Certificate of Analysis & Product Manual

Triple Repeat Disorders Genotyping
Fragile X, Myotonic Dystrophy, Friedreich's Ataxia, Huntington's disease
Fluorescent Probes, siRNA, Hybridization and Detection Reagents

Fragile X CGG Repeat Genotyping GeneProber™ GLFXDig1

Fragile X CGG triple repeat region chemiluminescent Southern blot genotyping

Catalog No. 40-2004-41

Storage Condition: -20°C

For Research Use Only. Not for use in diagnostic procedures for clinical purposes

Important Information

All Gene Link products are for research use only.
Not for use in diagnostic procedures for clinical purposes.
Product to be used by experienced researchers appropriately trained in performing molecular biology techniques following established safety procedures. Additional qualification and certification is required for interpretation of results.

Material Supplied

Fragile X CGG Repeat Genotyping GeneProber™ GLFXDig1

REF	Catalog No.	Description	Size
	40-2004-41	Fragile X GLFXDig1 GeneProber™ Digoxigenin labeled	110 µL

Certificate of Analysis & Product Specifications

One tube containing 110 µL of GeneProber™ GLFXDig1 probe at a concentration of ~40ng/ µL. This probe is digoxigenin labeled for non-radioactive detection. The quantity supplied is sufficient for at least 5 20x20 cm blots using 20 µL for each blot as probe. Experienced users with optimized hybridization conditions can save the hybridization solution containing the probe and re-use at least one more time for hybridization.

The Fragile X GeneProber™ GLFXDig1 probe supplied has been validated to hybridize to the CGG triple repeat spanning region of FMR-1.

Appropriate nuclease free handling, dispensing and storage conditions required.

Product Label Information

RUO Research Use Only	-10°C -20°C	LOT
Research Use Only	**Storage** Store at -20°C to -10°C	**Lot Number** Stated on product tube and packing slip
Expiry One year from Date of Shipment	**Instructions** Consult product manual	**QR Code** Visit Gene Link website for product details

GeneProber™ Related 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, Huntington's Disease, 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
FRAXE/FMR2/AFF2 GeneProber™ AFF2-AJ31Dig1	110 µL	40-2054-41
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
Mitochondrial DNA deletion GeneProber™ GLmtDNA2.5Dig1 Probe Digoxigenin labeled	110 µL	40-2055-41

GScan™ Related Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders. The kits contain optimized PCR amplification reagents and a wide array of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument. 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.

Product	Unit Size	Catalog No.
Fragile X GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2004-15XX
Fragile X GScan™ V2 Kit for fluorescent detection; 20 reactions kit	1 kit	40-2004-15FMS
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2054-15FM
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2054-15FMS
Huntington's Disease GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15XX
Huntington's Disease GScan™ V2 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

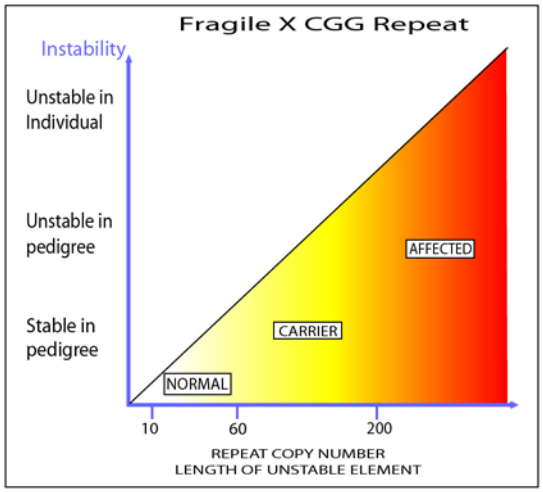
All Gene Link products are for research use only **Current pricing are posted at http://www.genelink.com/**

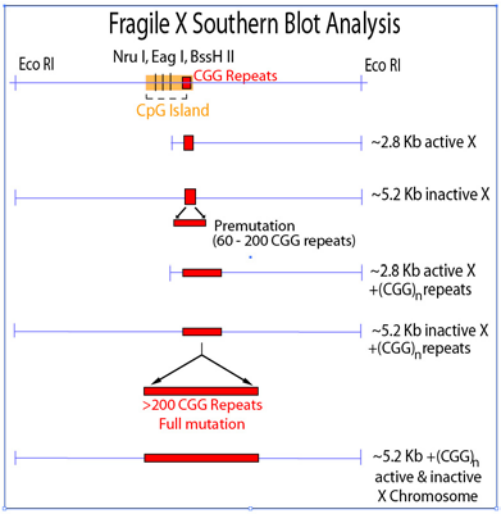
Fragile X Genotyping

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) 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- Trinucleotide Repeats (TNR's) 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 trinucleotide repeat, which is located in the 5' region of the cDNA. The most common allele in the normal population consists of 30 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).

Trinucleotide Repeats

To date, trinucleotide repeats expansion has been shown to be responsible for at least 15 different neuro degenerative disorders in humans. Table 1 lists these disorders. All share the instability of the repeats above a particular threshold. Once this threshold is exceeded the trinucleotide repeats become meiotically unstable and upon expansion exhibit the onset of disease symptoms.

Table 1. Trinucleotide Repeats in Human Genetic Disease

Disease	Repeat[a]	Normal Length[b]	Intermediate Length (Premulation)[a,b]	Full Disease Length[b]
Fragile XA (FRAXA)	$(CGG)_n$	6-52	59-230	230-2,000
Fragile XE (FRAXE)	$(CCG)_n$	4-39	? (31-61)	200-900
Fragile XF(FRAXF)	$(CGG)_n$	7-40	?	306-1,008
FRA16A	$(CCG)_n$	16-49	?	1,000-1,900
Jacobsen Syndrome (FRA11B)	$(CGC)_n$	11	80	100-1,000
Kennedy Syndrome (SMBA)	$(CAG)_n$	14-32	?	40-55
Myotonic Dstrophy (DM)	$(CTG)_n$	5-37	50-80	80-1,000; congenital, 2,000-3,000
Huntington disease (HD)	$(CAG)_n$	10-34	36-39	40-121
Spinocerebellar ataxia 1 (SCA1)	$(CAG)_n$	6-39	None Reported	40-81
Spinocerebellar ataxia 2 (SCA2)	$(CAG)_n$	14-31	None Reported	34-59
Spinocerebellar ataxia 3 (SCA3)/Machado Joseph disease (MJD)	$(CAG)_n$	13-44	None Reported	60-84
Spinocerebellar ataxia 6 (SCA6)	$(CAG)_n$	4-18	None Reported	21-28
Spinocerebellar ataxia 7 (SCA7)	$(CAG)_n$	7-17	28-35	38-130
Haw River syndrome (HRS; also DRPLA))	$(CAG)_n$	7-25	?	49-75
Friedreich ataxia (FRDA)	$(GAA)_n$	6-29	? (>34-40)	200-900

[a] Typically, repeats tracts contain sequence interruptions. See Pearson and Sinden (1998*a*) for a discussion of the sequence interruptions.

