Technical Sheet

GeneProber™ GLFX1

Catalog Number: 40-3201-01 500 ng

(old catalog number 40-2015-10)

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).

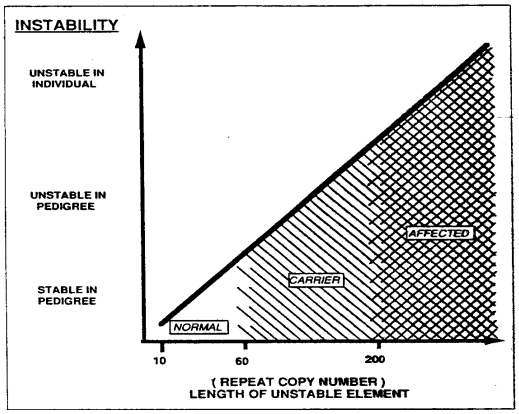
The inheritance pattern of fragile X puzzled geneticists as it did not follow a clear X linked pattern. Approximately 20% of males who are carriers based on pedigree analysis do not manifest any clinical symptoms and are thus termed as Normal Transmitting Males (NTM), mental retardation is rare among 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).

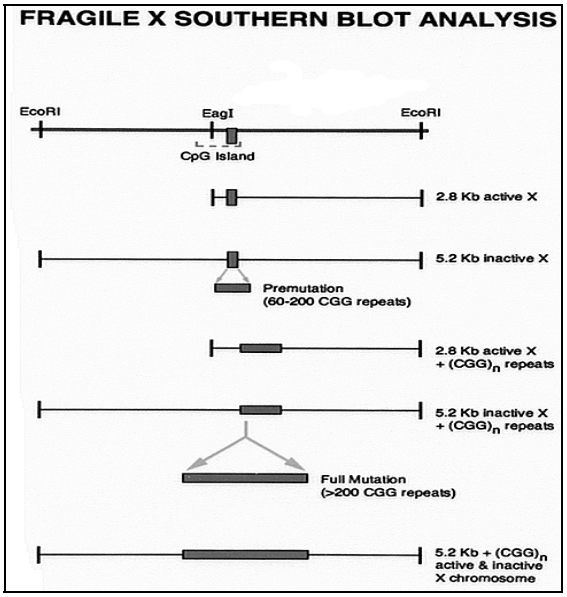
The fragile X syndrome gene (FMR-1, fragile X mental retardation -1) was cloned in 1991 simultaneously by three groups (3-6). Soon the peculiar genetic mode of transmission was established and a new class of mutation came into existence- Triple repeat amplification. This explained the clinical state 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'.





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.

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

Gene Link™

140 Old Saw Mill River Road Hawthorne, NY 10532
Tel: 914.769.1192 www.genelink.com Fax: 914.769.1193

Material Supplied

One tube containing 500 ng of lyophilized *GeneProber™* GLFX1 probe. The DNA probe is stable in dried state for extended period at room temperature. Upon reconstitution it should be stored at -20 °C. The quantity supplied is sufficient for at least 5 random prime labeling reactions using 100ng for each reaction.

Fragile X Southern Protocol

1. DNA digestion :

DNA	10 µg
10x Eag I Buffer	10 µl
10x EcoR I Buffer	10 µl
Eag I (10 U/µl)	4 µl
EcoR I(10 U/µl)	8 µl

	H_2O up to 100 µl

Precipitate the digests after over night digestion at 37°C, dissolve the pellets in 10 µl of 1x Loading buffer .

2 Load samples to 0.8% agarose gel , run over night at 45mA for ~24 hours. (1.6 kb fragment on the bottom of the gel).

3. Depurinate with 0.25N HCl (add 10 ml HCl to 500ml H_2O) for 10 minutes, denature the DNA with 0.4N NaOH/0.6M NaCl for 30 min. at RT, neutralize with 1.5M NaCl/0.5M Tris (pH 7.5) for 30 min. at RT, transfer to the MagnaCharge Nylon membrane (MSI) by 10xSSC and 10 pieces of SIGMA QuickDraw blotting paper over night. Wash the membrane with 2x SSC, bake it at 80°C for 2 hours.

4. Perform prehybridization at 50°C for 3 hours in 10 ml of hybrisol I buffer (Oncor) .

5. Label the probe as following: (BM Random Primer DNA Labeling Kit)

	GLFX1 GeneProber™ 25 -100 ng	
	H_2O	up to 9 µl
	Boil 10 minutes, and put on ice.	
Add :	Reaction mix	2 µl
	dNTP w/o dCTP	3 µl
	α^{32}PdCTP (3000 Ci/mmol)	5 µl (50 µCi)
	Klenow (2 U/µl)	1 µl

		Total 20 µl

Incubate at 37°C for 30 minutes. Add 500 µl of 5 x SSC to the reaction tube, boil 5 minutes, then add to the 50 ml Falcon tube containing the membrane and hybrisol I solution, mix well, incubate in shaking waterbath at 50°C over night.

6. Wash the membrane in 2 x SSC/ 0.1% SDS at RT twice (5 min per wash)., then wash with 0.1 x SSC/ 0.1% SDS at 60°C twice (30 min. per wash). Wrap the membrane and put X-ray film on it, expose at -80°C over night. Develop the film next morning.

7. Strip the membrane by incubating in 0.5 N NaOH for 1 hour at RT with constant agitation. Change the solution and incubate over night if necessary. Rinse the membrane with 2x SSC, air dry.

Ordering Information

GeneProber™

Product	Size	Catalog No.	Price, $
Fragile X GLFX1 Suitable for random primer labeling	500ng	40-3201-01 (old number 40-2015-10)	350.00
Fragile X GLFXDig1 Digoxigenin labeled probe, ready to use for southern hybridization	110µl	40-3202-01	400.00
Fragile X PCR Probe; GLFXPCRprober For non radioactive detection of Fragile X PCR products	5 blots	40-3101-01	400.00

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

All Gene Link products are for research use only.

Gene Link™

140 Old Saw Mill River Road Hawthorne, NY 10532
Tel: 914.769.1192 www.genelink.com Fax: 914.769.1193