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Product Manual

Blood Genomic DNA, Amniotic Fluid Genomic DNA,
Bodily Fluid Genomic DNA, Tissue Genomic DNA,
Viral DNA, Microbial DNA, Viral RNA

Omni-Pure™ Genomic DNA Purification Systems
Catalog No: 40-40XX-XX

Facile and Rapid Purification of Genomic DNA

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Materials Supplied

Omni-Pure™ Blood DNA Purification Systems

Product	Catalog No.	Size	Catalog No.	Size	Catalog No.	Size
Omni-Pure™ Blood DNA Purification System	40-4010-01	100	40-4010-05	500	40-4010-10	1000
	❑		❑		❑	
Materials Supplied						
GD1 Cell Suspension Solution	40-4011-01	100 ml	40-4011-05	500 ml	40-4011-10	1000 ml
GD2 Cell Lysis Solution	40-4012-03	30 ml	40-4012-15	150 ml	40-4012-30	300 ml
GD3 Protein Precipitation Solution	40-4013-01	10 ml	40-4013-05	50 ml	40-4013-10	100 ml
GD4 DNA Reconstitution Solution	40-4014-01	10 ml	40-4014-05	50 ml	40-4014-10	100 ml

Omni-Pure™ Tissue DNA Purification Systems

Product	Catalog No.	Size	Catalog No.	Size	Catalog No.	Size
Omni-Pure™ Tissue DNA Purification System	40-4050-01	100	40-4050-05	500	40-4050-10	1000
	❑		❑		❑	
Materials Supplied						
GD1 Cell Suspension Solution	40-4011-01	100 ml	40-4011-05	500 ml	40-4011-10	1000 ml
GD2 Cell Lysis Solution	40-4012-03	30 ml	40-4012-15	150 ml	40-4012-30	300 ml
GD3 Protein Precipitation Solution	40-4013-01	10 ml	40-4013-05	50 ml	40-4013-10	100 ml
GD4 DNA Reconstitution Solution	40-4014-01	10 ml	40-4014-05	50 ml	40-4014-10	100 ml
RNase A Solution; 2 mg/ml	40-5101-02	200 µl	40-5101-10	1 ml	40-5101-10	2 x 1 ml
Proteinase K; 10 mg/ml	40-5203-02	200 µl	40-5203-01	1 ml	40-5203-02	2 x 1 ml
Glycogen Solution; 10 mg/ml	40-5112-02	200 µl	40-5112-01	1 ml	40-5112-01	2 x 1 ml

Omni-Pure™ Plant DNA Purification Systems

Product	Catalog No.	Size	Catalog No.	Size	Catalog No.	Size
Omni-Pure™ Plant DNA Purification System	40-4060-01	100	40-4060-05	500	40-4060-10	1000
	❑		❑		❑	
Materials Supplied						
GD1 Cell Suspension Solution	40-4011-01	100 ml	40-4011-05	500 ml	40-4011-10	1000 ml
GD2 Cell Lysis Solution	40-4012-03	30 ml	40-4012-15	150 ml	40-4012-30	300 ml
GD3 Protein Precipitation Solution	40-4013-01	10 ml	40-4013-05	50 ml	40-4013-10	100 ml
GD4 DNA Reconstitution Solution	40-4014-01	10 ml	40-4014-05	50 ml	40-4014-10	100 ml
RNase A Solution; 2 mg/ml	40-5101-02	200 µl	40-5101-10	1 ml	40-5101-10	2 x 1 ml
Glycogen Solution; 10 mg/ml	40-5112-02	200 µl	40-5112-01	1 ml	40-5112-01	2 x 1 ml

Omni-Pure™ Universal DNA Purification Systems

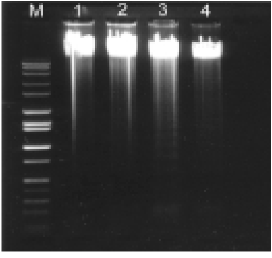
Product	Catalog No.	Size	Catalog No.	Size	Catalog No.	Size
Omni-Pure™ Universal DNA Purification System	40-4070-01	100	40-4070-05	500	40-4070-10	1000
	❑		❑		❑	
Materials Supplied						
GD1 Cell Suspension Solution	40-4011-01	100 ml	40-4011-05	500 ml	40-4011-10	1000 ml
GD2 Cell Lysis Solution	40-4012-03	30 ml	40-4012-15	150 ml	40-4012-30	300 ml
GD3 Protein Precipitation Solution	40-4013-01	10 ml	40-4013-05	50 ml	40-4013-10	100 ml
GD4 DNA Reconstitution Solution	40-4014-01	10 ml	40-4014-05	50 ml	40-4014-10	100 ml
RNase A Solution; 2 mg/ml	40-5101-02	200 µl	40-5101-10	1 ml	40-5101-10	2 x 1 ml
Proteinase K; 10 mg/ml	40-5203-02	200 µl	40-5203-01	1 ml	40-5203-01	2 x 1 ml
Lytic Enzyme; 4000 units/ml	40-5205-02	200 µl	40-5205-01	1 ml	40-5205-01	2 x 1 ml
Glycogen Solution; 10 mg/ml	40-5112-02	200 µl	40-5112-01	1 ml	40-5112-01	2 x 1 ml

Facile and Rapid Extraction and Purification of Genomic DNA

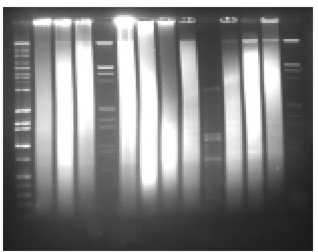
The Omni-Pure™ Genomic DNA Purification System is designed for a convenient volume of 300 µl whole blood (lower volumes can also be used) to yield an average of ~10 µg ultra pure DNA. This quantity is sufficient for restriction-based Southern blot analysis and hundreds of PCR-based analyses.

The Omni-Pure™ Genomic DNA purification system is designed for convenience and consistency. It is a universal genomic DNA purification system. Ultra pure genomic DNA can be purified from small amounts of almost all known sample types and sources. Samples from human blood, bodily fluids, animal and plant tissue and microbial and viral sources have been purified using the Omni-Pure™ Genomic DNA purification system. One purification is usually sufficient to yield enough DNA for all molecular biology applications.

APPLICATION:
Blood DNA Purification
Each purification sample volume is specially geared towards the desired downstream application. A sample volume of 300 µl is recommended for human blood samples yielding on average from 5–15 µg of high molecular weight and high quality genomic DNA for two restriction digestions for Southern blot analysis. The yield is sufficient for hundreds of PCR amplification reactions. An accompanying product manual contains a detailed protocol for the extraction of genomic DNA from tissues and bodily fluids.



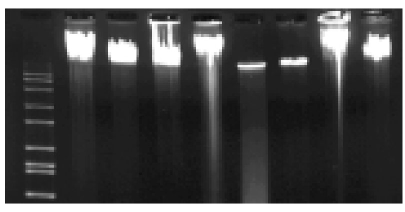
Purified genomic DNA (~200 ng) was electrophoresed on a 0.8% agarose gel and stained with ethidium bromide. Observe high quality genomic DNA that ranges from ~30 to 50 kb in size. Lane M contains molecular weight markers from 10 kb to 50 bp in length. Lanes 1-4 are genomic DNA samples obtained from blood samples of 4 different individuals using the Omni-Pure™ system.



Human blood genomic DNA from different individuals purified using the Omni-Pure™ Genomic DNA Purification System. Approximately 5 µg were digested with different restriction enzymes and the samples were electrophoresed on a 0.7% agarose gel. Note the high molecular weight DNA and the consistency between different samples. The gel was processed for Southern blot analysis and chemiluminescent detection.

APPLICATION:
Tissue DNA Purification

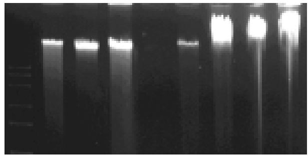
This product manual contains a detailed protocol for the purification of genomic DNA from animal tissue. The protocol has been tested and yields high quality DNA. The gel picture shows genomic DNA extracted from various animal sources. This system is geared towards minute tissue samples. From 2 mg of tissue an average yield of 2-10 µg is expected. The DNA is suitable for all molecular biology applications.



Samples from various animals were processed for DNA purification using the Omni-Pure™ Genomic DNA Purification System. Purified genomic DNA (~200 ng) was electrophoresed on a 0.7% agarose gel and stained with ethidium bromide. Observe high quality genomic DNA that ranges from ~30 to 50 kb in size. Lane 1 contains molecular weight markers followed by samples from human, rabbit, cat, mouse, guinea pig, sheep, pig and hamster.

APPLICATION:
Plant DNA Purification

This product manual contains a detailed protocol for the purification of genomic DNA from plant tissue. The protocol has been tested and yields high quality DNA. The gel picture shows genomic DNA extracted from plants such as ginger, green pepper, cilantro and carrot. This system is geared towards minute tissue samples. From 2 mg of tissue an average yield of 2-10 µg is expected. The DNA is suitable for all molecular biology applications.



Samples from various plant tissues were processed for DNA purification using the Omni-Pure™ Plant DNA Purification System. Purified genomic DNA (~200 ng) was electrophoresed on a 0.7% agarose gel and stained with ethidium bromide. Observe high quality genomic DNA that ranges from ~30 to 50 kb in size. Lane 1 contains molecular weight markers followed by plant samples from ginger, green pepper, cilantro, blank lane, carrot and animal genomic DNA comparison samples from human, mouse and pig.