[b] No. of triplet repeats.

[c] A question mark (?) indicates potential mutagenic intermediate length, and an ellipsis (…) indicates none. Not all disease are associated with a permutation length repeats tract or permutation disease condition.

Molecular Analysis

Fragile X genotyping can be done by direct PCR amplification of the CGG trinucleotide repeats 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 trinucleotide repeats is flanked by Eco RI sites; and an Eag I and Nru I site and three BssH II sites in the CpG region. Full mutation has been shown to methylate the active gene too and thus it prevents NruI, BssH II and Eag I restriction of DNA. Hybridization of southern blots of Eco RI and Nru I, BssH II or Eag I double digested DNA clearly can distinguish between normal, premutation and full mutation genotypes.

The detection of amplification/expansion of a region of DNA sequence can be detected by PCR and Southern, these methods can be used for all disorders involving increase in size of a region of DNA. DNA analysis for direct detection of fragile X mutation is based on enzymatic amplification of a fragment containing the CGG repeats sequence of the *FMR-1* gene. This test detects the fragile X mutation by the size of the amplified product; an increase in size is correlated with the corresponding number of CGG repeats and a risk factor calculated. The most common allele in the normal population consists of 30 repeats, the range varying from 6 to 54 repeats. Premutations in fragile X families showing no phenotypic effect range in size is from 52 to over 200 repeats. All alleles with greater than 52 repeats are meiotically unstable with a mutation frequency of one.

PCR based methods are fundamentally similar. The two primers are constructed such that they span the region of trinucleotide repeats expansion. In the case of Fragile X specifically, the nature of the mutation poses problems using normal PCR conditions. In Fragile X, the repeats is of CGG which can be hundreds to thousands bases long. All DNA polymerases, including Taq DNA polymerase do not copy long stretches of G residues efficiently. An analog of G called 7-deaza GTP functions better and is partially replaced in the PCR reaction to achieve amplification. The use of 7 deaza GTP instead of G precludes the staining of gels with ethidium bromide for visualization as 7 deaza GTP containing DNA does not stain well. This is resolved by using radioactively labeled nucleotide followed by autoradiography. Fragile X PCR still does not give accurate results for full mutations due to the inherent massive expansion and the inability of PCR to amplify very large fragments efficiently. All normal and pre-mutation PCR amplification is reliable, but still is coupled with a Southern blot analysis. The PCR results are obtained in 1-2 days followed by Southern blot results. All results from PCR are verifiable by Southern except full mutations which are not reliable with PCR.

Southern blot analysis for Fragile X mutation detection involves the cleavage of DNA with enzyme Eco R I and Nru I, BssH II or Eag I. This method detects the size of CGG repeats region by hybridization of probe GLFX1 or GLFXDig1 GeneProber™ to DNA that has been double digested with restriction enzymes Eco RI and Nru I, BssH II or Eag I and blotted onto a membrane. In normal females two fragments are seen, a 2.8kb corresponding to the active X and a 5.2kb fragment corresponding to the methylated inactive X chromosome. Normal males exhibit only the 2.8kb banding pattern. Affected males will have an amplified CGG repeats region with methylation thus giving rise to fragments larger than the normal 5.7kb. Premutations in males and females will be seen as fragments from 2.9-3.3kb (normal 2.8kb) derived from the X chromosome. Premutations in females derived from the inactive X will give fragments from 5.3-5.7kb. Mosaicism is characterized by fragments appearing as a mixture of full mutation (methylated, larger than 5.7kb) and unmethylated premutation (2.9-3.3kb).

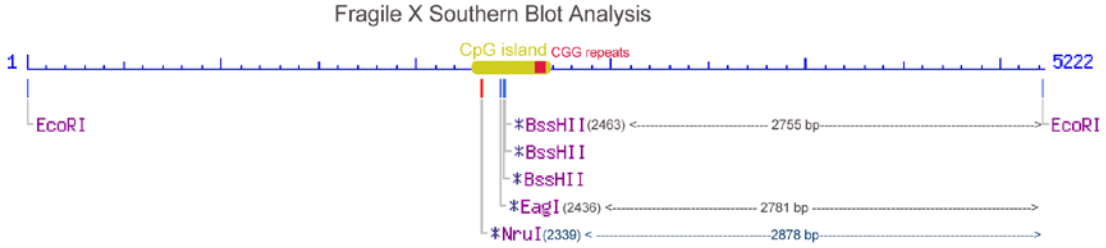


Table 2. CGG Trinucleotide Repeats and Fragment Size*

CGG	bp	Size	%	CGG	bp	Size	%	CGG	bp	Size	%
1	3	223		36	108	328	1.05	71	213	433	
2	6	226		37	111	331	0.35	72	216	436	
3	9	229		38	114	334	0.53	73	219	439	
4	12	232		39	117	337	1.23	74	222	442	
5	15	235		40	120	340	1.23	75	225	445	
6	18	238		41	123	343	0.35	76	228	448	
7	21	241		42	126	346	0.7	77	231	451	
8	24	244		43	129	349	0.7	78	234	454	
9	27	247		44	132	352	0.18	79	237	457	
10	30	250		45	135	355		80	240	460	
11	33	253		46	138	358		81	243	463	
12	36	256	0.18	47	141	361	0.18	82	246	466	
13	39	259		48	144	364	0.18	83	249	469	
14	42	262		49	147	367	0.18	84	252	472	
15	45	265	0.18	50	150	370		85	255	475	
16	48	268	0.35	51	153	373		90	270	490	
17	51	271		52	156	376	0.35	95	285	505	
18	54	274		53	159	379		100	300	520	
19	57	277		54	162	382		105	315	535	
20	60	280	6.32	55	165	385		110	330	550	
21	63	283	0.18	56	168	388		115	345	565	
22	66	286	0.88	57	171	391		120	360	580	
23	69	289	6.14	58	174	394		130	390	610	
24	72	292	2.63	59	177	397		140	420	640	
25	75	295	0.88	60	180	400		150	450	670	
26	78	298	1.4	61	183	403		160	480	700	
27	81	301	0.88	62	186	406		170	510	730	
28	84	304	2.28	63	189	409		180	540	760	
29	87	307	**18.8**	64	192	412		190	570	790	
30	90	310	**38.8**	65	195	415		200	600	820	
31	93	313	7.02	66	198	418		210	630	850	
32	96	316	3.51	67	201	421		220	660	880	
33	99	319	1.23	68	204	424		230	690	910	
34	102	322	0.53	69	207	427		240	720	940	
35	105	325	0.7	70	210	430		250	750	970	

***Fragment size is specific for Gene Link Genemer™ and GScan™ amplification and detection products.**

The size of the CGG repeats 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 an alkaline phosphatase conjugated probe for non-radioactive detection.

