Certificate of Analysis & Product Manual

Triple Repeat Disorders Genotyping
Fragile X, Myotonic Dystrophy, Friedreich's Ataxia, Huntington's disease
Fluorescent Probes, siRNA, Hybridization and Detection Reagents

mtDNA Deletion Genotyping GeneProber™ GL557

Mitochondrial DNA (mtDNA) deletion chemiluminescent Southern blot genotyping

Catalog No. 40-2055-41

Storage Condition: -20°C

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

Important Information

All Gene Link products are for research use only.
Not for use in diagnostic procedures for clinical purposes.
Product to be used by experienced researchers appropriately trained in performing molecular biology techniques following established safety procedures. Additional qualification and certification is required for interpretation of results.

20 years
results you can rely on
Gene Link™

Material Supplied

mtDNA Deletion Genotyping GeneProber™ GL557

Mitochondrial DNA deletion detection probe GL557 digoxigenin labeled probe for Southern blot genotyping

REF	Catalog No.	Description	Size
	40-2055-41	Mitochondrial DNA Deletion GeneProber™ GL557 Digoxigenin Labeled Probe	110 µL

Certificate of Analysis & Product Specifications

One tube containing 110 µL of mitochondrial DNA Deletion GeneProber™ GL557 digoxigenin labeled probe at a concentration of ~40ng/uL. This probe is digoxigenin labeled for non-radioactive detection. The quantity supplied is sufficient for at least 5 20x20 cm blots using 20µL for each blot as probe.

The mitochondrial DNA deletion GeneProber™ GL557 probe supplied has been validated to detect mtDNA deletion as restriction fragment length polymorphism by Southern blot.
Appropriate nuclease free handling, dispensing and storage conditions required.

Product Label Information

RUO Research Use Only	-10°C -20°C	LOT
Research Use Only	**Storage** Store at -20°C to -10°C	**Lot Number** Stated on product tube and packing slip
Expiry One year from Date of Shipment	**Instructions** Consult product manual	**QR Code** Visit Gene Link website for product details

GeneProber™ Related Product Ordering Information

The GeneProber™ product line is based on the chemiluminescent Southern blot detection method. Gene Link's non-radioactive detection systems for genotyping of triple repeat disorders are rapid, reliable and as sensitive as the ^{32}P labeled southern blots. No more decayed probes and radioactive exposure. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders.

Unlabeled GeneProber™ probes are also available for radio labeling and radioactive based detection. Gene Link strongly recommends the use of non-radioactive gene detection systems. Consider switching to Gene Link's product line of non-radioactive detection systems.

Product	Unit Size	Catalog No.
Mitochondrial DNA deletion GeneProber™ GL557 Digoxigenin labeled	110 µL	40-2055-41
Fragile X GeneProber™ GLFX1 Probe unlabeled	500 ng	40-2004-40
Fragile X GeneProber™ GLFXDig1 Probe Digoxigenin labeled	110 µL	40-2004-41
FRAXE/FMR2/AFF2 GeneProber™ AFF2-AJ31Dig1	110 µL	40-2054-41
Huntington's Disease GeneProber™ GLHD14 Probe unlabeled	500 ng	40-2025-40
Huntington's Disease GeneProber™ GLHDDig2X Probe Digoxigenin labeled	110 µL	40-2025-41
Myotonic Dystrophy GeneProber™ GLDM1 Probe unlabeled	500 ng	40-2026-40
Myotonic Dystrophy GeneProber™ GLDMDig2 Probe Digoxigenin labeled	110 µL	40-2026-41
Friedreich's Ataxia GeneProber™ GLFRDA21 Probe unlabeled	500 ng	40-2027-40
Friedreich's Ataxia GeneProber™ GLFRDADig21 Probe Digoxigenin labeled	110 µL	40-2027-41

GScan™ Related Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders. The kits contain optimized PCR amplification reagents and a wide array of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument. Included in these kits are ready-to-run control samples of various repeats of the triple repeat disorder kit. These control samples are for calibration with the molecular weight markers for accurate size determination of the amplified fragments.

The GScan™ kits are simple and robust for routine triple-repeat detection of greater than 100 repeats of all triple repeat disorders listed.

