis tube in an inverted position, slide the supplied float onto the dialysis tube. Pour at least 500 ml of TE buffer into a dialysis reservoir and float the dialysis tube in the reservoir. To dialyze multiple samples in a single dialysis reservoir, increase the volume of TE buffer proportionally.

- Ensure that the dialysis membrane is in contact with the dialysis buffer. If there are large air bubbles trapped underneath the dialysis membrane surface, tilt the tube or squirt buffer to remove the air bubble.Gently stir the dialysis buffer. For efficient and complete dialysis, Agilent recommends inverting or gently tapping the dialysis tube 1–2 time during dialysis to mix the sample.

- Allow the sample to dialyze for at least 5 days (120 hours). During this time, replace the dialysis buffer once a day to maximize the purity of the recovered DNA.

- After the 5-day dialysis period, remove the dialysis tube from the float. Put the dialysis tube in a 50-ml conical tube in an upright position (i.e., with the dialysis membrane facing up). Immediately spin the conical tube containing the dialysis tube for 5–6 seconds at 500–1000 $\times$ *g*. Do not spin longer than 6 seconds as this may cause the membrane to rupture.

- Discard the dialysis cap and replace with the supplied storage cap, or transfer the DNA sample to an appropriate sterile container using a wide-bore pipet tip.

Protocol Variations for Brain, Bone Marrow, Whole Testis, or Tissue Culture Tissue Samples

Liver, kidney, lung, heart, or spleen tissue samples	Protocol variations			
	Brain tissue samples	**Bone marrow tissue samples**	**Whole testis tissue samples**	**Tissue culture**
Use the appropriate amount of the tissue sample (see Table I)	Use ~100 mg of brain tissue	Use two mouse femurs, inject 4 ml of ice-cold lysis buffer through each femur, and combine the samples	Use ~50 mg of whole testis tissue	Use cells from one confluent T75 flask
		Vortex briefly and store on ice for 10 minutes, swirling occasionally		
Disaggregate with pestle B for 3–10 strokes in 5 ml of lysis buffer	Disaggregate with pestle B for 3–5 strokes only	Skip this step (do not perform)		Skip this step (do not perform)
Release the cell nuclei using pestle A for 8 strokes	Skip this step (do not perform)	Skip this step (do not perform)		Use pestle A for 5 strokes
Filter the homogenate through a sterile cell strainer		Skip this step (do not perform)		
Wash the tissue grinder with 3 ml of ice-cold lysis buffer, filter into the tube, and store on ice		Skip this step (do not perform)		
Centrifuge at 1100 × g for 12 minutes at 4°C		Centrifuge at 1100 × *g* for 15 minutes		
Carefully discard the supernatant, invert the conical tube to drain any excess liquid, and dry the tube with one of the sterile applicators provided	Do not dry the tube. Instead, add 10 ml of ice-cold lysis buffer, swirl to mix, centrifuge at 1100 × *g* for 5 minutes at 4°C, and repeat the step indicated on the left including drying the tube with one of the sterile applicators provided			
Add 70 µl of prepared digestion solution and gently rock the tube to allow the pellet to float freely			Add 100 µl of digestion solution and gently rock the tube to allow the pellet to float freely	
Place the tube in a 50°C water bath, add 70 µl of warmed proteinase K solution, and swirl gently			Use 100 µl of warmed proteinase K	
Incubate in a 50°C water bath for 45 minutes, swirling occasionally			Incubate in a 50°C water bath for 2½–3 hours, swirling occasionally	
Transfer the genomic DNA to a dialysis tube floating on the surface of TE buffer				
Dialyze at room temperature for 120 hours while stirring the buffer gently				
Transfer the DNA to a sterile microcentrifuge tube				