The Omni-Pure™ Genomic DNA Purification System

- Ultra Purified Genomic DNA
- No Toxic Reagents
- ~30 Minute Protocols
- Blood & Bodily Fluids
- Genomic DNA
- Animal Tissue
- Genomic DNA
- Plant Tissue Purification
- Yeast DNA Purification
- Gram Positive & Negative Bacterial DNA
- Suitable for All Molecular Biology Applications
- Convenient Optimized Systems

Product Description

Introduction

The Omni-Pure™ DNA Purification System provides an easy-to-use kit of optimized reagents and a rapid protocol to yield ultra purified DNA. The genomic DNA is suitable for all molecular biology applications and has been thoroughly tested. The Omni-Pure™ DNA purification system uses non-hazardous reagents and especially does not use the classic phenol-chloroform protocol.

Sample Type

Blood remains as the primary sample source to obtain 5–10 µg quantities of DNA from 300 µl sample volumes. This kit is particularly formulated to extract and purify DNA from 300 µl of blood and similar small sample sizes with almost all manipulations being carried out in 1.5 ml tubes. Multiple samples can be processed at the same time. Ultra pure genomic DNA is obtained in less than 30 minutes omitting the optional RNase A incubation. The RNase treatment is optional as most of the RNA is degraded and does not precipitate at the isopropanol step.

The Omni-Pure™ DNA Purification System can be used for DNA purification from all animal tissues, bodily fluid samples, plant tissues and pathogens. Slight modifications to the protocol can be instituted for the tissue types not listed in this manual based on the characteristics of the sample. For example, RNase treatment and proteinase K treatment are not required for blood samples but are essential for tissues containing high levels of RNA and nucleases, such as in the pancreas. Lytic enzyme should be used to lyse yeast, gram positive bacteria and particularly mycobacterium tuberculosis.

Protocol Principle

The basic principle used for the Omni-Pure™ DNA Purification System's first step is the preferential isolation of cells that contain DNA and disposal of the rest. In the case of blood, the white blood cells are separated and then lysed to release genomic DNA. For tissues, the tissue is homogenized and the cells are lysed. The lysed cells contain proteins and other macromolecules in addition to genomic DNA. In the next step the proteins are precipitated leaving the DNA (and RNA) in the supernatant solution phase. The DNA is then precipitated with isopropanol. Genomic DNA purified by this procedure yields un-sheared DNA of high molecular weight that requires a longer time to rehydrate and dissolve if totally dried.

Decontamination

All human and animal samples used for purification of DNA should be considered infectious. Proper decontamination protocols should be followed for eventual disposal. All waste materials should be properly decontaminated and disposed following institutional guidelines. A standard decontamination protocol is given in this manual for information only and is not a substitute for any other protocol established by the institution or OSHA. Household bleach is a readily available and effective disinfectant. Extended heating at 80°C to 100°C for 20 minutes or longer denatures and inactivates most pathogens.

Genotyping Method & Sample Requirements

PCR based genotyping requires low quantities of DNA and 10-20 ml of buccal wash sample is sufficient to yield DNA for hundreds of amplification reactions. A few milliliters of amniotic fluid or a few milligrams of chorionic villi sample will suffice most prenatal diagnosis requirements; these cells can be cultured to obtain more DNA.
Southern-based genotyping and linkage analysis requires more DNA and thus blood samples or transformed cell lines are ideal sources of DNA.

Omni-Pure™ Genomic DNA Purification System

Quick Protocol: Purification of Genomic DNA from Blood

Sample: 300 µl blood **Average Yield: 10 µg**

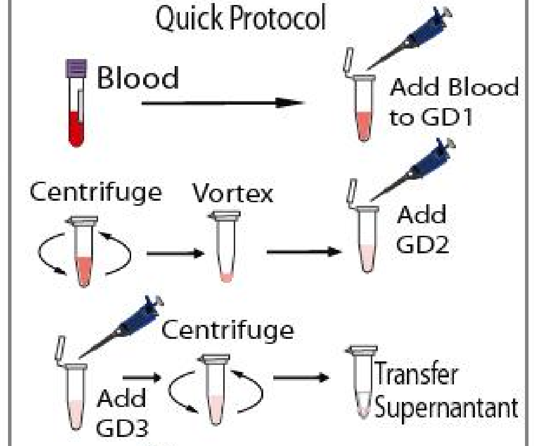
A. RBC & Cell Lysis
1. Using a filter tip pipet transfer 300 µl of whole blood to a 1.5 ml tube containing 900 µl of GD1 solution (Cell Suspension Solution). Mix gently. Incubate at room temperature for 10 minutes.
2. Centrifuge at 12K rpm for 20 seconds. Discard supernatant leaving a few residual drops.
3. Vortex vigorously to resuspend the white blood cell pellet in the residual liquid. To the resuspended cells add 300 µl of GD2 solution (Cell Lysis Solution).

B. Protein Precipitation
1. Add 100 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution. Vortex vigorously for 20 seconds. Brown particles should be visible at this stage.
2. Centrifuge at 12K rpm for 5 minutes.

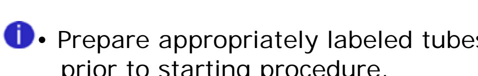
C. DNA Precipitation
1. Transfer the supernatant containing the DNA to a new appropriately labeled tube containing 300 µl 100% isopropanol (2-propanol). Mix the sample by inversion.
2. Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet.
3. Discard supernatant and place tube inverted on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
4. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
5. Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet.
6. Air dry the DNA pellet. Do not use vacuum.

D. DNA Reconstitution
1. Add 100 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. An average yield of 10 µg is expected from 300 µl of blood. The range is between 5 µg to 15 µg.

Omni-Pure™ Genomic DNA Purification System



Add Supernatant
Iso propanol
Centrifuge
Air Dry DNA
Add GD4
60°C
Purified Dissolved DNA



- Prepare appropriately labeled tubes prior to starting procedure.
- It is convenient to add blood sample to tubes containing pre-aliquoted GD1 solution.
- It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.

* **An optional step of RNase A treatment can be performed before protein precipitation. See detailed protocol.**

⚠ Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

Protocol: Purification of Genomic DNA from Blood & Bone Marrow

Sample: 300 µl blood **Average Yield: 10 µg**

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 900 µl of GD1 solution (Cell Suspension Solution) to one tube for each sample.
3. Add 300 µl isopropanol (2-propanol) to one tube for each sample. Cap the tubes.

- Blood samples preferably should be un-coagulated and collected in EDTA (purple top) or ACD (yellow top) tubes.
- Use filter barrier pipette tips to prevent cross contamination.

Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

B. Cell Suspension & Cell Lysis

1. To the tube containing 900 µl of GD1 solution (Cell Suspension Solution) transfer 300 µl of whole blood. Cap and gently mix by inversion. Incubate for 10 minutes at room temperature. Mix by inversion a few times during this incubation period. Incubate longer for fresh blood cells as they are intact and are not already lysed.
2. Centrifuge at 12K rpm for 20 seconds to pellet the white blood cells. A reddish white pellet should be clearly visible. Decant and discard supernatant leaving behind the last few droplets. Do not totally remove the supernatant.
3. Completely resuspend the white blood cell pellet by vigorously vortexing the tube. Ensure that the pellet is completely resuspended.
4. To the resuspended cells add 300 µl of GD2 solution (Cell Lysis Solution). Mix by gentle vortexing. You will notice release of DNA by the thickening of the liquid in the sample.
5. *Optional RNase A incubation.* Add 2 µl RNase A solution (2 mg/ml) and incubate at 37°C for 20 minutes.

- Prepare appropriately labeled tubes prior to starting procedure.
- It is convenient to add blood sample to tubes containing pre-aliquoted GD1 solution.

- White blood cell pellet should be completely resuspended.
- Samples may be stored at this stage for processing later.
- RNase treatment is optional.

C. Protein Precipitation

1. Add 100 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of brown color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible brown pellet containing proteins should be collected at the bottom of the tube.

- Vigorous vortexing is required at the protein precipitation step.
- Small brown particles should be visible as an indication of protein being precipitated.

D. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube (see initial preparation above) containing 300 µl 100% isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Mixing by inversion 30-40 times is usually sufficient.
3. Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet. Gently take out tubes to prevent dislodging of the pellet. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

- It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.
- 70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

E. DNA Reconstitution & Use

1. Add 100 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at –20°C or –80°C.
3. An average yield of 10 µg is expected from 300 µl of blood, ranging from 5 µg to 15 µg.
4. The 100 µl of purified DNA obtained will have an average concentration of ~100 ng/µl.
5. For PCR amplification use 1-2 µl.
6. Use 5-10 µl for restriction digestion followed by Southern blot analysis.
7. It is convenient to perform multiple 300 µl of blood DNA purifications instead of scaling up the procedure.

- Overnight DNA reconstitution is recommended if DNA is to be used for Southern blot analysis.
- All bodily fluids and tissue samples are to be considered infectious and hazardous.
- All waste materials should be properly decontaminated and disposed following institutional guidelines.

Protocol: Purification of DNA from Amniotic Fluid

Sample: 1-6 ml Amniotic Fluid | **Average Yield: 2-10 µg**

The number of exfoliated cells in amniotic fluid varies. This is dependent upon the subject and the timing of the fluid sampling. Standard amniotic fluid samples are generally taken within 15-18 weeks of gestation when the average cell number is about 200K cells per ml. Samples taken earlier will have drastically fewer cells. Culturing of the cells is recommended when it is not possible to obtain more samples. It is advised to spin down the fluid (cells) repeatedly in the same 1.5 ml tube to collect all the cells in one tube to be processed for DNA purification.