.Product	Unit Size	Catalog No.
Fragile X GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2004-15XX
Fragile X GScan™ V2 Kit for fluorescent detection; 20 reactions kit	1 kit	40-2004-15FMS
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2054-15FM
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2054-15FMS
Huntington's Disease GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15XX
Huntington's Disease GScan™ V2 Kit for fluorescent detection; 20 reactions kit	1 kit	40-2025-15FMS
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2026-15XX
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2026-15FMS
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2027-15XX
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2027-15FMS

All Gene Link products are for research use only **Current pricing are posted at http://www.genelink.com/**

Mitochondrial DNA (mtDNA) Deletion Genotyping

Background

For an excellent review visit the link below; the following background is excerpted from the review at the website link

Mitochondrial DNA Deletion Syndromes
Salvatore DiMauro, MD & Michio Hirano, MD
http://www.ncbi.nlm.nih.gov/books/NBK1203/

Molecular Genetic Pathogenesis

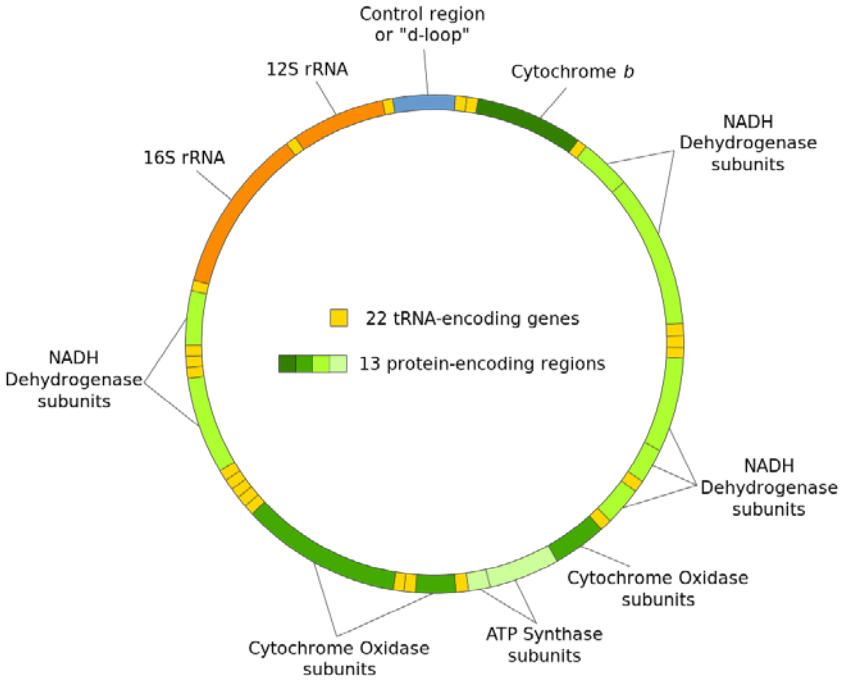
Mitochondrial DNA deletion syndromes are almost never inherited, suggesting that these disorders are caused by *denovo* mtDNA deletions that occur in the mother's oocytes during germline development or in the embryo during embryogenesis. Chen et al [1995] showed that the "common deletion" (m.8470_13446del4977) accounted for 0.1% of the approximately 150,000 mtDNAs in a human oocyte. A "bottleneck" between oocyte and embryo allows only a minority of maternal mtDNAs to populate the fetus. On rare occasions, a "deleted" mtDNA may slip through. From the blastocyst, deleted mtDNAs can enter all three germ layers and cause KSS, segregate predominantly to the hematopoietic lineage and cause Pearson syndrome, or segregate to muscle and cause PEO [DiMauro & Schon 2003]. The origin of mtDNA deletions is uncertain. However, it has been noted that deletions fall into two classes [Mita et al 1990]:

- Class I mutations are flanked by perfect direct repeats;
- Class II mutations are not flanked by any unique elements.

Homologous recombination or slipped mispairing (i.e., unequal crossing over) may explain the origin of class I deletions; the genesis of class II deletions remains unknown. The fact that a mtDNA deletion of a given length is found in a given individual implies that the population of deleted mtDNAs is a clonal expansion of a single mutation event that occurred early in oogenesis or in embryogenesis [Schon 2003]. The hypothesis of clonality implies that a single rearranged molecule present in the oocyte or the embryo multiplies wildly to form the trillions of deleted mtDNAs in the affected individual. How the selective amplification of deleted mtDNAs occurs is currently unknown, but the bottleneck concept described above may provide an answer.

Pathogenic allelic variants
Deletions can vary in size and abundance among affected individuals, but deleted mtDNA of a given length is present in each individual. Approximately 90% of individuals with KSS have a largescale (i.e., 1.1- to 10-kb) mtDNA deletion. The "common deletion" (m.8470_13446del4977) is present in about one third of affected individuals.

Abnormal gene product
The similarly deleterious effects of different mtDNA deletions can be explained by the fact that even the smallest mtDNA deletion encompasses several tRNA genes; thus, "deleted" mtDNAs are transcribed into RNA in the usual way, but the processed transcript encoding polypeptides is not translated because the deletions remove essential tRNAs needed for protein synthesis [Schon 2003].



