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 Genemer™

Primer Pair for PCR Amplification of Fragile X CGG triple region

Catalog No. 40-2004-10

Storage Condition: See Material Supplied List

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.

20 years
results you can rely on
Gene Link™

Material Supplied

Fragile X CGG Repeat Genotyping Genemer™ Primer Pair

Primer Pair for PCR Amplification of Fragile X CGG triple region

This product should be used by researchers who would like to develop their own amplification reaction conditions.

Catalog No.	Description	Size
40-2004-10	Fragile X CGG Repeat Genotyping Genemer™ Primer Pair	10 nmols

Storage Condition

Store at -20°C.

Certificate of Analysis & Product Specifications

The Fragile X Genemer™ Primer Pair supplied have been validated to amplify the CGG triple repeat spanning region of the FMR-1 gene. Special amplification conditions and the use of PCR additives are required to amplify the CGG triple repeat region. PCR additives are available for purchase from Gene Link.

Gene Link has optimized kit for Fragile X triple CGG repeat amplification and detection by conventional agarose or fluorescent methods and Southern blot analysis of large CGG repeats and confirmation of homozygous alleles.

Appropriate nuclease free handling, dispensing and storage conditions required.

Manufacturing lot numbers are stated on the label of each product and accompanying packing slip.

Genemer™ Kits Product Ordering Information

Gene Link's Genemer™ kits contain optimized PCR amplification components for convenient agarose or polyacrylamide genotyping of triple repeat disorders and other genetic disorders. These are safe, convenient and sensitive, and afford rapid screening of samples. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders. Included in these kits are ready-to-run control samples of various repeats of the specific triple repeat disorder.
The Genemer™ 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 Genemer™ V2 Kit for gel based detection; 100 reactions kit	1 kit	40-2004-11
FRAXE/FMR2/AFF2 Genemer ™ Kit for gel based detection; 100 reactions kit	1 kit	40-2054-11
Huntington's Disease Genemer ™ V2 Kit for gel based detection; 100 reactions kit kit	1 kit	40-2025-11
Myotonic Dystrophy Genemer ™ Kit for for gel based detection; 100 reactions kit	1 kit	40-2026-11
Friedreich's Ataxia Genemer ™ Kit for gel based detection; 100 reactions kit	1 kit	40-2027-11

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

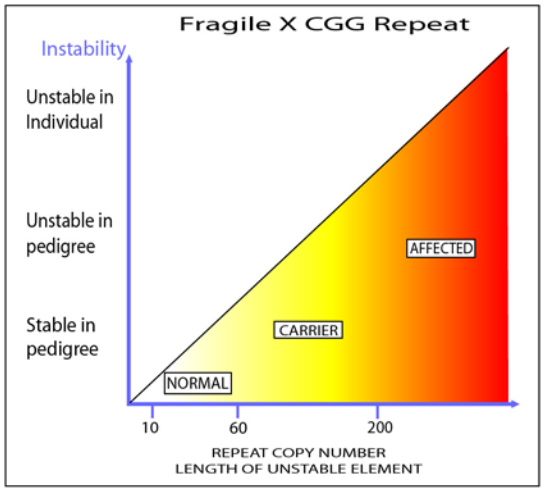
All Gene Link products are for research use only **Current pricing are posted at http://www.genelink.com/**

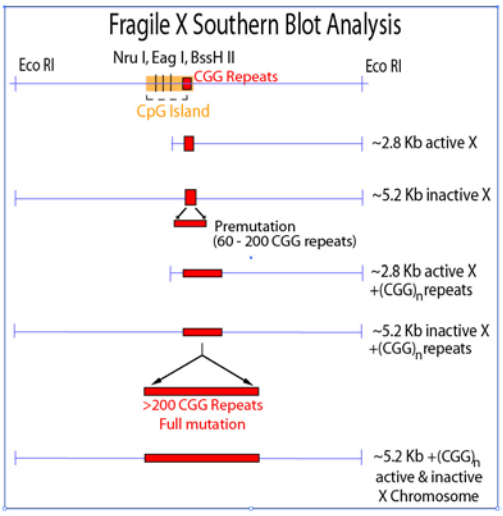
Fragile X Genotyping

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 patterns 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 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, 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).

Table 1. Trinucleotide Repeats in Human Genetic Disease

Disease	Repeat [a]	Normal Length [b]	Intermediate Length (Premutation)[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 Dystrophy (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 diseases are associated with a premutation length repeats tract or premutation 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 in the CpG region. Full mutation has been shown to methylate the active gene too and thus it prevents NruI and Eag I restriction of DNA. Hybridization of southern blots of Eco RI and Nru I or Eag I double digested DNA clearly can distinguish between normal, premutation and full mutation genotypes.

The size of the CGG repeats can be determined by PCR analysis and sizing preferably on sequencing gel. The PCR products can be labeled fluorescently by using fluorescently labeled primers or with ^{35}S or ^{32}P followed by autoradiography. Another attractive alternative is to run a cold PCR reaction followed by simple agarose gel or polyacrylamide electrophoresis followed by ethidium bromide staining. This method will yield results for alleles up to 100 CGG triple repeats for rapid genotyping of normal and permutation status. All samples that do not yield interpretable allele status should be processed for Southern blot analysis.