•Use filter barrier pipette tips to prevent cross contamination.

Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

A. Sample Preparation & Cell Lysis

1. Spin 1.5 ml of amniotic fluid at 12K rpm for 20 seconds to pellet the cells. Decant supernatant. If you have more sample volume to process, add more to the same tube and collect cells by repeated spinning.
2. After the last spin, decant and discard supernatant leaving behind the last few droplets. Do not totally remove the supernatant.
3. Completely resuspend the cell pellet by vigorously vortexing the tube. Ensure that the pellet is completely resuspended.
4. To the resuspended cells add 300 µl of GD2 solution (Cell Lysis Solution). Mix by gentle vortexing. You will notice release of DNA by the thickening of the liquid in the sample.
5. *Optional Step:* Perform this step if you observe that the cells are not completely lysed. You will notice release of DNA by the thickening of the liquid in the sample upon complete cell lysis. Add 4 µl of Proteinase K (10 mg/ml) and incubate at 55°C for 1 hour to complete lysis.
6. RNase A incubation. Add 2 µl RNase A solution (2 mg/ml) and incubate at 37°C for 20 minutes.

•Cell pellet should be completely resuspended in Cell Lysis Solution.
•Samples may be stored at this stage for processing later.

B. Protein Precipitation

1. Add 100 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of brown color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible off-white pellet containing proteins should be collected at the bottom of the tube.

•Vigorous vortexing is required at the protein precipitation step.
•Small particles of off-white color should be visible as an indication of protein being precipitated.

C. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube containing 300 µl 100% isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Mixing by inversion 30-40 times is usually sufficient.
3. Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet. Gently take out the tube so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

- It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.
- Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.
- 70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

D. DNA Reconstitution & Use

1. Add 50 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at -20°C or -80°C.
3. An average yield of 5 µg is expected from 3 ml of amniotic fluid.
4. The 50 µl of purified DNA obtained will have an average concentration of ~100 ng/µl.
5. For PCR amplification use 2-5 µl.

- Overnight DNA reconstitution is recommended if DNA is to be used for Southern blot analysis.
- All bodily fluids and tissue samples are to be considered infectious and hazardous.
- All waste materials should be properly decontaminated and disposed following institutional guidelines.

Protocol: Purification of DNA from Chorionic Villi

Sample: 5-10 mg Chorionic Villi **Average Yield: 2-10 µg**

A. Sample Preparation & Cell Lysis

1. Sample Washing: Transfer chorionic villi sample to a 1.5 ml tube and add 400 µl sterile water. Spin at 12K rpm for 20 seconds to pellet the sample. Decant supernatant. Repeat this step again.
2. Add 300 µl of GD2 solution (Cell Lysis Solution), vortex vigorously to break up the tissue. If required, crush the remaining tissue using a sterile pipet tip.
3. Incubate at 65°C for 10 minutes to facilitate dissolution and complete lysis.
4. *Optional Step:* Perform this step if you observe that the cells are not completely lysed. You will notice release of DNA by the thickening of the liquid in the sample upon complete cell lysis. Add 4 µl of Proteinase K (10 mg/ml) and incubate at 55°C for 1 hour to complete lysis.
5. *Optional Step:* Add 2 µl RNase A solution (2 mg/ml) and incubate at 37°C for 20 minutes.

- Use filter barrier pipette tips to prevent cross contamination.

Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

- Cell pellet should be completely resuspended in Cell Lysis Solution.
- Samples may be stored at this stage for processing later.

B. Protein Precipitation

1. Add 100 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of pale yellow color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible pale yellow pellet containing proteins should be collected at the bottom of the tube.

- Vigorous vortexing is required at the protein precipitation step.
- Small pale yellow particles should be visible as an indication of protein being precipitated.

- Cooling the sample tubes containing the protein precipitation solution on ice facilitates the formation of a tight pellet.

C. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube containing 300 µl 100% isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Mixing by inversion 30-40 times is usually sufficient.
3. Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet. Gently take out the tube so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

• It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.

• Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.

• 70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

D. DNA Reconstitution & Use

1. Add 50 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at -20°C or -80°C.
3. An average yield of 5 µg is expected from 5 mg chorionic villi sample.
4. The 50 µl of purified DNA obtained will have an average concentration of ~100 ng/µl.
5. For PCR amplification use 2-5 µl.
6. Use 5- 10 µg for restriction digestion followed by Southern blot analysis.

• Overnight DNA reconstitution is recommended if DNA is to be used for Southern blot analysis.

• All bodily fluids and tissue samples are to be considered infectious and hazardous.

• All waste materials should be properly decontaminated and disposed following institutional guidelines.

Protocol: Purification of DNA from other Bodily Fluids

Sample: 100 µl CSF, Plasma, Saliva, Milk, Semen, etc. **Average Yield: 2-5 µg**

A. Sample Preparation & Cell Lysis

1. Plan on the number of samples to be processed and the volume of samples. Consider that a maximum of 100 µl of sample can be accommodated in each 1.5 ml tube. Label two (duplicate) sets of tubes. Add 500 µl of GD2 (Cell Lysis Solution) to set 1 and 600 µl 100% isopropanol (2-propanol) to set 2.
2. Transfer 100 µl bodily fluid samples to each 1.5 ml tube of set 1 containing 500 µl of GD2 (Cell Lysis Solution). Vortex vigorously to break up the cells.
3. Incubate at 65°C for 10 minutes to facilitate dissolution and complete lysis.
4. *Optional Step:* Perform this step if you observe that the cells are not completely lysed. You will notice release of DNA by the thickening of the liquid in the sample upon complete cell lysis. Add 4 µl of Proteinase K (10mg/ml) and incubate at 55°C for 1 hour to complete lysis. Incubate overnight if required.
5. *Optional Step:* Add 2 µl RNase A solution (2 mg/ml) and incubate at 37°C for 20 minutes.

- Use filter barrier pipette tips to prevent cross contamination.

Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

- Cell pellet should be completely resuspended in Cell Lysis Solution.
- Samples may be stored at this stage for processing later.

B. Protein Precipitation

1. Cool the tubes to room temperature. Add 200 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution. Cool the tubes in ice for 1-2 minutes.
2. Vortex vigorously for 20 seconds. Depending on sample type, small particles of pale yellow to off-white color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible pale yellow to off-white pellet containing proteins should be collected at the bottom of the tube.

- Vigorous vortexing is required at the protein precipitation step.
- Small particles should be visible as an indication of protein being precipitated.

- Cooling the sample tubes containing the protein precipitation solution on ice facilitates the formation of a tight pellet.

C. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube containing 600 µl 100% isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Mixing by inversion 30-40 times is usually sufficient.
3. Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place tube inverted on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet. Gently take out the tube so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

• It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.

• Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.

• 70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

D. DNA Reconstitution & Use

1. Add 50 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at -20°C or -80°C.
3. An average yield of 2-5 µg is expected from 100 µl of bone marrow. Yield varies for other bodily fluid samples.
4. The purified DNA obtained will have an average concentration of ~50 ng/µl.
5. For PCR amplification use 2-5 µl.
6. Use 5 - 10 µg for restriction digestion followed by Southern blot analysis.

• Overnight DNA reconstitution is recommended if DNA is to be used for Southern blot analysis.

• All bodily fluids and tissue samples are to be considered infectious and hazardous.

• All waste materials should be properly decontaminated and disposed following institutional guidelines.

Protocol: Purification of DNA from Buccal Wash

Sample: 10-20 ml Buccal Wash **Average Yield: 2-4 µg**

A. Sample Preparation & Cell Lysis

1. Buccal wash samples can be conveniently collected using commercial mouth wash solutions. Alternatively, clean water can be used to obtain a buccal wash sample. It is important to rotate the wash solution in the mouth to get the maximum number of cells. Spin the buccal wash sample in a 50 ml tube at 5K rpm for 5 minutes to pellet the cells. Decant supernatant.
2. Resuspend the cells in 900 µl of GD1 (Cell Suspension solution) and transfer to a 1.5 ml tube. Spin at 12K rpm for 2 minutes to pellet the cells. Decant and discard supernatant leaving behind the last few droplets. Do not totally remove the supernatant.
3. Completely resuspend the cell pellet by vigorously vortexing the tube. Ensure that the pellet is completely resuspended.
4. To the resuspended cells add 300 µl of GD2 solution (Cell Lysis Solution). Mix by gentle vortexing. You will notice release of DNA by the thickening of the liquid in the sample
5. Incubate at 65°C for 10 minutes to facilitate dissolution and complete lysis.
6. *Optional Step:* Perform this step if you observe that the cells are not completely lysed. You will notice release of DNA by the thickening of the liquid in the sample upon complete cell lysis. Add 4 µl of Proteinase K (10mg/ml) and incubate at 55°C for 1 hour to complete lysis.
7. *Optional Step:* Add 2 µl RNase A solution (2 mg/ml) and incubate at 37°C for 20 minutes.

•Use filter barrier pipette tips to prevent cross contamination.

Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

•Cell pellet should be completely resuspended in Cell Lysis Solution.
•Samples may be stored at this stage for processing later.

B. Protein Precipitation

1. Add 100 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of off-white color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible off-white pellet containing proteins should be collected at the bottom of the tube.

•Vigorous vortexing is required at the protein precipitation step.
•Small particles should be visible as an indication of protein being precipitated.

•Cooling the sample tubes containing the protein precipitation solution on ice facilitates the formation of a tight pellet.

C. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube containing 300 µl 100% isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Mixing by inversion 30-40 times is usually sufficient.
3. Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet. Gently take out the tubes so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

• It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.

• Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.

• 70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

D. DNA Reconstitution & Use

1. Add 50 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at -20°C or -80°C.
3. An average yield of 2-4 µg is expected from 10-20 ml of buccal wash sample.
4. The 50 µl of purified DNA obtained will have an average concentration of ~50 ng/µl.
5. For PCR amplification use 2-5 µl.
6. Use 5 - 10 µg for restriction digestion followed by Southern blot analysis.

• Overnight DNA reconstitution is recommended if DNA is to be used for Southern blot analysis.

• All bodily fluids and tissue samples are to be considered infectious and hazardous.

• All waste materials should be properly decontaminated and disposed following institutional guidelines.

Protocol: Purification of DNA from Cultured Cells

Sample: Cultured Cells (maximum 2 x 10^6 cells)	Average Yield: 5-15 µg

A. Harvest cells

1. Cells grown in Suspension
   Spin down the appropriate number of cells (maximum 2 x 10^6 cells) for 5 minutes at 3K rpm in a 1.5 ml tube. Decant and discard the supernatant, taking care not to dislodge the cell pellet. Leave approximately 10-20 µl of residual supernatant in the tube, as this will be used to resuspend the cells in step B. Go to step B.
2. Cells grown in a Monolayer
   Cells grown in a monolayer can be detached from the culture flask by either i) trypsinization or ii) using a cell scraper.
   i) Trypsinization: Remove the medium and wash cells with PBS. Remove the PBS and add trypsin solution. After cells have detached from the dish or flask, transfer the appropriate number of cells (maximum 2 x 10^6 cells) to a 1.5 ml tube. Centrifuge for 5 minutes at 300x g. Decant and discard the supernatant taking care not to aspirate the cell pellet. Go to step B.
   ii) Use a cell scraper to detach cells from the dish or flask. Transfer the appropriate number of cells (maximum 2 x 10^6 cells) to a 1.5 ml tube and centrifuge for 5 minutes at 300x g. Decant and discard the supernatant being careful not to dislodge the cell pellet. Go on to step B.

B. Cell Lysis

1. Resuspend the cells in residual supernatant by vortexing. Resuspension facilitates the subsequent lysis step by minimizing the formation of cell clumps.
2. Add 600 µl of solution GD2 (Cell Lysis Solution). Mix by pipeting up and down several times. You will notice release of DNA by the thickening of the liquid in the sample. Samples may be stored at this stage for processing later.
3. *Optional Step:* Perform this step if you observe that the cells are not completely lysed. You will notice release of DNA by the thickening of the liquid in the sample upon complete cell lysis. Add 4 µl of Proteinase K (10mg/ml) and incubate at 55°C for 1 hour to complete lysis.
4. *Optional Step:* Add 2 µl RNase A solution (2 mg/ml) and incubate at 37°C for 20 minutes.

•Use filter barrier pipette tips to prevent cross contamination.

Treat all bodily fluids and waste as hazardous material. Use appropriate safety procedures. Dispose following institutional guidelines. Refer to decontamination protocol in this manual.

•Cell pellet should be completely resuspended in Cell Lysis Solution.
•Samples may be stored at this stage for processing later.

C. Protein Precipitation

1. Add 200 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of pale yellow color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible pale yellow pellet containing proteins should be collected at the bottom of the tube.

•Vigorous vortexing is required at the protein precipitation step.
•Small pale yellow particles should be visible as an indication of protein being precipitated.

•Cooling the sample tubes containing the protein precipitation solution on ice facilitates the formation of a tight pellet.

D. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube containing 600 µl 100% isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Inverting the tube 30-40 times is usually sufficient.
3. Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet. Gently take out the tube so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

•It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.

•Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.

•70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

E. DNA Reconstitution & Use

1. Add 100 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at -20°C or -80°C.
3. An average yield of 10 µg is expected from 2 x 10^6 cells. The range is between 5 µg to 15 µg.
4. The 100 µl of purified DNA obtained will have an average concentration of 100 ng/µl.

•Overnight DNA reconstitution is recommended if DNA is to be used for Southern blot analysis.

•All bodily fluids and tissue samples are to be considered infectious and hazardous.

•All waste materials should be properly decontaminated and disposed following institutional guidelines.

Protocol: Purification of DNA from Tissue

Sample: Animal Tissue (5-10 mg) **Average Yield: 2-10 μg**

A. Tissue Lysis

1. Place 5-10 mg fresh or frozen animal tissue into a 1.5 ml tube containing 600 μl of GD2 (Cell Lysis Solution). If possible, fresh tissue should be finely minced and frozen tissue crushed (preferably in liquid nitrogen) with a mortar and pestle to facilitate the lysis procedure.
2. To the lysate, add 4 μl Proteinase K Solution (10 mg/ml) and mix by gentle inversion 25-30 times. Incubate at 55°C overnight or until tissue has dissolved. If possible, the tube should be mixed periodically by inversion during the incubation. Some undigested material may be present at the end of the incubation, which will be removed during the protein precipitation step.
3. RNase Treatment
   - i.) Add 2 μl RNase A solution (2 mg/ml).
   - ii.) Mix sample by gently inverting the tube 25-30 times and incubate at 37°C for 30 minutes.

•Use filter barrier pipette tips to prevent cross contamination.

•Cell pellet should be completely resuspended in Cell Lysis Solution.
•Samples may be stored at this stage for processing later.

•All bodily fluids and tissue samples are to be considered infectious and hazardous.
• All waste materials should be properly decontaminated and disposed following institutional guidelines.

B. Protein Precipitation

Allow sample to cool to room temperature.
Add 200 μl of GD3 solution (Protein Precipitation Solution) to the tissue lysate and vortex vigorously for at least 20 seconds to ensure that the tissue lysate and Protein Precipitation Solution are completely mixed.

3. Pellet the precipitated protein by centrifugation at 12K rpm for 5 minutes. Depending on the type of tissue and the amount of blood present, a pale yellow to light brown pellet should be visible.

•Vigorous vortexing is required at the protein precipitation step.
•Small particles should be visible as an indication of protein being precipitated.

•Cooling the sample tubes containing the protein precipitation solution on ice facilitates the formation of a tight pellet.

C. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube containing 600 µl 100% isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Inverting the tube 30-40 times is usually sufficient.
3. Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet. Gently take out the tube so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

•It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.

•Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.

•70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

D. DNA Reconstitution & Use

1. Add 50 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at -20°C or -80°C.
3. An average yield of 5 µg is expected. The range is between 2 µg to 10 µg.
4. The 50 µl of purified DNA obtained will have an average concentration of 100 ng/µl.

•Overnight DNA reconstitution is recommended if DNA is to be used for Southern blot analysis.

•All bodily fluids and tissue samples are to be considered infectious and hazardous.

•All waste materials should be properly decontaminated and disposed following institutional guidelines.

Protocol: Purification of DNA from Plant Tissue

Sample: Plant Tissue (5-10 mg) **Average Yield: 2-5 µg**

A. Tissue Lysis

Samples may be fresh, frozen or dried. Fresh tissue can be stored for up to 5 days at 4°C. Frozen tissue should be stored at -70°C. Dried tissue can be stored at room temperature. Fresh or frozen tissue should be finely crushed with a mortar and pestle in liquid nitrogen prior to DNA purification. If liquid nitrogen is not available, then the tissue can be homogenized during the cell lysis step using 30-50 strokes with a 1.5 ml tube pestle. Dried tissue can be ground with a mortar and pestle at room temperature. From 10-30 mg of fresh/frozen tissue or 5-10 mg of dried tissue the expected yield of DNA is 2-15 µg.

1. Add the tissue to a 1.5 ml tube.
2. Add 600 µl of GD2 (Cell Lysis Solution) to the sample. When using dried tissue, vortex for a few seconds to give the tissue a chance to rehydrate.
3. Incubate the tissue lysate at 65°C for 60 minutes. At 15 minute intervals, mix the contents of the tube by inversion 10 times.
4. RNase Treatment; Add 2 µl RNase A solution (2 mg/ml) and incubate at 37°C for 20 minutes.

- Use filter barrier pipette tips to prevent cross contamination.

- Cell pellet should be completely resuspended in Cell Lysis Solution.
- Samples may be stored at this stage for processing later.

B. Protein Precipitation

1. Cool sample to room temperature.
2. Add 200 µl of GD3 (Protein Precipitation Solution) to the tissue lysate and mix by vortexing at high speed for at least 20 seconds. If the tissue sample is known to have high polysaccharide content, place the tube on ice for 30 minutes.
3. Centrifuge at 12K rpm for 5 minutes. A compact, green pellet should be observed.

- Vigorous vortexing is required at the protein precipitation step.
- Small green particles should be visible as an indication of protein being precipitated.

- Cooling the sample tubes containing the protein precipitation solution on ice facilitates the formation of a tight pellet.

C. DNA Precipitation

Decant the supernatant containing the DNA to a new appropriately labeled tube (see initial preparation above) containing 300 µl 100% isopropanol (2-propanol).

Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Inverting the tube 30-40 times is usually sufficient.

Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.

Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.

To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.

Centrifuge at 12 K rpm for 2 minutes to collect the DNA as a pellet. Gently take out the tubes so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.

Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.

Air dry the DNA pellet. Do not use vacuum.

• It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.

• Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.

• 70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

D. DNA Reconstitution & Use

1. Add 50 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at -20°C or -80°C.
3. An average yield of 2-5 µg is expected.
4. The 50 µl of purified DNA obtained will have an average concentration of 50 ng/µl.