Gene Link offers safe and reliable chemiluminescent detection methods as an alternate to radioactive based detection methods. PCR-Prober™, GScan™ and GeneProber™ line of products replaces radioactive based methods. Gene Link's GScan Ver2 kit is for PCR amplification followed by fluorescent detection of the specific triple repeat fragment size and routinely detects greater than 120 CGG repeats.

Genemer™ Kit Agarose Gel Analysis

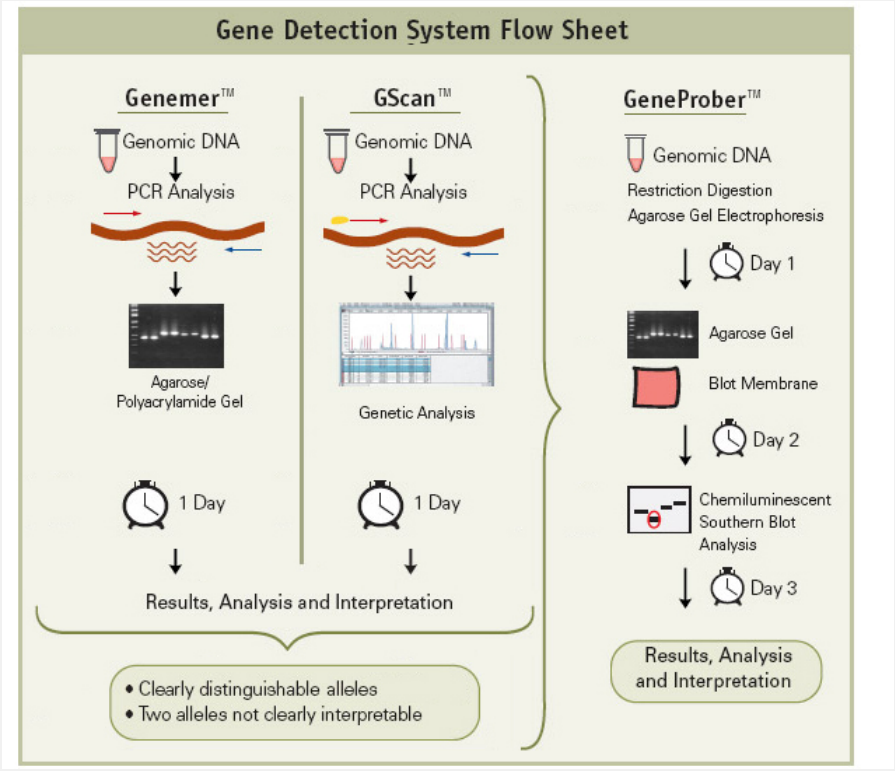
Optimized fragile X Genemer™ kit with components for PCR amplification of up to 130 CGG repeats using standard Taq polymerase. Amplified samples are resolved by agarose gel electrophoresis. This Genemer™ method or GScan™ fluorescent detection is recommended for initial screening of all samples.

GScan™ Kit

Optimized fragile X GScan™ kit with components for PCR amplification of up to 100 CGG repeats using standard Taq polymerase. Amplified samples are resolved by genetic analyzers capable of fluorescent detection or agarose gel electrophoresis. This Genemer™ Kit or GScan™ kit for fluorescent detection is recommended for initial screening of all samples.

GeneProber™ Probes for Southern Blot Analysis

Digoxigenin labelled probes for chemiluminescent Southern blot detection method or unlabeled probe for end user to perform radioactive label. Gene Link offers safe and reliable chemiluminescent detection methods as an alternate to radioactive based detection methods.



Procedure: Fragile X Chemiluminescent Southern Blot Protocol

Material Supplied

One tube containing 110 µL of GeneProber™ GLFXDig1 probe at a concentration of ~40ng/ µL. This probe is digoxigenin labeled for non-radioactive detection. The quantity supplied is sufficient for at least 5 20x20 cm blots using 20 µL for each blot as probe. Experienced users with optimized hybridization conditions can save the hybridization solution containing the probe and re-use at least one more time for hybridization.

A. Chromosomal DNA digestion

GLFXDig1 and GLFX1 Probe Southern Blot Fragment Detection Double Digestion with Eco RI and Eag I or Nru I or BssH II				
Enzyme	**Specificity**	**CpG methylation Sensitive**	**Normal Male Fragment Size**	**Normal Female Fragment Size**
EcoRI and EagI double digest				
EcoRI	G^AATT_C	No	2781 bp	5216 & 2781 bp
EagI	C^GGCC_G	Yes		
EcoRI and NruI double digest				
EcoRI	G^AATT_C	No	2878 bp	5216 & 2878 bp
NruI	TCG_CGA	Yes		
EcoRI and BssH II double digest				
EcoRI	G^AATT_C	No	2755 bp	5216 & 2755 bp
BssH II	G^CGCG_C	Yes		

Important Note
-Double digest genomic DNA with Eco RI and Nru I, BssH II or Eag I.

Restriction Digestion	
Component	Volume|Quantity
Genomic DNA	10µg
10x Eco RI Buffer	10 µL
Nru I, BssH II or Eag I (10 u/ µL)	4 µL
Eco RI (40 u/ µL)	4 µL
H_2O to	**100** µL
Overnight digestion at 37^{O}C	

Ethanol precipitate the digests, dissolve the pellets in 10 µL of 1x Loading buffer.

Refer to Reagent preparation section for composition and preparation instructions. Abridged Southern blot protocol is given below. It is assumed the researcher has extensive experience in Southern blot procedure.

