Genomic DNA Isolation Kit (Flexible)

Genomic DNA Isolation Protocol For 3 ml Whole Blood

15 ml Tube Prep - Expected Yield Range 50-150 ug DNA

Cell Lysis

1. **Add 3 ml whole blood (or bone marrow) to a 15 ml tube containing 9 ml RBC Lysis Solution. Invert to mix and incubate 5 minutes at room temperature. Invert again at least once during the incubation.**
2. **Centrifuge at 2,000 x g for 5 minutes**.
   Remove supernatant leaving behind the white cell pellet and about 100-200 ul of the residual liquid.
3. **Vortex the tube vigorously to resuspend the cells in the residual liquid.**
   This greatly facilitates cell lysis in Step 4 below.
4. **Add 3 ml Cell Lysis Solution to the resuspended cells and pipet up and down to lyse the cells.**
   Usually no incubation is required; however, if cell clumps are visible, incubate at 37°C until the solution is homogeneous. Samples are stable in **Cell Lysis Solution** for at least 2 years at room temperature.

RNase Treatment (Optional)

1. **Add 15 ul RNase A Solution to the cell lysate.**
2. **Mix the sample by inverting the tube 25 times and incubate at 37°C for 15 minutes.**

Protein Precipitation

1. **Cool sample to room temperature.**
2. **Add 1 ml Protein Precipitation Solution to the cell lysate.**
3. **Vortex at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.**
4. **Centrifuge at 2,000 x g for 5 minutes**. The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, repeat Step 3 followed by incubation on ice for 5 minutes and then repeat Step 4.

DNA Precipitation

1. **Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a 15 ml tube containing 3 ml 100% Isopropanol (2-propanol).**
2. **Mix the sample by inverting gently 50 times until the white threads of DNA form a visible clump.**
3. **Centrifuge at 2,000 x g for 3 minutes**. The DNA will be visible as a small white pellet.
4. **Pour off supernatant and drain tube briefly on clean absorbant paper.**
   Add 3 ml 70% Ethanol and invert tube several times to wash the DNA pellet.
5. **Centrifuge at 2,000 x g for 1 minute. Carefully pour off the ethanol.**
   Pellet may be loose so pour slowly and watch pellet.
6. **Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.**

DNA Hydration

1. **Add 250 ul DNA Hydration Solution**
   (250 ul will give a concentration of 400 ug/ml if the total yield is 100 ug DNA).
2. **Rehydrate DNA by incubating at 65°C for 1 hour and overnight at room temperature.**
   If possible, tap tube periodically to aid in dispersing the DNA.
3. **For storage, sample may be centrifuged briefly and then transferred to a 1.5 ml tube.**
   Store DNA at 4°C. For long-term storage, store at -20°C or -80°C.

Genomic DNA Isolation Kit (Flexible) Reagent Volumes

(0.05 ml to 10 ml Blood or 0.35 to 70 Million Cells)

Blood or Bone Marrow Nolume (ml)	0.10	0.30	0.50	0.70	1.00	2.00	5.0	7.0	10.0
Number of White Cells (Million) (1)	0.70	2.10	3.50	4.90	7.00	14.00	35.0	49.0	70.0
Red Blood Cell Lysis Solution (ml)	0.30	0.90	1.50	2.10	3.00	6.00	15.0	21.0	30.0
Cell Lysis Solution (ml)	0.10	0.30	0.50	0.70	1.00	2.00	5.0	7.0	10.0
RNase A, Optional (ul)	0.50	1.50	2.50	3.50	5.00	10.00	25.0	35.0	50.0
Protein Precipitation Solution (ml)	0.033	0.100	0.167	0.233	0.333	0.667	1.67	2.33	3.33
100% Isopropanol (ml)	0.10	0.30	0.50	0.70	1.00	2.00	5.0	7.0	10.0
70% Ethanol (ml)	0.10	0.30	0.50	0.70	1.00	2.00	5.0	7.0	10.0
DNA Hydration Solution (ul) (2)	8.3	25.0	41.7	58.3	83.3	166.7	417	583	833
Theoreticak DNA Yield (ug) (3)	3.4	10.1	16.8	23.5	33.6	67.2	168	235	336

1) Cell number estimates assume an average of seven million white cells per ml of whole blood.
2) Hydration Solution volume gives a DNA concentration of approximately 400 ug/ml.
3) DNA yield assumes 6 pg DNA per diploid nucleus and 80% recovery.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise.

