# Technical Sheet

# Non-radioactive Fragile X CGG repeat detection by PCR

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|---|---|---|---|---|
| ❑ | **Genemer™** | **GLFX1C/GLFX1F** | **Catalog No.: 40-2004-10** | **10 nmoles** |

**Fragile X CGG repeat spanning region amplification primers**

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| ❑ | **GeneProber™** | **GLFXPCRprober** | **Catalog No.: 40-3101-01** | **12µl** |

**Fragile X CGG repeat probe for non-radioactive detection**

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| ❑ | **GeneProber™** | **GLFXPCRprober Kit** | **Catalog No.: 40-3102-00** | **5 blots** |

**Kit for performing Fragile X CGG repeat spanning region PCR and probe for non-radioactive detection**

**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 repeat which 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).

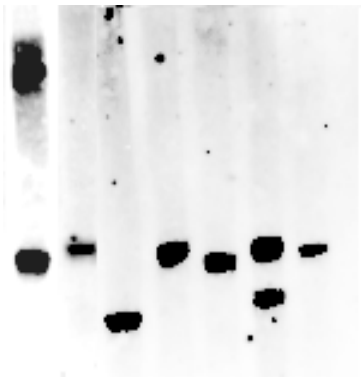
## Genotyping

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' .

**CGG Repeat Analysis by PCR**

The size of the CGG repeat 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. Alkaline phosphatase labeled CGG repeat specific probe and chemiluminescent detection. This yields reliable result within 2-3 hrs. Gene Link probes are developed for rapid and reliable non-radioactive genotyping.



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

### Fragile X CGG repeat interpretation

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|---|---|
| **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.
8. Richards, R and Sutherland, G.R (1992) TIG 8: 249-255.




*Prices subject to change without notice*

**All Gene Link products are for research use only.**


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*Appendix*

**Non-radioactive Fragile X CGG repeat detection by PCR**

- ❑ **Genemer™ GLFX1C/GLFX1F Catalog No.: 40-2004-10**
- ❑ **GeneProber™ GLFXPCRprober Catalog No.: 40-3101-01**
- ❑ **GeneProber™ GLFXPCRprober Kit Catalog No.: 40-3102-00**

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❑ **Genemer™ GLFX1C/GLFX1F Catalog No.: 40-2004-10**

Material Supplied: 10 nmoles. Sufficient for 100 reactions

❑ **GeneProber™ GLFXPCRprober Catalog No.: 40-3101-01**

Material Supplied: 12µl Sufficient for five 16x16 cm blot

❑ **GeneProber™ GLFXPCRprober Kit Catalog No.: 40-3102-00**

Material Supplied

**Genemer™ GLFX1C/GLFX1F Catalog No.: 40-2004-10**

**GeneProber™ GLFXPCRprober Catalog No.: 40-3101-01**

**PCR Premix**

**Hybridization Reagents and Wash solutions**

**Detection Reagents**

**Detailed protocol included with product. Call Gene Link Technical service for more information**.

1-800-Gene Link (1-800-436-3546)

## Ordering Information

***Fragile X Genemer™ and GeneProber™ products.***

| Product | Size | Catalog No. | Price, $ |
|---|---|---|---|
| **GLFX1 GeneProber™** Probe spanning the Fragile X CGG triple repeat region. Suitable for random primer labeling for southern analysis. | 500ng | 40-3201-01 (old number 40-2015-10) | 350.00 |
| **GLFXDig1 GeneProber™** Digoxigenin labeled probe spanning the Fragile X CGG triple repeat region. Ready to use for non-radioactive southern analysis. | 110µl | 40-3202-01 | 400.00 |
| **GLFX PCRprober™** Probe for non-radioactive detection of Fragile X CGG triple repeat region amplified PCR products. Contains Probe only. | 5 blots | 40-3101-01 | 400.00 |
| **GLFX PCRprober™ Kit** Kit for amplification and **non-radioactive** detection of Fragile X CGG triple repeat region amplified PCR products. | 5 blots | 40-3102-00 | 650.00 |
| **GLFX Genemer™ Kit** Kit for amplification and **radioactive** detection of Fragile X CGG triple repeat region amplified PCR products using $^{35}S$ or $^{32}P$. | 100 reactions | 40-3103-00 | 650.00 |
| **GLFX Genemer™** Primers for Fragile X CGG triple repeat region amplification. Contains primers only. | 10 nmoles | 40-2004-10 | 100.00 |

**GENEMER™**

| Product | Size | Catalog No. | Price, $ |
|---|---|---|---|
| Sickle Cell SC2/SC5 primer pair | 10nmoles | 40-2001-10 | 100.00 |
| RhD (Rh D gene exon 10 specific) | 10nmoles | 40-2002-10 | 100.00 |
| Rh EeCc (Rh Ee and Cc exon 7 specific) | 10nmoles | 40-2003-10 | 100.00 |
| Fragile X (spanning triple repeat region) | 10nmoles | 40-2004-10 | 100.00 |
| Gaucher 1226G mutation specific | 10nmoles | 40-2005-10 | 100.00 |
| Gaucher 1448C mutation specific | 10nmoles | 40-2006-10 | 100.00 |
| Gaucher 84GG mutation specific | 10nmoles | 40-2007-10 | 100.00 |
| Gaucher IVS2 mutation specific | 10nmoles | 40-2008-10 | 100.00 |
| Cystic Fibrosis ΔF508 | 10nmoles | 40-2009-10 | 100.00 |
| Cystic Fibrosis G542X | 10nmoles | 40-2010-10 | 100.00 |
| Cystic Fibrosis W1282X | 10nmoles | 40-2011-10 | 100.00 |
| Cystic Fibrosis G551D/R553X | 10nmoles | 40-2012-10 | 100.00 |
| Cystic Fibrosis N1303K | 10nmoles | 40-2013-10 | 100.00 |
| Cystic FibrosisCT3849 | 10nmoles | 40-2014-10 | 100.00 |
| SRY (sex determining region on Y) | 10nmoles | 40-2020-10 | 100.00 |
| X alphoid repeat | 10nmoles | 40-2021-10 | 100.00 |
| Y alphoid repeat | 10nmoles | 40-2022-10 | 100.00 |

**Please inquire about other GENEMER™ not listed here**

Revised 10/27/1997

**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*

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