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

Gene Detection System

FRAXE/FMR2/AFF2 Genotyping GScan™ V3 Kits

FMR2 CCG triple repeat fluorescent genotyping

Catalog No. 40-2054-15

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.
Genotyping using this kit requires use of the appropriate fluorescent genetic analyzer instrument and software capable of detection of fluorescently labeled fragments of varying lengths.
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

FRAXE/FMR2/AFF2 CCG Repeat Genotyping GScan™ V3 Kits HEX Labeled

FRAXE/FMR2/AFF2 CCG Repeat Genotyping GScan™ V3 Kits			
Kit	**Catalog No.**	**Description**	**Size**
□	40-2054-15FM	FRAXE/FMR2/AFF2 CCG Repeat Genotyping GScan™ V3 Hex Kit, 100 rxns.	100 rxns
□	40-2054-15FMS	FRAXE/FMR2/AFF2 CCG Repeat Genotyping GScan™ V3 Hex Kit, 20 rxns.	20 rxns

FRAXE/FMR2/AFF2 CCG Repeat Genotyping GScan™ V3 Kit 100 Reactions Components			
Content	**Catalog No.**	**Kits Components 100 X 25 µL Reactions**	**Size**
□	40-2054-15Q	FRAXE/FMR2/AFF2 GScan™ V3 Hex Component Q	425 µL
□	40-2054-15E	FRAXE/FMR2/AFF2 GScan™ Component E	1.2 mL
□	40-2054-15D	FRAXE/FMR2/AFF2 GScan™ Component D	250 µL
□	40-2054-15P	FRAXE/FMR2/AFF2 GScan™ Hex amplified 14 CCG repeats control	10 µL

FRAXE/FMR2/AFF2 CCG Repeat Genotyping GScan™ V3 Kits Kit 100 Reactions Components			
Content	**Catalog No.**	**Kit components 100 X 25 µL Reactions**	**Size**
□	40-2054-15QS	FRAXE/FMR2/AFF2 GScan™ V3 Hex Component Q	90 µL
□	40-2054-15ES	FRAXE/FMR2/AFF2 GScan™ Component E	250 µL
□	40-2054-15DS	FRAXE/FMR2/AFF2 GScan™ Component D	60 µL
□	40-2054-15PS	FRAXE/FMR2/AFF2 GScan™ Hex amplified 14 CCG repeats control	5 µL

Storage Condition

Store at -20°C.

Certificate of Analysis & Product Specifications

The FRAXE/FMR2/AFF2 CCG Repeat Genotyping GScan™ V2 kit components supplied have been validated to amplify the CCG triple repeat spanning region of the FMR-2 gene. The length of CCG triple repeat amplification routinely obtained by using standard Taq polymerase is greater than 60 CCG repeats.
Appropriate nuclear-free handling, dispensing and storage conditions required.

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

Product Label Information

REF	Catalog No.	Description	Size
	40-2054-15FM	FRAXE/FMR2/AFF2 CCG Repeat Genotyping GScan™ V3 6-Fam Kit. 100 X 25 µL rxns.	100 rxns
	40-2054-15FMS	FRAXE/FMR2/AFF2 CCG Repeat Genotyping GScan™ V3 6-Fam Kit. 20 X 25 µL rxns.	20 rxns

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

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/**

FRAXE/FMR2/AFF2 Genotyping

Background

FRAXE mental retardation (OMIM 309548) is a form of mild to moderate intellectual disability generally associated with learning difficulties, communication deficits, attention problems, hyperactivity and autistic behavior. FRAXE mental retardation is the cause of a non-syndromic X-linked mental retardation affecting 1/50,000 newborn males. The CCG repeat of FRAXE can either expand or contract and is equally unstable when transmitted through the male or the female germ line (1–3).

