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

Friedreich's Ataxia GAA Repeat Genotyping
GeneProber™ FRDA-GLDig21

Friedreich's Ataxia GAA Triple Repeat Chemiluminescent Southern Blot Genotyping

Catalog No. 40-2027-41

Storage Condition: See Material Supplied List

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.

Material Supplied

Friedreich's Ataxia GeneProber™ FRDA-GLDig21 Digoxigenin Labeled Probe

Friedreich's Ataxia GAA triple repeat spanning region digoxigenin labeled probe for Southern blot genotyping

REF	Catalog No.	Description	Size
	40-2027-41	Friedreich's Ataxia GeneProber™ FRDA-GLDig21 Digoxigenin Labeled	110 µL

Certificate of Analysis & Product Specifications

One tube containing 110 µL of GeneProber™ FRDA-GLDig21 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 20 µL for each blot as probe. Experienced users with optimized hybridization conditions can save the hybridization solution containing the probe and re-use at least one more time for hybridization.

The FRDA GeneProber™ FRDA-GLDig21 probe supplied has been validated to hybridize to the GAA triple repeat within the first intron of the X25 gene (frataxin).

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.
Friedreich's Ataxia GeneProber™ FRDA-GL21 Probe unlabeled	500 ng	40-2027-40
Friedreich's Ataxia GeneProber™ FRDA-GLDig21 Probe Digoxigenin labeled	110 µL	40-2027-41
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
Mitochondrial DNA deletion GeneProber™ GL557 Digoxigenin labeled	110 µL	40-2055-41

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.
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
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

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

Friedreich's Ataxia Genotyping

Background

Friedreich's ataxia (FRDA [MIM 229300], NM_181425) is an autosomal recessive neurodegenerative disorder characterized by a progressive loss of voluntary muscle coordination (ataxia). The disorder affects upper and lower limbs, and the head and neck. FRDA is characterized clinically by progressive gait and limb ataxia; signs of upper motoneuron dysfunction including dysarthria, areflexia, and loss of the senses of position and vibration; cardiomyopathy; diabetes mellitus; and secondary skeletal abnormalities. Most patients develop hypertrophic cardiomyopathy and skeletal abnormalities, and some become diabetic (1,2). These symptoms progress with age, such that most patients become wheelchair-bound by their late twenties and die by their mid-thirties—most commonly of congestive heart failure. Some of the other symptoms include muscle weakness, loss of pressure and position sense in the arms and legs, speech problem and heart disease. Unlike some neurological diseases, FRDA does not affect mental capacity. See recent reviews (3,4).

Although rare, FRDA is the most prevalent inherited ataxia, affecting about 1-2 in every 50,000 individuals. It is usually diagnosed in childhood between the ages of 5 and 15. The majority (~98%) of patients with FRDA are homozygous for a GAA repeat expansion within the first intron of frataxin gene. The remaining patients are compound heterozygotes for the GAA expansion and for point mutations within the X25 gene. In normal alleles, the repeat varies in size between 7 and 30 units, whereas in mutated alleles the repeat length ranges from 66 to more than 1000 units. Generally, the milder forms or late onset of the disease are associated with shorter expansions.

FRDA is caused by degeneration of nerve tissue in the spinal cord and of nerves that extend to peripheral areas such as the arms and legs. The disorder is associated with an unstable expansion of GAA repeats in the first intron of the FRDA gene, called X25, on chromosome 9q13. The encoded protein, frataxin, is located in mitochondria and reduced in FRDA patients. It is suggested that FRDA is the result of mitochondrial iron overload leading to excess production of free radicals, which results in cellular damage and death.

The majority (>95%) of patients with FRDA are homozygous for large expansions of a GAA triplet—repeat sequence (66—1800 triplets) located within the first intron of the gene *X25,* which encodes the protein frataxin (2). The expansion causes a severe reduction in the levels of frataxin, a 210—amino acid protein that is targeted to mitochondrial matrix and that appears to play a crucial role in iron homeostasis. The severity of the disease is directly correlated with the length of the expansion. A very small minority of patients are compound heterozygotes for the GAA expansion and for point mutations within the *X25* gene. Chamberlain and coworkers have recently summarized all point mutations described to date (5).