Table 2. CGG Trinucleotide repeats and fragment size*

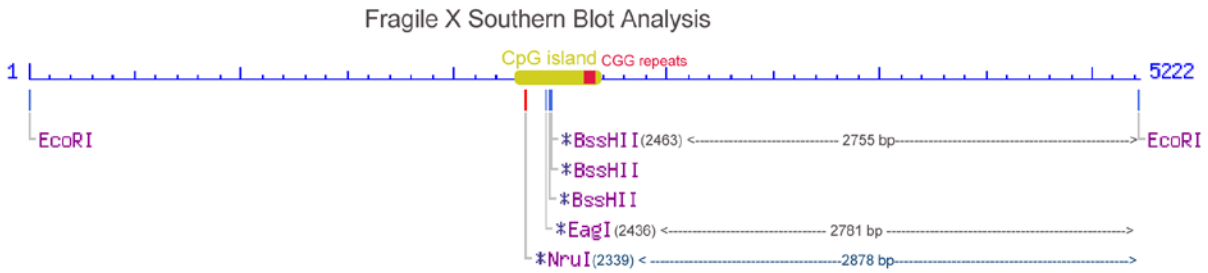
CGG	bp	Size	%	CGG	bp	Size	%	CGG	bp	Size	%
1	3	223		36	108	328	1.05	71	213	433	
2	6	226		37	111	331	0.35	72	216	436	
3	9	229		38	114	334	0.53	73	219	439	
4	12	232		39	117	337	1.23	74	222	442	
5	15	235		40	120	340	1.23	75	225	445	
6	18	238		41	123	343	0.35	76	228	448	
7	21	241		42	126	346	0.7	77	231	451	
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 sizes based on Gene Link Fragile X Genemer™ & GScan genotyping products.

The detection of amplification/expansion of a region of DNA sequence can be detected by PCR and Southern blot analysis, 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 repeat 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 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 repeat expansion. In the case of Fragile X specifically, the nature of the mutation poses problems using normal PCR conditions. In Fragile X, the repeat 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 has been classically 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 premutation PCR amplification is reliable, but still is coupled with a Southern blot analysis. In our laboratory PCR is performed in addition to Southern blot analysis. The PCR results are obtained in 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 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 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).