B. Electrophoresis and Transfer

1. Load samples to a 0.8% agarose gel. Electrophorese over night at 45mA for 14-16 hours. (1.6 kb fragment on the bottom of the gel).
2. Depurinate with 0.25N HCl (add 10 mL HCl to 500 mL H_2O) for 10 minutes.
3. Denature the DNA with 0.4N NaOH/0.6M NaCl for 30 min. at room temperature (RT).
4. Neutralize with 1.5M NaCl/0.5M Tris (pH 7.5) for 30 min. at RT.
5. Transfer overnight by Southern blot procedure to positively charged nylon membrane using 10xSSC.
6. Wash the membrane with 2x SSC and then bake at 80^{O}C for 2 hours.

C. Hybridization

Gene Link recommends using Roche Digoxigenin based washing and detection system reagents.

1. Perform prehybridization at 55^{o}C for 3 hours in 10 mL of Easy Hyb buffer (Roche Biochemicals).
2. Boil 20 µL GeneProber™ probe in 500µL of Easy Hyb for 10 minutes. Chill directly on ice.
3. Add the above probe to 10mL of Easy Hyb.
4. Discard the prehybridization buffer and replace it with the hybridization buffer containing the boiled probe. Hybridize overnight at 55^{o}C.
5. Washing. Wash the membrane in Hyb Wash I (2xSSC/0.1% SDS) at room temperature twice (5 min/wash) followed by washing twice with Hyb Wash II (0.5xSSC, 0.1%SDS) twice at 60^{o}C (15 min/wash).
6. Warm the blocking reagent at this point. Prepare fresh 100 mL of Buffer MB by adding 10 mL of 10% blocking reagent [Gene Link Catalog no: 40-5026-10; Blocking solution for hybridization (10%)] and 10 mL of Maleic acid buffer 10X (Buffer M 10X) [Gene Link Catalog no: 40-5025-20; Maleic acid buffer 10X (Buffer M 10X)] to 80 mL of sterile water. Use 80 mL for blocking, the rest of 20 mL for making Anti-DIG-AP conjugate.

D. Anti-Dig Alkaline Phosphatase Binding

1. Equilibrate the membrane in 100 mL of 1X Buffer M for 1 minute.
2. Incubate the membrane in 80 mL of Buffer MB (prepared in step 6 above) blocking solution at room temperature for 30 min.
3. Prepare 1:10000 Anti-DIG-AP conjugate at this point. e.g. add 2 µL to 20 ml Buffer MB (prepared in Section C. step 6 above).
4. Incubate the membrane in 20 mL of Anti-DIG-AP conjugate solution at RT for 30 min.
5. Wash the membrane twice, 15 min/wash in 200 mL of 1x Buffer M at RT.
6. Equilibrate the membrane in 50 mL of 1x Detection buffer for 2 min. Repeat 2 times. Total of three washes.

E. Detection

Detection with CDP star as substrate will yield reliable result by exposing to Kodak X-OMAT or XAR X-ray film for 1 hour to overnight at room temperature.

1. Transfer blot to a plastic sheet, (sheet protector cut from two sides to open up) and drain off excess buffer. Wipe off edges with paper towel. Blot should not be allowed to dry.
2. Spray CDP-star ready-to-use substrate evenly to cover the blot. DO NOT OVER SPRAY. Cover the blot with plastic sheet and wipe entire surface of the covered blot to expel any excess substrate and air bubbles. Expose the film at room temperature for 1 hr. or for shorter or longer time as required.
3. Luminescence continues for at least 24 hours and signal intensity remains almost constant during the first few hours. Multiple exposures can be taken to achieve the desired signal strength.

F. Stripping

Wash the membrane in water to remove the substrate. Then wash the membrane in 0.2N NaOH/0.1% SDS at 37^{o}C for 30 minutes. Rinse the membrane in 2XSSC. Air dry. Blot is ready for re-hybridization.

Required reagents with recommended suppliers

Roche Applied Science	http://www.roche-applied-science.com
Product Description	**Catalog Number**
Nylon Membranes, positively charged ; 20 sheets 10 x 15 cm	11209272001
DNA Molecular Weight Marker III, DIG-labeled ; 500 µl 10 µg/mL 5 µg	11218603910
DIG Easy Hyb ; 500 mL	11603558001
DIG Wash and Block Buffer Set ; 1 set 30 blots	11585762001
Anti-Digoxigenin-AP, Fab fragments from sheep; 200 µl 150 U	11093274910
CDP Star Ready to use; 2X 50 mL	12041677001

Gene Link	**http://www.genelink.com/geneprodsite/category.asp?c=44**	
Non-radioactive Southern Blot Reagents		
Product Description	**Catalog No.**	**Unit Size**
Agarose LE Molecular Biology Grade 100 gms	40-3010-10	100 gms
TAE Buffer 50 X Concentrate 1000 mL	40-3007-10	1 L
TBE Buffer 5 X Concentrate; 1L	40-3008-10	1 L
Loading buffer 10X BPB/XC non-denaturing; 1mL	40-3003-10	1 mL
Loading buffer 10X BPB/XC non-denaturing ; 15 mL	40-3003-15	15 mL
Lumisol II, Hybridization Solution; 200 mL	40-5023-20	200 mL
Depurination Solution (2X) for Southern Blotting; 1 L	40-5034-10	1 L
Denaturation Solution (2X) for Southern Blotting; 1L	40-5035-10	1 L
Neutralization Solution (2X) for Southern Blotting; 1L	40-5036-10	1 L
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC); 250 mL	40-5020-25	250 mL
Hybwash B, Hybridization Wash Solution (10% SDS) ; 100 mL	40-5021-10	100 mL
Maleic acid buffer 10X (Buffer M 10X); 200 mL	40-5025-20	200 mL
10% Blocking Reagent; 100 mL	40-5026-10	200 mL
Detection Buffer 10X; Alkaline Phosphatase detection buffer; 100 mL	40-5031-10	100 mL
CDP-Star® Substrate; Ready-to-Use 0.25 mM in spray bottle; 10 mL	40-5010-10	10 mL

Reagent Preparation

Most reagents with composition listed below are available in a molecular biology laboratory or these can be prepared in house. Gene Link catalog numbers are also listed if you like to purchase these common reagents.