Clinical characteristics

Mitochondrial DNA (mtDNA) deletion syndromes predominantly comprise three overlapping phenotypes that are usually simplex (i.e., a single occurrence in a family), but rarely may be observed in different members of the same family or may evolve in a given individual over time. The three phenotypes are Kearns-Sayre syndrome (KSS), Pearson syndrome, and progressive external ophthalmoplegia (PEO). Rarely Leigh syndrome can be a manifestation of a mtDNA deletion. KSS is a multisystem disorder defined by the triad of onset before age 20 years, pigmentary retinopathy, and PEO. In addition, affected individuals have at least one of the following: cardiac conduction block, cerebrospinal fluid protein concentration greater than 100 mg/dL, or cerebellar ataxia. Onset is usually in childhood. Pearson syndrome is characterized by sideroblastic anemia and exocrine pancreas dysfunction and is usually fatal in infancy. PEO, characterized by ptosis, paralysis of the extraocular muscles (ophthalmoplegia), oropharyngeal weakness, and variably severe proximal limb weakness, is relatively benign.

Mitochondrial DNA deletion syndromes are caused by deletion of mtDNA and, when inherited, are transmitted by maternal inheritance.

• The father of a proband is not at risk of having the disease-causing mtDNA mutation.
• The mother of a proband with a mtDNA deletion syndrome is usually unaffected and does not have mtDNA deletions in her tissues.
• The mtDNA deletion is usually *de novo* in the proband, occurring either in the mother's oocyte or during embryogenesis.

Genotyping: Molecular Analysis

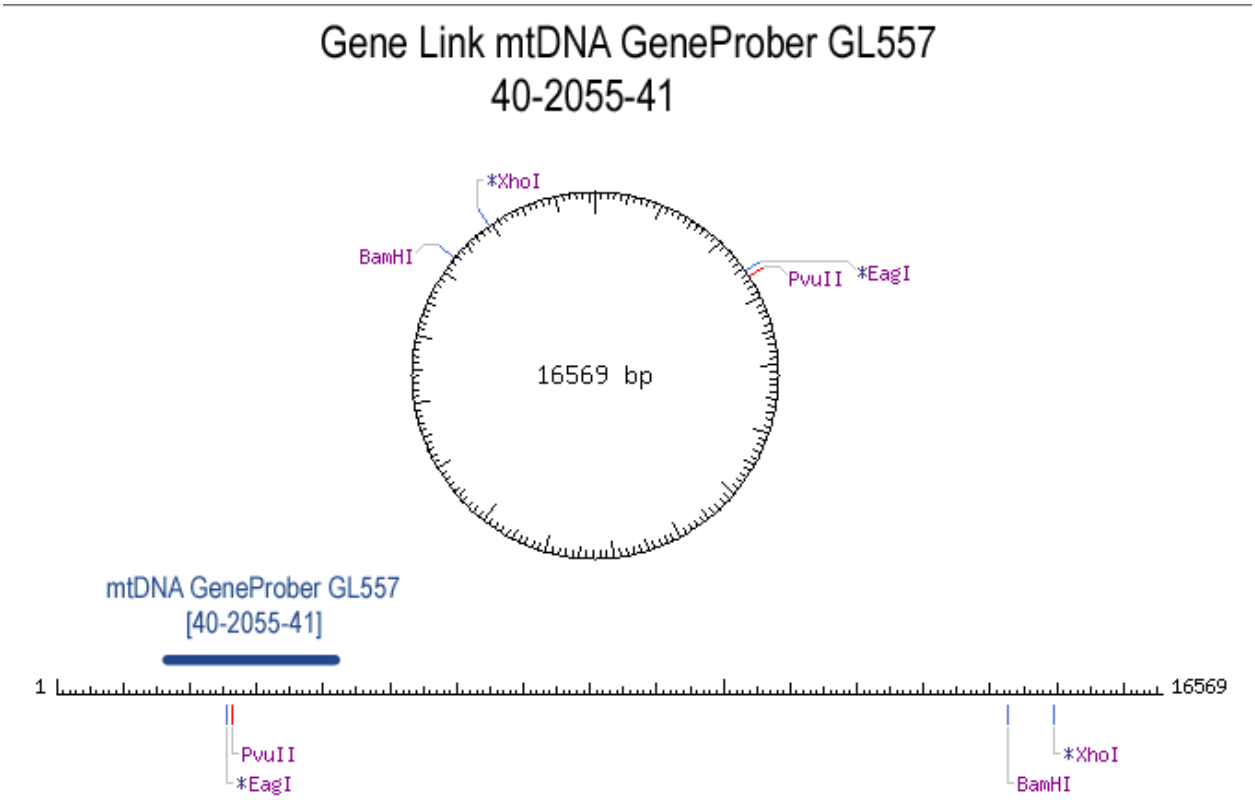
Mitochondrial DNA deletion syndromes are caused by mtDNA deletions ranging in size from two to ten kilobases. Approximately 90% of individuals with KSS have a large-scale (i.e., 1.1- to 10-kb) mtDNA deletion that is usually present in all tissues; however, mutant mtDNA is often undetectable in blood cells, necessitating examination of

muscle. In Pearson syndrome, mtDNA deletions are usually more abundant in blood than in other tissues. In PEO, mtDNA deletions are confined to skeletal muscle.

