Product Sheet

Friedreich Ataxia

Genemer™ Control DNA*

***Specific control DNA for use with Gene Link Genemer™& GeneProber™ product lines.**

Catalog No. 40-2027-0X

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

Product		Catalog Number	Unit Size
☐	GLFRDA ~64 GAA repeat Genemer™ Control DNA	40-2027-01	500 ng
☐	GLFRDA ~102 GAA repeat Genemer™ Control DNA	40-2027-02	500 ng
☐	GLFRDA ~110 GAA repeat Genemer™ Control DNA	40-2027-03	500 ng
☐	GLFRDA ~125 GAA repeat Genemer™ Control DNA	40-2027-04	500 ng
☐	GLFRDA ~9 GAA repeat Genemer™ Control DNA	40-2027-05	500 ng

Background

Friedreich ataxia (FRDA) 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. Some of the 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.

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.

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 heterozygote 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 100 to more than 1000 units. Generally, the milder forms or late onset of the disease are associated with shorter expansions.

Genotyping

Molecular tests to confirm the diagnosis of FRDA and to detect carriers are usually performed by PCR amplification of the GAA repeat region, followed by agarose gel electrophoresis of the amplified fragments to determine their size. The FRDA Genemer (F1B/R3C) flanks the GAA repeat region. The formula for determining PCR fragment size using F1B/R3C primer set is 322 + 3n, where n= the number of GAA repeats.

Material Supplied

A tube containing 500ng of lyophilized control DNA segment. The above control DNA is an ideal genotyping template 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.
The quantity supplied is sufficient for 1000 regular 50μl PCR** reaction.

Reconstitution

Stock Solution: Add 100μl sterile water to the tube containing the lyophilized DNA to yield a solution of 5 ng/μl.
Working Solution: Dilute 1:10 an aliquot of the stock solution.

Usage: Initially use 1μl each of the stock and working template solution for amplification and optimization of the reaction. Perform further dilution if necessary. Based on the results, use 1μl of template at the lowest concentration.

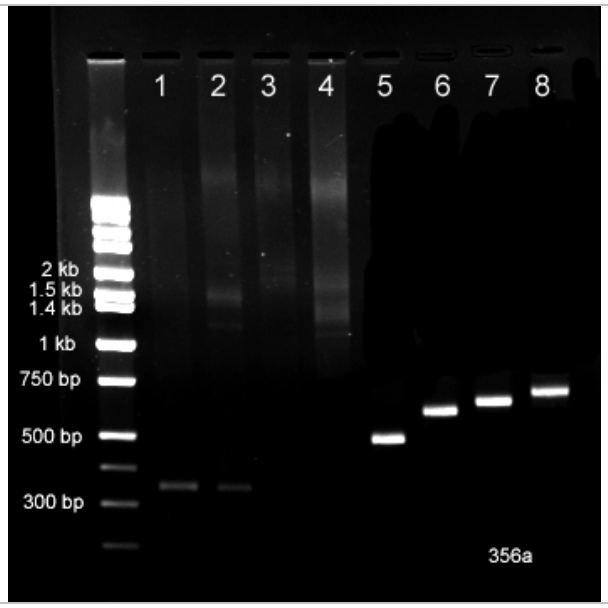
Protocol for PCR Analysis of Triple Repeat Size
Follow protocol supplied for the appropriate Genemer™ or GeneScan™ product.

Results and Interpretation

The results obtained after electrophoretic separation using gels or 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. 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
200-900 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 ~502 bp fragment. Lane 6 (Catalog #: 40-2027-02) 102 GAA repeats ~616 bp fragment. Lane 7 (Catalog #: 40-2027-03) 110 GAA repeats ~640 bp fragment and lane 8 (Catalog #: 40-2027-04) 125 GAA repeats ~685 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 appear as a broad smear.**

References:

1. Campuzano, V. et al. (1996) Science 271: 1423-7.
2. Monrós, E. et al. (1997) Am. J. Hum. Genet. 61: 101-110.
3. Castro, M. et al. (2000) Hum. Genet. 106: 86-92.

GScan™ Kits Product Ordering Information

Kits 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.

Product	Size	Catalog No.	Price, $
Fragile X GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2004-15XX	650.00
Fragile X GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2004-15FMS	250.00
Huntington's Disease GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15XX	650.00
Huntington's Disease GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2025-15FMS	250.00
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2026-15XX	650.00
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2026-15FMS	250.00
Friedreich's Ataxia GScan™Kit for fluorescent detection; 100 reactions kit	1 kit	40-2027-15XX	650.00
Friedreich's Ataxia GScan™Kit for fluorescent detection; 20 reactions kit	1 kit	40-2027-15FMS	250.00
Kennedy Disease GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2032-15XX	650.00
Kennedy Disease GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2032-15FMS	250.00

Freidreich Ataxia Product Ordering Information

Product	Size	Catalog No.	Price, $
Friedreich Ataxia Genemer™ Primer pair 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	$100.00
Friedreich Ataxia GeneProber™ GLFRDA Probe unlabeled Friedreich Ataxia GAA triple repeat spanning region unlabeled probe for radioactive labeling and Southern blot detection. Suitable for random primer labeling.	500 ng	40-2027-40	$350.00
Friedreich Ataxia GeneProber™ GLFRDA Probe Digoxigenin labeled Friedreich Ataxia GAA triple repeat spanning region digoxigenin labeled probe for Southern blot non-radioactive detection.	110 µl	40-2027-41	$425.00
Friedreich Ataxia PCRProber ™ AP labeled probe Alkaline phosphatase labeled probe	12 µl	40-2027-31	$400.00
Friedreich Ataxia PCRProber ™ Kit. Kit for performing non-radioactive PCR amplification based detection. 5 blots (50 rxns)	5 blots	40-2027-32	$650.00

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	175.00
GLFRDA ~102 GAA repeat Genemer Control DNA	500 ng	40-2027-02	175.00
GLFRDA ~110 GAA repeat Genemer Control DNA	500 ng	40-2027-03	175.00
GLFRDA ~125 GAA repeat Genemer Control DNA	500 ng	40-2027-04	175.00
GLFRDA ~9 GAA repeat Genemer Control DNA	500 ng	40-2027-05	175.00

Please visit www.genelink.com for other Genemer™ control DNA not listed here

Genemer™ Control DNA (Selected List) Control DNA for use with gene or mutation specific Genemer™

Product	Size	Catalog No.	Price, $
Fragile X, various CGG triple repeat region control DNA	500 ng	40-2004-XX	175.00
Huntington Disease various CAG triple repeat region control DNA	500 ng	40-2025-XX	175.00
Myotonic Dystrophy various CTG triple repeat region control DNA	500 ng	40-2026-XX	175.00
Friedreich's Ataxia, various GAA triple repeat region control DNA	500 ng	40-2027-XX	175.00

***Please visit www.genelink.com for other Genemer™ not listed here**

**The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.

Prices subject to change without notice All Gene Link products are for research use only