Frataxin RNA levels were severely reduced lymphoblast cell lines of patients with FRDA who were homozygous for the GAA expansion. Several groups have demonstrated that the GAA-repeat expansion interferes with transcription. It has been show by various groups that the GAA Triplet—Repeat Expansion acts as an Impediment to Transcription (3).

Genetically, FRDA belongs to a class of neurodegenerative disorders in which the underlying gene, *FRDA,* carries an unstable trinucleotide-repeat sequence. At least eight other members of this class have been identified, including HD and many types of spinocerebellar ataxia. However, key genetic features separate FA from the other trinucleotide-repeat disorders. First, the sequence of the trinucleotide repeat in the *FRDA1* gene is GAA (2), whereas a CAG repeat occurs in the other trinucleotide-associated ataxias, and other repeats (CTG or CGG) are seen in other trinucleotide diseases. Second, the GAA repeat of *FRDA* is located in the first intron and is therefore noncoding, whereas the CAG repeat in HD and the spinocerebellar ataxias always occurs within an exon and encodes glutamine.

The third difference is that FRDA is inherited in a recessive manner, and multiple lines of evidence suggest that loss of function leads to the disease. In contrast, in the other trinucleotide-repeat disorders, whether the repeat occurs in an expressed DNA sequence or in a 3' untranslated sequence, the mutation is inherited in a dominant manner, and it is a gain of function of the affected protein or RNA that perturbs cell physiology.

Table 1: Trinucleotide Repeats in Human Genetic Disease

Disease	Repeat[a]	Normal Length[b]	Intermediate Length (Premutation)[a,b]	Full Disease Length[b]
Fragile XA (FRAXA)	$(CGG)_n$	6-52	59-230	230-2,000
Fragile XE (FRAXE)	$(CCG)_n$	4-39	? (31-61)	200-900
Fragile XF(FRAXF)	$(CGG)_n$	7-40	?	306-1,008
FRA16A	$(CCG)_n$	16-49	?	1,000-1,900
Jacobsen Syndrome (FRA11B)	$(CGC)_n$	11	80	100-1,000
Kennedy Syndrome (SMBA)	$(CAG)_n$	14-32	?	40-55
Myotonic Dstrophy (DM)	$(CTG)_n$	5-37	50-80	80-1,000; congenital, 2,000-3,000
Huntington disease (HD)	$(CAG)_n$	10-34	36-39	40-121
Spinocerebellar ataxia 1 (SCA1)	$(CAG)_n$	6-39	…	40-81 (Pure)
Spinocerebellar ataxia 2 (SCA2)	$(CAG)_n$	14-31	…	34-59 (Pure)
Spinocerebellar ataxia 3 (SCA3)/Machado Joseph disease (MJD)	$(CAG)_n$	13-44	?	60-84
Spinocerebellar ataxia 6 (SCA6)	$(CAG)_n$	4-18	?	21-28
Spinocerebellar ataxia 7 (SCA7)	$(CAG)_n$	7-17	?	38-130
Haw River syndrome (HRS; also DRPLA))	$(CAG)_n$	7-25	?	49-75
Friedreich ataxia (FRDA)	$(GAA)_n$	6-29	? (>34-40)	66-1800

[a] Typically, repeats tracts contain sequence interruptions. See Pearson and Sinden (1998*a*) for a discussion of the sequence interruptions.
[b] No. of triplet repeats.
[c] A question mark (?) indicates potential mutagenic intermediate length, and an ellipsis (…) indicates none. Not all disease are associated with a permutation length repeats tract or permutation disease condition.-