• Overnight DNA reconstitution is recommended if DNA is to be used for Southern blot analysis.

Protocol: Purification of Genomic DNA from Yeast

Sample: 1 ml Yeast Culture — **Average Yield: 5 µg**

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 300 µl isopropanol (2-propanol) to one tube for each sample. Cap the tubes, will use in Step D (DNA precipitation).
3. Spin 1 ml of yeast culture in a 1.5 ml tube at 4K rpm for 10 seconds. Discard supernatant by decanting.

- Prepare appropriately labeled tubes prior to starting procedure.

B. Cell Suspension & Cell Lysis

1. Add 300 µl of GD1 solution (Cell Suspension Solution). Cap and gently mix by inversion or pipeting up and down until all the cells are resuspended. Incubate for 1-3 minutes at room temperature. Mix by inversion a few times during this incubation period.
1. To the resuspended cells add 2 µl of Lytic Enzyme Solution. Mix by inversion and incubate at 37°C for 30 minutes to digest cell wall. Mix intermittently during incubation by inversion. This process will speed the digestion of the cell wall.
2. Spin at 12K rpm for 4 minutes to pellet the cells. Discard supernatant by decanting.
3. Add 300 µl of GD2 solution (Cell Lysis Solution). Mix by gentle vortexing. You will notice release of DNA by the thickening of the liquid in the sample.

- Cell pellet should be completely resuspended in Cell Lysis Solution.
- Samples may be stored at this stage for processing later.

C. Protein Precipitation

1. Add 100 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of white color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible white pellet containing proteins should be collected at the bottom of the tube.

- Vigorous vortexing is required at the protein precipitation step.
- Small white particles should be visible as an indication of protein being precipitated.

- Cooling the sample tubes containing the protein precipitation solution on ice facilitates the formation of a tight pellet.

D. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube (see initial preparation above) containing 300 µl 100% isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Inverting the tube 30-40 times is usually sufficient.
3. Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 12K rpm for 2 minute to collect the DNA as a pellet. Gently take out the tubes so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

- It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.
- Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.
- 70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

E. DNA Reconstitution & Use

1. Add 50 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at –20°C or –80°C.
3. An average yield of 5 µg is expected from 1 ml yeast culture. The range is between 2 µg to 8 µg.
4. The 50 µl of purified DNA obtained will have an average concentration of ~100 ng/µl.
5. For PCR amplification use 1-2 µl.
6. It is convenient to perform multiple 1 ml yeast culture DNA purifications instead of scaling up the procedure.

- Overnight DNA reconstitution is recommended if DNA is to be used for Southern blot analysis.

Protocol: Purification of Genomic DNA from Gram Negative Bacteria

Sample: 0.5 ml Gram Negative Bacteria **Average Yield: 25 µg**

Example: Escherichia coli, Vibrio cholerae, Rickettsia, Haemophilus influenzae, etc.

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 300 µl isopropanol (2-propanol) to one tube for each sample. Cap the tubes, will use in Step D (DNA precipitation)
3. Spin 0.5 ml of bacterial culture in 1.5 ml tube at 4K rpm for 20 seconds. Discard supernatant by decanting.

B. Cell Suspension & Cell Lysis

1. Add 300 µl of GD1 solution (Cell Suspension Solution). Cap and gently mix by inversion or pipeting up and down until all the cells are resuspended. Incubate for 1-3 minutes at room temperature. Mix by inversion a few times during this incubation period.
2. Spin at 12K rpm for 1 minute to pellet the cells. Discard supernatant by decanting.
4. Add 300 µl of GD2 solution (Cell Lysis Solution). Mix by gentle vortexing. Incubate at 80°C for 5 minutes to lyse the cell. You will notice release of DNA by the thickening of the liquid in the sample.
3. Add 2 µl RNase A Solution to cell lysate. Mix the sample by inversion 25 times and incubate at 37°C for 30 minutes.

Always use filter barrier pipette tips to prevent cross contamination.

Pathogen sample decontamination and DNA extraction should be performed in a biological safety cabinet with unidirectional work flow for all procedures.

- Samples may be stored at this stage for processing later.
- RNase treatment is optional.

C. Protein Precipitation

1. Add 100 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of pale yellow color will be appear and be visible at this stage.
3. Centrifuge at 12K rpm for 4 minutes to pellet the precipitated proteins. A clearly visible pale yellow pellet containing proteins should be collected at the bottom of the tube.

- Vigorous vortexing is required at the protein precipitation step.
- Small pale yellow particles should be visible as an indication of protein being precipitated.

- Cooling the sample tubes containing the protein precipitation solution on ice facilitates the formation of a tight pellet.

D. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube (see initial preparation above) containing 300 µl 100% isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Inverting the tube 30-40 times is usually sufficient.
3. Centrifuge at 12K rpm for 4 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet. Gently take out the tubes so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

- It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.
- Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.
- 70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

E. DNA Reconstitution & Use

1. Add 100 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at –20°C or –80°C.
3. An average yield of 25 µg is expected from 0.5 ml bacterial culture. The range is between 15 µg to 30 µg.
4. The 100 µl of purified DNA obtained will have an average concentration of ~250 ng/µl.
5. For PCR amplification use 1-2 µl.
6. It is convenient to perform multiple 0.5 ml bacterial culture DNA purifications instead of scaling up the procedure.

- All waste materials should be properly decontaminated and disposed following institutional guidelines.

Protocol: Purification of Genomic DNA from Gram Positive Bacteria

Sample: 0.5 ml Bacterial Culture **Average Yield: 10 µg**

Example: Streptococcus pneumoniae, Staphylococcus aureus, Corynebacterium diphtheriae, Anthrax, etc.

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 300 µl isopropanol (2-propanol) to one tube for each sample. Cap the tubes, will use in Step D (DNA precipitation)
3. Spin 0.5 ml of bacterial culture in 1.5 ml tube at 4K rpm for 20 seconds. Discard supernatant by decanting.

Always use filter barrier pipette tips to prevent cross contamination.

Pathogen sample decontamination and DNA extraction should be performed in a biological safety cabinet with unidirectional work flow for all procedures.

B. Cell Suspension & Cell Lysis

1. Add 300 µl of GD1 solution (Cell Suspension Solution). Cap and gently mix by inversion or pipeting up and down until all the cells are resuspended. Incubate for 1-3 minutes at room temperature. Mix by inversion a few times during this incubation period.
4. To the resuspended cells add 2 µl of Lytic Enzyme Solution. Mix by inversion and incubate at 37°C for 30 minutes to digest cell wall. Mix intermittently during incubation by inversion. This process will speed the digestion of the cell wall.
2. Spin at 12K rpm for 4 minutes to pellet the cells. Discard supernatant by decanting.
5. Add 300 µl of GD2 solution (Cell Lysis Solution). Mix by gentle vortexing. Incubate at 80°C for 5 minutes to lyse the cell. You will notice release of DNA by the thickening of the liquid in the sample.
3. Add 2 µl RNase A Solution to cell lysate. Mix the sample by inversion 25 times and incubate at 37°C for 30 minutes.

- Samples may be stored at this stage for processing later.
- RNase treatment is optional.

C. Protein Precipitation

1. Add 100 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of pale yellow color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible pale yellow pellet containing proteins should be collected at the bottom of the tube.

- Vigorous vortexing is required at the protein precipitation step.
- Small pale yellow particles should be visible as an indication of protein being precipitated.

- Cooling the sample tubes containing the protein precipitation solution on ice facilitates the formation of a tight pellet.

D. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube containing 300 µl 100% isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Inverting the tube 30-40 times is usually sufficient.
3. Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet. Gently take out the tubes so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

- It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.
- Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.
- 70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

E. DNA Reconstitution & Use

1. Add 100 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at –20°C or –80°C.
3. An average yield of 10 µg is expected from 0.5 ml bacterial culture. The range is between 5 µg to 15 µg.
4. The 100 µl of purified DNA obtained will have an average concentration of ~100 ng/µl.
5. For PCR amplification use 1-2 µl.
6. It is convenient to perform multiple 0.5 ml culture DNA purifications instead of scaling up the procedure.

- All waste materials should be properly decontaminated and disposed following institutional guidelines.

Protocol: Purification of *Mycobacterium tuberculosis* DNA from Sputum

Sample: 600 µl Sputum **Average Yield: 5 µg**

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 600 µl of 2% (wt/vol) NaOH-1.45%(wt/vol) trisodium citrate to each tube.
3. Add 300 µl isopropanol (2-propanol) to one tube for each sample. Cap the tubes, will use in Step D (DNA precipitation).
4. Add 600 µl of sample sputum to the tubes containg 2% (wt/vol) NaOH-1.45%(wt/vol) trisodium citrate. Vortex and incubate at room temperature for 30 minutes.
5. Centrifuge at 12K rpm for 1 minute. Discard supernatant by decanting.

Always use filter barrier pipette tips to prevent cross contamination.

Pathogen sample decontamination and DNA extraction should be performed in a biological safety cabinet with unidirectional work flow for all procedures.

