

# 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 GScan™ V2 Kit

Friedreich's Ataxia GAA Triple Repeat Fluorescent Genotyping

Catalog No. 40-2027-15XX

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 GAA Repeat Genotyping GScan™ V2 Kit****Friedreich's Ataxia GAA Triple Repeat Fluorescent Genotyping****Note: Components of this version kit are not compatible with components of previous versions**

Friedreich's Ataxia GAA Repeat Genotyping GScan™ Kits			
Kit	Catalog No.	Description	Size
<input type="checkbox"/>	40-2027-15FM	Friedreich's Ataxia GAA Repeat Genotyping GScan™ V2 6-Fam Kit. 100 Rxns	100 rxns
<input type="checkbox"/>	40-2027-15FMS	Friedreich's Ataxia GAA Repeat Genotyping GScan™ V2 6-Fam Kit. 20 Rxns	20 rxns

Friedreich's Ataxia GAA Repeat Genotyping GScan™ Kit 100 Reactions Components			
Content	Catalog No.	Description	Size
<input type="checkbox"/>	40-2027-15FFM	FRDA GScan™ V2 6-Fam Component FM	900 µL
<input type="checkbox"/>	40-2027-15E	PCR Component E; PCR Additive	800 µL
<input type="checkbox"/>	40-2027-15D	PCR Component D; PCR Additive	150 µL
<input type="checkbox"/>	40-2027-15S	FRDA GScan™ 6-Fam amplified 64 GAA Repeats control	10 µL

Friedreich's Ataxia GAA Repeat Genotyping GScan™ Kit 20 Reactions Components			
Content	Catalog No.	Description	Size
<input type="checkbox"/>	40-2027-15FFMS	FRDA GScan™ V2 6-Fam Component FM	180 µL
<input type="checkbox"/>	40-2027-15ES	PCR Component E; PCR Additive	200 µL
<input type="checkbox"/>	40-2027-15DS	PCR Component D; PCR Additive	40 µL
<input type="checkbox"/>	40-2027-15SS	FRDA GScan™ 6-Fam amplified 64 GAA Repeats control	5 µL

**Storage Condition**

Store at -20°C

## Certificate of Analysis & Product Specifications

The FRDA GScan™ kit contains optimized components and Genemer™ FRDA-F1B/R3C primer pair that has been validated to amplify the GAA triple repeats within the first intron of the X25 gene (frataxin).


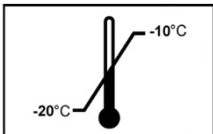
Appropriate nuclease free handling, dispensing and storage conditions required.




### Lot Number:

Manufacturing lot number is stated on the label of product and accompanying packing slip.

### Product Label Information

<b>REF</b>	<b>Catalog No.</b>	<b>Description</b>	<b>Size</b>
	40-2027-15FM	Friedreich's Ataxia GAA Repeat Genotyping GScan™ V2 6-Fam Kit. 100 rxns	100 rxns
	40-2027-15FMS	Friedreich's Ataxia GAA Repeat Genotyping GScan™ V2 6-Fam Kit. 20 rxns	20 rxns

		<b>LOT</b>
<b>Research Use Only</b>	<b>Storage</b> Store at -20°C to -10°C	<b>Lot Number</b> Stated on product tube and packing slip

		
<b>Expiry</b> One year from Date of Shipment	<b>Instructions</b> Consult product manual	<b>QR Code</b> 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 <sup>32</sup>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™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2027-15XX
Friedreich's Ataxia GScan™ V2 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™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2026-15XX
Myotonic Dystrophy GScan™ V2 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 shown 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.

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
66-1800 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 <sup>35</sup>S or <sup>32</sup>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.

