Product Profile

Genemer™ Fragile X GLFXPCR Kit

Amplification of the Fragile X CGG repeat region and Radioactive Labeling with ^{35}S or ^{32}P dATP

Catalog Number: 40-3103-00 100 Reactions

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'.

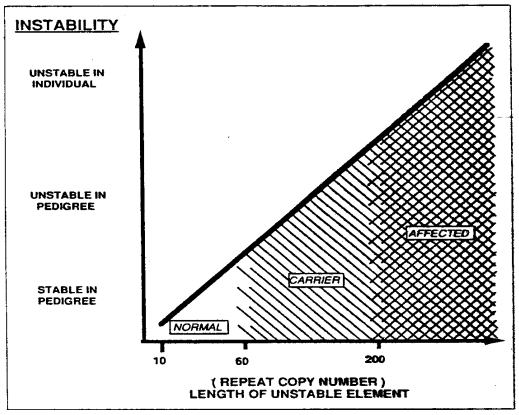
Triple Repeat Size Analysis

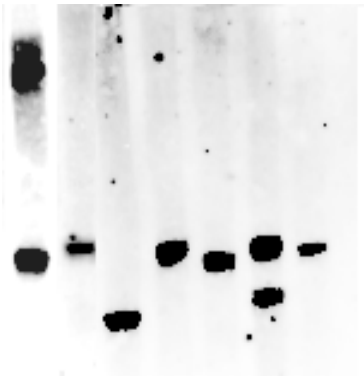
Important Note:

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.

The conditions given below using ^{35}S has routinely amplified DNA samples with up to 50 CGG repeats.

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.





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.
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.

Manual

Gene Link™

140 Old Saw Mill River Road Hawthorne, NY 10532
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Material Supplied

Component A	520 µl contains dNTP, primers and buffer
Component B	42 µl contains buffer and 7-deaza GTP
Component C	200 µl DMSO
Sequencing Loading Buffer 1ml	

Material NOT supplied

Taq DNA Polymerase
^{35}S dATP; Recommended Supplier NEN
Catalog Number NEG-034H 250 µCi

Protocol

A. PCR premix.

Given below is a protocol for preparing a PCR premix for ten 25 µl reactions. This can be scaled up or down as required.

Component A	51.25 µl
Component B	3.75 µl
Component C	25 µl
Sterile water	170 µl

B. Enzyme premix (for 10 reaction)

PCR premix (above)	20 µl
^{35}S dATP	10µl
Taq. Polymerase	2.5 µl (5 units)

Total	32.5 µl

C. PCR reaction (25 µl)

Use 22 µl of premix for each PCR reaction + 1 µl DNA (~100 ng), denature, then add 3 µl enzyme premix.

Denaturation	95^{O}C	1 min.30sec.
Annealing	65^{O}C	1 min.
Elongation	72^{O}C	4 min.

30 cycles, 7 min. 72^{O}C fillup, 4^{O}C soak.

Precipitate and dissolve pellet in 4 µl H_2O and 4 µl of the provided Sequencing Loading buffer.

D. Electrophoresis

Pre-warm the 6% sequencing gel at 1500 volts about 1 hour, denature the samples at 70^{O}C at least 2 minutes and load 4 µl to the gel. Electrophorese for 1 hour more after second dye line (xylene cyanol) comes out. Vacuum dry the gel at 80^{O}C for 1 hour. Expose the film at RT over night.

Call Gene Link Technical service for more information.
1-800-Gene Link (1-800-436-3546)

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
Fragile X PCR Probe; GLFXPCRprober Kit For amplification and non radioactive detection of Fragile X PCR products	5 blots	40-3102-00	650.00
Fragile X GLFXPCR Genemer™ Kit For radioactive detection of PCR products using ^{35}S or ^{32}P	100 reactions	40-3103-00	650.00
Fragile X GLFXPCR Genemer™ Primers Only	10 nmoles	40-2004-10	100.00

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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

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Prices subject to change without notice

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