FRAXE (AFF2/ FMR2 gene) a folate-sensitive fragile site in Xq28 ~600 kb distal to the FRAXA (FMR1 gene) site is the most common form of inherited mental retardation (4-5). FMR2 is a large gene with a major 8.75-kb transcript in placenta, fibroblasts adult and brain and a longer 13.7-kb FMR2 isoform in fetal brain (6). The FMR2 gene is organized in 22 exons, showing several possibilities of alternative splicing for exons 2, 3, 5, 7 and 21. The longest of the FMR2 isoforms is composed of 1272 amino acids and contains two nuclear localization signal (NLS) sequences that are both able to direct GFP into the nucleus (6). Molecular characterization revealed that individuals expressing FRAXE had amplifications of a CCG repeat adjacent to a CpG island. Normal individuals showed 4–39 copies of the polymorphic FRAXE CCG repeat, while individuals expressing the fragile site had >200 copies and their CpG island was fully methylated. These findings are similar to those found for folate-sensitive fragile X site FRAXA. Reports of FRAXE full expansions and pre-mutations are rarely documented. In this respect, it has been very difficult to determine to what extent the alleles, with CCG repeats in the range of 36 and 199, have a pathogenic effect (7-8). Intellectually disabled individuals are primarily referred for FRAXA screening and individuals who are negative for FRAXA are possible candidates for FRAXE screening. Traditionally in some laboratories FRAXE/FMR2/AFF2 molecular analysis is performed by PCR, it is known that CCG repeats in the range of ~80 and above are not reliably amplified. We embarked on an effort to supplement our PCR analysis by Southern blot and cloned a segment of the AFF2 gene that can be used by appropriate labeling as a probe to determine expansion of the CCG repeats in the AFF2 gene. We have developed a probe to be used for Southern blot analysis that reliably detects the AFF2 CCG triple repeat amplification. We have presented data of AFF2 molecular analysis in a subpopulation of 5,000 individuals referred for FRAXA screening (7-8). The presence of pre-mutated and fully expanded alleles in either gender, were confirmed by Southern blot analysis, which also enabled exclusion of methylation or repeat number mosaics as well as PCR failure. The use of this probe has been recommended as suitable for genotyping of pre-mutations, full mutations, and mosaics specifically for individuals presented for FRAXA screening with negative results to determine FRAXE status (7-8).

CCG repeats	Genotype/Phenotype	Clinical Condition	Comments
≤ 30 repeats	Normal allele	unaffected	Normal. Alleles stable on gene transmission
31-60 repeats	Intermediate allele	Unaffected (?)	Intermediate allele, mutable normal allele generating a normal phenotype
61-200 repeats	premutation	normal or mild phenotype	Generating a normal or mild FRAXE phenotype
≥ 200 CCG	Full mutation	Affected	Generating a FRAXE phenotype.

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

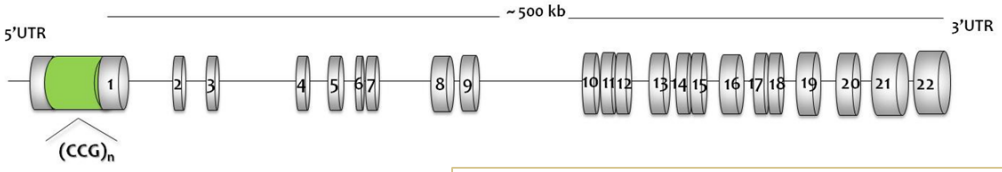
Disease	Repeat[a]	Normal Length[b]	Intermediate Length (Premulation)[a,b]	Full Disease Length[b]
Fragile XA (FRAXA)	$(CGG)_n$	6-52	59-230	230-2,000
Fragile XE (FRAXE)	$(CCG)_n$	4-39	? (31-61)	200-900
Fragile XF(FRAXF)	$(CGG)_n$	7-40	?	306-1,008
FRA16A	$(CCG)_n$	16-49	?	1,000-1,900
Jacobsen Syndrome (FRA11B)	$(CGC)_n$	11	80	100-1,000
Kennedy Syndrome (SMBA)	$(CAG)_n$	14-32	?	40-55
Myotonic 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

