



## Product Manual

### **Factor VIII (F8, Hemophilia A) Genemer™** **F8 Genemer™ for F8 Exon Specific Amplification and Mutation** **Scanning by SSCP or Sequence Analysis**

Catalog No.: 40-2036-10; 40-2036-XX

Store at -20 °C

**For research use only.** Not for use in diagnostic procedures for clinical purposes

## Material Supplied

### Factor VIII (F8, Hemophilia A) Genemer™

F8 Genemer™ for F8 Exon Specific Amplification and Mutation Scanning by SSCP or Sequence Analysis

#### Storage Instructions:

1. Shipped lyophilized at room temperature.
2. Store at -20 °C upon receipt.
3. Store at -20 °C after reconstitution.

<input type="checkbox"/>	40-2036-10	Factor 8 Genemer™ Pack, containing F8 Exon specific amplification primers.	10 nmols
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F8 Genemer™		
<input type="checkbox"/>	40-2036-51	F8-Exon 01 Genemer™
<input type="checkbox"/>	40-2036-52	F8-Exon 02 Genemer™
<input type="checkbox"/>	40-2036-53	F8-Exon 03 Genemer™
<input type="checkbox"/>	40-2036-54	F8-Exon 04 Genemer™
<input type="checkbox"/>	40-2036-55	F8-Exon 05 Genemer™
<input type="checkbox"/>	40-2036-56	F8-Exon 06 Genemer™
<input type="checkbox"/>	40-2036-57	F8-Exon 07 Genemer™
<input type="checkbox"/>	40-2036-58	F8-Exon 08 Genemer™
<input type="checkbox"/>	40-2036-59	F8-Exon 09 Genemer™
<input type="checkbox"/>	40-2036-60	F8-Exon 10 Genemer™
<input type="checkbox"/>	40-2036-61	F8-Exon 11 Genemer™
<input type="checkbox"/>	40-2036-62	F8-Exon 12 Genemer™
<input type="checkbox"/>	40-2036-63	F8-Exon 13 Genemer™
<input type="checkbox"/>	40-2036-64A	F8-Exon 14A Genemer™
<input type="checkbox"/>	40-2036-64B	F8-Exon 14B Genemer™
<input type="checkbox"/>	40-2036-64C	F8-Exon 14C Genemer™
<input type="checkbox"/>	40-2036-65	F8-Exon 15 Genemer™
<input type="checkbox"/>	40-2036-66	F8-Exon 16 Genemer™
<input type="checkbox"/>	40-2036-67	F8-Exon 17-18 Genemer™
<input type="checkbox"/>	40-2036-69	F8-Exon 19 Genemer™
<input type="checkbox"/>	40-2036-70	F8-Exon 20 Genemer™
<input type="checkbox"/>	40-2036-71	F8-Exon 21 Genemer™
<input type="checkbox"/>	40-2036-72	F8-Exon 22 Genemer™
<input type="checkbox"/>	40-2036-73	F8-Exon 23 Genemer™
<input type="checkbox"/>	40-2036-74	F8-Exon 24 Genemer™
<input type="checkbox"/>	40-2036-75	F8-Exon 25 Genemer™
<input type="checkbox"/>	40-2036-76	F8-Exon 26 Genemer™

## Factor VIII, F8 or Hemophilia A

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### Background

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Inherited factor VIII deficiency, commonly known as Hemophilia A, is an X-linked bleeding disorder affecting 1:5,000 men and results from a deficiency or abnormality in the activity of factor VIII (FVIII). FVIII is a protein cofactor for the intrinsic activation of factor X by factor IXa in the presence of phospholipids and calcium ions. FVIII is synthesized as a large single chain protein with structural domains represented as A1-A2-B-A3-C1-C2. In plasma, FVIII circulates in a noncovalent complex with von Willebrand factor as a heterodimer consisting of a light chain (A3-C1-C2 domains) linked to a heavy chain (variable lengths of the B domain and A1-A2 domains). Thrombin converts FVIII to activated FVIII by cleaving at residues 372, 740, and 1689. The activated form of FVIII is markedly unstable, with the decay in activity being due to a dissociation of the A2 subunit from the A1-A3-C1-C2 dimers.