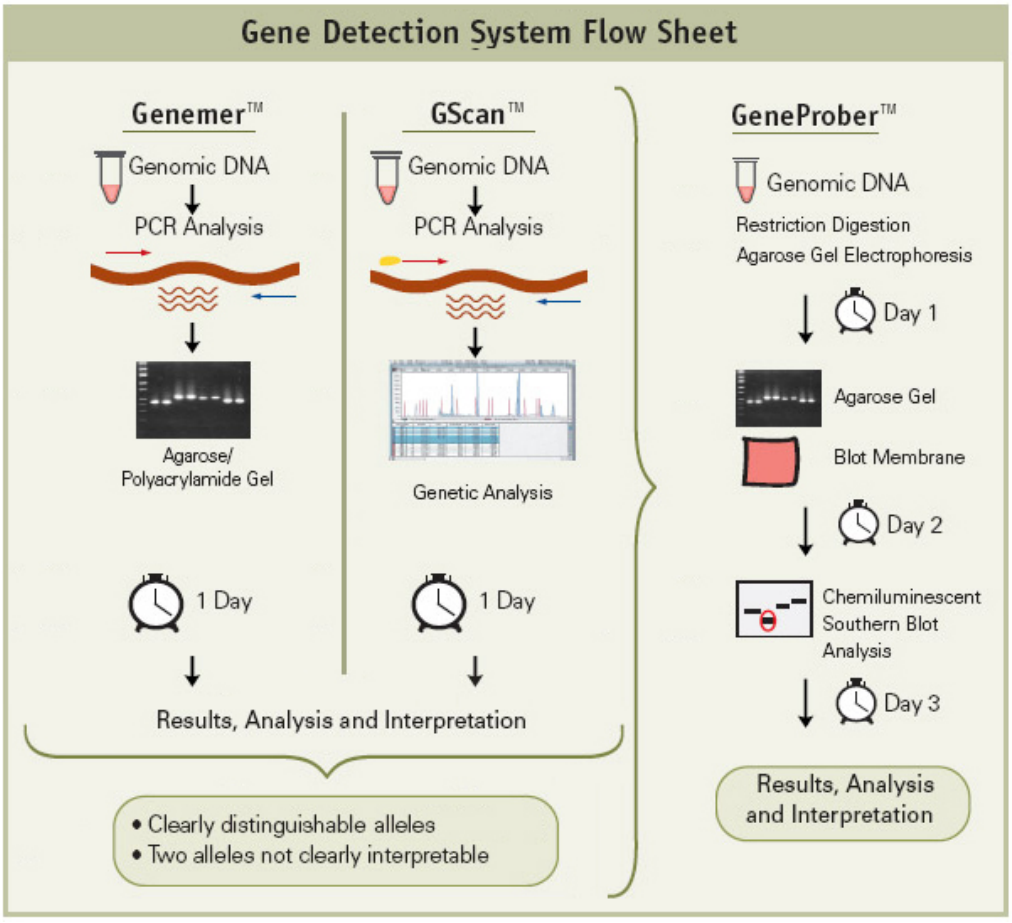


Gene Link offers safe and reliable chemiluminescent detection methods as an alternate to radioactive based detection methods. PCR-Prober™, GScan™ and GeneProber™ line of products replaces radioactive based methods. Gene Link's GScan Ver2 kit is for PCR amplification followed by fluorescent detection 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

Important Information

This product contains primer pair for amplification of the Fragile X CGG triple repeat region. Specialized amplification conditions are required to amplify large triple repeat regions.

This product should be used by researchers who would like to develop their own amplification reaction conditions. Special amplification conditions and the use of PCR additives are required to amplify the CGG triple repeat region. PCR additives are available for purchase from Gene Link.

Material Supplied: One tube containing 10 nmole lyophilized primers (FX-F1/R1). The quantity supplied is sufficient for 400 regular 50 µL PCR reactions.

Reconstitution

1. **Stock Primer solution**: Add 50 µL sterile pH 7.0 water to the tube containing the primers. The 10 nmole of primer when dissolved in 50 µL will give a solution of 200 µM, i.e. 200 pmole/µL.
2. **Primer Mix**: Prepare a 10 µM (10 pmole/µL) Primer Mix solution. Example: Transfer 10µL of stock primer solution to a new tube. Add 190 µL sterile water to this tube. Label this tube as **Primer Mix 10 µM.**

Fragile X CGG Repeats Genotyping Recommended PCR Cycling Conditions

PCR Thermal Cycler Files

Program two PCR thermal cycler files as follows

Hot Start File		
Step	Time and Temperature	Cycles
Denaturation	5 minutes at 98°C	1
Hold	Hold for infinity at 62°C	Hold
Add Taq enzyme mix (EM) while on hold.		

Fragile X CGG Amplification File		
Step	Time and Temperature	Cycles
Denaturation	2 minutes at 94°C	1
Denaturation	30 seconds at 94°C	30
Annealing	30 seconds at 60°C	
Extension	3 minutes at 72°C	
Fill up	7 minutes at 72°C	1
Hold	Hold for infinity at 4°C	Hold

Results and Interpretation

The Genemer™ fragile X gene detection system is optimized to give results. Fragile X PCR based genotyping is considered difficult due to the extensive stretch of CGG repeats in fragile X mutation that causes a strong secondary structure in the DNA. This secondary structure prevents DNA polymerases, even Taq polymerase used at elevated temperature, from replicating the CGG repeat region. The Genemer™ kit includes PCR additives and enhancers to reduce the secondary structure and increase the length of the CGG repeats that the Taq polymerase can replicate. The Genemer™ kit system can reliably detect up to 120 CGG repeats. Detection of more than ~100 repeats is not reliable. A DNA sample that does not yield a PCR product on duplicate analysis indicates either a failure of the PCR reaction, or a DNA sample with extensive CGG expansion. In the latter case, we recommend Southern analysis using the GeneProber™ Fragile X Detection System to clearly determine the genotype.

Using the reagents provided, the Genemer™ kit will yield an amplified Fragile X CGG triple repeat spanning PCR product with 30 CGG repeats of ~310 base pairs. All other repeats can be tabulated based on this size. Please refer to Table 2 of this manual. Note that the amplified product can only be resolved on a suitable genetic analyzer. The mobility of the amplified samples cannot be exactly correlated to the size markers due to the extensive CGG repeats. Based on sequenced CGG repeat DNA samples, we observe that the amplified CGG repeat fragments runs slower and is thus, when compared to the molecular weight markers run on genetic analyzers is approximately 3 base or one CGG repeat shorter. Example, a 29 CGG repeat fragment is 307 bp and travels as a 304 bp fragment when compared to commercially available genotyping molecular weight markers. It is advisable to run several control samples of known CGG repeats to ascertain the approximate size by comparison.

All normal samples except female homozygous samples will yield interpretable results. The normal pattern is one amplified fragment with CGG repeat size below 45 from male DNA samples and two amplified fragments with CGG repeat size below 45 from female DNA samples. Keep in mind that more than 40% of the population is homozygous for the 29-30 CGG allele and thus may give only one amplified fragment from female DNA in PCR results but are actually normal.

From our experience, less than 5% of the samples referred for Fragile X are actually Fx cases. PCR amplification and detection is a way to ascertain genotype of almost 90% of the samples. *It is essential to perform Southern blot analysis for ALL samples that does not give NORMAL results in duplicate PCR analysis.* PCR analysis is to screen bulk of the samples and then perform Southern blot analysis only on those samples that do not yield 'normal' amplification pattern.