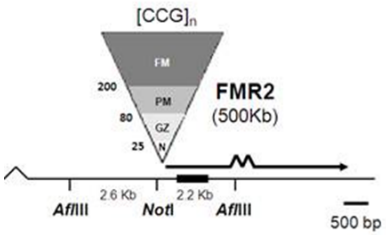
[a] Typically, repeats tracts contain sequence interruptions. See Pearson and Sinden (1998*a*) for a discussion of the sequence interruptions.
[b] No. of triplet repeats.
[c] A question mark (?) indicates potential mutagenic intermediate length, and an ellipsis (…) indicates none. Not all disease are associated with a permutation length repeats tract or permutation disease condition.

Molecular Analysis

FRAXE/AFF2/FMR2 genotyping can be done by direct PCR amplification of the CCG 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 FRAXE gene region containing the CCG trinucleotide repeats is flanked by Not I and Afl III sites. Full mutation has been shown to methylate the active gene too and thus it prevents Not I restriction of DNA. Hybridization of southern blots Not I and Afl III double digested DNA clearly can distinguish between normal, premutation and full mutation genotypes

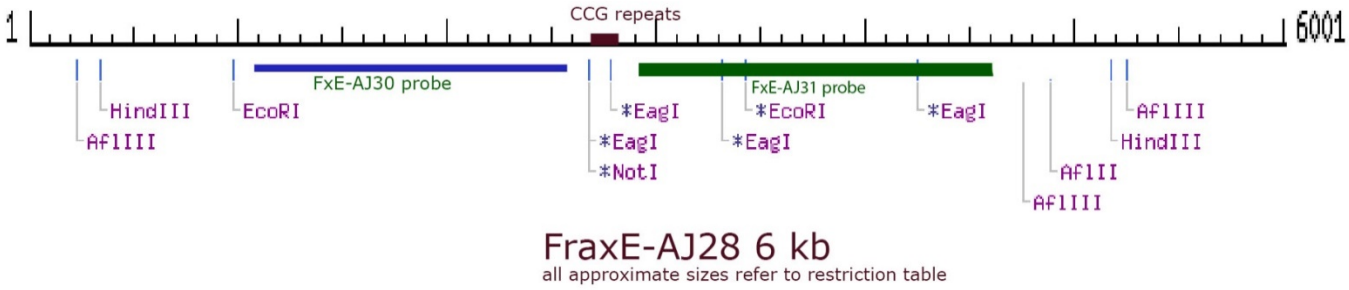
The size of the CCG repeats can be determined by PCR analysis and sizing preferably on sequencing gel. The PCR products can be either labeled with ^{35}S or ^{32}P followed by autoradiography. Another attractive alternate is to run a cold PCR reaction followed by blotting and hybridization with an alkaline phosphatase conjugated probe for non-radioactive detection.





Genomic Summary

- Approved symbol is AFF2
- Approved names are AF4/FMR2 family, member 2
- Previous symbol & names are FMR2, "fragile X mental retardation 2"
- HGNC:3776
- Synonym is FRAXE
- AFF2 gene is on the X chromosome at q28
- Comprises 22 exons spanning approximately 500 kb.
- The AFF2 CCG repetitive region is located in the 5'-UTR



FraxE-AJ28 6 kb
all approximate sizes refer to restriction table

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.

Southern blot analysis for FRAXE mutation detection involves the cleavage of DNA with enzyme Not I and Afl III. This method detects the size of CCG repeats region by hybridization of AFF2 AJ31-Dig1 GeneProber™ to DNA that has been double digested with restriction enzymes Not I and Afl III and blotted onto a membrane. In normal females two fragments are seen, a 2.2kb corresponding to the active X and a 4.8kb fragment corresponding to the methylated inactive X chromosome. Normal males exhibit only the 2.2kb banding pattern. Affected males will have an amplified CCG repeats region with methylation thus giving rise to fragments larger than the normal 4.8kb. Premutations in males and females will be seen as fragments from 2.3-3.3kb (normal 2.2kb) derived from the X chromosome. Premutations in females derived from the inactive X will give fragments from 4.9- ~6kb. Mosaicism is characterized by fragments appearing as a mixture of full mutation (methylated, larger than 4.8kb) and unmethylated premutation (2.2-3.3kb).

