Product Specification

Fragile X Genemer™ Kit

For PCR amplification of the Fragile X CGG triple repeat region*
*Special optimized conditions required for amplification

Catalog No. 40-2004-11 1 Kit Shipped at ambient temperature. Store at -20°C

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

Background

Fragile X syndrome is the most common form of inherited mental retardation. It affects approximately 1 in 1200 males and 1 in 2500 females. As suggested by the name, it is associated with a fragile site under specific cytogenetic laboratory conditions at position Xq27.3 (1).

The inheritance patterns of fragile X puzzled geneticists, as it did not follow a clear X linked pattern. Approximately 20% of males who are carriers based on pedigree analysis do not manifest any clinical symptoms and are thus termed as Normal Transmitting Males (NTM), mental retardation is rare among the daughters of male carriers. Approximately 35% of female carriers have some mental impairment. Based on the above it has been proposed that there are two states of the mutation, one mutation range in which there is no clinical expression (premutation), which could change to the disease causing state predominantly when transmitted by a female (full mutation)(2).

The fragile X syndrome gene (FMR-1, fragile X mental retardation -1) was cloned in 1991 simultaneously by three groups (3-6). Soon the peculiar genetic mode of transmission was established and a new class of mutation came into existence- Triple repeat amplification. This explained the clinical state of 'premutation' and 'full mutation' as well as 'anticipation'. The fragile X syndrome is caused by the amplification of CGG repeats that is located in the 5' region of the cDNA. The most common allele in the normal population consists of 29 repeats, the range varying from 6 to 54 repeats. Premutations in fragile X families showing no phenotypic effect range in size from 52 to over 200 repeats. All alleles with greater than 52 repeats are meiotically unstable with a mutation frequency of one. In general repeats up to 45 are considered normal; repeats above 50 to 200 are considered as premutation and above 200 as full mutation (3-7). The range between 40-55 is considered even by most experienced clinical geneticists and molecular geneticists very difficult to interpret and is considered as a 'gray zone' with interpretations made on a case-by-case basis (8).

Genotyping

Fragile X genotyping can be done by direct PCR amplification of the CGG triple repeat region or by southern analysis. In most cases both methods are used to complement the results, full mutations usually cannot be identified by PCR by most investigators and southern analysis is the preferred method to distinguish full mutations. The FMR-1 gene region containing the CGG triple repeat is flanked by Eco RI sites and a Eag I site in the region. Full mutation has been shown to methylate the active gene too and thus it prevents Eag I restriction of DNA. Hybridization of southern blots of Eco RI and Eag I double digested DNA clearly can distinguish between normal, premutation and full mutation genotypes (2).

Southern analysis *can not* determine the exact number of repeats or the identification of genotypes corresponding to the 'gray zone'.

Triple Repeat Size Analysis

Important Note: PCR* amplification of the CGG triple repeat region is not amplified using regular PCR reaction conditions due to the long stretch of CGG in the target amplification fragment. The inclusion of deaza GTP considerably overcomes this limitation. Long expansion of the CGG repeat on some DNA sample may still fail to amplify. Proper optimization needs to be carried out for such DNA samples. PCR amplification can be achieved by direct label incorporation of ^{35}S or ^{33}P dATP during PCR or by using ^{32}P end labeled primers.

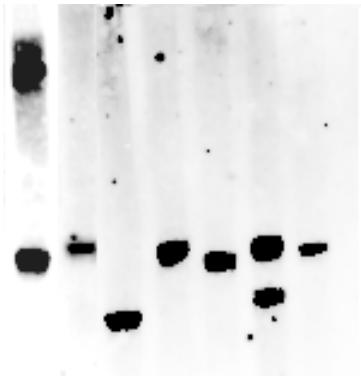
Initial screening of samples can be performed by PCR using Gene Link's PCRProber™ non-radioactive detection system (Catalog Number 40-2004-32). Only those samples whose genotype can not be conclusively established should be processed for Southern blot analysis. Gene Link recommends the use of non-radioactive Fragile X GeneProber™ gene detection system (Catalog Number 40-2004-41).

Product Components

40-2004-10 Fragile X Genemer™ 10 nmole; lyophilized.
40-2004-02 Fragile X Genemer™ Control DNA 500 ng; lyophilized.
Please refer to enclosed product specifications sheet.

40-2004-10 Fragile X Genemer™ 10 nmole; lyophilized
This product contains one tube containing 10 nmole of forward and reverse lyophilized primer. The quantity supplied is sufficient for 400 regular 50µl PCR reaction. The 10 nmole of primer when dissolved in 50µl water will give a solution of 200 µMolar i.e. 200 pmole/µl.