The severity of the disease correlates with decreased *FRDA* expression and with the length of the hyperexpansive repeat. Normally, this gene, which encodes the protein frataxin, contains <39 GAA repeats, but in patients with FRDA, this locus contains 66—1,700 repeat units. This hyperexpansion results in marked decreases in frataxin mRNA levels, thought to result from the formation of an unusual non-β DNA structure inhibiting transcription (3). More than 95% of patients with FRDA are homozygous for the GAA hyperexpansion, although the alleles are polymorphic in the number of GAA repeats. Studies have shown a correlation between the length of the GAA expansion on the smaller allele and severity of disease (1). An inverse correlation between GAA expansion size and frataxin protein levels has been observed in lymphoblast cell lines from patients with FRDA (3). Together, these findings suggest that lack of frataxin protein in critical tissues leads to FRDA. The remaining 5% of patients with FRDA are compound heterozygotes for the GAA expansion on one allele and carry point mutations within *FRDA1* on the other allele.

The most common disease-causing point mutation in frataxin is I154F (numbering based on the initiator methionine of the predicted open reading frame [ORF]), prevalent in some southern Italian families. Those individuals carrying this missense mutation on one allele, together with the hyperexpansion on the other allele, are indistinguishable in disease severity when compared with homozygous relatives who carry the GAA triplet expansion on both alleles (4). Another missense mutation in frataxin, G130V, compounded with a hyperexpansive allele, is associated with a milder and more slowly progressive disease course (3).

Meiotic instability and Somatic Variation in GAA Expansion Length

The GAA expansion shows intergenerational variation in length, with evidence for changes in the prezygotic and postzygotic stages. Studies have shown that the expanded alleles seen in patients arose from a small pool of uninterrupted "large normal" alleles referred to as "premutations." Interruptions within the pure GAA triplet repeats impeded these large normal alleles from expanding into disease-causing alleles. De Michele et al. (10) have noted that premutation alleles can undergo large expansions in a single generation. Expanded GAA repeats can expand or contract when transmitted through the female germline. In contrast, contractions are favored in male transmission. This is attributed to postzygotic mechanisms, because shorter expansions are seen in sperm DNA when compared with lymphocyte DNA (prezygotic mechanism). However, evidence for postzygotic variation in repeat number has also been suggested, because the degree of repeat contraction in the sperm is greater than that actually seen in intergenerational transmission and because the overall length of expanded alleles is shorter in homozygous versus heterozygous carriers. The formation of unexpected parallel duplex has been shown in GAA and TTC trinucleotide repeats of Friedreich's ataxia (11). This presumably interferes with normal transcription activity.

Number of GAA repeats	Clinical Condition	Symptoms
5-30 repeats	Unaffected	Normal
?34-40 repeats	Mild	Premutation
66-900 repeats	Severe	Full mutation

Genotyping

Molecular diagnosis of Friedreich's Ataxia is available. It involves a combination of direct PCR analysis and Southern blotting tests to determine the GAA-repeat number within the FRDA gene. PCR can identify GAA expansions between 5-200 GAA repeats.

With larger expansions, Southern blot analysis of restriction fragments can be used for an approximate measure of large repeat size. Genomic DNA is digested with BsiHKAI. The DNA blot is then hybridized with FRDA-GL probe.