You should perform Southern blot analysis for the following sample types

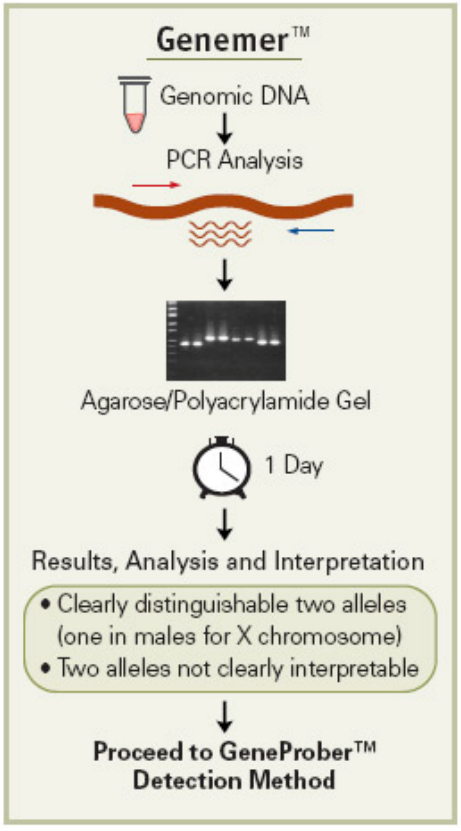
a. Only one fragment amplified for female samples. Realize that more than 40% of the population is homozygous for the 29-30 CGG allele and thus may give only one fragment in PCR results but are actually normal with two alleles.
b. No amplification products on duplicate amplification.
c. Samples with amplification over ~45 CGG repeats.

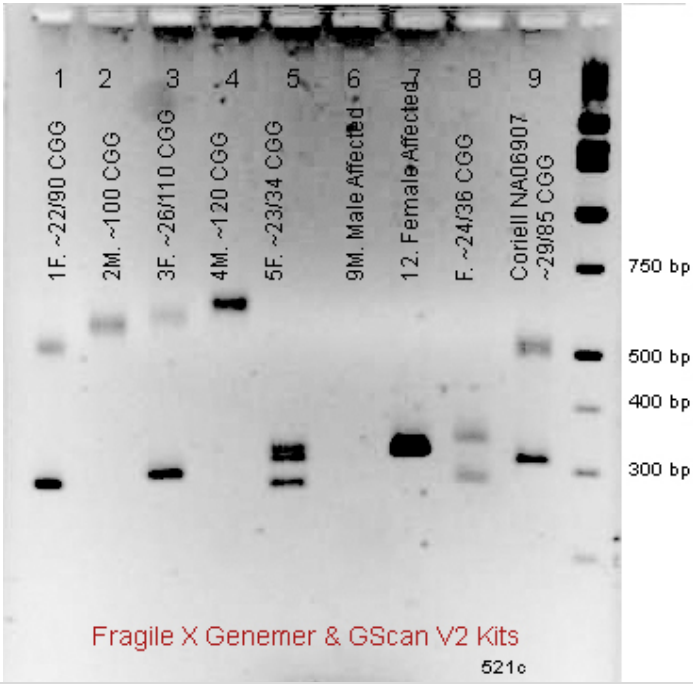
Genemer™ results should be easy to interpret based on the size of the fragment amplified, a guideline is provided in Table 3.

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

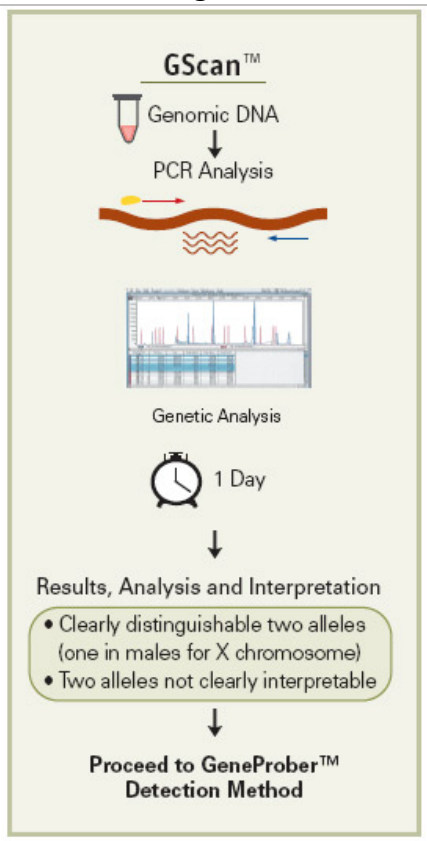
Fragile X Genemer™ V2 Kit [40-2004-11] PCR Amplification & Agarose Gel Detection





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 an 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