Gene Link recommends the use of non-radioactive gene detection systems.



Fragile X PCR blot. Lane 1 pre-mutation female; 30/60 CGG repeats.
Non-radioactive detection, ~2 hr. exposure.

Fragile X CGG repeat interpretation

Normal Male/Female	**6-40**
Female Carrier with small amplification	**41-70**
Carrier Male (NTM)	**41-200**
Full mutation Male/Female	**>200**

References

1. Nelson, D.L. (1993) Growth Genetics and Hormone. 9:1-4.
2. Rousseau, F. et al. (1991) NEJM 325:1673-1681.
3. Verkerk, A. et al. (1991) Cell 65:905-914
4. Fu, Y.H et al. (1991) Cell 67:1047-1058.
5. Oberle, I. et al. (1991) Science 252:1097-1102.
6. Yu, S. et al. (1991) Science 252: 1179-1181.
7. Nelson, D.L. (1996) Growth Genetics and Hormone. 12:1-4.
8. Richards, R and Sutherland, G.R (1992) TIG 8: 249-255.

Product Specification

Fragile X Genemer™ Control DNA

For PCR amplification of the Fragile X CGG triple repeat region**
*Special optimized conditions required for amplification

Shipped at ambient temperature Store at -20°C

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Product Supplied	Catalog Number	Product Description	Size
❑	40-2004-01	GLFX ~16 CGG repeat Genemer Control DNA	500 ng
❑	40-2004-02	GLFX ~29 CGG repeat Genemer Control DNA	500 ng
❑	40-2004-03	GLFX ~40 CGG repeat Genemer Control DNA	500 ng
❑	40-2004-04	GLFX ~60 CGG repeat Genemer Control DNA	500 ng
❑	40-2004-05	GLFX ~90 CGG repeat Genemer Control DNA	500 ng

**Important Product Description & Use Limitation

The Fragile X CGG repeat region control DNA's are cloned products. These have been developed by inserting varying number of CGG repeats to serve as control DNA specifically for use with the Fragile X Genemer™ detection system. The above control DNA is an ideal genotyping template for optimizing and performing control amplification.

The size of the CGG 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 CGG repeats. These control DNA's are sold with the express condition that these NOT be used for exact CGG size determination of DNA of unknown genotype. The control DNA should be used for determining the performance of specific Gene Link products listed below.

Fragile X PCRProber™ Kit Catalog No. 40-2004-32
Fragile X Genemer™ Catalog No. 40-2004-10
Fragile X GScan™ Kit Catalog No. 40-2004-15XX

Material Supplied

A tube containing 500 ng of lyophilized DNA segment of the specified CGG repeat fragment spanning the CGG repeat . The quantity supplied is sufficient for 1000 regular 50µl PCR** reaction.

Reconstitution

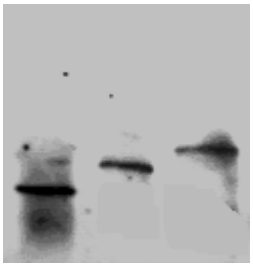
Stock Solution: Add 100µl sterile water to the tube containing the lyophilized DNA to yield a solution of 5 ng/µl.
Working Solution: Dilute 1:10 an aliquot of the stock solution.
Usage: Initially use 1µl each of the stock and working template solution for amplification and optimization of the reaction. Dilute further based on results obtained. Use 1µl of template at the lowest concentration.

Product Use

This product is specifically for use with products listed. Please refer to the Instruction Manual provided with the product. The instruction manuals are available online at www.genelink.com

Triple Repeat Size Analysis

Important Note: PCR** amplification of the CGG triple repeat region is not amplified using regular PCR reaction conditions due to the long stretch of CGG in the target amplification fragment. The inclusion of deaza GTP considerably overcomes this limitation. Long expansion of the CGG repeat on some DNA sample may still fail to amplify. Proper optimization needs to be carried out for such DNA samples. PCR amplification can also be achieved by direct label incorporation of ^{35}S or ^{33}P dATP during PCR or by using ^{32}P end labeled primers.



Fragile X PCR blot. CGG repeats control DNA
Lane 1, 16 CGG repeats; lane 2, 29 CGG and lane 3, 40 CGG repeats
Chemiluminescent detection, ~20 minute exposure.