## References

1. Durr, A, Cossee M, Agid Y, Campuzano V, Mignard C, Penet C, Mandel JL, et al (1996) Clinical and genetic abnormalities in patients with Friedreich's ataxia. *New Engl J Med* 335:1169–1175.
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.
4. Simon A. B. Knight, S.A.B; Kim, R; Pain, D and Dancis, A. (1999) The Yeast Connection to Friedreich Ataxia. *Am. J. Hum. Genet.*, 64:365-371.
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6. Castro, M. et al. (2000) *Hum. Genet.* 106: 86-92.
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.
9. Ohshima K, Montermini L, Wells RD, Pandolfo M (1998) Inhibitory effects of expanded GAA.TTC triplet repeats from intron I of the Friedreich ataxia gene on transcription and replication in vivo. *J Biol Chem* 273:14588–14595
10. Bidichandani SI, Ashizawa T, Patel PI (1998) The GAA triplet-repeat expansion in Friedreich ataxia interferes with transcription and may be associated with an unusual DNA structure. *Am J Hum Genet* 62:111–121.
11. 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.
12. De Michele G, Cavalcanti F, Criscuolo C, Pianese L, Monticelli A, Filla A, Coccozza 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 GScan™ V2 Kit. FRDA GAA Triple Repeat Fluorescent Genotyping

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GAA Repeat PCR Amplified Fragment Size Using Primer Set F1B/R3C*			
GAA <sub>(n)</sub>	PCR Fragment (bp)	GAA <sub>(n)</sub>	PCR Fragment (bp)
5	337	45	457
6	340	50	472
7	343	55	487
8	346	60	502
9	349	65	517
10	352	70	532
11	355	75	547
12	358	80	562
13	361	85	577
14	364	90	592
15	367	95	607
16	370	100	622
17	373	105	637
18	376	110	652
19	379	115	667
20	382	120	682
21	385	125	697
22	388	130	712
23	391	135	727
24	394	140	742
25	397	145	757
26	400	150	772
27	403	155	787
28	406	160	802
29	409	165	817
30	412	170	832
31	415	175	847
32	418	180	862
33	421	185	877
34	424	190	892
35	427	195	907
40	442	200	922

\*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



## Procedure

### Important Information

Genotyping using this kit requires use of the appropriate fluorescent genetic analyzer instrument(s) and software capable of detection of fluorescently labeled fragments of varying lengths. This kit has been optimized for an ABI310 genetic analyzer.

### PCR Premix Preparation

Thaw individual components. *Promptly store at -20°C after use.* Prepare **fresh** before use enough PCR premix for the number of reactions to be performed. Prepare 10% more for pipetting allowance. Prepare premix following the volumes given below. Follow the same ratio for preparing other final volumes.

**Material Supplied:** Please see page 2 of this manual.

**PCR Thermal Cycler Files :** Prepare the following PCR thermal cycler files

Hot Start File	
Step	Time and Temperature
Denaturation	5 minutes at 94°C
Hold	62°C

FRDA Amplification File		
Step	Time and Temperature	Cycles
Denaturation	30 seconds at 94°C	30 Cycles
Annealing	30 seconds at 60°C	
Extension	3 minute at 72°C	
Fillup	7 minutes at 72°C	1 Cycle
Hold	Hold for infinity at 4°C	Hold for infinity

### PCR Amplification

#### A. PCR premix preparation

Given below is a protocol for preparing a PCR premix for 25 µL reactions. Prepare 10% more for pipetting allowance This can be scaled up as required.

PCR Premix Preparation			
Component	1 x 25 µL rxn.	10 x 25 µL rxns.	Worksheet
FRDA GScan™ V2 Component FM	8 µL	80 µL	
GScan™ Component E	7 µL	70 µL	
GScan™ Component D	1 µL	10 µL	
Sterile Water	9 µL	90 µL	
<b>Total</b>	<b>25 µL</b>	<b>250 µL</b>	

#### B. Enzyme premix preparation

Enzyme Mix Preparation			
Component	1 x 25 µL rxn.	10 x 25 µL rxns.	Worksheet
PCR premix (above)	3.0 µL	30 µL	
Taq. Polymerase	0.5 µL	3.5 µL	
<b>Total</b>	<b>3.5 µL</b>	<b>33.5 µL</b>	