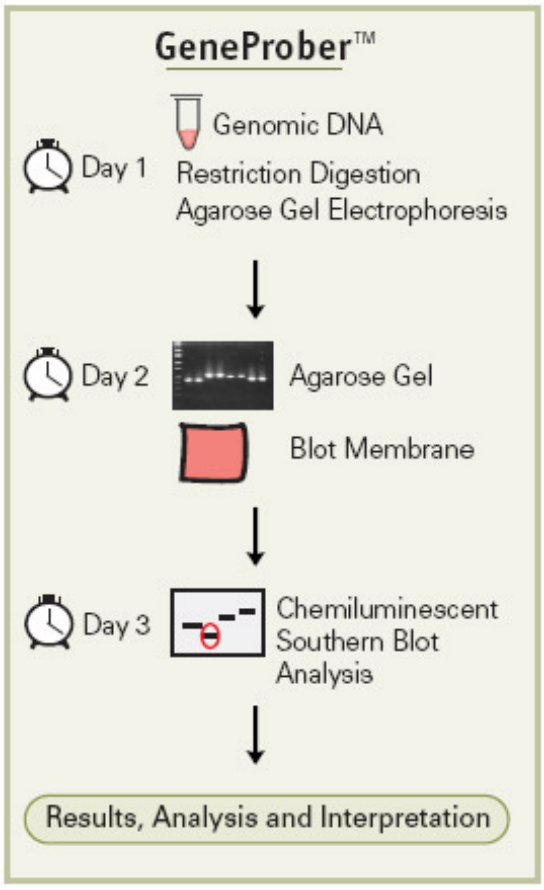
Fragile X GScan™ V2 [40-2004-15FM] Kit was used to amplify human genomic samples representing various CGG triple length pre-mutations, full mutations and normal Fragile X genotype. Fragment analysis results are shown, each panel denotes the fragile X CGG repeat genotype

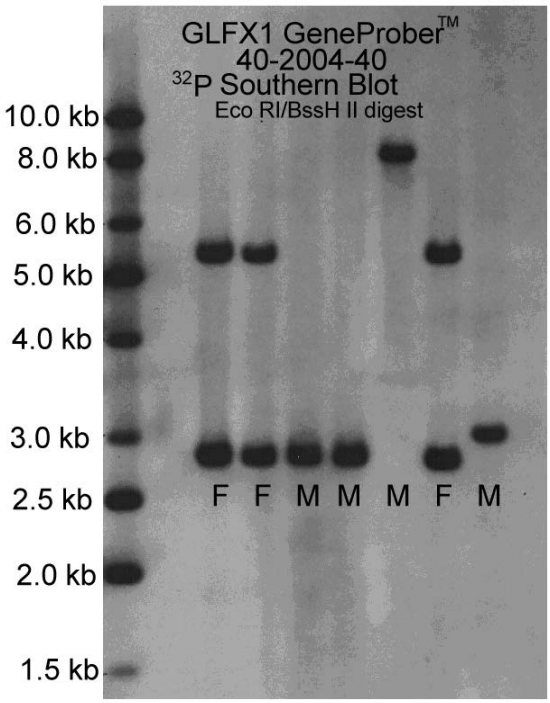
Recommendation for Southern Blot Analysis

Southern blot analysis can be performed using the non-radioactive digoxigenin labeled GeneProber™ probe (Catalog Number 40-2004-41) or for radioactive based detection use the unlabeled GeneProber™ (Catalog Number 40-2004-40). These probes are specific and deliver reliable results.

The GeneProber™ Fragile X gene detection system is for Southern blot genotyping based on size of the 2.8kb and 5.2 kb Eco RI/Eag I digests (or other appropriate restriction enzymes) of genomic DNA. This is similar to the radioactive blot which we are all used to, instead of having to label the probe with ^{32}P every week and the associated hazards. In the GeneProber™ system the probe is simply replaced with the non-radioactive GLFXDig1 digoxigenin labeled probe. All manipulations can thus be carried safely on the bench top. After washing, the detection system requires an added step of incubation with anti-Dig alkaline phosphatase for 30 minutes and after a few wash, detection using CSPD star as the chemiluminescent substrate. The blot is exposed to X-ray film from 2 hrs and onwards depending on the signal achieved. For a Southern blot using 5µg genomic DNA a 2hr exposure is normally sufficient.

Fragile X GeneProber™ [40-2004-41] Southern Blot & Chemiluminescent Detection





Fragile X southern blot analysis. Genomic DNA digested with Eco RI/BssH II and GLFX1 GeneProber [40-2004-40] labeled with ^{32}P as the probe. Normal pattern expected is as follows. Female: 5.2 kb and 2.7kb; Males; 2.7kb

Fragile X Frequently Asked Questions/Troubleshooting

1. **General Comment.** Fragile X genotyping is not easy. Fragile X PCR based genotyping is difficult due to the extensive stretch of CGG. Even Taq polymerase at elevated temperature cannot replicate the long stretch of CGG repeats. Our kit is optimized to give results for up to at least 120 CGG repeats. 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. **Reliable Detection.** The system will be able to detect reliably the CGG repeats greater than 120 repeats. Detection beyond this is dependent on the PCR conditions and is NOT reliable. Any DNA sample which does not give a reliable CGG repeat analysis on this system clearly indicates either a failure of the PCR reaction or a DNA with extensive CGG repeats. A particular DNA sample not yielding a PCR product on duplicate analysis clearly indicates the possibility of long CGG repeats. In cases like these we suggest that Southern analysis should be done using the Fragile X GeneProber™ [40-2004-41] gene detection system to clearly determine the genotype. Southern analysis is also strongly advised when both the alleles are not reliably genotyped.