AFF2/FMR2/FRAXE CCG Trinucleotide repeats and fragment size*													
CGG	bp	Size	%		CGG	bp	Size	%		CGG	bp	Size	%
1	3	210			36	108	316			71	213	421	
2	6	213			37	111	319			72	216	424	
3	9	216			38	114	322			73	219	427	
4	12	219			39	117	325			74	222	430	
5	15	222			40	120	328			75	225	433	
6	18	225			41	123	331			76	228	436	
7	21	228			42	126	334			77	231	439	
8	24	231			43	129	337			78	234	442	
9	27	234			44	132	340			79	237	445	
10	30	237			45	135	343			80	240	448	
11	33	240	3		46	138	346			81	243	451	
12	36	243	4		47	141	349			82	246	454	
13	39	247	12		48	144	352			83	249	457	
14	42	250	58		49	147	355			84	252	460	
15	45	253	18		50	150	358			85	255	463	
16	48	256			51	153	361			90	270	466	
17	51	259			52	156	364			95	285	469	
18	54	262			53	159	367			100	300	472	
19	57	265			54	162	370			105	315	475	
20	60	268	5		55	165	373			110	330	478	
21	63	271			56	168	376			115	345	481	
22	66	274			57	171	379			120	360	484	
23	69	277			58	174	382			130	390	487	
24	72	280			59	177	385			140	420	490	
25	75	283			60	180	388			150	450	493	
26	78	286			61	183	391			160	480	496	
27	81	289			62	186	394			170	510	499	
28	84	292			63	189	397			180	540	502	
29	87	295			64	192	400			190	570	505	
30	90	298			65	195	403			200	600	508	
31	93	301			66	198	406			210	630	511	
32	96	304			67	201	409			220	660	514	
33	99	307			68	204	412			230	690	517	
34	102	310			69	207	415			240	720	520	
35	105	313			70	210	418			250	750	523	

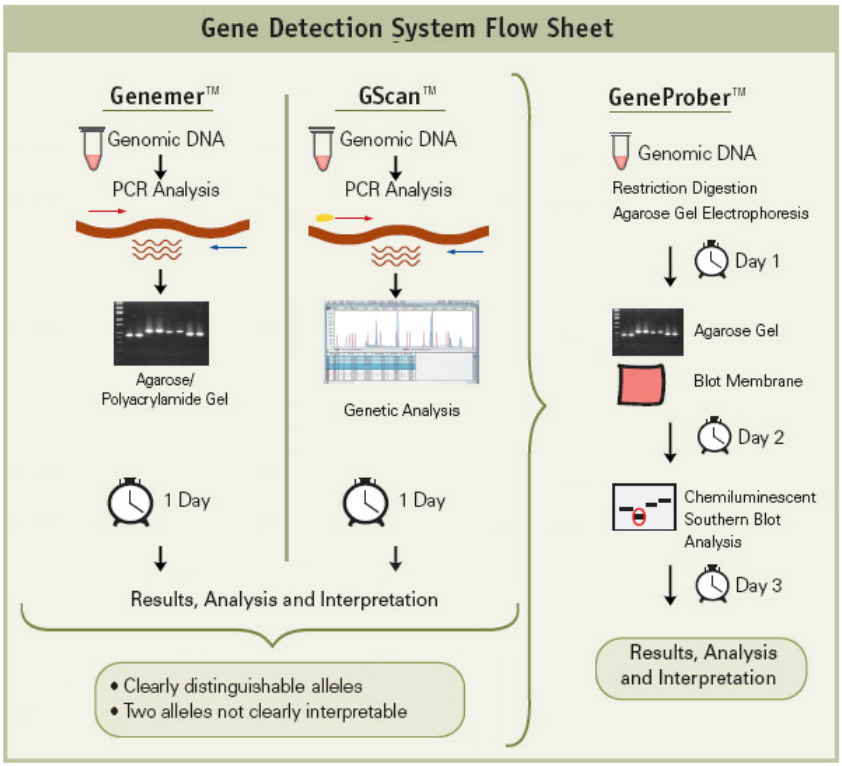
*Fragment size obtained using Gene Link AFF2 F1/R1 GScan™ & Genemer™ products. The formula for determining PCR fragment size is 207 + 3n, where n= the number of CCG repeats