### C. PCR reaction

'Hot Start' PCR

For each sample add the following

Hot Start PCR	
Component	Quantity
PCR premix (above)	21 $\mu$ L
DNA Template (~100ng chromosomal DNA)	1 $\mu$ L
<b>Total</b>	<b>22 <math>\mu</math>L</b>

**Start "Hot Start" file.**

After initial denaturation while thermal cycler is 'holding' at 60°C

**Add 3  $\mu$ L of Enzyme premix to each tube and start FRDA amplification PCR file.**

### E. Post PCR Processing

1. Process sample for [Omni-Clean](#) purification of amplified products. This purification method is preferred over Sephadex G-50 spin column chromatography or ethanol precipitation as it is the most effective way to reduce the fluorescent primers and shorter primer dimers if present. [Omni-Clean Kit](#) is available from Gene Link, catalog number 40-4130-10 or 40-4140-10.
2. Process sample for Sephadex G-50 spin column chromatography. G-50 columns should be equilibrated with sterile water.
3. Ethanol precipitate and dissolve pellet in 20  $\mu$ L sterile water. OR
4. Collected sample is ready for genetic analysis.

### F. Fragment Analysis

#### IMPORTANT NOTE

The expected amplified size range is 330 bp and above. A 200 GAA repeat sample will yield a ~922 bp fragment. Appropriate molecular weight size standards to span the expected region are required for approximate genotyping. The FRDA GScan™ Kit should not be used to interpret GAA repeat sizes above ~100 GAA repeats (600 bp fragment). Southern blot analysis is recommended for confirmation of large repeats. Gene Link non-radioactive digoxigenin labeled probe (40-2027-41) is recommended for Southern blot analysis.

Follow genetic analyzer manufacturer's instructions. The amplified fragments are labelled with 6-Fam as the fluorophore. Use appropriate filter set for detection of 6-Fam emission. Molecular weight markers preferable should be 500 Liz or other compatible dye set for particular instrument type.

Run two aliquots of sample at different concentrations. A brief protocol is given below for ABI3100 instrument.

Follow genetic analyzer manufacturer's instructions. Run two aliquots of sample at different concentrations. A brief protocol is given below for ABI3100 instrument.

1. Add 0.5  $\mu$ L of appropriate size standards.
2. Add 1 & 2  $\mu$ L of sample in duplicate tubes.
3. Add 12  $\mu$ L of formamide loading buffer.
4. Mix and heat denature at 95°C for 5 minutes.
5. Transfer to ice.
6. Samples ready to load on instrument.

## G. Pre-run GAA Control DNA Sample Analysis

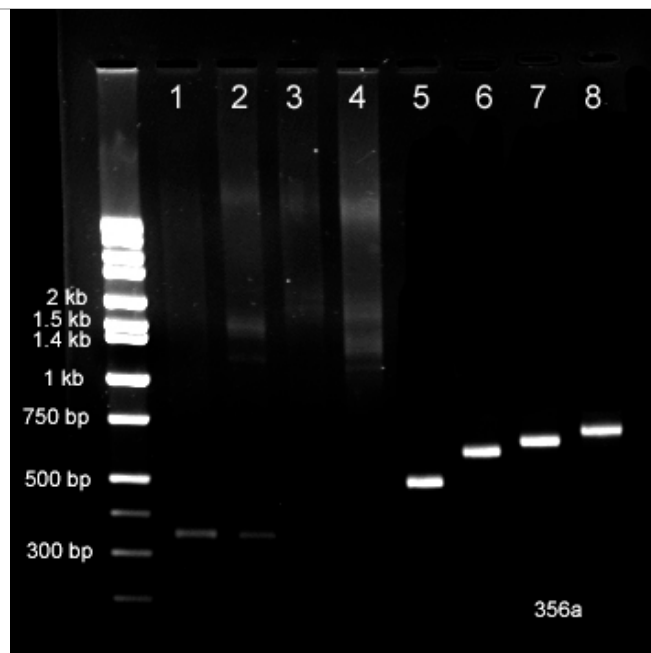
Due to their high GA content and the increased probability of secondary structure, fragments containing the GAA expansion tend to migrate faster during gel electrophoresis than corresponding molecular weight standards that usually have random GC contents. Furthermore, electrophoretic mobility increases with the size of the GAA expansion. To correct for the anomalous migration rate of the GAA fragments, a FRDA pre-run GScan Control DNA that contains 64 GAA repeats is provided with the kit. We recommend that, along with your sample DNA, a PCR reaction containing 1 µL of this the positive control DNA be included. The sample is provided in water. Follow genetic analyzer manufacturer's instructions relating to running samples, data recovery, and interpretation.