Factor VIII deficiency is the result of heterogeneous mutations within the factor VIII gene (F8) located at Xq28. The molecular genetic analysis of hemophilia A has been complicated by the large size of the gene, its complex genomic organization, and the mutational heterogeneity exhibited by this disease. The F8 gene is 186-kb long, has 26 exons, and encodes a 9-kb mRNA. Due to the large size of the gene a high mutation frequency is observed resulting in approximately 20 to 30% of the sporadic cases (1-4).

A mutation hot spot in intron 22 resulting in inversion mutations accounts for approximately 45% of severe hemophilia A cases. Inversions within the factor VIII gene cause almost half of all cases of severe hemophilia A. A 9.5-kb region of intron 22 (*Int22h1*) is repeated twice near the Xq telomere (*Int22h2* and *Int22h3*). These repeated sequences are more than 99% identical to one another. The inversion occurs by intra-chromosomal homologous recombination between *Int22h1* and an extragenic homolog (either *Int22h2* or *Int22h3*, known as Types I and II inversions, respectively) (1-4).

The diagnosis of hemophilia A is established in individuals with a low factor VIII clotting activity in the presence of a normal von Willebrand factor level. Molecular genetic testing of the factor VIII *F8* gene (chromosomal locus Xq28) identifies disease-causing mutations in up to 90% of patients with severe hemophilia A and about 80-95% of patients with mild to moderately severe hemophilia A. Such testing is available clinically from reference laboratories. Molecular genetic testing is used occasionally in the diagnosis of mild cases; it is primarily used for carrier detection and prenatal diagnosis.

### Molecular Analysis

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*F8* gene inversions occur through recombination between a sequence located within intron 22 with one of usually two additional copies of homologous sequences that are located far from the *F8* gene near the telomere of the long arm of the X chromosome. These telomeric sequences are in the opposite orientation relative to the intron 22 sequence. Crossovers can be distinguished by size patterns on agarose gels of Southern blots of genomic DNA digested with BclI. Of the two most frequent types, crossover with the distal telomeric sequence is more frequent than with the proximal sequence. Infrequently, a third telomeric additional copy can exist and can lead to a variant inversion pattern. Gene inversions account for about 45% of the *F8* mutations in severe hemophilia A. These mutations are the result of inversions resulting from homologous intrachromatid or intrachromosome (ie, intranemic) recombination between a 9503-base pair (bp) sequence (*int22h-1*) in intron 22 of the *F8* gene and one or other of 2 inverted copies of this sequence (*int22h-2*,

*int22h-3*) located, respectively, 500 and 600 kb more telomeric (1-3). A successful polymerase chain reaction (PCR) amplification spanning these regions has been reported, despite a 3.5-kb GC island of 65% GC content and a 1-kb region of 79% GC content within the GC island. This PCR amplification protocol requires specialized reaction and cycling conditions (5,6).

The remaining mutations span the entire spectrum of mutations, including complete or partial gene deletions, large insertions, sequence duplications, frameshifts, splice junction alterations, non-sense mutations, and mis-sense mutations.

Non-gene inversion mutations can be screened by amplification of all the 26 exons and the promoter region followed by SSCP analysis by direct staining or silver staining and by direct sequencing (4).

**Southern Based Intron 22 Inversion Analysis**

Gene Link’s GLF8-1 GeneProber™ detects F8 Intron 22 inversions. The GLF8-1 GeneProber™ is available unlabeled (Catalog Number 40-2036-40) for radioactive labeling by researcher for Southern based analysis. Gene Link strongly recommends the use of non-radioactive gene detection systems. Consider using Gene Link’s GLF8Dig1 GeneProber™ (Catalog Number 40-2036-41) for non-radioactive Southern blot gene detection system.