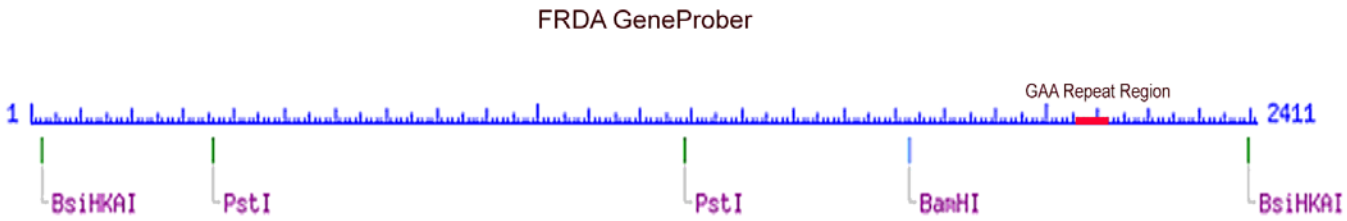
Molecular Analysis

The direct analysis of GAA repeats in the *FRDA* gene (chromosomal locus 9q13) is clinically available. An increased number of GAA repeats is identified in essentially 100% of patients with FRDA. The number of GAA repeats ranges from 5 to <30 in normal alleles. GAA repeat lengths in the range from about >30-40 are considered "premutations." Persons with GAA expansions in the premutation range have not been reported as having developed severe symptoms, but their children are at risk of inheriting a larger repeat size. Persons with GAA repeat length greater than 50 are frequently symptomatic.

Friedreich's Ataxia genotyping can be done by direct PCR amplification of the GAA trinucleotide repeats region or by Southern analysis. In most cases both methods are used to complement the results. Full mutations usually cannot be identified by PCR and southern analysis is the preferred method to distinguish full mutations.

The size of the GAA repeats can be determined by PCR analysis and sizing preferably on a sequencing gel. The PCR products can be either labeled with ^{35}S or ^{32}P followed by autoradiography. Another attractive alternate is to run a cold PCR reaction followed by blotting and hybridization with an alkaline phosphatase conjugated probe for non-radioactive detection

Southern blot analysis for Friedreich's Ataxia mutation detection involves the cleavage of genomic DNA with BsiHKAI enzyme. This method detects the size of GAA repeats region by hybridization of probe FRDA-GL to genomic DNA that has been digested with the appropriate restriction enzyme and blotted onto a membrane. The GAA repeat in the normal range yields a ~2374 bp.



The following table lists the size of PCR fragment in base pairs (bp) that can be expected when using the GAA primer mix (F1B/R3C) that has been provided. The formula for determining PCR fragment size is 322 + 3n, where n= the number of GAA repeats.

GAA Repeat PCR Amplified Fragment Size Using Primer Set F1B/R3C*			
$GAA_{(n)}$	PCR Fragment (bp)	$GAA_{(n)}$	PCR Fragment (bp)
5	337	45	445
6	340	50	460
7	343	55	475
8	346	60	490
9	349	65	505
10	352	70	520
11	355	75	535
12	358	80	550
13	361	85	565
14	364	90	580
15	367	95	595
16	370	100	610
17	373	105	625
18	376	110	640
19	379	115	655
20	382	120	670
21	385	125	685
22	388	130	700
23	391	135	715
24	394	140	730
25	397	145	745
26	400	150	760
27	403	155	775
28	406	160	790
29	409	165	805
30	412	170	820
31	415	175	835
32	418	180	850
33	421	185	865
34	424	190	880
35	427	195	895
40	430	200	910
*The size of PCR fragment that can be expected when using the FRDA GAA primer mix (F1B/R3C) that has been provided in this kit. The formula for determining PCR fragment size is 322 + 3n, where n= the number of GAA repeats			

References

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2. Campuzano V, Montermini L, Moltó MD, Pianese L, Cossée M, Cavalcanti F, Monros E, et al (1996) Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 271:1423—1427.
3. Patel PI and Grazia Isaya G (2001) Friedreich Ataxia: From GAA Triplet—Repeat Expansion to Frataxin Deficiency. Am. J. Hum. Genet., 69:15-24.
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5. Monrós, E. et al. (1997) Am. J. Hum. Genet. 61: 101-110.
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7. Pook MA, Al Mahdawi SA, Thomas NH, Appleton R, Norman A, Mountford R, Chamberlain S (2000) Identification of three novel frameshift mutations in patients with Friedreich's ataxia. J Med Genet 37:E38.
8. Bradley JL, Blake JC, Chamberlain S, Thomas PK, Cooper JM, Schapira AH (2000) Clinical, biochemical and molecular genetic correlations in Friedreich's ataxia. Hum Mol Genet 9:275—282.
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12. De Michele G, Cavalcanti F, Criscuolo C, Pianese L, Monticelli A, Filla A, Cocozza S (1998) Parental gender, age at birth and expansion length influence GAA repeat intergenerational instability in the X25 gene: pedigree studies and analysis of sperm from patients with Friedreich's ataxia. Hum Mol Genet 7:1901—1906
13. LeProust EM, Pearso CE, Sinden RR, Gao X (2000) Unexpected formation of parallel duplex in GAA and TTC trinucleotide repeats of Friedreich's ataxia. J Mol Biol 302:1063—1080.