## Results and Interpretation

The results obtained from the genetic analyzer will approximately show the fragment size amplified, based on these results an interpretation can be made about the genotype of the sample. It is known that there is an overlap between the normal and FRDA allele sizes. The repeat sizes obtained falling in the overlap region should be preferably repeated and possibly run with more samples from other family members. Refer to the table 2 for determining the GAA repeats and fragment size expected using the primers provided in this kit. The formula for determining PCR fragment size with the amplification primer F1B/R3C is  $322 + 3n$ , where  $n$  = the number of GAA repeats.

Number of GAA repeats	Clinical Condition	Symptoms
5-30 repeats	Unaffected	Normal
34-40 repeats	Mild	Premutation
66-1800 repeats	Severe	Full mutation

### FRDA GAA Repeat Genotyping with Primer Set F1B/R3C\*



**Lanes 1-4 human genomic DNA samples.** Lane 1 normal FRDA DNA GAA repeat fragment sizes of ~335 bp (6 & 8 GAA repeats) ; lane 2 heterozygote FRDA DNA Coriell ID NA16213 of ~335 and 1579 bp fragments (6 GAA and 420 GAA repeats); lane 3 homozygote FRDA DNA Coriell ID NA 16203 of ~2239 and 2809 bp fragments (670 and 830 GAA repeats) and lane 4 homozygote FRDA DNA Coriell ID NA04079 of ~1339 and 1579 bp fragments (340 and 420 GAA repeats).