References

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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 Catalog Number: 40-4010-01

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 for 20 seconds. Small particles of brown color will 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. Mixing by inversion 30-40 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.

PCR Enhancers & Additives

DNA polymerases need to elongate rapidly and accurately to function effectively *in vivo* and *in vitro*, yet certain DNA regions appear to interfere with their progress. One common problem is pause sites, at which DNA polymerase molecules cease elongation for varying lengths of time. Many strong DNA polymerase pauses are at the beginnings of regions of strong secondary structure such as template hairpins (1). Taq polymerase used in PCR suffers the same fate and GC-rich DNA sequences often require laborious work to optimize the amplification assay. The GC-rich sequences possess high thermal and structural stability, presumably because the high duplex melting temperature that permits stable secondary structures to form, thus preventing completion of a faithful replication (2).

Nucleotide analog 7-deaza dGTP is effective in reducing the secondary structure associated with GC rich region by reducing the duplex stability (4). Betaine, DMSO and formamide reduces the Tm and the complex secondary structure, thus the duplex stability (1-5). Tetramethyl ammonium chloride (TMAC) actually increases the specificity of hybridization and increases the Tm. The use of TMAC is recommended in PCR conditions using degenerate primers.

These PCR additives and enhancing agents have been used to increase the yield, specificity and consistency of PCR reactions. These additives may have beneficial effects on some amplification and it is impossible to predict which agents will be useful in a particular context and therefore they must be empirically tested for each combination of template and primers.

PCR Additives		
Additive	**Purpose & Function**	**Concentration**
7-deaza-2'-deoxyguanosine; 7-deaza dGTP	GC rich region amplification. Reduce the stability of duplex DNA	Totally replace dGTP with 7-deaza dGTP; or use 7-deaza dGTP: dGTP at 3:1
Betaine (N,N,N-trimethylglycine = [carboxymethyl]trimethylammonium)	Reduces Tm facilitating GC rich region amplification. Reduces duplex stability	Use 3.5M to 0.1M betaine. Be sure to use Betaine or Betaine (mono)hydrate and **not** Betaine HCl.
BSA (bovine serum albumin)	BSA has proven particularly useful when attempting to amplify ancient DNA or templates, which contain PCR inhibitors such as melanin.	BSA concentration of 0.01 µg/µl to 0.1 µg/ µl can be used.
DMSO (dimethyl sulfoxide)	DMSO is thought to reduce secondary structure and is particularly useful for GC rich templates.	DMSO at 2-10% may be necessary for amplification of some templates; however 10% DMSO can reduce *Taq* polymerase activity by up to 50% so it should not be used routinely.
Formamide	Reduces secondary structure and is particularly useful for GC rich templates.	Formamide is generally used at 1-5%. Do not exceed 10%.
Non-ionic detergents e.g. Triton X-100, Tween 20 or Nonidet P-40 (NP-40)	Non-ionic detergents stabilize *Taq* polymerase and may also suppress the formation of secondary structure.	0.1-1% Triton X-100, Tween 20 or NP-40 may increase yield but may also increase non-specific amplification. As little as 0.01% SDS contamination of the template DNA (left-over from the extraction procedure) can inhibit PCR by reducing *Taq* polymerase activity to as low as 10%, however, inclusion of 0.5% Tween-20 or -40 will effectively neutralize this effect.
TMAC (tetramethylammonium chloride)	TMAC is used to reduce potential DNA-RNA mismatch and improve the stringency of hybridization reactions. It increases Tm and minimizes mis-pairing.	TMAC is generally used at a final concentration of 15-100 mM to eliminate non-specific priming.

Purification of PCR Product

Various purification methods are available for the purification of PCR products. The selection of a particular method over another is based on the downstream application and the initial robustness of the amplification. Usually no further purification is required for most cloning experiments if a single fragment is amplified, whereas for sequencing applications the amplified product should be purified from the primers and any other minor amplification products.

For fragment analysis of PCR products the preferred method of purification to eliminate primers, primer dimers and salts is the Omni-Clean™ Purification System available from Gene Link. Catalog No. 40-4130-10 for bead based system; 40-4140-10 for spin column based system. Gene Link recommends the beads system as recovery of the amplified PCR product is critical. Please refer to product insert for detailed protocol or visit www.genelink.com

A. **Purification of DNA from solution using glass beads. Provides removal of salts, primers and dNTP.**
[Omni-Clean™ DNA Beads Concentration System; Catalog No. 40-4130-10]

Protocol

1. Determine volume of DNA solution and add 2 volumes of OCC-2 solution and mix by vortexing.
2. Add 1 µl of glass bead suspension per µg of DNA and mix by vortexing.
3. Centrifuge at 4K rpm for 20 seconds to pellet glass bead/DNA complex. Discard all traces of supernatant.
4. Re-suspend pellet in 400 µl Omni-Clean™ G3 wash buffer.
5. Centrifuge at 4K rpm for 20 seconds and discard wash buffer.
6. Pipet out any remaining buffer in the tube.
7. Repeat steps 4-6 twice.
8. Add 20 µl water or TE; re-suspend pellet by vortexing and centrifuge at 4K rpm for 20 seconds.
9. The supernatant contains the purified DNA. Using a pipet, collect the supernatant and transfer to a new appropriately labeled tube.

