



## Product Specification

### Fragile X Genemer™

For PCR amplification of the Fragile X CGG triple repeat region\*

\*Special optimized conditions required for amplification

Catalog No. 40-2004-10      10 nmole      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 pattern 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

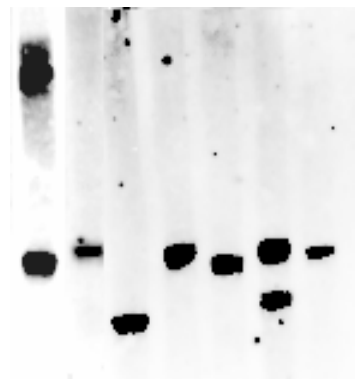
**Important Note:** PCR\* amplification of the CGG triple repeat region is not amplified using regular PCR reaction conditions due to the long stretch of CGG in the target amplification fragment. The inclusion of deaza GTP considerably overcomes this limitation. Long expansion of the CGG repeat on some 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 <sup>35</sup>S or <sup>33</sup>P dATP during PCR or by using <sup>32</sup>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

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 Gen. and Hormone. 12:1-4.
- Richards, R and Sutherland, G.R (1992) TIG 8: 249-255.

## 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, \$
<b>Fragile X GScan™ Kit</b> for fluorescent detection	1 kit	40-2004-15XX	650.00
<b>Huntington's Disease GScan™ Kit</b> for fluorescent detection	1 kit	40-2025-15XX	650.00
<b>Myotonic Dystrophy GScan™ Kit</b> for fluorescent detection	1 kit	40-2026-15XX	650.00
<b>Friedreich's Ataxia GScan™ Kit</b> for fluorescent detection	1 kit	40-2027-15XX	650.00
<b>Kennedy Disease GScan™ Kit</b> for fluorescent detection	1 kit	40-2032-15XX	650.00
<b>SCA 1 GScan™ Kit</b> for fluorescent detection	1 kit	40-2037-15XX	650.00
<b>SCA 2 GScan™ Kit</b> for fluorescent detection	1 kit	40-2038-15XX	650.00
<b>SCA 3 GScan™ Kit</b> for fluorescent detection	1 kit	40-2039-15XX	650.00
<b>SCA 6 GScan™ Kit</b> for fluorescent detection	1 kit	40-2040-15XX	650.00
<b>SCA 7 GScan™ Kit</b> for fluorescent detection	1 kit	40-2041-15XX	650.00
<b>DRPLA GScan™ Kit</b> for fluorescent detection	1 kit	40-2042-15XX	650.00

## Fragile X Product Ordering Information

Product	Size	Catalog No.	Price, \$
<b>Fragile X Genemer™ Primer pair</b> 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	100.00
<b>Fragile X GeneProber™ GLFX1 Probe unlabeled</b> 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	350.00
<b>Fragile X GeneProber™ GLFXDig1 Probe Digoxigenin labeled</b> Fragile X CGG triple repeat spanning region digoxigenin labeled probe for non-radioactive Southern blot detection.	110 µl	40-2004-41	400.00
<b>Fragile X PCRProber™ AP labeled probe</b> Alkaline phosphatase labeled probe	12 µl	40-2004-31	400.00
<b>Fragile X PCRProber™ Kit for chemiluminescent detection</b> Kit for performing PCR amplification and chemiluminescent based detection.	5 blots [50 rxns]	40-2004-32	650.00
<b>Fragile X Genemer™ Kit for Radioactive Detection</b> Kit for amplification and radioactive detection of Fragile X CGG triple repeat region amplified PCR products using <sup>35</sup> S or <sup>32</sup> P. 100 Reactions.	100 [rxns]	40-2004-20	650.00
<b>Fragile X GScan™ 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]	40-2004-15XX	650.00
<b>Genemer™ control DNA</b> 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	175.00
Fragile X ~29 CGG repeat Genemer Control DNA	500 ng	40-2004-02	175.00
Fragile X ~40 CGG repeat Genemer Control DNA	500 ng	40-2004-03	175.00
Fragile X ~60 CGG repeat Genemer Control DNA	500 ng	40-2004-04	175.00
Fragile X ~90 CGG repeat Genemer Control DNA	500 ng	40-2004-05	175.00

Please visit [www.genelink.com](http://www.genelink.com) for other Genemer™ control DNA not listed here

<b>Genemer™ Control DNA (Selected List)</b> 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](http://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.  
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