B. Cell Suspension & Cell Lysis

1. Add 300 µl of GD2 solution (Cell Lysis Solution). Mix by gentle vortexing. Incubate at 80°C for 5 minutes to lyse the cell. Cap and gently mix by inversion or pipeting up and down until all the cells are resuspended.
2. To the resuspended cells add 2 µl of Lytic Enzyme Solution. Mix by inversion and incubate at 37°C for 60 minutes to digest cell wall. Mix intermittently during incubation by inversion. This process will speed the digestion of the cell wall.
3. To the lysate, add 4 µl Proteinase K Solution (10 mg/ml) and mix by gentle inversion 25-30 times. Incubate at 55°C overnight. If possible, the tube should be mixed periodically by inversion during the incubation. Some undigested material may be present at the end of the incubation, which will be removed during the protein precipitation step.
4. Add 2 µl RNase A Solution to cell lysate. Mix the sample by inversion 25 times and incubate at 37°C for 30 minutes.

- Samples may be stored at this stage for processing later.
- RNase treatment is optional.

C. Protein Precipitation

1. Add 100 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of white color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible white pellet containing proteins should be collected at the bottom of the tube.

- Vigorous vortexing is required at the protein precipitation step.
- Small white particles should be visible as an indication of protein being precipitated.

D. DNA Precipitation

Decant the supernatant containing the DNA to a new appropriately labeled tube (see initial preparation above) containing 300 µl 100% isopropanol (2-propanol).

Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Inverting the tube 30-40 times is usually sufficient.

Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.

Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.

To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.

Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet. Gently take out the tubes so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.

Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.

Air dry the DNA pellet. Do not use vacuum.

- It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.
- Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.
- 70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

E. DNA Reconstitution & Use

1. Add 50 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, keep sample at –20°C or –80°C.
3. An average yield of 5 µg is expected from 0.6 ml sputum, ranging between 2 µg to 10 µg.
4. The 50 µl of purified DNA obtained will have an average concentration of ~100 ng/µl.
5. For PCR amplification use 1-2 µl.
6. It is convenient to perform multiple 0.6 ml sputum DNA purifications instead of scaling up the procedure.

- All waste materials should be properly decontaminated and disposed following institutional guidelines.

Omni-Pure™ Genomic DNA Purification System

Protocol: Purification of Viral DNA from Serum

Sample: 200 µl Serum **Average Yield: 2 µg**

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 300 µl of GD2 solution (Cell Lysis Solution) to each tube of set 1.
3. Add 600 µl isopropanol (2-propanol) to each tube for set 2. Cap the tubes, will use in Step D (DNA precipitation).

B. Cell Suspension & Cell Lysis

6. Add 200 µl of serum to tubes containing GD2 solution (Cell Lysis Solution). Mix by gentle vortexing. Incubate at 60°C for 5 minutes.
1. To the lysate, add 4 µl Proteinase K Solution (10 mg/ml) and mix by gentle inversion 25-30 times. Incubate at 55°C for 30 minutes.
2. *Optional:* Add 2 µl RNase A Solution to cell lysate. Mix the sample by inversion 25 times and incubate at 37°C for 30 minutes.

Always use filter barrier pipette tips to prevent cross contamination.

Pathogen sample decontamination and DNA extraction should be performed in a biological safety cabinet with unidirectional work flow for all procedures.

- Samples may be stored at this stage for processing later.
- RNase treatment is optional.

C. Protein Precipitation

1. Add 200 µl of GD3 solution (Protein Precipitation Solution) to the sample in Cell Lysis Solution.
2. Vortex vigorously for 20 seconds. Small particles of white color will become visible at this stage.
3. Centrifuge at 12K rpm for 5 minutes to pellet the precipitated proteins. A clearly visible white pellet containing proteins should be collected at the bottom of the tube.

- Vigorous vortexing is required at the protein precipitation step.
- Small white particles should be visible as an indication of protein being precipitated.

- Cooling the sample tubes containing the protein precipitation solution on ice facilitates the formation of a tight pellet.

D. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube (see initial preparation above) containing 600 µl 100% isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Inverting the tube 30-40 times is usually sufficient.
3. Centrifuge at 12K rpm for 5 minutes to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 12K rpm for 2 minutes to collect the DNA as a pellet. Gently take out the tubes so that the pellet is not dislodged. While holding the tube, rotate it so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place inverted tube on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

•It is convenient to transfer supernatant after protein precipitation to tubes containing pre-aliquoted isopropanol.

•Add 1 µl of glycogen (10 mg/ml) as carrier to the isopropanol when the expected yield of DNA is below 2 µg.

•70% ethanol rinse step can be omitted if all the supernatant is clearly drained.

E. DNA Reconstitution & Use

2. Add 50 µl of GD4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
3. Store DNA at 4°C. For long-term storage, keep sample at –20°C or –80°C.
4. An average yield of 2 µg is expected from 200 µl serum. The range will vary based on the viral load.
5. The 50 µl of purified DNA obtained will have an average concentration of ~40 ng/µl.
6. For PCR amplification use 1-2 µl.
6. It is convenient to perform multiple 200 µl serum DNA purifications instead of scaling up the procedure.

•All waste materials should be properly decontaminated and disposed following institutional guidelines.

Troubleshooting

Problem	Protocol Step	Reasons and Suggestions
Incomplete lysis of red blood cells	Cell Suspension Procedure	Sample was not adequately mixed with buffer GD1. Repeat the purification procedure with a new sample, being careful that the sample and Solution GD1 are mixed immediately and completely. The number of red blood cells in the sample was higher than average. Repeat the lysis step by adding 3 parts of solution GD1 to 1 part of sample and incubating for 10 minutes at room temperature. Following the incubation, centrifuge as described in the protocol.
Blood clots are present in the blood sample.		When removing the sample from the collection tube, remove the clots with forceps and use only the non-coagulated portion for purification of DNA. If large clots are observed in the white cell pellet, resuspend the pellet in PBS and remove the clots with a pipet tip. Recover the white blood cells by centrifugation and discard the supernatant. Proceed to next step.
Lysis solution is very viscous and cells appear to clump.	Cell Lysis Procedure	Too many cells were present for the amount of lysis buffer. Add more solution GD2 and continue the incubation, with frequent mixing. Raising the incubation temperature to 37°C also helps to dissolve the cell clumps. To prevent this from occurring in the future, it is usually a good idea to measure the cell number with a hemacytometer prior to cell lysis.
Protein does not form a tight pellet.	Protein Precipitation	Be sure the cell lysate has cooled to room temperature before the addition of solution GD3. To increase the tightness of the protein pellet, resuspend the pellet by vortexing and incubate on ice for 5-15 minutes. Centrifuge according to the precipitation protocol. Be sure that solution GD3 is mixed completely with the cell lysate by vigorous vortexing for the full 20 seconds as described in the protocol. Check to make certain that the centrifuge speed and time of centrifugation was set correctly.
DNA pellet is difficult to dissolve.	DNA Reconstitution Step	Samples should be mixed frequently during the rehydration step. Do not overdry samples. DNA pellets should not be open to the air at room temperature for longer than 15 minutes. Pellets that have been exposed to the air for longer periods of time or samples that have been dried in a vacuum tend to be much more difficult to reconstitute. Hydration can be facilitated by incubating for 1 hour at 65°C and then leaving overnight at room temperature.
Purified DNA is less than 50 kb in size		DNA may be degraded due to improper sample collection or storage of starting material. Care must be taken to collect and store samples using methods that preserve DNA integrity. For long-term sample storage, samples should be stored in aliquots at -80°C. Alternatively, samples can be stored in Cell Lysis Solution at room temperature. Over-homogenizing samples either at the Lysis step or the Reconstitution step can lead to mechanical shearing of DNA. When collecting tissue samples, freezing immediately will minimize enzyme activity that leads to DNA breakdown. If samples cannot be frozen immediately, they should be placed directly into Tissue Lysis Buffer and homogenized directly.
DNA yield is low		The amount of starting material was either insufficient or was present in excess. Too little starting material will yield low concentrations of DNA that may not be efficiently precipitated during the DNA Precipitation step. If it is known beforehand that DNA yields will be low, it is recommended that during DNA precipitation a carrier such as 1-2 µl of glycogen (20 mg/ml) or 3-5 µl of linear acrylamide (5 mg/ml) be added to the DNA solution. If an excess of starting material is present, then the Lysis step may be overloaded, resulting in incomplete lysis and low yields of DNA. The procedures have been optimized for the amount of cells and tissue described in the protocols. Cells should be counted and tissue weighed prior to the Lysis step.

Decontamination of Bodily Fluids and Tissue Samples

All human and animal samples used for purification of DNA should be considered infectious and proper decontamination protocol should be followed for eventual disposal. The following protocol is an easy and tested decontamination protocol.

Bodily Fluids

1. Prepare 1 L of 1x bleach solution in a large narrow mouth bottle. Keep the bottle capped. See recipe.
2. Transfer all liquid waste to this bottle. You can add up to 300 ml waste to this 1 L bleach solution.
3. At the end of the DNA purification protocol and after at least 1 hour decontamination, this bleach solution can be safely discarded in a regular sink/sewer. Precipitates appear after longer storage.
4. Let cold water run for 3-5 minutes to completely rinse, dilute and wash the sink.

Solid Waste

1. All solid wastes should be disposed of in orange biohazard bags for eventual autoclaving and disposal.
2. All sharps should be disposed in sharps container and disposed of after autoclaving.
3. Paper towels, pipet tips and disposable plasticware should be treated as solid waste.

- All bodily fluids and tissue samples are to be considered infectious and hazardous.
- Wear gloves and protective clothing to prevent any exposure.
- All waste materials should be properly decontaminated and disposed following institutional guidelines.
- The decontamination protocol given here is for information only and is not a substitute for any other protocol established by your institution or OSHA.