1X Depurination Solution (0.25M HCl)		
Product Description	**Catalog No.**	**Volume**
Depurination Solution (2X) for Southern Blotting	40-5034-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

1X Denaturation Solution (0.4M NaOH, 0.6M NaCl)		
Product Description	**Catalog No.**	**Volume**
Denaturation Solution (2X) for Southern Blotting	40-5035-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

1X Neutralization Solution (0.5M Tris-HCl pH 7.5, 1.5M NaCl)		
Product Description	**Catalog No.**	**Volume**
Neutralization Solution (2X) for Southern Blotting	40-5036-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

Hybwash I (2xSSC, 0.1% SDS)		
Product Description	**Catalog No.**	**Volume**
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC)	40-5020-25	35 mL
Sterile water		311 mL*
Hybwash B, Hybridization Wash Solution (10% SDS)	40-5021-10	4 mL*
Total Volume		**350 mL**
*** Volumes adjusted to whole numbers**		

Hybwash II (0.5xSSC, 0.1%SDS)		
Product Description	**Catalog No.**	**Volume**
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC)	40-5020-25	9 mL*
Sterile water		337 mL
Hybwash B, Hybridization Wash Solution (10% SDS)	40-5021-10	4 mL*
Total Volume		**351 mL**
*** Volumes adjusted to whole numbers**		

1X Buffer M (Maleic Acid Buffer, 1X) (100 mM Maleic acid, 150 mM NaCl pH7.5)		
Product Description	**Catalog No.**	**Volume**
Buffer M 10X (Maleic acid buffer 10X)	40-5025-20	10 mL
Sterile water		90 mL
Total Volume		**100 mL**

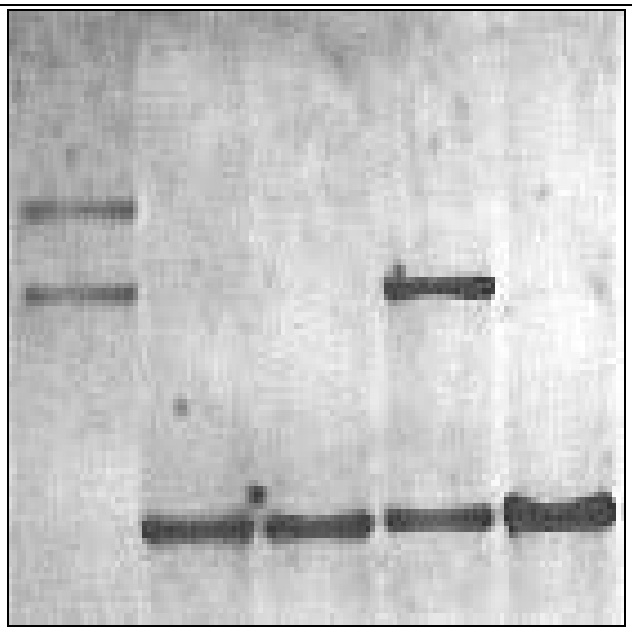
Buffer MB (1 x Buffer M (Maleic acid buffer, 1X with Blocking Reagent) Always prepare fresh!		
Product Description	**Catalog No.**	**Volume**
Buffer M 10X (Maleic acid buffer 10X)	40-5025-20	10 mL
Sterile water		80 mL
10% Blocking Reagent*	40-5026-10	10 mL
Total Volume		**100 mL**

The prepared reagent will be turbid yellow in color
* The 10% Blocking Reagent is turbid yellow in color and will form precipitates on storage.
Warm to 50°C and shake well before aliquoting. DO NOT SHAKE VIGOROUSLY

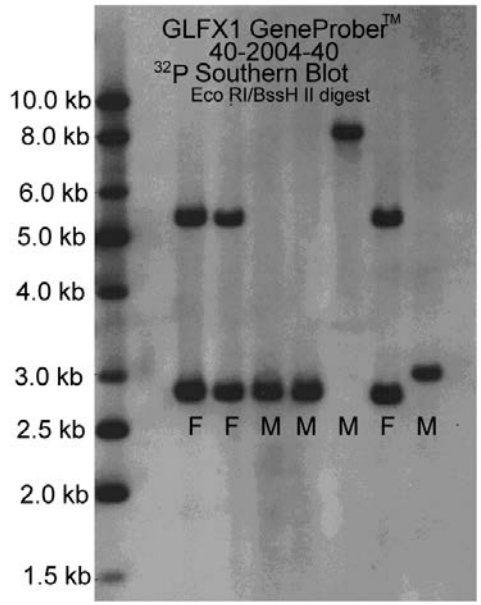
1X Detection Buffer, Alkaline phosphatase detection buffer (100mM Tris-HCl pH 9.5, 100mM NaCl)		
Product Description	**Catalog No.**	**Volume**
Detection Buffer 10X; Alkaline phosphatase detection buffer	40-5031-10	10 mL
Sterile water		90 mL
Total Volume		**100 mL**

Results and Analysis

Southern blot analysis for Fragile X mutation detection involves the cleavage of DNA with enzyme Eco R I and Nru I or Eag I. This method detects the size of CGG repeats region by hybridization of probe GLFX1 or GLFXDig1 GeneProber™ to DNA that has been double digested with restriction enzymes Eco RI and Nru I or Eag I and blotted onto a membrane. In normal females two fragments are seen, a ~2.8kb corresponding to the active X and a ~5.2kb fragment corresponding to the methylated inactive X chromosome. Normal males exhibit only the ~2.8kb banding pattern. Affected males will have an amplified CGG repeats region with methylation thus giving rise to fragments larger than the normal ~2.8kb. Premutations in males and females will be seen as fragments from 2.9-3.3kb (normal 2.8kb) derived from the X chromosome. Premutations in females derived from the inactive X will give fragments from 5.3-5.7kb. Mosaicism is characterized by fragments appearing as a mixture of full mutation (methylated, larger than 5.7kb) and unmethylated premutation (2.9-3.3kb).