Deletions of mitochondrial DNA (mtDNA), ranging in size from 1.1 to 10 kb, are associated with Kearns-Sayre syndrome, Pearson syndrome, progressive external ophthalmoplegia, and rarely Leigh syndrome.

• More than 150 different mtDNA deletions have been associated with KSS. A deletion of 4977 bp known as m.8470_13446del4977 is encountered most frequently ("common deletion") [Schon 2003].
• The same m.8470_13446del4977 and numerous other types of deletions of varying length have been identified in Pearson syndrome and PEO.

Southern blot analysis of restriction fragments can be used to determine the presence of deletions. Genomic DNA is digested with single site cutters e.g. PvuII, BamHI, XhoI or EagI that will linearize the circular mtDNA to determine the RFLP compared to normal DNA pattern. The DNA blot is hybridized with Gene Link's mitochondrial deletion GeneProber™ GL557 Digoxigenin labelled probe for non-radioactive chemiluminescent detection.



References

1. DiMauro S, and Hirano, M. Mitochondrial DNA deletion syndromes. http://www.ncbi.nlm.nih.gov/books/NBK1203/
2. Chen X, Prosser R, Simonetti S, Sadlock J, Jagiello G, Schon EA. Rearranged mitochondrial genomes are present in human oocytes. Am J Hum Genet. 1995;57:239–47. [PMC free article: PMC1801549] [PubMed: 7668249].
3. DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. N Engl J Med. 2003;348:2656–68. [PubMed: 12826641].
4. Mita S, Rizzuto R, Moraes CT, Shanske S, Arnaudo E, Fabrizi GM, Koga Y, DiMauro S, Schon EA. Recombination via flanking direct repeats is a major cause of large-scale deletions of human mitochondrial DNA. Nucleic Acids Res. 1990;18:561–7. [PMC free article: PMC333462] [PubMed: 2308845].
5. Schon EA. Rearrangements of mitochondrial DNA. In: Holt I, ed. *Genetics of Mitochondrial Diseases.* Oxford, UK: Oxford University Press; 2003:111-24.

Procedure: Chemiluminescent Southern Protocol

Material Supplied

One tube containing 110 µL of mtDNA deletion GeneProber™ GL557 probe at a concentration of ~40ng/µL. This probe is digoxigenin labeled for non-radioactive detection. The quantity supplied is sufficient for at least 5 20x20 cm blots using 20µL for each blot as probe. Experienced users can optimize hybridization conditions for use of 2-5 µL GeneProber™ GL557 probe and/or save and reuse the hybridization solution 2-3 times.

A. Chromosomal DNA digestion

-Digest genomic DNA with your laboratory's preferred fragment analysis restriction enzyme to generate a linear single normal fragment pattern to compare with the deletion fragments.

Restriction Digestion	
Component	Volume|Quantity
Genomic DNA	5 to 10µg
10x Restriction enzyme buffer	10 µL
PvuII, BamHI, XhoI or EagI (~40 u/µL)	4 µL
H_2O to	**100 µL**

♦ **Incubate over night at 37^{O}C**

♦ **Ethanol Precipitate the digests**

-To 100 µL DNA add 10 µL of 3M Na Acetate pH 5.2
-Add 2 volumes (250 µL) of 100% ethanol
-Put in the freezer (-20 ^{o}C) for 20-30 minutes
-Spin at -10 ^{o}C for 5 minutes
-Discard the supernatant
-Add 100 µL of 70% ethanol, vortex.
-Spin again at -10 ^{o}C for 5 minutes
-Dry samples

♦ **Dissolve the pellets in 10 µL of 1x loading buffer**

B. Electrophoresis and Transfer

1. Load samples to a 0.8% agarose gel. Electrophorese over night at 45mA for 20-24 hours. (1.6 kb fragment on the bottom of the gel).
2. Depurinate with 0.25N HCl (add 10 mL HCl to 500 ml H_2O) for 10 minutes.
3. Denature the DNA with 0.4N NaOH/0.6M NaCl for 30 min. at room temperature (RT).
4. Neutralize with 1.5M NaCl/0.5M Tris (pH 7.5) for 30 min. at RT.
5. Transfer overnight by Southern blot procedure to positively charged nylon membrane using 10xSSC.
6. Wash the membrane with 2x SSC and then bake at 80^{O}C for 2 hours.