**F8 Mutation Scanning**

Gene Link’s F8 Genemer™ pack contains primer pairs for all the exons. All non-gene inversion mutations can be screened by amplification of all the 26 exons and the promoter region followed by SSCP analysis by direct staining or silver staining followed by sequence analysis of the exon fragment showing polymorphic electrophoretic mobility in SSCP gel analysis.

Molecular Analysis Used in Hemophilia A			
Severe Hemophilia A	Mild to Moderately Severe Hemophilia A	Genetic Mechanism	Test Type
45%		<i>F8</i> gene intron 22 inversion	Southern Analysis
Up to 45%		Gene deletions or rearrangements, frameshift, splice junction, nonsense or missense mutations	Mutation scanning or DNA sequencing of all amplified exons and promoter sequence.
	80-95%	Missense or occasionally splicing or in-frame deletion mutations	PCR amplification and mutation scanning or DNA sequencing
80-90% of families		Linkage analysis for F8	RFLP or PCR based linkage analysis

**References**

1. Hemophilia A review at <http://www.geneclinics.org/>
2. Antonarakis, S.E; Rossiter, J.P; Young, M; Horst, J; et al (1995) Factor VIII gene inversions in severe hemophilia A: results of an international consortium study. *Blood* 86:2206-12.
3. Windsor, S.; Taylor, S.M; and David Lillicrap, D. (1994) Direct Detection of a Common Inversion Mutation in the Genetic Diagnosis of Severe Hemophilia A. *Blood* 84:2202-2205.
4. Lakich, D.; Kazazian, H.H, Antonarakis, S.E, and Gitschier, J. (1993) Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. *Nat Genet.*;5:236-241.
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6. Liu, Q.; Nozari, G.; Sommer, S.S.; (1998) Single-tube polymerase chain reaction for rapid diagnosis of the inversion hotspot of mutation in hemophilia A [letter]. [published erratum in *Blood* 1999 Mar 15;93(6):2141] *Blood* 92:1458-9.
7. Liu, Q; Sommer, S.S; (1998) Subcycling-PCR for multiplex long-distance amplification of regions with high and low GC content: application to the inversion hotspot in the factor VIII gene. *Biotechniques* 25:1022-8.

## Procedure

Single Strand Conformation Polymorphism (SSCP). This technique reveals differences in electrophoretic mobility of wild type and mutant single strands. Conventional SSCP is performed by labeling the PCR fragment during amplification using [ $\alpha$ 32P] dCTP. SSCP can also be reliably observed by silver staining. Gene Link recommends the use of non-radioactive methods and techniques. This manual provides protocols for radioactive as well as silver staining of gels.

### Single Exon Amplification

Scanning for mutations in the F8 gene using the supplied Genemer™ pair is recommended by initially performing single Exon amplification. Ascertaining that the correct fragment size is amplified is by Agarose gel electrophoresis and ethidium bromide staining. This initial validation is advised for all the Genemer™ pairs. Reproducible results are obtained by using fresh high quality genomic DNA and the PCR amplification protocol and buffer conditions provided in this manual. Table 1 provides the fragment size obtained for each Exon Genemer™ pair.

NOTE: On occasion it has been observed that certain Exons are not amplified or amplified at a very low yield using old DNA which has gone through numerous freeze and thaw cycles. It is recommended that these 'exon dropout' amplifications be repeated with more template DNA and/or with fresh DNA. The cause for this is possibly due to the large gene size and fragmentation of genomic DNA.