Fragile X southern blot.. Lane 1 affected female. Lanes 2, 3 & 5 are normal males. Lane 4 normal female. **Chemiluminescent detection**, ~2hr. exposure



Fragile X southern blot analysis. Genomic DNA digested with Eco RI/BssH II and GLFX1 GeneProber labeled with ^{32}P as the probe. Normal pattern expected is as follows. Female: 5.2 kb and 2.7kb; Males; 2.7kb

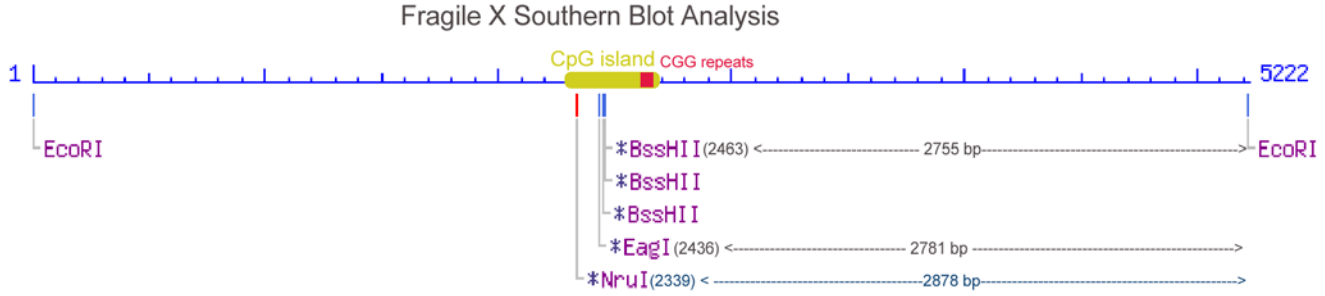
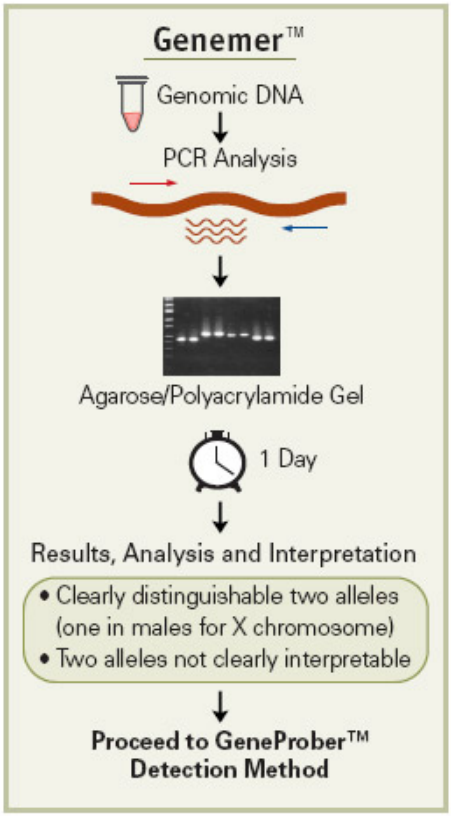
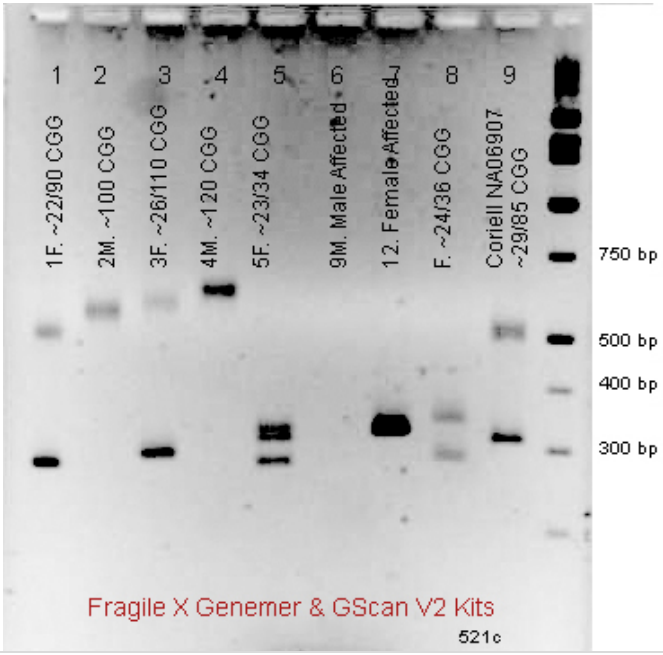


Table 3. Fragile X Molecular Analysis Results Interpretation

Clinical Category	Normal (male/female)	Female Carrier with small amplification	Female carrier with significant amplification	Female carrier with Large amplification	Carrier male with premutation (NTM)	Full Mutatiom (Male/Female)	Carrier with Fragile X Mosaicism
Risk mutation will become full mutation in next generation	0%	moderate	significant	high	0%	moderate to high	can vary from 0-100%
Number of CGG repeats	6-45	46-69	70-86	87-200	40-200	>200	40-200/ >200
Size of CGG repeats; bp	18-135	138-207	210-258	260-600	120-600	>600	120-600/ >600
Total Fragment Size; bp	221-338	341-410	413-461	464-803	323-803	>803	323-803/ >803

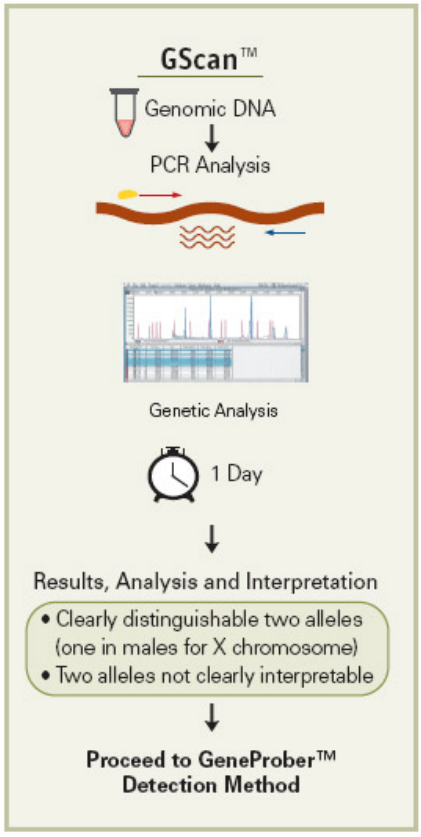
Fragile X Genemer™ V2 Kit [40-2004-11] PCR Amplification & Agarose Gel Detection