- Household bleach is a readily available and effective disinfectant.
- Common household bleach contains 5% sodium hypochlorite. This is a convenient 10X solution.
- Extended heating at 80°C to 100°C for 20 minutes or longer denatures and inactivates most pathogens.

Recipe

1x Bleach Solution	
Dilution of household bleach	
10x Bleach	Water
100 ml	900 ml

DNA Content	
Source	**DNA Content**
One human cell	~7 pg
One plucked hair	~300 ng
One shed hair	~100 pg
One drop blood	~1.5 µg
One drop semen	~10 µg
One ml blood	~40 µg
One ml amniotic fluid	~0.3 µg
One mg chorionic villi	~1 µg
One T25 flask or 60 mm plate cultured cells (approx. 2-7 x 10^6 cells)	~30 µg

DNA Yield	
Sample	**Average Yield**
Human Samples	
Whole blood	10 µg/0.3 ml
Bone marrow	10 µg/0.3 ml
Amniotic fluid	10 µg/6 ml
Chorionic Villi	2 µg/mg
Bodily Fluid (CSF, Semen, etc.)	2-5 µg/100 µl
Buccal wash	4 µg/10 ml
Cultured Cells, fibroblasts, amniocytes, lymphoblastiod cells	10 µg/million cells
Solid Tissue	5 µg/mg
Other Samples	
Plant tissue	1 µg/mg
Yeast	5 µg/ml culture
Gram negative bacteria	50 µg/ml culture
Gram positive bacteria	20 µg/ml culture
MTB from sputum	5 µg/600 µl
Viral DNA from serum	2 µg/200 µl

Size and MW of Various Nucleic Acids

Nucleic acid	Length in bases or base pairs	MW, Daltons
RNA		
tRNA (E.coli)	75	2.5×10^{4}
5S rRNA	120	3.6×10^{4}
16S rRNA	1700	5.5×10^{5}
18S rRNA	1900	6.1×10^{5}
23S rRNA	3700	1.2×10^{6}
28S rRNA	4800	1.6×10^{6}
DNA		
pBR322 DNA	4361	2.8×10^{6}
SV40	5243	3.5×10^{6}
PhiX174	5386	3.6×10^{6}
Adenovirus 2 (Ad2)	35937	2.8×10^{7}
Lambda phage	48502	3.1×10^{7}
Escherichia coli	4.7×10^{6}	3.1×10^{9}
Saccharomyces cerevisiae	1.5×10^{7}	9.9×10^{10}
Dictyostelium discoideum	5.4×10^{7}	3.6×10^{10}
Arabidopsis thaliana	7.0×10^{7}	4.6×10^{10}
Caenorhabditis elegans	8.0×10^{7}	5.3×10^{10}
Drosophila melanogaster	1.4×10^{8}	9.2×10^{10}
Gallus domesticus (chicken)	1.2×10^{9}	7.9×10^{11}
Mus musculus (mouse)	2.7×10^{9}	1.8×10^{12}
Rattus norvegicus (rat)	3.0×10^{9}	2.0×10^{12}
Xenopus laevis	3.1×10^{9}	2.0×10^{12}
Homo sapiens	3.3×10^{9}	2.2×10^{12}
Zea mays	3.9×10^{9}	2.6×10^{12}
Nicotiana tabacum	4.8×10^{9}	3.2×10^{12}

Reference

1. *Ausubel, F.M., et al., Current Protocols in Molecular Biology, John Wiley and Sons, New York, 1988.*

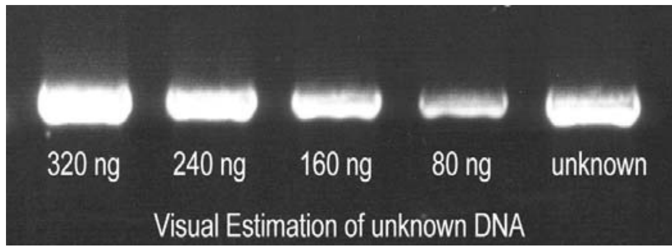
Spectrophotometric Determination of DNA Concentration

Measuring the optical density (OD) or absorbance at 260 nm (A_{260}) in a UV spectrophotometer is a relatively accurate method for calculating the concentration of DNA in an aqueous solution if a standard curve is meticulously prepared. An A_{260} of 1.0, using a 1 cm path length, corresponds to a DNA concentration of 50 µg/ml for double stranded DNA, 40 µg/ml for single stranded DNA and RNA, and 33 µg/ml for oligonucleotides. However, this method is not suitable for determining concentrations of dilute solutions of DNA, as the sensitivity of this method is not very high. For reliable readings, the concentration of double stranded DNA must be greater than 1 µg/ml.

A simple, inexpensive method for the estimation of nanogram quantities of DNA is described in the following section. We recommend the use of agarose gel electrophoresis for routine approximate determination of DNA concentration.

Estimation of DNA Concentration by Agarose Gel Electrophoresis

The amount of DNA in a sample may be estimated by running the sample along side of standards containing known amounts of the same-sized DNA fragment. In the presence of ethidium bromide staining, the amount of sample DNA can be visually estimated by comparing the band intensity with that of the known standards.



An unknown amount of a 4 kb DNA fragment was run alongside known quantities (indicated in nanograms) of the same DNA fragment. As estimated by visual comparison with the known standards, the unknown sample contained 240-320 ng of DNA.

Ethidium bromide is a carcinogen. Follow Health and Safety Procedures established by your institution. Follow proper Hazardous Material Disposal procedures established by your institution.

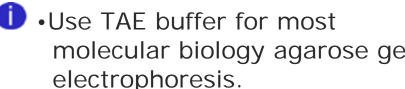
- Use 0.1 µg of ethidium bromide solution for each ml of gel volume.

Agarose Gel Electrophoresis of DNA

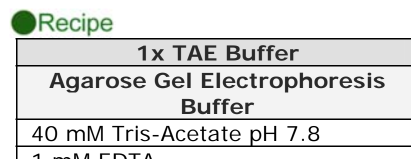
Agarose gels are typically run at 20 to 150V. The upper voltage limit is the amount of heat produced. At room temperature about 5 Watts is correct for a mini-gel (Volts x Amps = Watts). At low voltages migration is linearly proportional to voltage, but long DNA molecules migrate relatively faster in stronger fields. Migration is inversely proportional to the log of the fragment length; a log function also governs migration rate and gel concentration (0.5 to 2% for most purposes). Furthermore, supercoiled/circular DNA molecules migrate at different rates from linear molecules; single-stranded DNA and RNA migrate at similar rates, but usually faster than double-stranded DNA of the same length. Salt in the samples increases conductivity and, hence, migration rate. The most common buffers used for electrophoresis are 1x TAE and 1x TBE.

- Use TAE buffer for most molecular biology agarose gel electrophoresis.

Recipe



1x TAE Buffer Agarose Gel Electrophoresis Buffer
40 mM Tris-Acetate pH 7.8
1 mM EDTA



1x TBE Agarose and Polyacrylamide Gel Electrophoresis Buffer
0.089 M Tris
0.089 M Boric Acid
0.002 M EDTA

Polymerase Chain Reaction

PCR Components and Analysis

PCR buffer conditions vary and it is imperative to optimize buffer conditions for each amplification reaction. At Gene Link most amplification reactions have been optimized to work with the following standard buffer conditions, unless otherwise indicated.

Recipe

Standard Gene Link PCR Buffer Composition	
10x PCR buffer	**1x PCR buffer**
100 mM Tris-HCl pH 8.3	10 mM
500 mM KCl	50 mM
15 mM $MgCl_2$	1.5 mM
0.01% Gelatin	0.001%

dNTP Concentration

A standard dNTP concentration of 0.2 mM of each base is used. See section on PCR additives when dNTP concentration is changed.

Recipe

2.0 mM dNTP Stock Solution Preparation*	
Component	**Volume**
100 mM dGTP	100 µl
100 mM dATP	100 µl
100 mM dTTP	100 µl
100 mM dCTP	100 µl
Water	4.6 ml
Total Volume	5 ml
*Aliquot and freeze	

$MgCl_2$ Concentration

The concentration of Mg^{2+} will vary from 1-5 mM, depending upon primers and substrate. Since Mg^{2+} ions form complexes with dNTPs, primers and DNA templates, the optimal concentration of $MgCl_2$ has to be selected for each experiment. Low Mg^{2+} ion concentration results in a low yield of PCR product, and high concentrations increase the yield of non-specific products and promote mis-incorporation. Lower Mg^{2+} concentrations are desirable when fidelity of DNA synthesis is critical. The recommended range of $MgCl_2$ concentration is 1-4 mM, under the standard reaction conditions specified. At Gene Link, using the standard PCR buffer with KCl, a final dNTP concentration of 0.2 mM, a $MgCl_2$ concentration of 1.5 mM is used in most cases. If the DNA samples contain EDTA or other chelators, the $MgCl_2$ concentration in the reaction mixture should be raised proportionally. Given below is a $MgCl_2$ concentration calculation and addition table using a stock solution of 25 mM $MgCl_2$.

$MgCl_2$ Concentration & Addition Table								
Final concentration of $MgCl_2$ in 50 µl reaction mix, (mM)	1.0	1.25	1.5	1.75	2.0	2.5	3.0	4.0
Volume of 25 mM $MgCl_2$, (µl)	2	2.5	3	3.5	4	5	6	8

Primer Concentration

The final concentration of primers in a PCR reaction is usually 0.5 to 1 µM (micromolar). This is equivalent to 0.5 to 1 pmol/µl. For a 100 µl reaction you would add 50 to 100 pmols. At Gene Link we use 0.5 pmol/µl in the final PCR.