Friedreich's Ataxia Chemiluminescent Southern Blot Analysis

Protocol

Material Supplied

One tube containing 110 µL of FRDA-GLDig *GeneProber ™* 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 20 µL for each blot as probe. . Experienced users with optimized hybridization conditions can save the hybridization solution containing the probe and re-use at least one more time for hybridization.

A. Chromosomal DNA digestion

-Digest genomic DNA with BsiHKAI when using FRDA-GLDig21 GeneProber™ as labeled probe.

Restriction Digestion	
Component	Volume|Quantity
Genomic DNA	5 to 10µg
10x BsiHKAI Buffer	10 µL
BsiHKAI (~40 u/µl)	4 µL
Water to	**100 µL**
Overnight digestion at 37^{O}C	

Ethanol precipitate the digests, dissolve the pellets in 10 µL of 1x Loading buffer.

Refer to Reagent preparation section for composition and preparation instructions. Abridged Southern blot protocol is given below. It is assumed the researcher has extensive experience in Southern blot procedure.

B. Electrophoresis and Transfer

1. Load samples to a 0.8% agarose gel. Electrophorese over night at 45mA for 14-16 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

Gene Link recommends using Roche Digoxigenin based washing and detection system reagents.

1. Perform prehybridization at 55^{o}C for 3 hours in 10 mL of Easy Hyb buffer (Roche Biochemicals).
2. Boil 20 µL GeneProber™ probe in 500µL of Easy Hyb for 10 minutes. Chill directly on ice.
3. Add the above probe to 10 mL of Easy Hyb.
4. Discard the prehybridization buffer and replace it with the hybridization buffer containing the boiled probe. Hybridize overnight at 55^{o}C.

5. Washing. Wash the membrane in Hyb Wash I (2xSSC/0.1% SDS) at room temperature twice (5 min/wash) followed by washing twice with Hyb Wash II (0.5xSSC, 0.1%SDS) twice at 60°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, 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 Buffer M for 1 minute.
2. Incubate the membrane in 80 mL of Buffer MB (prepared in step 6 above) blocking solution at room temperature for 30 min.
3. Prepare 1:10000 Anti-DIG-AP conjugate at this point. e.g. add 2 μL to 20 ml Buffer MB (prepared in Section C. 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 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 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.

1. 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.
2. 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.
3. 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. Blot is ready for re-hybridization.

G. Results & Interpretation

1. Normal hybridization pattern is ~2374 bp fragment with genomic DNA digested with BsiHKAI using FRDA-GLDig GeneProber™ as labeled probe.
2. Larger fragment size is attributable to expanded GAA repeat region. See FRDA probe region figure below.

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 (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 (10% SDS)	40-5021-10	4 mL*
Total Volume		**351 mL**
*** Volumes adjusted to whole numbers**		

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

Buffer MB (1 x Buffer M (Maleic acid buffer, 1X with Blocking Reagent) Always prepare fresh!		
Product Description	**Catalog No.**	**Volume**
Buffer M 10X (Maleic acid buffer 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 Gene Link 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.