C. Hybridization

1. Perform prehybridization at 55°C for 3 hours in 10 mL of Easy Hyb buffer (Roche Biochemicals).
2. Boil 20µL GeneProber™ probe in 500µL of Easy Hyb for 10 minutes. Chill directly on ice.
3. Add the above probe to 10mL of Easy Hyb.
4. Discard the prehybridization buffer and replace it with the hybridization buffer containing the boiled probe. Hybridize overnight at 55°C.
5. Washing. Wash the membrane in 2xSSC/0.1% SDS at RT twice (5 min/wash), 0.5xSSC, 0.1%SDS twice at 65°C to 70°C (15 min/wash).
6. Warm the blocking reagent at this point. Prepare fresh 100 mL of Buffer MB by adding 10 mL of 10% blocking reagent [Gene Link Catalog no: 40-5026-10; Blocking solution for hybridization (10%)] and 10 mL of Maleic acid buffer 10X (Buffer M 10X) [Gene Link Catalog no: 40-5025-20; Maleic acid buffer 10X (Buffer M 10X)] to 80 mL of sterile water. Use 80 mL for blocking and the rest of 20 mL for making Anti-DIG-AP conjugate.

D. Anti-Dig Alkaline Phosphatase Binding

1. Equilibrate the membrane in 100 mL of 1x washing buffer M for 1 minute.
2. Incubate the membrane in 80 mL of Buffer MB (prepared in step 6 above) blocking solution at RT for 30 min.
3. Prepare 1:10000 Anti-DIG-AP conjugate at this point. Example, add 2 µL to 20 ml Buffer MB (prepared in step 6 above).
4. Incubate the membrane in 20 mL of Anti-DIG-AP conjugate solution at RT for 30 min.
5. Wash the membrane twice, 15 min/wash in 200 mL of 1x washing buffer M at RT.
6. Equilibrate the membrane in 50 mL of 1x Detection buffer for 2 min. Repeat 2 times. Total of three washes.

E. Detection

Detection with CDP star (Tropix) as substrate will yield reliable result by exposing to Kodak X-OMAT or XAR X-ray film for 1 hour to overnight at room temperature.

Transfer blot to a plastic sheet, (sheet protector cut from two sides to open up) and drain off excess buffer. Wipe off edges with paper towel. Blot should not be allowed to dry.

Spray CDP-star ready-to-use substrate evenly to cover the blot. DO NOT OVER SPRAY. Cover the blot with plastic sheet and wipe entire surface of the covered blot to expel any excess substrate and air bubbles. Expose the film at room temperature for 1 hr. or for shorter or longer time as required.

Luminescence continues for at least 24 hours and signal intensity remains almost constant during the first few hours. Multiple exposures can be taken to achieve the desired signal strength.

F. Stripping

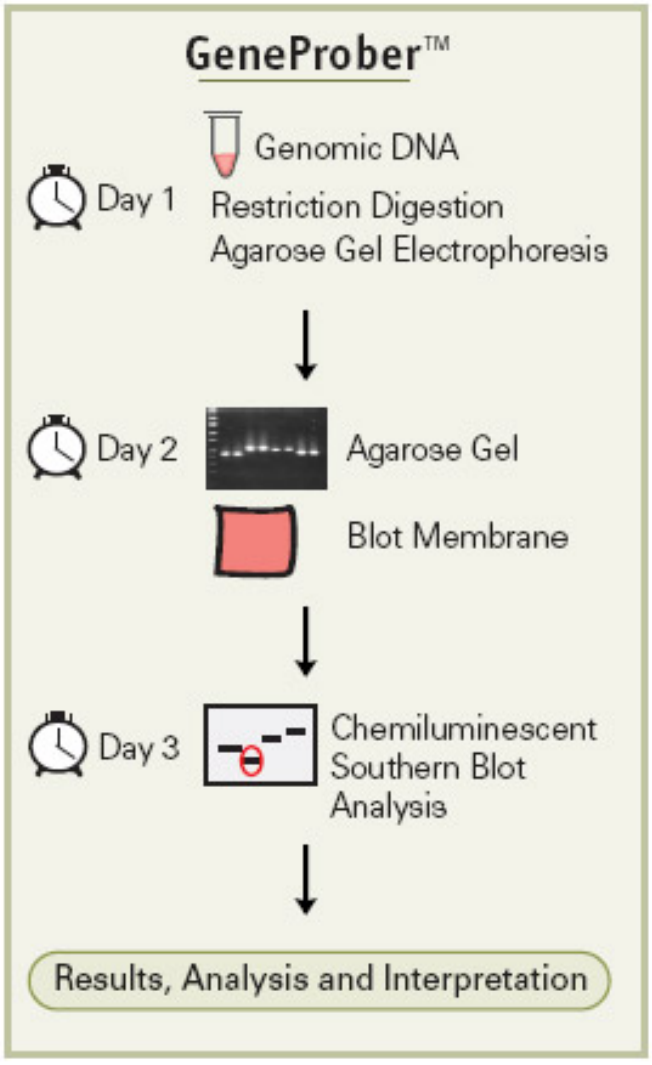
Wash the membrane in water to remove the substrate. Then wash the membrane in 0.2N NaOH/0.1% SDS at 37°C for 30 minutes. Rinse the membrane in 2XSSC. Air dry.