Always use filter barrier pipette tips to prevent cross contamination.

Genemer™ Reconstitution

Stock Primer Mix: Dissolve the supplied 10 nmols of lyophilized Genemer™ in 100 µl sterile TE. The 10 nmols of primers when dissolved in 100 µl will give a solution of 100 µM, i.e., 100 pmols/µl.

Recipe

TE Buffer pH 7.5 Composition
1x TE Buffer pH 7.5
10 mM Tris-HCl pH 7.5
1 mM EDTA

Primer Mix: Prepare a 10 pmols/µl Primer Mix solution by a ten fold dilution of the stock primer mix.
Example: Add 180 µl sterile TE to a new tube, to this tube add 20 µl of primer stock solution. Label this tube as Primer Mix 10 pmols/µl.

Amplification Thermal Cycling

Hot Start: It is essential to have a 'Hot Start' profile for amplification of any fragment from a complex template like human genomic DNA. Taq polymerase has low activity at room temperature and it is essential to minimize any mis-priming in the first cycle of amplification. Various

- Program your thermal cycler instrument with an amplification profile prior to beginning the amplification protocol.
Consult your appropriate instrument manufacturer's manual.

enzyme preparations are available which are activated by heat in the first cycle. A simple hot start protocol is given below that can be used with regular Taq polymerase. See the section on PCR additives for amplification of products from high GC content templates.

Hot Start		
Step	Time & Temperature	Cycles
Initial Denaturation	95°C for 5 minutes	1
Annealing	60°C Hold Infinity	Hold
Comments: Add Taq premix while on hold.		

Amplification File
The initial denaturation step at 94°C for 30 seconds is sufficient for all templates. The number of cycles is usually set to 30 and is sufficient to amplify 1-10 µg of product depending on the initial concentration of template. A higher number of cycles from 35-45 cycles may be used, but internal priming on the product and over amplification of unwanted bands often result from over-cycling. Generally, it is better to focus on optimizing reaction conditions than to go beyond 35 cycles.

Typical Amplification File			
Step	Temperature	Time	Cycles
Denaturation	94°C	30 sec.	30
Annealing	*	30 sec.	
Elongation	72°C	30 sec.	
Fill in Extension	72°C	7 minutes	1
Hold	4°C	Infinity	Hold
Based on the T_m of the primers. Usually varies from 50°C to 65°C			

PCR Premix Preparation (PP)		
Component	1x 50 µl Rxn.	10x 50 µl Rxns.
Sterile Water	32 µl	320 µl
10x PCR Buffer	4.5 µl	45 µl
2.0 mM dNTP	5 µl	50 µl
10 pmol/µl Primer Mix	2.5 µl	25 µl
Taq Enzyme Mix (EM) See below for preparation	5 µl	50 µl
Template DNA (~500 ng)	1-2 µl	Add 1-2 µl DNA to each tube
Total Volume	50 µl	
Keep on ice during set up. After adding template start PCR file		

Recipe

Typical PCR Premix (/50 µl)	
Component	Volume
10x PCR Buffer	5 µl
2.0 mM dNTP mix (each)	5 µl
Primer Mix (10 pmol/µl each) or 2.5 µl of 10 pmol/µl of individual primer (final 25 pmol of each primer/50 µl)	2.5 µl
H_2O	37.5 µl
Total Volume	50 µl

Recipe

PCR reaction (/50 µl)	
Component	Volume
PCR premix	45 µl
100 ng/µl diluted DNA	1 µl
Hot start and then add	
Taq premix	5 µl

Recipe

Taq Premix (/50 µl)	
Component	Volume
PCR Premix	6 µl
Taq polymerase (5 u/µl)	0.25 µl
Add 5 µl/50 µl rxn after initial denaturation	
Use 2.5 units of Taq for 100 µl reactions. Taq is usually supplied at a concentration of 5 units/µl	

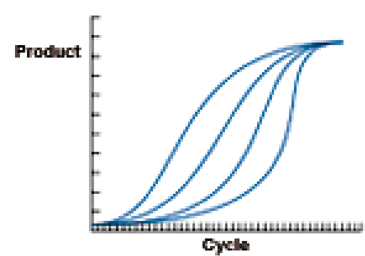
- The PCR premix preparation protocol is written considering that more than one amplification reaction will be performed at the same time. If only one reaction is planned then there is no need to prepare the Taq Enzyme Mix (EM).

Recipe

Gene Link PCR Buffer
1x PCR Buffer
10 mM Tris-HCl pH 8.3
50 mM KCl
1.5 mM $MgCl_2$
0.001% Gelatin

Yield and Kinetics

The target will be amplified by up to 10^6 fold in a successful reaction, but the amplification will usually plateau at 1-10 µg. Thus, 1 pg of target sequence in the reaction is a good place to begin.



PCR reactions produce product in a nonlinear pattern. Amplification follows a typical exponential curve until some saturation point is reached. Generally products will not be further amplified once 1-5 µg has been generated. Saturation by one product of a reaction does not always prevent further amplification of other generally unwanted products. Over-cycling may decrease the quality of an otherwise good reaction. When first optimizing a reaction, it is advisable to take samples every 5 or 10 cycles to determine the number of cycles actually needed.

Gel Electrophoresis of PCR Products

Gel electrophoresis of PCR products is the standard method for analyzing reaction quality and yield. PCR products can range up to 10 kb in length, but the majority of amplifications are at 1 kb and below. Agarose electrophoresis is the classical method to analyze amplification products from 150 bp to greater than 10 kb. Polyacrylamide gel electrophoresis should be used for resolution of short fragments in the range of 100 bp to 500 bp when discrimination of as small as a 10 bp difference is required.

PAGE gels for PCR products can be formulated with the amount of cross-linker chosen to give pore sizes optimal for the size of DNA fragment desired. Gels are most often stained in ethidium bromide, even though the fluorescence of this stain is quenched by polyacrylamide, which decreases sensitivity 2-5 fold. This decrease in sensitivity generally does not present a problem, because most PCR reactions yield product levels in the microgram range, and ethidium will detect as little as 1/10 of this amount. Polyacrylamide gels can be stained by silver staining for more sensitive detection.

PCR Additives

DNA polymerases need to elongate rapidly and accurately to function effectively *in vivo* and *in vitro*, yet certain DNA regions appear to interfere with their progress. One common problem is pause sites, at which DNA polymerase molecules cease elongation for varying lengths of time. Many strong DNA polymerase pauses are at the beginnings of regions of strong secondary structure such as template hairpins (1). Taq polymerase used in PCR suffers the same fate and GC-rich DNA sequences often require laborious work to optimize the amplification assay. The GC-rich sequences possess high thermal and structural stability, presumably because the high duplex melting temperature that permits stable secondary structures to form, thus preventing completion of a faithful replication (2).

Nucleotide analog 7-deaza dGTP is effective in reducing the secondary structure associated with the GC-rich region by reducing the duplex stability (4). Betaine, DMSO and formamide reduce the T_m and the complex secondary structure, thus the duplex stability (1-5). Tetramethyl ammonium chloride (TMAC) actually increases the specificity of hybridization and increases the T_m. The use of TMAC is recommended in PCR conditions using degenerate primers.

These PCR additives and enhancing agents have been used to increase the yield, specificity and consistency of PCR reactions. These additives may have beneficial effects on some amplification and it is impossible to predict which agents will be useful in a particular context and therefore they must be empirically tested for each combination of template and primers.

PCR Additives		
Additive	**Purpose & Function**	**Concentration**
7-deaza-2'-deoxyguanosine; 7-deaza dGTP	GC-rich region amplification. Reduce the stability of duplex DNA.	Totally replace dGTP with 7-deaza dGTP; or use 7-deaza dGTP: dGTP at 3:1.
Betaine (N,N,N-trimethylglycine = [carboxymethyl]trimethylammonium)	Reduces T_m facilitating GC-rich region amplification. Reduces duplex stability.	Use 3.5M to 0.1M Betaine. Be sure to use Betaine or Betaine (mono)hydrate and *not* Betaine HCl.
BSA (bovine serum albumin)	BSA has proven particularly useful when attempting to amplify ancient DNA or templates which contain PCR inhibitors such as melanin.	BSA concentration of 0.01 µg/µl to 0.1 µg/ µl can be used.
DMSO (dimethyl sulfoxide)	DMSO is thought to reduce secondary structure and is particularly useful for GC-rich templates.	DMSO at 2-10% may be necessary for amplification of some templates, however 10% DMSO can reduce *Taq* polymerase activity by up to 50% so it should not be used routinely.
Formamide	Reduces secondary structure and is particularly useful for GC-rich templates.	Formamide is generally used at 1-5%. Do not exceed 10%.
Non-ionic detergents e.g.,Triton X-100, Tween 20 or Nonidet P-40 (NP-40)	Non-ionic detergents stabilize *Taq* polymerase and may also suppress the formation of secondary structure.	0.1-1% Triton X-100, Tween 20 or NP-40 may increase yield but may also increase non-specific amplification. As little as 0.01% SDS contamination of the template DNA (left-over from the extraction procedure) can inhibit PCR by reducing *Taq* polymerase activity to as low as 10%, however, inclusion of 0.5% Tween-20 or -40 will effectively neutralize this effect.
TMAC (tetramethyl ammonium chloride)	TMAC is used to reduce potential DNA-RNA mismatch and improve the stringency of hybridization reactions. It increases T_m and minimizes mis-pairing.	TMAC is generally used at a final concentration of 15-100 mM to eliminate non-specific priming.

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