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.



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Procedure

Important Information

Genotyping using this kit requires use of the appropriate fluorescent genetic analyzer instrument and software capable of detection of fluorescently labeled fragments of varying lengths. This kit has been optimized for ABI3100 genetic analyzer.

Procedure: AFF2/FRAXE/FMR2 CCG Repeats Analysis by PCR

PCR Premix Preparation

Thaw individual components. Promptly store at -20°C after use. Prepare ***fresh*** before use enough PCR premix for the number of reactions to be performed. Prepare 10% more for pipeting allowance. Prepare premix following the volumes given below. Follow the same ratio for preparing other final volumes.

PCR Thermal Cycler Files

Program PCR thermal cycler files as follows

AFF2 CCG Amplification File		
Step	Time and Temperature	Cycles
Initial Denaturation	2 minutes at 94°C	1
Denaturation	15 seconds at 94°C	42
Annealing	20 seconds at 58°C	
Extension	3 minutes at 72°C	
Fill up	7 minutes at 72°C	1
Hold	Hold for infinity at 12°C	Hold

Protocol: PCR Amplification. Note the amplified fragment will be labeled with HEX fluorescent dye. Assure that the fragment analyzer is calibrated for Hex dye.

A. Example: PCR premix for 25 µL reaction. Label tube as "PP" (PCR premix).

Given below is a protocol for preparing a PCR premix for one and ten 20 µL reaction. This can be scaled up or down as required. Prepare 10% more for pipetting loss.

PCR Premix Preparation-PP			
Component	Volume/25 µL rxn.	11X 25 µL rxns.*	Worksheet
Sterile water	6 µL	66 µL	
AFF2 GScan™ Component Q	4 µL	44 µL	
AFF2 GScan™ Component E	10 µL	110 µL	
AFF2 GScan™ Component D	2 µL	22 µL	
Taq Polymerase	1 µL	11 µL	
Total	**23 µL**	**253 µL**	
*Preparation of 10% more to account for pipetting loss.			

The kit is optimized for regular Taq Polymerase. Any good quality Taq polymerase that is regularly used in the laboratory can be used. Hi Fidelity and long template Taq polymerase may perform better.

B. PCR

Final PCR			
Component	Volume/25 µL rxn.		
PCR premix (from above)	23 µL		
Template DNA (~100 ng)	2 µL		
Total	**25 µL**		

Start AFF2 CCG amplification PCR file.

E. Post PCR Processing

Process sample for Omni-Clean purification of amplified products. This purification method is preferred over Sephadex G-50 spin column chromatography or ethanol precipitation as it is the most effective way to reduce the fluorescent primer and primer dimers if present. Omni-Clean Kit is available from Gene Link, catalog number 40-4130-10 or 40-4140-10.

OR

Process sample for Sephadex G-50 spin column chromatography. G-50 columns should be equilibrated with sterile water.

OR

Process sample for ethanol precipitation. Dissolve purified fragments in 20 µL water. Ready for fragment analysis.

F. Fragment Analysis

Follow genetic analyzer manufacturer's instructions. Run two aliquots of sample at different concentrations. A brief protocol is given below for ABI3100 instrument.