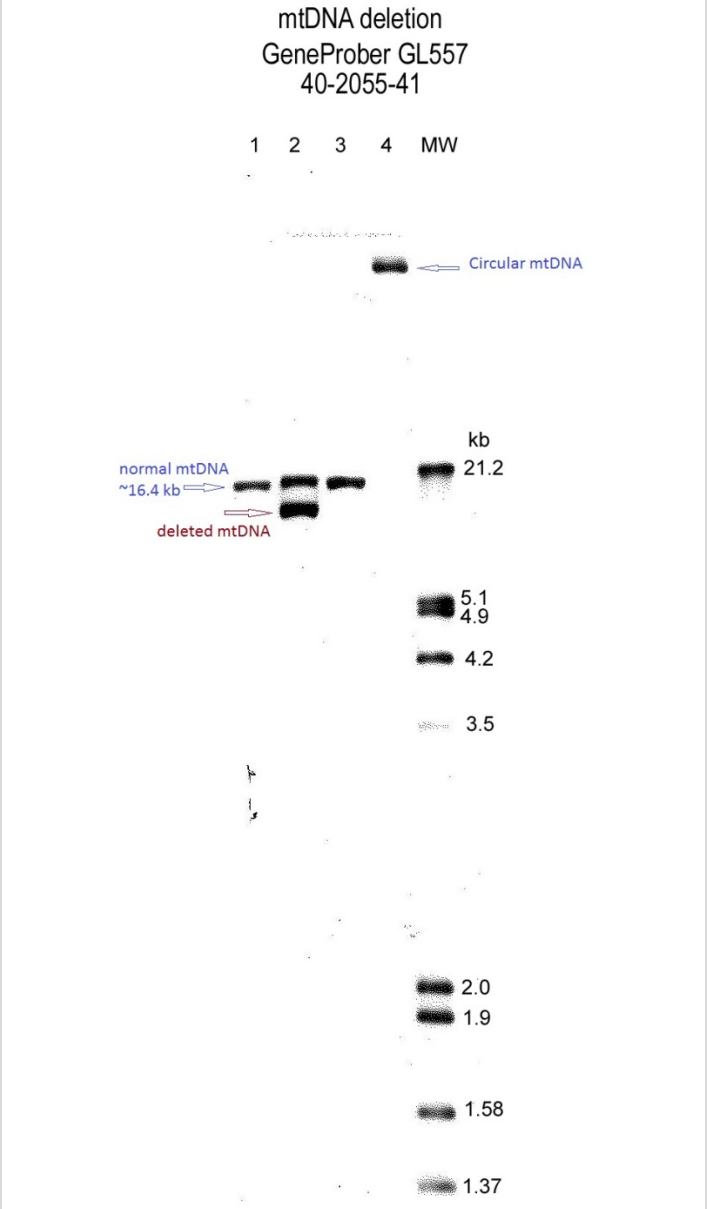
Results and Analysis

Gene Link's mtDNA deletion Southern blot genotyping GeneProber™ GL557 is a derived from a 2.5 kb fragment and labelled with digoxigenin for chemiluminescent Southern blot analysis.

Traditionally Southern blot analysis for mtDNA deletion genotyping involves the cleavage of DNA with restriction enzyme PvuII to generate a single linear normal mtDNA fragment of 16.5 kb, other enzymes e.g. BamHI, XhoI or EagI can also be used to compare with normal and deleted fragment length polymorphism.

Gene Link mtDNA deletion GeneProber™ GL557 [40-2055-41] Southern Blot Fragment Analysis





Non-radioactive Southern blot analysis was performed with human genomic DNA digested with PvuII and probed with Gene Link's mtDNA deletion GeneProber™ GL557 followed by chemiluminescent detection. Lanes 1 and 3 represent normal mtDNA PvuII fragment of ~16.5 kb. Lane 2 represents a sample with a mtDNA deletion and the appearance of a smaller deleted fragment of ~11 kb and the normal fragment of ~16.5 kb. Lane 4 is an undigested DNA with an expected higher mobility circular mtDNA.