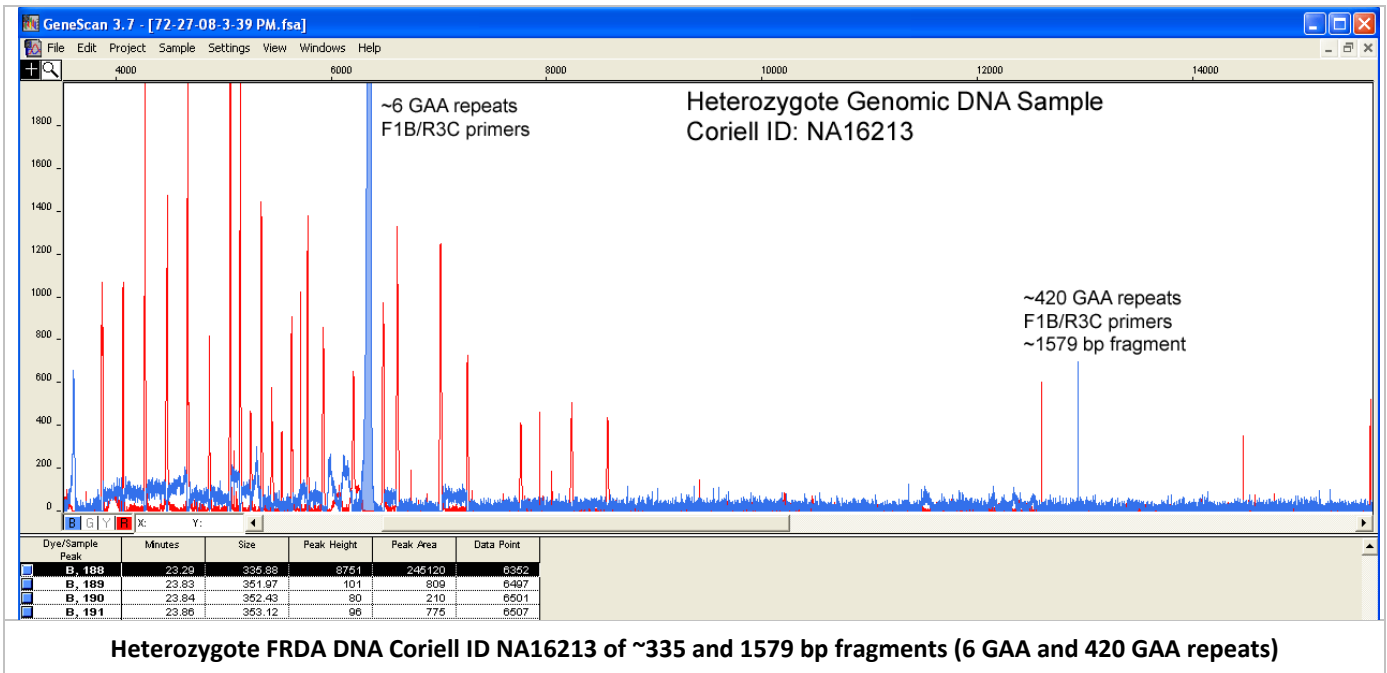
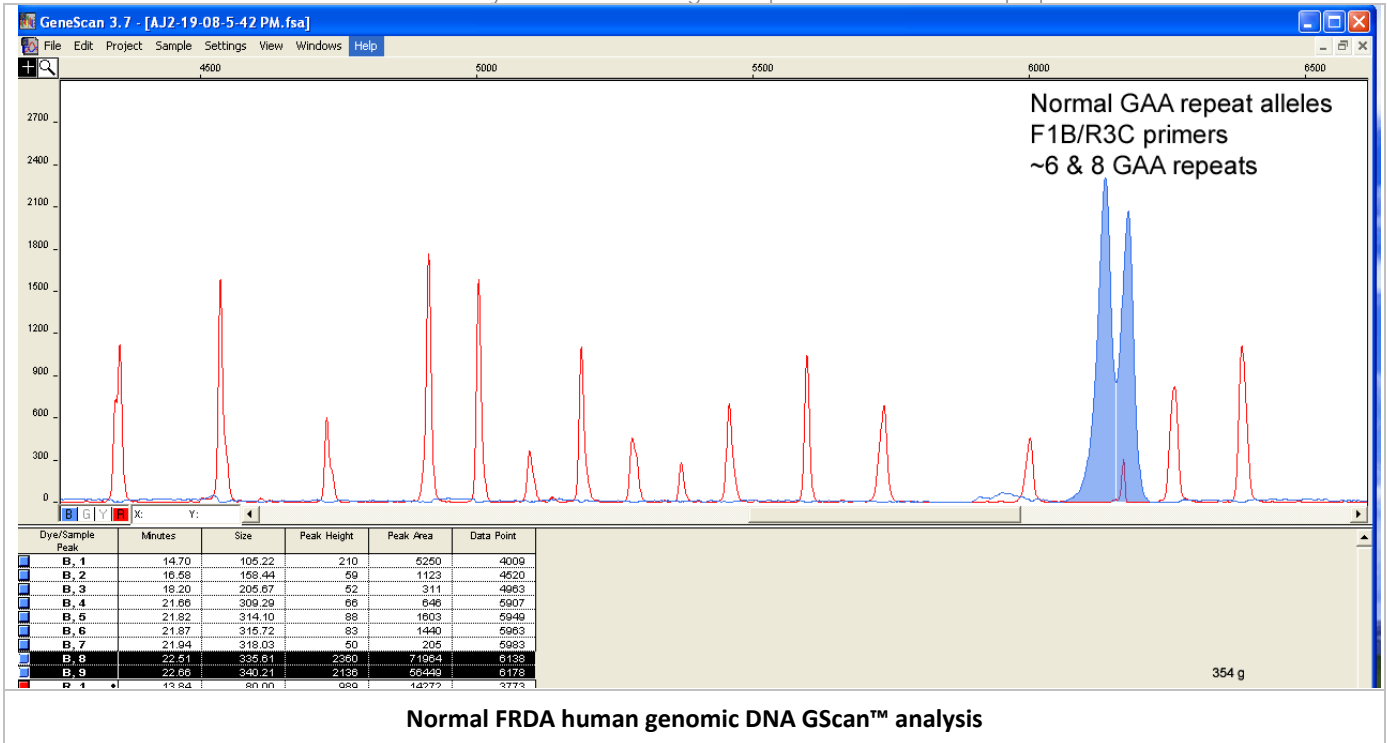
**Lanes 5-8 cloned Gene Link FRDA Genemer™ control DNA.**