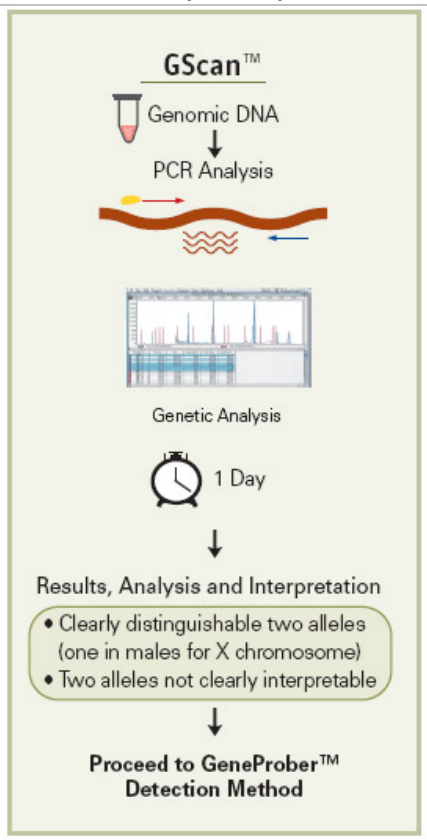
1. Add 0.5 μL of appropriate size standards.
2. Add 2 & 4μL of sample in duplicate tubes.
3. Add 12 μL of formamide loading buffer.
4. Mix and heat denature at 95°C for 5 minutes.
5. Transfer to ice.
6. Samples ready to load on instrument.

G. Amplified Control Sample Analysis

A FRAXE/FMR2/AFF2 ready to load sample is provided in the kit. This is an aliquot of a GScan kit amplified genomic DNA sample containing ~14 CCG repeats. This amplified sample can be run as a control to yield the appropriate size fragment. We recommend that 2 and 4 μL of this sample be loaded with appropriate molecular weight markers. The sample is provided in water. Follow genetic analyzer manufacturer's instructions relating to running samples, data recovery and interpretation.

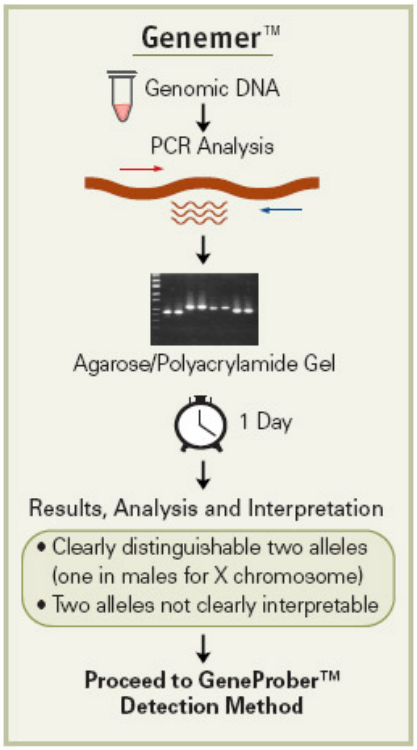
Results and Analysis

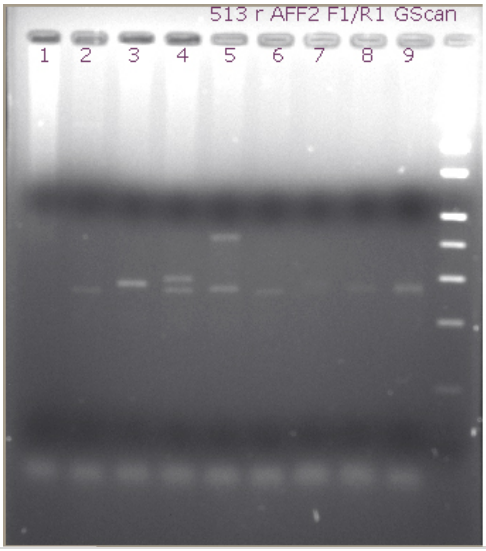
FRAXE/FMR2/AFF2 GScan™ Kit [40-2054-15] PCR Amplification & Fluorescent Detection



FRAXE/FMR2/AFF2 GScan™ [40-2054-15FM] Kit was used to amplify human genomic samples representing various CCG triple length pre-mutations, full mutations and normal genotype. Fragment analysis results are shown, each panel denotes the AFF2 CCG triple repeat genotype.