Required reagents with recommended suppliers

Roche Applied Science	http://www.roche-applied-science.com
Product Description	**Catalog Number**
Nylon Membranes, positively charged ; 20 sheets 10 x 15 cm	11209272001
DNA Molecular Weight Marker III, DIG-labeled ; 500 µl 10 µg/ml 5 µg	11218603910
DIG Easy Hyb ; 500 mL	11603558001
DIG Wash and Block Buffer Set ; 1 set 30 blots	11585762001
Anti-Digoxigenin-AP, Fab fragments from sheep; 200 µL 150 U	11093274910
CDP Star Ready to use; 2X 50 mL	12041677001

Gene Link	**http://www.genelink.com/geneprodsite/category.asp?c=44**	
Non-radioactive Southern Blot Reagents		
Product Description	**Catalog No.**	**Unit Size**
Agarose LE Molecular Biology Grade 100 gms	40-3010-10	100 gms
TAE Buffer 50 X Concentrate 1000 mL	40-3007-10	1 L
TBE Buffer 5 X Concentrate; 1L	40-3008-10	1 L
Loading buffer 10X BPB/XC non-denaturing; 1mL	40-3003-10	1 mL
Loading buffer 10X BPB/XC non-denaturing ; 15 mL	40-3003-15	15 mL
Lumisol II, Hybridization Solution; 200 mL	40-5023-20	200 mL
Depurination Solution (2X) for Southern Blotting; 1 L	40-5034-10	1 L
Denaturation Solution (2X) for Southern Blotting; 1L	40-5035-10	1 L
Neutralization Solution (2X) for Southern Blotting; 1L	40-5036-10	1 L
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC); 250 mL	40-5020-25	250 mL
Hybwash B, Hybridization Wash Solution (10% SDS) ; 100 mL	40-5021-10	100 mL
Maleic acid buffer 10X (Buffer M 10X); 200 mL	40-5025-20	200 mL
10% Blocking Reagent; 100 mL	40-5026-10	200 mL
Detection Buffer 10X; Alkaline Phosphatase detection buffer; 100 mL	40-5031-10	100 mL
CDP-Star® Substrate; Ready-to-Use 0.25 mM in spray bottle; 10 mL	40-5010-10	10 mL

Reagent Preparation

Most reagents with composition listed below are available in a molecular biology laboratory or these can be prepared in house. Gene Link catalog numbers are also listed if you like to purchase these common reagents.

Depurination Solution (0.25M HCl)		
Product Description	**Catalog No.**	**Volume**
Depurination Solution (2X) for Southern Blotting	40-5034-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

Denaturation Solution (0.5M NaOH, 1.5M NaCl)		
Product Description	**Catalog No.**	**Volume**
Denaturation Solution (2X) for Southern Blotting	40-5035-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

Neutralization Solution (0.5M Tris-HCl pH 7.5, 1.5M NaCl)		
Product Description	**Catalog No.**	**Volume**
Neutralization Solution (2X) for Southern Blotting	40-5036-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

Hybwash I (2xSSC, 0.1% SDS)		
Product Description	**Catalog No.**	**Volume**
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC)	40-5020-25	35 mL
Sterile water		311 mL*
Hybwash B, Hybridization Wash Solution Concentrate (10% SDS)	40-5021-10	4 mL*
Total Volume		**350 mL**
*** Volumes adjusted to whole numbers**		

Hybwash II (0.5xSSC, 0.1%SDS)		
Product Description	**Catalog No.**	**Volume**
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC)	40-5020-25	9 mL*
Sterile water		337 mL
Hybwash B, Hybridization Wash Solution Concentrate (10% SDS)	40-5021-10	4 mL*
Total Volume		**351 mL**
*** Volumes adjusted to whole numbers**		

1X Maleic Acid Buffer (Buffer M 1X) (100 mM Maleic acid, 150 mM NaCl pH7.5)		
Product Description	**Catalog No.**	**Volume**
Maleic acid buffer 10X (Buffer M 10X)	40-5025-20	10 mL
Sterile water		90 mL
Total Volume		**100 mL**

Buffer MB (1 x Maleic acid buffer (Buffer M) with Blocking Reagent) Always prepare fresh!		
Product Description	**Catalog No.**	**Volume**
Maleic acid buffer 10X (Buffer M 10X)	40-5025-20	10 mL
Sterile water		80 mL
10% Blocking Reagent*	40-5026-10	10 mL
Total Volume		**100 mL**
The prepared reagent will be turbid yellow in color * The 10% Blocking Reagent is turbid yellow in color and will form precipitates on storage. Warm to 50°C and shake well before aliquoting. DO NOT SHAKE VIGOROUSLY		

1X Detection Buffer, Alkaline phosphatase detection buffer (100mM Tris-HCl pH 9.5, 100mM NaCl)		
Product Description	**Catalog No.**	**Volume**
Detection Buffer 10X; Alkaline phosphatase detection buffer	40-5031-10	10 mL
Sterile water		90 mL
Total Volume		**100 mL**

Appendix: **Protocols**

Genomic DNA Purification

Genomic DNA is usually extracted from blood. A simple procedure is given below that purifies ~10 µg DNA from 300 µl blood using a 30 minute procedure.