	Comments and suggestions
Low or no yield	
a) No DNA precipitate visible after addition of isopropanol	Ensure that the sample is completely mixed with isopropanol. Invert the tube at least 20 times after addition of isopropanol, until the strands of DNA clump together.
b) DNA was over-dried	Avoid over-drying DNA pellet after removal of 70% (v/v) ethanol, as over-dried genomic DNA is difficult to dissolve. At DNA Hydration step, prolong the incubation time used to resuspend the DNA to 2 hours, then leave the solution overnight at room temperature.
c) DNA not completely dissolved	Ensure that the DNA is dissolved completely by incubating for 1 hour at 65°C, followed by incubation overnight at room temperature. If the DNA is still not fully dissolved, vortex at low speed for 5 seconds and incubate again for 10 minutes at 65°C.
Purified DNA does not perform well in downstream enzymatic applications	
a) Final DNA solution contaminated with ethanol	Ensure that all the ethanol has evaporated before adding DNA Hydration Solution. Leave the tube at DNA Precipitation step 6 inverted on a clean piece of absorbent paper for at least 10-15 minutes to minimize backflow of ethanol from the rim and sides of the tube onto the pellet.
b) Wrong amount of DNA used in the downstream application	Amplification reactions are often inhibited by excess DNA. Reduce the amount of DNA used — 20–50 ng is usually sufficient for PCR.

Additional Protocols

Protocol for 25 mg Solid Tissue

Expected Yield 35 ug DNA

Cell Lysis

1. **Dissect tissue sample quickly and freeze in liquid nitrogen. Store at -70° to -80°C.**
   Fresh tissue may also be used. Work very quickly and keep tissue on ice at all times including when tissue is weighed.
2. **Add 25 mg frozen ground tissue or fresh tissue to a 2 ml centrifuge tube containing 750 ul Cell Lysis Solution, remove from ice, and homogenize thoroughly using a microfuge tube pestle. Place sample back on ice until next step.**
3. **Incubate lysate at 65°C for 15-60 minutes. Alternatively, if maximum yield is required, 3.75 ul Proteinase K Solution (20 mg/ml) may be added to the lysate.**
   Mix by inverting 25 times and incubate at 55°C for 3 hours to overnight, until tissue particulates have dissolved. If possible, invert tube periodically during the incubation.

RNase Treatment

1. **Add 3.75 ul RNase A Solution (4 mg/ml) to the cell lysate.**
2. **Mix the sample by inverting the tube 25 times and incubate at 37°C for 15-60 minutes.**

Protein Precipitation

1. **Cool sample to room temperature.**
2. **Add 250 ul Protein Precipitation Solution to the RNase A-treated cell lysate.**
3. **Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.**
4. **Centrifuge at 13,000-16,000 x g for 3 minutes.**
   The precipitated proteins will form a tight pellet.
   If the protein pellet is not visible, repeat Step 3 followed by incubation on ice for 5 minutes, then repeat Step 4.

DNA Precipitation

1. **Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 2 ml centrifuge tube containing 750 ul 100% Isopropanol.**
2. **Mix the sample by inverting gently 50 times.**
3. **Centrifuge at 13,000-16,000 x g for 1 minute; the DNA will be visible as a white pellet.**
4. **Pour off supernatant and drain tube on clean absorbent paper. Add 750 ul 70% Ethanol and invert tube several times to wash the DNA pellet.**
5. **Centrifuge at 13,000-16,000 x g for 1 minute. Carefully pour off the ethanol.**
   Pellet may be loose so pour slowly and watch pellet.
6. **Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.**

DNA Hydration

1. **Add 175 ul DNA Hydration Solution.**
   (175 ul will give a concentration of 200 ug/ml if the total yield is 35 ug DNA).
2. **Rehydrate DNA by incubating sample 1 hour at 65ºC and/or overnight at room temperature.**
   If possible, tap tube periodically to aid in dispersing the DNA.
3. **Store DNA at 4°C.** For long-term storage, store at -20°C or -80°C.

Protocol for 15-30 mg Mouse Tail Tissue

Expected Yield Range 37-225 ug DNA

Cell Lysis

1. **Chill a 15 ml tube containing 900 ul Cell Lysis Solution on ice.**
   Please note that the solution will turn cloudy.
2. **Place 15 mm (15-30 mg) fresh or frozen tail tissue (minced if possible) into the chilled Cell Lysis Solution.**
3. **Add 4.5 ul Proteinase K Solution to the sample and mix by inverting 25 times. Incubate at 55°C overnight or until tissue has dissolved.**
   If possible, invert tube periodically during the incubation.