Table 1. F8 Genemer™ Fragment Size		
Catalog No.	Genemer™	Amplified Fragment Size*
40-2036-51	F8-Exon 01	608 bp
40-2036-52	F8-Exon 02	207
40-2036-53	F8-Exon 03	208
40-2036-54	F8-Exon 04	295
40-2036-55	F8-Exon 05	187
40-2036-56	F8-Exon 06	220
40-2036-57	F8-Exon 07	225
40-2036-58	F8-Exon 08	356
40-2036-59	F8-Exon 09	284
40-2036-60	F8-Exon 10	347
40-2036-61	F8-Exon 11	334
40-2036-62	F8-Exon 12	283
40-2036-63	F8-Exon 13	379
40-2036-64A	F8-Exon 14A	1252
40-2036-64B	F8-Exon 14B	1085
40-2036-64C	F8-Exon 14C	1029
40-2036-65	F8-Exon 15	314
40-2036-66	F8-Exon 16	456
40-2036-67	F8-Exon 17-18	778
40-2036-69	F8-Exon 19	197
40-2036-70	F8-Exon 20	155
40-2036-71	F8-Exon 21	181
40-2036-72	F8-Exon 22	288
40-2036-73	F8-Exon 23	214
40-2036-74	F8-Exon 24	249
40-2036-75	F8-Exon 25	322
40-2036-76	F8-Exon 26	317
* The F8 Genemer™ fragments in most cases span intron/exon boundaries and include non-coding sequences. Most sequences obtained from Arruda et al (5) and GenBank Accession Number: <a href="#">AY769950</a>		

## Multiplex Amplification

Possible multiplexing of various exons are listed in Table 2. All multiplex amplifications prior to SSCP analysis should be electrophoresed in a 1.6% agarose gel to confirm faithful amplification of all of the fragments. An aliquot of the correctly amplified product should then be subjected to SSCP analysis.

Table 2. F8 Exon Amplification and Mutation Scanning				
PCR	Exons	Fragment(s); bp	Detection Method*	Notes
Multiplex F8-A	5, 12, 22, 23, 24, 25	187, 283, 288, 214, 249, 322	Direct SSCP	
Multiplex F8-B	2, 3, 4, 6, 7	207, 208, 295, 220, 225	Direct SSCP	
Multiplex F8-C	11, 15, 16, 20, 21	334, 314, 456, 155, 181	Direct SSCP	
Multiplex F8-D	10, 19, 26	347, 197, 317	Direct SSCP	
F8-1	1	608	Restriction>SSCP	
F8-8	8	356	Restriction>SSCP	
F8-9	9	284	Restriction>SSCP	
F8-13	13	368	Restriction>SSCP	
F8-14A	14A	1252	Restriction>SSCP	
F8-14B	14B	1085	Restriction>SSCP	
F8-14C	14C	1029	Restriction>SSCP	
F8-17-18	17 & 18	778	Restriction>SSCP	

## Restriction Digestion of Amplified Fragments

Table 3. F8 Amplified Exon Fragments Restriction Endonuclease Digestion			
Exon	Endonucleases	Restriction Fragments Sizes (bp)	Amplified Fragment Size (bp)
1	<i>Mva</i> I	283,147, 101,70	608
8-9	<i>Dde</i> I	227,185,177, 163,77	356 & 284
14 A	<i>Ava</i> II, <i>Hind</i> III, <i>Bst</i> N I	290, 248, 245, 166, 138, 87, 69	1252
14 B	<i>Ava</i> II, <i>Rsa</i> I	343, 279, 199, 169, 66, 29	1085
14 C	<i>Bst</i> N I	321, 253, 169, 160, 78, 48	1029
17-18	<i>Taq</i> I	320, 248, 209	778

## Brief Product Protocol

### Material Supplied

Each tube supplied contains the lyophilized primer Genemer™ pair for that exon. Please refer to label on the specific tube. Each tube contains 10 nmols. The quantity supplied is sufficient for 400 regular 50 µl PCR reaction.

### A. Genemer™ Reconstitution

**Stock Primer Mix:** Dissolve the supplied lyophilized Genemer™ in 100 µl sterile TE. The 10 nmols of primers when dissolved in 100 µl will give a solution of 100 µM i.e. 100 pmols/µl.

**Primer Mix:** Prepare a 10 pmols/µl Primer Mix solution by a ten fold dilution of the stock primer mix. Example: Add 180 µl sterile TE to a new tube, to this tube add 20 µl of primer stock solution. Label this tube as Primer Mix 10 pmols/µl.