Omni-Pure™ Genomic DNA Purification System Catalog Number: 40-4010-01
Rapid DNA Purification Protocol for 300 µl Whole Blood

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 900 µl GD-1 solution (RBC Lysis Solution) to one tube for each sample.
3. Add 300 µl Isopropanol (2-propanol) to one tube for each sample. Cap the tubes.

B. Cell Lysis

1. To the tube containing 900 µl GD-1 solution (RBC Lysis Solution) using a filter tip pipet transfer 300 µl whole blood. Cap and gently mix by inversion. Incubate for 1-3 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 not lysed already.
2. Centrifuge at 3 K 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 GD-2 solution (Cell Lysis Solution). Mix by gentle vortexing. 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. It has been shown that the samples are stable in Cell Lysis Solution for at least 2 years at room temperature.

C. Protein Precipitation

1. Add 100 µl GD-3 solution (Protein Precipitation Solution) to the sample in cell lysis solution.
2. Vortex vigorously for 20 seconds. Small particles of brown color will appear and be visible at this stage.
3. Centrifuge at 5 K rpm for 1 minute to pellet the precipitated proteins. A clearly visible brown pellet containing proteins should be collected at the bottom of the tube.

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 is usually sufficient.
3. Centrifuge at 6 K rpm for 1 minute 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 6 K rpm for 1 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 tube 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 tube inverted on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

E. DNA Reconstitution & Use

1. Add 100 µl of GD-4 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, place sample at –20 °C or –80 °C.
3. Average yield of 10 µg is expected from 300 µl blood DNA. 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. Use 100 µl for restriction digestion followed by Southern blot analysis.
7. It is convenient to perform multiple 300 µl blood DNA purification instead of scaling up the procedure.

Southern Blot Buffers& Reagents

Product	Catalog No.	Unit Size
Hybwash A, Hybridization Wash Solution (20X SSC); 200 mL	40-5020-20	200 mL
Hybwash B, Hybridization Wash Solution (10% SDS); 100 mL	40-5021-10	100 mL
TAE Buffer; 50 X Concentrate; 100 mL	40-3007-01	100 mL
TAE Buffer; 50 X Concentrate; 1 L	40-3007-10	1 L
TBE Buffer; 5 X Concentrate; 1 L	40-3008-10	1 L
Buffer M 10X (Maleic Acid buffer); 100 mL	40-5025-10	100 mL
10% Blocking solution; 100 mL	40-5026-10	100 mL
Loading Buffer 2X BPB/XC Denaturing for Sequencing; 1 mL	40-5027-10	1 mL
10x AP Detection buffer (alkaline phosphatase detection buffer); 100 mL	40-5031-10	100 mL
Lumisol™ I Hybridization Solution; contains formamide; 200 mL	40-5022-20	200 mL
Lumisol™ II Hybridization Solution; for non-toxic hybridizations; 200 mL	40-5023-20	200 mL
Lumisol™ III Hybridization Solution; for oligo probes; 200 mL	40-5024-20	200 mL
CDP-Star® Substrate; Ready-to-Use 0.25 mM in spray bottle; 10 mL	40-5010-10	10 mL

Omni-Pure™ DNA & RNA Purification Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Pure™ Blood DNA Purification System	40-4010-01	100
Omni-Pure™ Blood DNA Purification System	40-4010-05	500
Omni-Pure™ Blood DNA Purification System	40-4010-10	1000
Omni-Pure™ Tissue DNA Purification System	40-4050-01	100
Omni-Pure™ Tissue DNA Purification System	40-4050-05	500
Omni-Pure™ Tissue DNA Purification System	40-4050-10	1000

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Clean™ Gel DNA Purification and Concentration Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Clean™ Gel DNA Beads Purification System	40-4110-10	100
Omni-Clean™ Gel DNA Beads Purification System	40-4110-50	500
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-10	100
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-50	500
Omni-Clean™ DNA Beads Concentration System	40-4130-10	100
Omni-Clean™ DNA Beads Concentration System	40-4130-50	500
Omni-Clean™ DNA Spin Column Concentration System	40-4140-10	100
Omni-Clean™ DNA Spin Column Concentration System	40-4140-50	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

All Gene Link products are for research use only Current pricing are posted at http://www.genelink.com/

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