B. **Purification of DNA from solution using spin column. Provides removal of salts, primers and dNTP.**
[Omni-Clean™ DNA Spin Column Concentration System; Catalog No. 40-4140-10]

Protocol

1. Determine volume of DNA solution and add 2 volumes of OCC-2 solution and mix by vortexing.
2. Add the above solution to the spin column assembled on a collection tube.
3. Let the solution flow by gravity or centrifuge at 2K rpm for 20 seconds. Discard flow through collected in the collection tube.
4. Add 400 µl Omni-Clean™ G3 wash buffer to the spin column. Centrifuge at 2K rpm for 2 minutes and discard wash buffer collected in the collection tube.
5. Replace the collection tube with a new appropriately labeled 1.5ml tube.
6. Add 25 µl water or TE to the spin column. Let sit for 3 minutes.
7. Centrifuge at 2K rpm for 2 minutes.
8. The collection tube contains the purified DNA.

PEG Precipitation

Primers and salts are efficiently removed by a simple PEG precipitation. This method is recommended for downstream DNA sequencing application. This method is generally used for plasmid DNA.

Protocol

1. To 50 µl of amplified PCR reaction add 6.0 µl of 5 M NaCl and 40 µl of 13% (w/v) PEG 8000. Incubate the mixture on ice for 20-30 minutes.
2. Collect the DNA precipitate by centrifugation at maximum speed for 15 minutes at 4 °C in a microfuge. Carefully remove the supernatant by gentle aspiration.
   The pellet of DNA is translucent and generally invisible at this stage.
3. Rinse the pellet with 500 µl of 70% ethanol.
   The precipitate changes to a milky-white color and becomes visible.
4. Carefully pour off the 70% ethanol. Rinse the DNA pellet once more with 70% ethanol. Store the tube in an inverted position at room temperature until the last visible traces of ethanol have evaporated.
5. Dissolve the DNA in 20 µl of H_20.
6. Run an aliquot on an agarose gel to confirm the presence of the correct amplified product. The purified DNA is sequence grade and can be used directly for sequencing.

Gel Filtration

Primers and salts are efficiently removed by gel filtration using Sephadex G-50. This method is recommended for downstream DNA sequencing application.

Protocol

1. Hydrate Sephadex G-50 ahead of time in sterile water or TE (10mM Tris pH 8, 1 mM EDTA). Take out from fridge if already stored hydrated. Bring to room temperature.
2. Assemble a spin column on a collection tube.
3. Add 700 µl of hydrated Sephadex G-50 to each spin column, initiate flow using rubber bulb or any other method.
4. Allow flowing by gravity till there is no more fluid left above the Sephadex G-50 bed. Discard flow through from the collection tube.
5. Spin the spin column placed inside the collection tube for 2 minutes at 3 K rpm.
6. Change collection tube to new 1.5 ml tube appropriately labeled with sample name.
7. Apply up to 50 µl sample gently to the G-50 bed of the column.
8. Spin for 2 minutes at 3 K rpm.
9. Purified sample is collected in the collection tube. The eluent collected in the 1.5 ml tube is free of salts and primers shorter than 35-40mer.

References

1. Kovarova, M; and Draber, P; (2000) New Specificity and yield enhancer for polymerase chain reactions (2000) Nucl. Acids. Res. 28: e70.
2. Henke, W., Herdel, K., Jung, K., Schnorr, D. and Stefan A. Loening, S. (1997) Betaine improves the PCR amplification of GC-rich DNA sequences. Nucl. Acids Res. 25: 3957-3958.
3. Daniel S. Mytelka, D.S., and Chamberlin, M.J.,(1996) Analysis and suppression of DNA polymerasepauses associated with a trinucleotide consensus. Nuc. Acids Res.,. 24:2774–278.
4. Keith, J. M., Cochran, D.A.E., Lala, G.H., Adams, P., Bryant, D.and Mitchelson, K.R. (2004) Unlocking hidden genomic sequence. Nucl. Acids Res. 32: e35.
5. Owczarzy, R., Dunietz, I., Behlke, M.A., Klotz, I.M. and Joseph A. Walder. (2003) Thermodynamic treatment of oligonucleotide duplex–simplex equilibria. PNAS, 100:14840-14845.