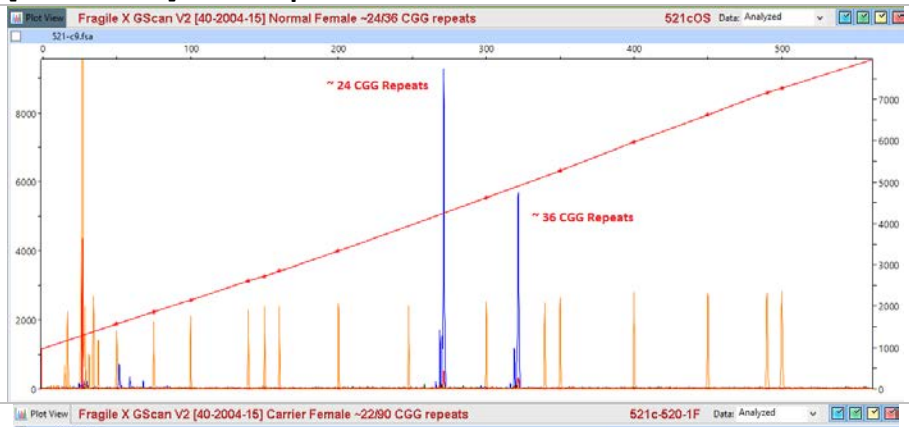


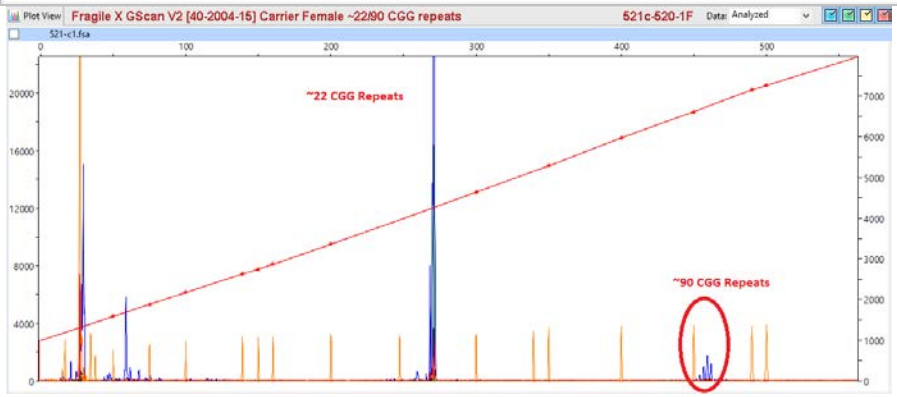


Fragile X GScan™ V2 [40-2004-15FM] and Genemer™ V2 Kit [40-2004-11] was used to amplify human genomic samples representing various CGG triple length pre-mutations, full mutations and normal Fragile X genotype. After PCR 10 µL samples were applied to a 2.5 agarose gel for electrophoresis. Gel picture is of a ethidium bromide stained gel. Gel lanes denote briefly the fragile X CGG repeat genotype.

Fragile X GScan™ V2 Kit [40-2004-15] PCR Amplification & Fluorescent Detection







Fragile X GScan™ V2 [40-2004-15FM] Kit was used to amplify human genomic samples representing various CGG triple length pre-mutations, full mutations and normal Fragile X genotype. Fragment analysis results are shown, each panel denotes the fragile X CGG repeat genotype

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.

Fragile X Frequently Asked Questions/Troubleshooting

1. **General Comment** Fragile X genotyping is not easy. A lab really has to optimize conditions. Following the protocol exactly works. A few initial rounds of optimization may be required. Once the investigator is experienced with all the manipulations, getting good results should be routine.

2. **High Background** Background problems may be due to various reasons and has to be optimized in each lab. Here at Gene Link we use Roche positively charged nylon membrane and other products, a list of recommended products with catalog numbers is given in the methods. Other positively charged membranes work but do not give consistently low background. The main reason for background is inadequate blocking and/or the membrane itself is curled, folded or has scratches and creases which trap the probe. We advise using glass trays or bottles for all washing and hybridization procedures. Plastic dishes inherently have small surface variations and can scratch the membrane. We would also advise increasing the washing and stringency and exposure to x-ray film for one hour initially. Wash again if you observe too much background and no real signal in an hour. Expose for longer time if the one hour exposure gives nearly no background. We get good signal in a 2 hr. exposure.

Again, to summarize, the background problem varies from lab to lab and has to be optimized. Once optimized, you will consistently get excellent signal in 1-2 hr. exposure. On some occasion an overnight exposure may be required based on original low quantity of DNA used for gel and Southern blot.

Appendix: **Protocols**

Genomic DNA Purification

Genomic DNA is usually extracted from blood. A simple procedure is given below that purifies ~10 µg DNA from 300 µl blood using a 30 minute procedure.

Omni-Pure™ Genomic DNA Purification System Gene Link Catalog Number: 40-4010-01
Rapid DNA Purification Protocol for 300 µL Whole Blood

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 900 µl GD-1 solution (RBC Lysis Solution) to one tube for each sample.
3. Add 300 µl Isopropanol (2-propanol) to one tube for each sample. Cap the tubes.

B. Cell Lysis

1. To the tube containing 900 µl GD-1 solution (RBC Lysis Solution) using a filter tip pipet transfer 300 µl whole blood. Cap and gently mix by inversion. Incubate for 1-3 minutes at room temperature. Mix by inversion a few times during this incubation period. Incubate longer for fresh blood cells as they are intact and not lysed already.
2. Centrifuge at 3 K rpm for 20 seconds to pellet the white blood cells. A reddish white pellet should be clearly visible. Decant and discard supernatant leaving behind the last few droplets. Do not totally remove the supernatant.
3. Completely resuspend the white blood cell pellet by vigorously vortexing the tube. Ensure that the pellet is completely resuspended.
4. To the resuspended cells add 300 µl GD-2 solution (Cell Lysis Solution). Mix by gentle vortexing. You will notice release of DNA by the thickening of the liquid in the sample. Samples may be stored at this stage for processing later. It has been shown that the samples are stable in Cell Lysis Solution for at least 2 years at room temperature.

C. Protein Precipitation

1. Add 100 µl GD-3 solution (Protein Precipitation Solution) to the sample in cell lysis solution.
2. Vortex vigorously at for 20 seconds. Small particles of brown color will be appear and be visible at this stage.
3. Centrifuge at 5 K rpm for 1 minute to pellet the precipitated proteins. A clearly visible brown pellet containing proteins should be collected at the bottom of the tube.

D. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube (see initial preparation above) containing 300 µl 100% Isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. 30-40 mixing by inversion is usually sufficient.
3. Centrifuge at 6 K rpm for 1 minute to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place tube inverted on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 6 K rpm for 1 minute to collect the DNA as a pellet. Gently take out the tubes so that the pellet is not dislodged. While holding the tube, rotate tube so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place tube inverted on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

E. DNA Reconstitution & Use

1. Add 100 µl of GD-4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4 °C. For long-term storage, place sample at –20 °C or –80 °C.
3. Average yield of 10 µg is expected from 300 µl blood DNA. The range is between 5 µg to 15 µg.
4. The 100 µl of purified DNA obtained will have an average concentration of ~ 100 ng/µl.
5. For PCR amplification use 1-2 µl.
6. Use 100 µl for restriction digestion followed by Southern blot analysis.
7. It is convenient to perform multiple 300 µl blood DNA purification instead of scaling up the procedure.