Fragile X Product Ordering Information

Product	Size	Catalog No.
Fragile X Genemer™ Primer pair Primers for amplification of CGG triple repeat spanning region. The quantity supplied is sufficient for 400 regular 50 µl PCR reactions.	10 nmole	40-2004-10
Fragile X GeneProber™ GLFX1 Probe unlabeled Fragile X CGG triple repeat spanning region unlabeled probe for radioactive labeling and Southern blot detection. Suitable for random primer labeling.	500 ng	40-2004-40
Fragile X GeneProber™ GLFXDig1 Probe Digoxigenin labeled Fragile X CGG triple repeat spanning region digoxigenin labeled probe for non-radioactive Southern blot detection.	110 µl	40-2004-41
Fragile X PCRProber ™ AP labeled probe Alkaline phosphatase labeled probe	12 µl	40-2004-31
Fragile X PCRProber ™ Kit for chemiluminescent detection Kit for performing PCR amplification and chemiluminescent based detection.	5 blots [50 rxns]	40-2004-32
Fragile X Genemer™ Kit for Radioactive Detection Kit for amplification and radioactive detection of Fragile X CGG triple repeat region amplified PCR products using ^{35}S or ^{32}P. 100 Reactions.	100 [rxns]	40-2004-20
Fragile X GScan™ Kit 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]	40-2004-15XX

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.

Fragile X ~16 CGG repeat Genemer Control DNA	500 ng	40-2004-01
Fragile X ~29 CGG repeat Genemer Control DNA	500 ng	40-2004-02
Fragile X ~40 CGG repeat Genemer Control DNA	500 ng	40-2004-03
Fragile X ~60 CGG repeat Genemer Control DNA	500 ng	40-2004-04
Fragile X ~90 CGG repeat Genemer Control DNA	500 ng	40-2004-05

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

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

GeneProber™ 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, 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.
Fragile X GeneProber™ GLFX1 Probe unlabeled	500 ng	40-2004-40
Fragile X GeneProber™ GLFXDig1 Probe Digoxigenin labeled	110 µl	40-2004-41
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™ Products Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data. The kits contain optimized PCR amplification reagents and a wide array of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument(s). 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, except Fragile X. The CGG repeat in Fragile X can be detected up to ~50 repeats.

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Product	Unit Size	Catalog No.
Fragile X GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2004-15XX
Fragile X GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2004-15FMS
Huntington's Disease GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15XX
Huntington's Disease GScan™ 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

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Southern Blot Buffers& Reagents

Product	Catalog No.	Unit Size
Agarose Tablets, 0.5 gm each	40-3011-10	100 tablets
Agarose LE Molecular Biology Grade; 100 gms	40-3010-10	100 gms
Agarose LE Molecular Biology Grade; 500 gms	40-3010-50	500 gms
Hybwash A, Hybridization Wash Solution (20X SSC)	40-5020-20	200 mL
Hybwash B, Hybridization Wash Solution (10% SDS)	40-5021-10	100 mL
TAE Buffer; 50 X Concentrate; 100 ml	40-3007-01	100 mL
TAE Buffer; 50 X Concentrate; 1000 ml	40-3007-10	1000 mL
TBE Buffer; 5 X Concentrate	40-3008-10	1000 mL
Buffer M 10X (Maleic Acid buffer)	40-5025-10	100 mL
10% Blocking solution	40-5026-10	100 mL
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-10	1 mL
10x AP Detection buffer (alkaline phosphatase detection buffer)	40-5031-10	100 mL
Lumisol™ I Hybridization Solution; contains formamide	40-5022-20	200 mL
Lumisol™ II Hybridization Solution; for non-toxic hybridizations	40-5023-20	200 mL
Lumisol™ III Hybridization Solution; for oligo probes	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	40-3002-10	1 mL
Gel Loading Buffer 5X BPB/XC non-denaturing	40-3002-15	15 mL
Gel Loading Buffer 10X BPB/XC non-denaturing	40-3003-10	1 mL
Gel Loading Buffer 10X BPB/XC non-denaturing	40-3003-15	15 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing	40-3004-10	1 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing	40-3004-15	15 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-10	1 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-15	15 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer	40-5028-10	1 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer	40-5028-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide	40-5029-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide	40-5029-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide	40-5030-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide	40-5030-15	15 mL

Omni-Marker™

Product	Catalog No.	Size
Omni-Marker™ Universal unlabeled	40-3005-10	1 mL
Omni- Marker™ Low unlabeled	40-3006-10	1 mL
Omni-Marker™ GScan™-2 Tamra labeled 50 bp - 600 bp	40-3062-05	500 µL

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-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.

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.

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