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

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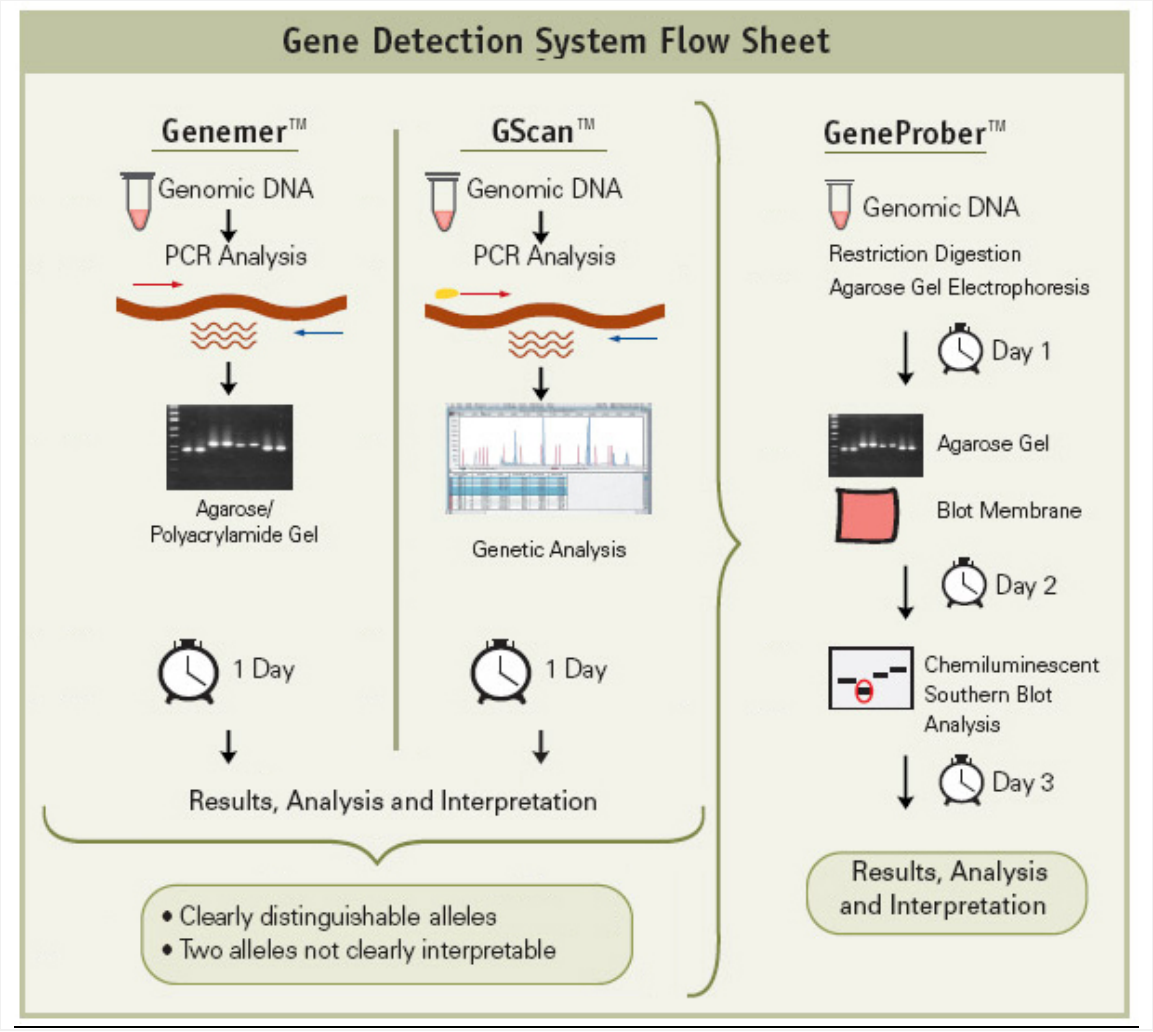

Genemer™ Kits Product Ordering Information

Gene Link's Genemer™ kits contain optimized PCR amplification components for convenient agarose or polyacrylamide genotyping of triple repeat disorders and other genetic disorders. These are safe, convenient and sensitive, and afford rapid screening of samples. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders. Included in these kits are ready-to-run control samples of various repeats of the specific triple repeat disorder.

The Genemer™ 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 Genemer™ V2 Kit for gel based detection; 100 reactions kit	1 kit	40-2004-11
FRAXE/FMR2/AFF2 Genemer ™ Kit for gel based detection; 100 reactions kit	1 kit	40-2054-11
Huntington's Disease Genemer ™ V2 Kit for gel based detection; 100 reactions kit kit	1 kit	40-2025-11
Myotonic Dystrophy Genemer ™ Kit for for gel based detection; 100 reactions kit	1 kit	40-2026-11
Friedreich's Ataxia Genemer ™ Kit for gel based detection; 100 reactions kit	1 kit	40-2027-11

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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

GScan™ Kits 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

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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

Omni-Marker™

Product	Catalog No.	Size*
Omni-Marker™ Universal unlabeled; 1 mL	40-3005-10	1 mL
Omni- Marker™ Low unlabeled; 1 mL	40-3006-10	1 mL
Omni-Marker™ GScan™-2 Tamra labeled 50 bp - 600 bp; 500 µL	40-3062-05	500 µL

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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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Notes:

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