Gel Electrophoresis of DNA

Gel electrophoresis of PCR products is the standard method for analyzing reaction quality and yield. PCR products can range up to 10 kb in length, but the majority of amplifications are at 1 kb and below. Agarose electrophoresis is the classical method to analyze amplification products from 150 bp to greater than 10 kb. Polyacrylamide gel electrophoresis should be used for resolution of short fragments in the range of 100 bp to 500 bp when discrimination of as small as a 10 bp difference is required. PAGE gels for PCR products formulated with the amount of cross-linker chosen to give pore sizes optimal for the size of DNA fragment desired. Gels are most often stained in ethidium bromide, even though the fluorescence of this stain is quenched by polyacrylamide, which decreases sensitivity 2-5 fold. This decrease in sensitivity generally does not present a problem, because most PCR reactions yield product levels in the microgram range, and ethidium will detect as little as 1/10 of this amount. Polyacrylamide gels can be stained by silver staining for more sensitive detection.

Agarose Gel Electrophoresis of DNA

Agarose gels are typically run at 20 to 150V. The upper voltage limit is the amount of heat produced. At room temperature about 5 Watts is correct for a minigel (Volts x Amps = Watts). At low voltages migration is linearly proportional to voltage, but long DNA molecules migrate relatively faster in stronger fields. Migration is inversely proportional to the log of the fragment length; a log function also governs migration rate and gel concentration (0.5 to 2% for most purposes). Furthermore, supercoiled / circular DNA molecules migrate at different rates from linear molecules; single-stranded DNA and RNA migrate at similar rates, but usually faster than double-stranded DNA of the same length. Salt in the samples increases conductivity and, hence, migration rate.

The buffers used for most neutral agarose gels (the gel itself and the solution in which it lies) is 1 x TAE or 1 x TBE. Agarose powder is added to the buffer at room temperature, heated in a microwave and boiled slowly until the powder has dissolved. Cast the gel on a horizontal surface once the agarose has been cooled to ca. 60° C (just cool enough to hold) and add 0.1 µg of ethidium bromide solution for each ml of gel volume. At times, during removal of the comb, it is possible to tear the bottom of the sample wells gels, which results in sample leakage upon loading. This can be avoided by removing the comb after the gel has been placed in the running buffer.

- Use TAE buffer for most molecular biology agarose gel electrophoresis.

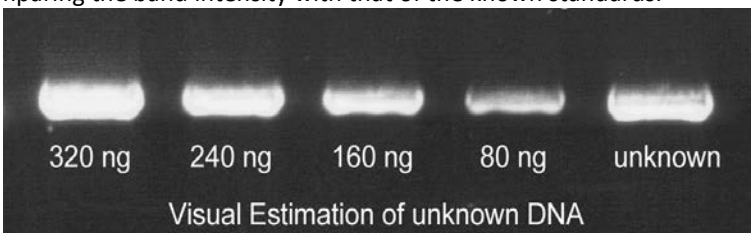
Recipe

1 X TAE Buffer
Agarose Gel Electrophoresis Buffer
40 mM Tris-Acetate pH 7.8
1 mM EDTA

1 X TBE
Agarose and Polyacrylamide Gel Electrophoresis Buffer
0.089 M Tris
0.089 M Boric Acid
0.002 M EDTA

Spectrophotometric Determination of DNA Concentration & Estimation by Agarose Gel Electrophoresis

Measuring the optical density (OD) or absorbance at 260 nm (A_{260}) in a UV spectrophotometer is a relatively accurate method for calculating the concentration of DNA in an aqueous solution if a standard curve is meticulously prepared. An A_{260} of 1, using a 1 cm path length, corresponds to a DNA concentration of 50 µg/ml for double stranded DNA, 40 µg/ml for RNA and 33 µg/ml for oligonucleotides. However, this method is not suitable for determining concentrations of dilute solutions of DNA, as the sensitivity of this method is not very high. For reliable readings, the concentration of double stranded DNA must be greater than 1 µg/ml. A simple, inexpensive method for the estimation of nanogram quantities of DNA is described in the following section. We recommend the use of agarose gel electrophoresis for routine approximate determination of DNA concentration.
The amount of DNA in sample may be estimated by running the sample alongside standards containing known amounts of the same-sized DNA fragment. In the presence of ethidium bromide staining, the amount of sample DNA can be visually estimated by comparing the band intensity with that of the known standards.



An unknown amount of a 4 kb DNA fragment (U) was run alongside known quantities (indicated in nanograms) of the same DNA fragment. As estimated by visual comparison with the known standards, the unknown sample contained 240-320 ng of DNA.

Ethidium bromide is a carcinogen. Follow Health and Safety Procedures established by your institution.
Follow proper Hazardous Material Disposal procedures established by your institution.

- Use 0.1 µg of ethidium bromide solution for each ml of gel volume.

Fragile X Genotyping Product Ordering Information

Product	Unit Size	Catalog No.
Fragile X Genemer™ Kit for gel based detection. Kit for performing PCR amplification and gel based detection.	1 Kit [100 rxns]	40-2004-11
Fragile X GScan™ Kits 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
Fragile X GeneProber™ GLFX1 Probe unlabeled Probe for radioactive labelling and Southern blot analysis	500 ng	40-2004-40
Fragile X GeneProber™ GLFX1 Probe Digoxigenin labeled Probe for non-radioactive chemiluminescent Southern blot analysis	110 µL	40-2004-41
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 nmols	40-2004-10
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

FRAXE/FMR2/AFF2 Genotyping Product Ordering Information

Product	Unit Size	Catalog No.
FRAXE/FMR2/AFF2 GeneProber™ AFF2-AJ31Dig1 Probe Digoxigenin labeled Probe for non-radioactive chemiluminescent Southern blot analysis	110 µL	40-2054-41
FRAXE/FMR2/AFF2 Genemer™ Kit for gel based detection Kit for performing PCR amplification & gel based detection	1 Kit [100 rxns]	40-2054-11
FRAXE/FMR2/AFF2 GScan™ Kits for fluorescent detection Kit for performing fluorescent PCR amplification based detection, Fam labeled	1 Kit [100 rxns]	40-2054-15FM

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

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

Southern Blot Buffers& Reagents

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

Related Products Ordering Information

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-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.

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.

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