### B. Thermal Cycler Files

#### Protocol for F8 Exon Fragment Amplification

The following amplification profile has been optimized for F8 exon specific product amplification using the supplied Genemer™. The protocol given below is for simplex amplification of each exon fragment separately. Multiplexing protocol is not supplied. Multiplexing reactions have to be optimized by the investigator.

Program the following thermal cycler files.

#### 1. Hot Start

Hot Start

For research use only. Not for use in diagnostic procedures for clinical purposes.

Step	Time & Temperature	Cycles
Initial Denaturation	95 °C for 5 minutes	1
Annealing	50 °C Hold Infinity	Hold
Comments: Add Taq premix while on hold.		

**2. Amplification File**

F8 Exon Amplification File			
Step	Temperature	Time	Cycles
Denaturation	94 °C	30 sec.	30
Annealing	52 °C	30 sec.	
Elongation	72 °C	1 minute	
Fill in Extension	72 °C	7 minutes	1
Hold	4 °C	Infinity	Hold

**C. PCR**

**1. PCR Premix Preparation (PP). Label tube “PP”**

PCR Premix Preparation (PP)		
Component	1 X 50 µl Rxn.	10 X 50 µl Rxns.
Sterile Water	32 µl	320 µl
10 X PCR Buffer	4.5 µl	45 µl
2.0 mM dNTP	5 µl	50 µl
10 pmol/µl Primer Mix	2.5 µl	25 µl
Template DNA (~500 ng)	1-2 µl	Add DNA to each tube
Total Volume	45 µl	
After adding template start hot start PCR File		

Standard Gene Link PCR Buffer Composition	
10 X PCR buffer	1 X PCR buffer
100 mM Tris-HCl pH 8.3	10 mM
500 mM KCl	50 mM
15 mM MgCl <sub>2</sub>	1.5 mM
0.01% Gelatin	0.001%

Dispense 44 µl of the above PCR premix to individual PCR tubes for each amplification reaction and then add the template DNA. Start “Hot Start” thermal cycler file. While holding at 50 °C add 5 µl of the Taq Enzyme Mix (EM). Start amplification file.

**2. Taq Polymerase mix Preparation (EM). Label tube “EM”**

Taq Enzyme Mix Preparation (EM)		
Component	1 X 50 µl Rxn.	10 X 50 µl Rxns.
Sterile Water	5 µl	50 µl
10 X PCR Buffer	0.5 µl	5 µl
Taq Polymerase	0.5 µl	5 µl
Add 5 µl to each reaction after holding after hot start		

**D. Agarose Electrophoresis**

Load 10 to 15 µl samples to 1.8% agarose gel. Run at 90 mAmps. Confirm correct amplification fragment size.

**E. Single Strand Confirmation Polymorphism (SSCP) Analysis**

## Non-Radioactive Detection of Mutations by Single-Strand Conformational Polymorphism

**Single-strand Conformational Polymorphism (SSCP).** Under certain conditions, single-stranded (ss) nucleic acids form secondary structure in solution. The secondary structure depends on the base composition and may be altered by a single nucleotide substitution, causing differences in electrophoretic mobility under non-denaturing conditions. Fragments for detection are labeled radioactively, or silver staining is used. Initially SSCP was described for the analysis of DNA; however analysis of RNA is also possible. Distinct secondary structures are formed more frequently by RNA than by DNA molecules. (The additional step of in vitro transcription is required for RNA-SSCP). With RNA, larger fragments can be analyzed.

- **Fragment size:** For optimal results, fragment size should be in the range of 150 to 200 bp. For larger fragments acceptable sensitivities may be achieved by RNA-SSCP.
- **Detectable mutations:** Under optimal conditions ~ 80-90% of potential base exchanges are detectable by SSCP.
- **Detection limit:** Approximately one mutant cell is detectable in the presence of 10 normal cells.