RNase Treatment (optional)

1. **Add 4.5 ul RNase A Solution to the cell lysate.**
2. **Mix sample by inverting the tube 25 times and incubate at 37°C for 15-60 minutes.**

Protein Precipitation

1. **Cool sample to room temperature.**
2. **Add 300 ul Protein Precipitation Solution to the RNase A-treated cell lysate.**
3. **Vortex at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.**
4. **Centrifuge at 2,000 x g for 10 minutes.**
   The precipitated proteins will form a tight pellet. If the protein pellet is not visible, repeat Step 3 followed by incubation in an ice bath for 5-15 minutes, then repeat Step 4.

DNA Precipitation

1. **Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 15 ml tube containing 900 ul 100% Isopropanol.**
2. **Mix sample by inverting gently 50 times.**
3. **Centrifuge at 2,000 x g for 3 minutes; the DNA will be visible as a small white pellet.**
4. **Pour off the supernatant and drain tube on clean absorbent paper. Add 900 ul 70% Ethanol and invert tube several times to wash the DNA pellet.**
5. **Centrifuge at 2,000 x g for 1 minute. Carefully pour off the ethanol.**
   Pellet may be loose so pour slowly and watch pellet.
6. **Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.**

DNA Hydration

1. **Add 150 ul DNA Hydration Solution.**
   (150 ul will give a concentration of 500 ug/ml if the total yield is 75 ug DNA).
2. **Rehydrate DNA by incubating at 65°C for 1 hour and overnight at room temperature.**
   If possible, tap tube periodically to aid in dispersing the DNA.
3. **For storage, vortex sample briefly, pulse spin, and transfer to a 1.5 ml tube. Store DNA at 4°C.**
   For long-term storage, store at -20°C or -80°C.

Protocol for Leaf Tissue
(10-20 mg Dried or 20-60 mg Fresh or Frozen)
Expected Yield Range 3-30 ug DNA

Cell Lysis

1. **Add 10-20 mg dried tissue (finely ground), 20-60 mg frozen tissue (may be finely ground with a mortar and pestle in liquid nitrogen), or 20-60 mg fresh leaf tissue (2-5 disks) to a 1.5 ml tube.** A leaf disk (7 mm diameter) may be prepared by placing the leaf between the microfuge tube and its cap and then snapping the cap closed.
   Work quickly and keep tissue cold to minimize DNase activity.
   Note: it may be necessary to vary the amount of starting material depending upon the species, age, tissue preparation and genome size.
2. **Add 600 ul Cell Lysis Solution to the leaf tissue.** For dried tissue, vortex 1-3 seconds to wet the tissue. For unground tissue, homogenize using 30-50 strokes with a microfuge tube pestle.
3. **Incubate cell lysate at 65°C for 60 minutes**. After 30 and 60 minutes invert tube 10 times.

RNase Treatment (Optional)

1. **Add 3 ul RNase A Solution to the cell lysate.**
2. **Mix the sample by inverting the tube 25 times and incubate at 37°C for 15 minutes.**

Protein Precipitation

1. **Cool sample to room temperature.**
2. **Add 200 ul Protein Precipitation Solution to the cell lysate.**
3. **Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.**
   Alternatively, invert rack containing the samples 150 times (approximately 2 minutes) to mix the Protein Precipitation Solution uniformly with the cell lysate. For species with high polysaccharide content, it may be necessary to incubate sample on ice for 5-15 minutes.
4. **Centrifuge at 13,000-16,000 x g for 3 minutes.** The precipitated proteins should form a tight, green pellet. The supernatant may range in appearance from brown to green depending on the sample. If the pellet is not tight, repeat Step 3, followed by incubation on ice for 5 minutes, and then repeat Step 4.