Lane 5 (Catalog #: 40-2027-01) 64 GAA repeats ~514 bp fragment. Lane 6 (Catalog #: 40-2027-02) 102 GAA repeats ~628 bp fragment. Lane 7 (Catalog #: 40-2027-03) 110 GAA repeats ~652 bp fragment and lane 8 (Catalog #: 40-2027-04) 125 GAA repeats ~697 bp fragment.

\*1% agarose gel electrophoresis of FRDA GAA repeats genotyping with primer set F1B/R3C. Long GAA repeats are not amplified with high fidelity as discrete fragments and appears as a broad smear.

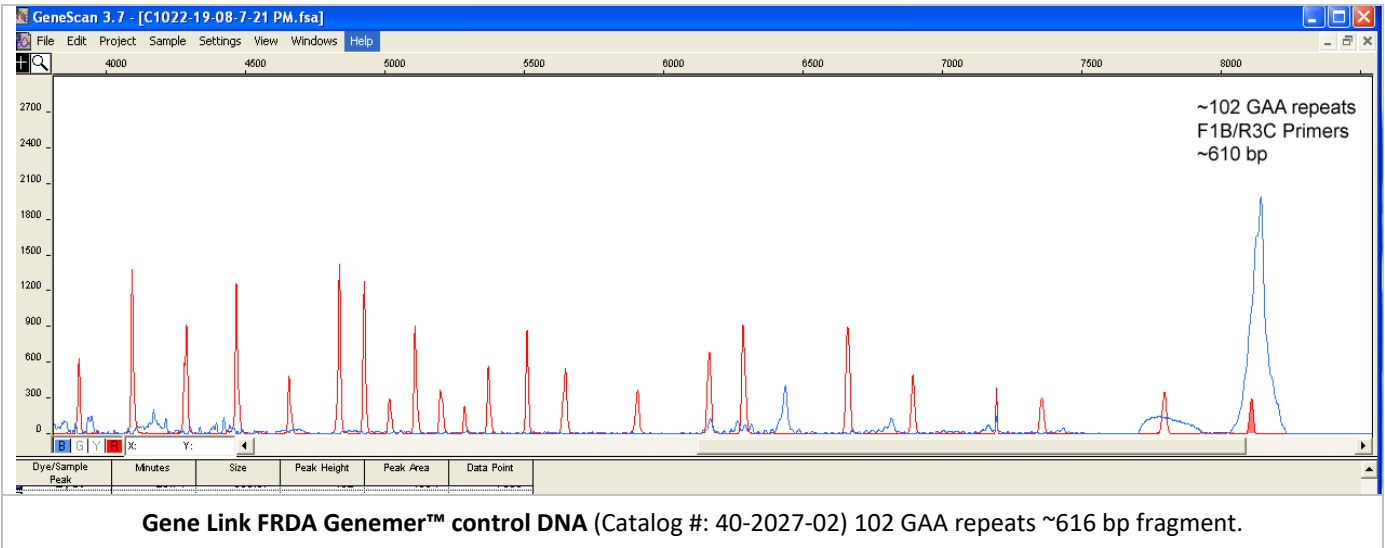
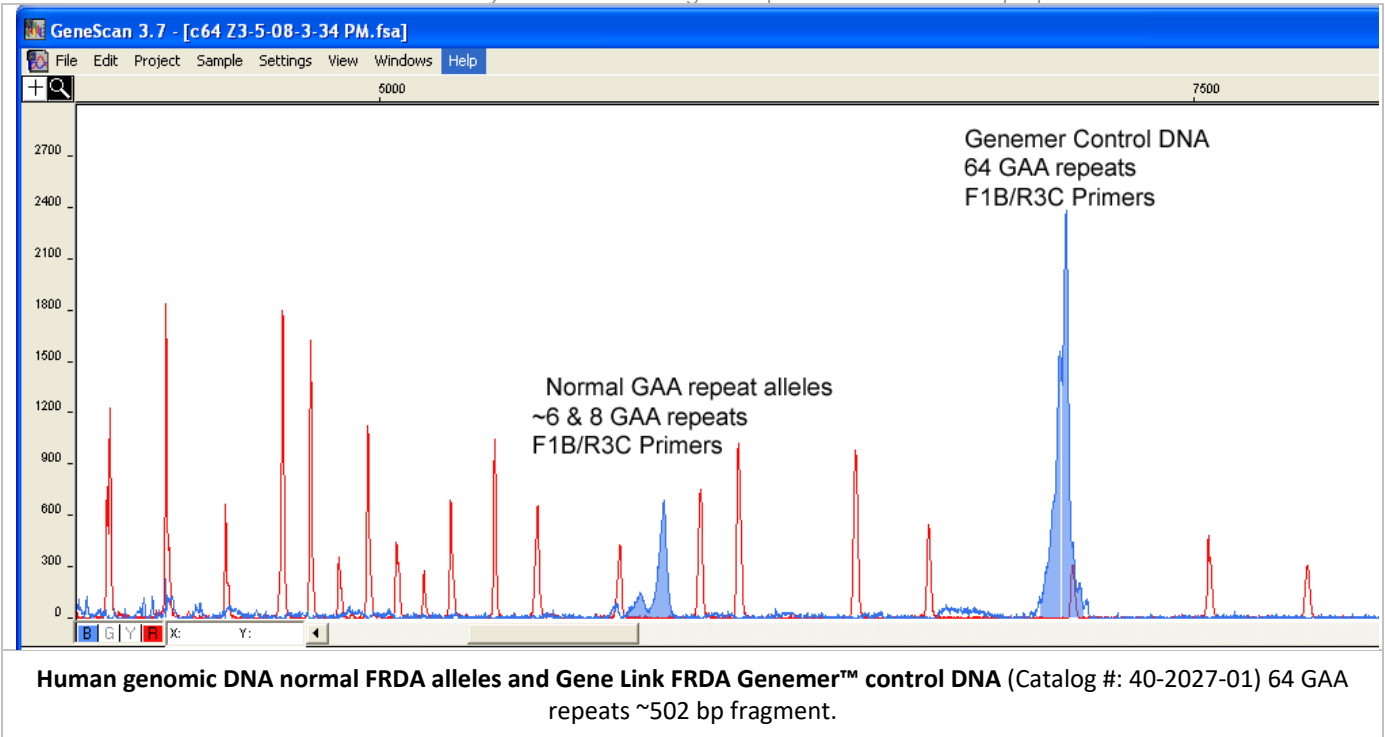
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## Appendix: Protocols

### 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.

## Friedreich's Ataxia Product Ordering Information

Product	Unit Size	Catalog No.
<b>Friedreich's Ataxia Genemer™ Primer pair</b> 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
<b>Friedreich's Ataxia Genemer™ Kit</b> 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
<b>Friedreich's Ataxia GScan™V2 Kit for fluorescent detection</b> 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]	40-2027-15
<b>Friedreich's Ataxia GeneProber™ FRDA-GL21 Probe unlabeled</b> Probe for radioactive labelling and Southern blot analysis	500 ng	40-2027-40
<b>Friedreich's Ataxia GeneProber™ FRDA-GLDig21 Probe Digoxigenin labeled</b> 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

All Gene Link products are for research use only

Current pricing are posted at <http://www.genelink.com/>

## Friedreich's Ataxia GScan™ V2 Kit. FRDA GAA Triple Repeat Fluorescent Genotyping

For research use only. Not for use in diagnostic procedures for clinical purposes.

### 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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Current pricing are posted at <http://www.genelink.com/>



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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