Friedreich's Ataxia Product Ordering Information

Product	Unit Size	Catalog No.
Friedreich's Ataxia Genemer™ Primer pair Primers for amplification of GAA triple repeat spanning region. The quantity supplied is sufficient for 400 regular 50 µL PCR reactions.	10 nmols	40-2027-10
Friedreich's Ataxia Genemer™ Kit Kit for amplification of GAA triple repeat spanning region. The quantity supplied is sufficient for 400 regular 25 µL PCR reactions.	100 rxns	40-2027-11
Friedreich's Ataxia GScan™ Kits for fluorescent detection Kit for performing fluorescent PCR amplification based detection. Various dye kits. XX=FM for 6-Fam; HX for Hex; TT for Tet; C3 for Cy3 and C5 for Cy5.	1 Kit [100 rxns]	40-2027-15XX
Friedreich's Ataxia GeneProber™ FRDA-GL21 Probe unlabeled Probe for radioactive labelling and Southern blot analysis	500 ng	40-2027-40
Friedreich's Ataxia GeneProber™ FRDA-GLDig21 Probe Digoxigenin labeled Probe for non-radioactive chemiluminescent Southern blot analysis	110 µL	40-2027-41

Genemer™ control DNA Cloned fragment of the mutation region of a particular gene. These control DNA's are ideal genotyping templates for optimizing and performing control amplification with unknown DNA. The size of the triple repeats has been determined by sequencing and gel electrophoresis. The stability of size repeats upon cloning and amplification has NOT been determined. Thus, the size should be considered approximate and there is no claim for each fragment to contain the exact number of triple repeats. These control DNA's are sold with the express condition that these NOT be used for exact triple repeat size determination of DNA of unknown genotype. The control DNA should be used for determining the performance of specific Genemer™ and PCRProber™ Gene Link products.

GLFRDA ~64 GAA repeat Genemer Control DNA	500 ng	40-2027-01
GLFRDA ~102 GAA repeat Genemer Control DNA	500 ng	40-2027-02
GLFRDA ~110 GAA repeat Genemer Control DNA	500 ng	40-2027-03
GLFRDA ~125 GAA repeat Genemer Control DNA	500 ng	40-2027-04
GLFRDA ~9 GAA repeat Genemer Control DNA	500 ng	40-2027-05

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Southern Blot Buffers& Reagents

Product	Catalog No.	Unit Size
Agarose Tablets, 0.5 gm each; 100 tablets	40-3011-10	100 tablets
Agarose LE Molecular Biology Grade; 100 g	40-3010-10	100 g
Agarose LE Molecular Biology Grade; 500 g	40-3010-50	500 g
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

Loading Buffers

Product	Catalog No.	Size
Gel Loading Buffer 5X BPB/XC non-denaturing; 1 mL	40-3002-10	1 mL
Gel Loading Buffer 5X BPB/XC non-denaturing; 15 mL	40-3002-15	15 mL
Gel Loading Buffer 10X BPB/XC non-denaturing; 1 mL	40-3003-10	1 mL
Gel Loading Buffer 10X BPB/XC non-denaturing; 15 mL	40-3003-15	15 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing; 1 mL	40-3004-10	1 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing; 15 mL	40-3004-15	15 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing; 1 mL	40-5027-10	1 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing; 15 mL	40-5027-15	15 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer ; 1 mL	40-5028-10	1 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer; 15 mL	40-5028-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide; 1 mL	40-5029-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide; 15 mL	40-5029-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide ; 1 mL	40-5030-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide; 15 mL	40-5030-15	15 mL

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Related Products Ordering Information

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
Omni-Pure™ Plant DNA Purification System	40-4060-01	100
Omni-Pure™ Plant DNA Purification System	40-4060-05	500
Omni-Pure™ Plant DNA Purification System	40-4060-10	1000
Omni-Pure™ Viral DNA Purification System	40-3720-01	100
Omni-Pure™ Viral DNA Purification System	40-3720-05	500
Omni-Pure™ Microbial DNA Purification System	40-3700-01	100
Omni-Pure™ Microbial DNA Purification System	40-3700-05	500
Omni-Pure™ Viral RNA Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Purification System	40-3650-05	500

*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.

Omni-Pure™ Plasmid DNA Purification Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Pure™ Plasmid DNA Purification System	40-4020-01	100
Omni-Pure™ Plasmid DNA Purification System	40-4020-05	500

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

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