DNA Precipitation

1. **Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a 1.5 ml centrifuge tube containing 600 ul 100% Isopropanol.**
2. **Mix the sample by inverting gently 50 times.**
3. **Centrifuge at 13,000-16,000 x g for 1 minute.** The DNA will be visible as a pellet that ranges in color from off-white to light green.
4. **Pour off supernatant and drain tube briefly on clean absorbent paper.**
   Add 600 ul 70% Ethanol and invert tube several times to wash the DNA pellet.
5. **Centrifuge at 13,000-16,000 x g for 1 minute. Carefully pour off the ethanol.**
   Pellet may be loose so pour slowly and watch pellet.
6. **Invert and drain the tube on clean absorbent paper and allow to air dry for 10-15 minutes.**

DNA Hydration

1. **Add 100 ul DNA Hydration Solution.**
   (100 ul will give a concentration of 100 ug/ml if the total DNA yield is 10 ug).
2. **Rehydrate DNA by incubating sample 1 hour at 65ºC and/or overnight at room temperature.**
   If possible, tap tube periodically to aid in dispersing the DNA.
3. **If particulates are present in the rehydrated DNA sample, centrifuge at 13,000-16,000 x g for 5-10 minutes and then transfer the supernatant containing the DNA to a clean tube. Store DNA at 4°C.** For long-term storage, store at -20°C or -80°C.

Protocol for 1 ml Yeast

Expected Yield 3-6 ug DNA

Cell Lysis

1. **Add 1 ml cell suspension (*e.g.*, overnight culture containing approximately 1-2 x 10^8 cells) to a 1.5 ml tube on ice.**
2. **Centrifuge at 13,000-16,000 x g for 5 seconds to pellet cells and remove supernatant.**
3. **Add 300 ul 50 mM EDTA (pH 8.0) to cell pellet and gently pipet up and down until cells are suspended.**
4. **Add 7.5µl of 20mg/ml lyticase and invert tube 25 times to mix.**
5. **Incubate at 37°C for 30 minutes to digest cell walls. Invert sample occasionally during the incubation.**
6. **Centrifuge at 13,000-16,000 x g for 1 minute to pellet the cells. Remove supernatant.**
7. **Add 300 ul Cell Lysis Solution to the cell pellet and gently pipet up and down to lyse the cells.**

Protein Precipitation

1. **Add 100 ul Protein Precipitation Solution to the cell lysate.**
2. **Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.**
3. **Centrifuge at 13,000-16,000 x g for 3 minutes.** The precipitated proteins will form a tight white pellet. If the protein pellet is not visible, repeat Step 2 followed by incubation on ice for 5 minutes, then repeat Step 3.

DNA Precipitation

1. **Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a 1.5 ml microfuge tube containing 300 ul 100% Isopropanol.**
2. **Mix the sample by inverting gently 50 times.**
3. **Centrifuge at 13,000-16,000 x g for 1 minute;** the DNA should be visible as a small white pellet.
4. **Pour off supernatant and drain tube briefly on clean absorbent paper.**
   Add 300 ul 70% Ethanol and invert tube several times to wash the DNA pellet.
5. **Centrifuge at 13,000-16,000 x g for 1 minute. Carefully pour off the ethanol.**
6. **Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.**

DNA Hydration and RNase Treatment

1. **Add 50 ul DNA Hydration Solution.**
   (50 ul will give a concentration of 100 ug/ml if the yield is 5 ug DNA).
2. **Add 1.5 ul RNase A Solution to the purified DNA sample.**
3. **Mix the sample by vortexing 1 second. Pulse spin to collect liquid, and incubate at 37°C for 15 minutes.**
4. **Rehydrate DNA by incubating sample 1 hour at 65ºC and/or overnight at room temperature.**
   If possible, tap tube periodically to aid in dispersing the DNA.
5. **Store DNA at 4°C.** For long-term storage, store at -20°C or -80°C.

Protocol for 1 ml Gram-negative Bacteria

Expected Yield 25-75 µg DNA

Cell Lysis

1. **Add 1 ml cell suspension (*e.g.*, overnight culture containing approximately 1-3 billion cells) to a 1.5 ml tube.**
2. **Centrifuge at 13,000-16,000 x g for 5 seconds to pellet cells.**
   For some species centrifugation for up to 60 seconds may be required to obtain a tight cell pellet. Remove as much supernatant as possible using a pipet.
3. **Add 600 ul Cell Lysis Solution and gently pipet up and down until the cells are suspended.**
4. **Incubate samples at 80°C for 5 minutes to lyse cells.**

RNase Treatment

1. **Add 3 ul RNase A Solution to the cell lysate.**
2. **Mix the sample by inverting the tube 25 times and incubate at 37°C for 15-60 minutes.**

Protein Precipitation

1. **Cool sample to room temperature.**
2. **Add 200 ul Protein Precipitation Solution to the RNase A-treated cell lysate.**
3. **Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.** For species with a high mucopolysaccharide content, placing the sample on ice for 5 minutes may be required.
4. **Centrifuge at 13,000-16,000 x g for 3 minutes.**
   For species with a high mucopolysaccharide content, centrifugation at 4°C may be required. The precipitated proteins should form a tight pellet. If the protein pellet is not tight, repeat Step 3 followed by incubation on ice for 5 minutes, then repeat Step 4.