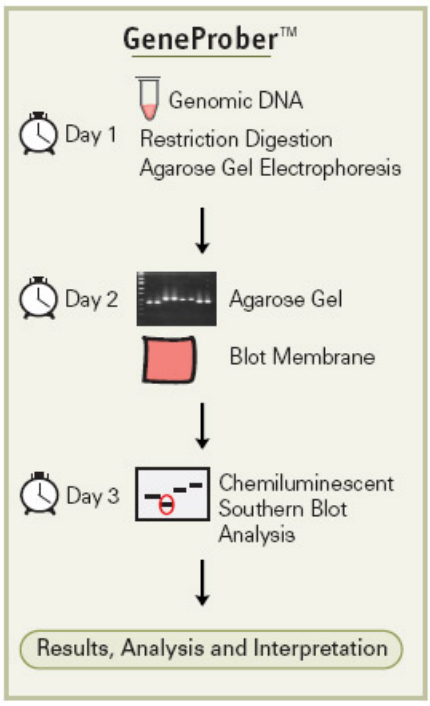
FRAXE/FMR2/AFF2 GScan™ Kit [40-2054-11] PCR Amplification & Agarose Gel Detection

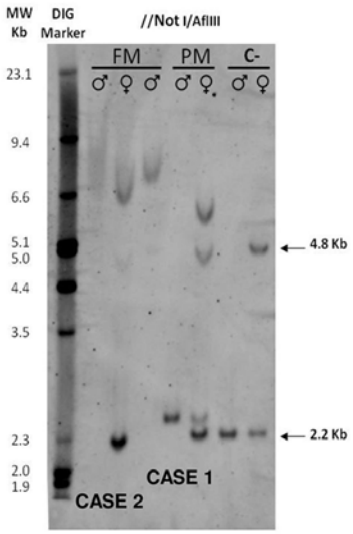




FRAXE/FMR2/AFF2 GScan™ Kit [40-2004-15] and Genemer™ Kit [40-2054-11] was used to amplify human genomic samples representing various CCG triple length pre-mutations, full mutations and normal FRAXE genotype. After PCR 10 µL samples were applied to a 2.5 agarose gel for electrophoresis. Gel picture is of an ethidium bromide stained gel. Gel lanes denote briefly the FRAXE CCG repeat genotype.

FRAXE/FMR2/AFF2 GScan™ Kit [40-2054-41] Southern Blot & Chemiluminescent Detection





Southern Blot Restriction Pattern & Fragments

- Lane 1 – DNA Molecular Weight Marker
- Lanes 2, 3, and 4 are from the CASE 2 with a full mutation allele (FM) with ~500 CCG, mother , and brother.
- Lanes 5 and 6 represent results from the CASE 1 showing a "pure" premutation with ~68CGGs and his mother.
- Lanes 7 and 8 are the normal controls (C-).

Frequently Asked Questions/Troubleshooting

1. **General Comment** FRAXE genotyping is not easy. A lab 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.

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™ 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 Genemer™ Kit Primers for amplification of CGG triple repeat spanning region. The quantity supplied is sufficient for 400 regular 25 µL PCR reactions.	100 rxns	40-2004-11
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
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

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 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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GeneProber™ 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, 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™ Products Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data. The kits contain optimized PCR amplification reagents and a wide array of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument(s). 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, except Fragile X. The CGG repeat in Fragile X can be detected up to ~50 repeats.

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Product	Unit Size	Catalog No.
Fragile X GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2004-15XX
Fragile X GScan™ 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™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15XX
Huntington's Disease GScan™ 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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