DNA Precipitation

1. **Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a 1.5 ml microfuge tube containing 600 ul 100% Isopropanol.**
2. **Mix the sample by inverting gently 50 times.**
3. **Centrifuge at 13,000-16,000 x g for 1 minute;** the DNA should be visible as a small white pellet.
4. **Pour off supernatant and drain tube on clean absorbent paper. Add 600 ul 70% Ethanol and invert the tube several times to wash the DNA pellet.**
5. **Centrifuge at 13,000-16,000 x g for 1 minute. Carefully pour off the ethanol.**
   Pellet may be loose so pour slowly and watch pellet.
6. **Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.**

DNA Hydration

1. **Add 100 ul DNA Hydration Solution**.
   (100 ul will give a DNA concentration of 500 ug/ml if the yield of DNA is 50 ug).
2. **Rehydrate DNA by incubating sample 1 hour at 65ºC and/or overnight at room temperature.**
   If possible, tap tube periodically to aid in dispersing the DNA.
3. **Store DNA at 4°C.** For long-term storage, store at -20°C or -80°C.

Protocol for 1 ml Gram-Positive Bacteria

Expected Yield 6-60 ug DNA

Cell Lysis

1. **Add 1.0 ml cell suspension (*e.g.*, overnight culture containing approximately 1-3 billion cells) to a 1.5 ml tube on ice.**
2. **Centrifuge at 13,000-16,000 x g for 5 seconds to pellet cells.**
   For some species centrifugation for up to 60 seconds may be required to obtain a tight cell pellet.
   Remove as much supernatant as possible using a pipet.
3. **Add 500 ul 50 mM EDTA (pH 8.0) to cell pellet and gently pipet up and down until cells are suspended.**
4. **Add 120 ul Lytic Enzyme Solution (60 ul 10mg/ml lysozyme + 60 ul 10mg/ml lysostaphin) and invert tube 25 times to mix.**
5. **Incubate at 37°C for 30 minutes to digest cell walls. Invert sample occasionally during the incubation.**
6. **Centrifuge at 13,000-16,000 x g for 1 minute to pellet the cells. Remove supernatant.**
7. **Add 600 ul Cell Lysis Solution to the cell pellet and gently pipet up and down to lyse the cells.**
8. **For some species heating the sample to 80°C for five minutes may be required to complete cell lysis.**

RNase Treatment

1. **Add 3.0 ul RNase A Solution to the cell lysate.**
2. **Mix the sample by inverting the tube 25 times and incubate at 37°C for 15-60 minutes.**

Protein Precipitation

1. **Cool sample to room temperature.**
2. **Add 200 ul Protein Precipitation Solution to the cell lysate.**
3. **Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.** For species with a high polysaccharide content, placing the sample on ice for 15-60 minutes may be required.
4. **Centrifuge at 13,000-16,000 x g for 3 minutes.**
   The precipitated proteins will form a tight white pellet. If the protein pellet is not tight, repeat Step 3 followed by incubation on ice for 5 minutes, then repeat Step 4.

DNA Precipitation

1. **Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml microfuge tube containing 600 ul 100% Isopropanol.**
2. **Mix the sample by inverting gently 50 times.**
3. **Centrifuge at 13,000-16,000 x g for 1 minute;** the DNA should be visible as a small white pellet.
4. **Pour off supernatant and drain tube briefly on clean absorbent paper.**
   Add 600 ul 70% Ethanol and invert tube several times to wash the DNA pellet.
5. **Centrifuge at 13,000-16,000 x g for 1 minute. Carefully pour off the ethanol.**
6. **Invert and drain the tube on clean absorbent paper and allow to air dry 10-15 minutes.**

DNA Hydration

1. **Add 200 ul DNA Hydration Solution.**
   (200 ul will give a concentration of 100 ug/ml if the yield is 20 ug DNA).
2. **Rehydrate DNA by incubating sample 1 hour at 65ºC and/or overnight at room temperature.**
   If possible, tap tube periodically to aid in dispersing the DNA.
3. **Store DNA at 4°C.** For long-term storage, store at -20°C or -80°C.