### Buffers and Solutions

#### Formamide Loading Buffer

80% (w/v) deionized formamide  
10 mM EDTA (pH 8.0)  
1 mg/ml xylene cyanol FF  
1 mg/ml bromophenol blue

#### 6x Sucrose Gel-loading Buffer

0.25% (w/v) bromophenol blue  
0.25% (w/v) xylene cyanol FF  
40% (w/v) sucrose in H<sub>2</sub>O  
Store at 4°C.

### Enzymes and Buffers

Restriction enzymes (optional)  
Thermostable DNA polymerase  
Taq *DNA polymerase is recommended*

### Method

1. While the thermal cycler program is running, prepare a 20% polyacrylamide gel containing 10% (v/v) glycerol in 1x TBE gel buffer.
2. Dilute either 1.5 µl of the original PCR or 5 µl of the restriction-enzyme-digested PCR into 20 µl of sucrose gel-loading buffer. Dilute similar aliquots into 20 µl of formamide loading buffer.
3. Boil the formamide-containing samples for 6 minutes, and then plunge the tubes directly into ice.
4. Use a Pasteur pipette or a Hamilton syringe to wash out the wells of the polyacrylamide gel with 1x TBE gel buffer. With a drawn-out glass capillary tube or a micropipettor equipped with a gel-loading tip, load 2 µl of each sample on the polyacrylamide gel.
5. Apply 6-7 V/cm (approx. 250 V [and 15 mA] for a 40 x 40-cm gel) to the gel for approx. 14 hours.
6. At the completion of electrophoresis, process for silver staining.



## Silver staining

### Buffers and Solutions

#### Fixer

10% acetic acid

Prepare by adding 50 ml glacial acetic acid to 450 ml distilled water.

#### Silver Stain

To 500 ml distilled water add the following  
3 ml 1N silver nitrate solution  
0.75ml formamide

#### Developer

To 500 ml distilled water add the following  
15 g sodium carbonate  
Store at 4°C

#### Sodium thiosulphate Solution

0.1 N Sodium thiosulphate  
Store at 4°C

#### Developer Mix-Prepare Fresh Before Use

To 500ml chilled Developer add the following  
75 µl 0.1N sodium thiosulphate solution  
0.75 ml formamide  
Store at 4°C

### Method

1. Remove the gel and separate the plates carefully.
2. Transfer the gel to the tray with 500 ml fixer. Leave shaking in a fume hood for 30 minutes. Pour off fixer. (Can save fixer and reuse 3-4 times).
3. Wash with 500 ml water for 15 minutes, on shaker. Rinse again and pour off the water.
4. Add 500 ml silver-stain and leave shaking for 30 minutes. Pour off silver stain. (Can save silver stain and reuse up to 10 times).
5. While gel is staining, prepare fresh Developer Mix and transfer to tray.
6. After silver-stain (step 4), rinse the gel in 50 ml water twice for 10 seconds each.
7. Immediately transfer the gel to the Developer Mix until band development progress sufficiently.
8. Stop the reaction by adding the fixer saved earlier (step 2). Agitate until bubbles cease.
9. Rinse gel in water for 20 minutes.
10. Leave to surface dry by standing vertically for 10 min.
11. Scan the gel with a computer flatbed scanner, or photograph.

### Radioactive Detection of Mutations by Single-strand Conformational Polymorphism

All reagents listed above for non-radioactive SSCP analysis plus the radioactive compound for radioactive labeling is required.

## Radioactive Compounds

[ $\alpha$ -<sup>32</sup>P]dCTP (3000 Ci/mmole, 10 mCi/ml)

## Method

1. Perform PCR amplification as noted in the methods section of the manual. In addition add 1  $\mu$ l of 10  $\mu$ Ci/ $\mu$ l [ $\alpha$ -<sup>32</sup>P]dCTP to each PCR.
2. [ $\alpha$ -<sup>32</sup>P]dCTP is incorporated in the PCR to label the amplified DNA uniformly. <sup>32</sup>P-labeled oligonucleotide primers can be used in place of [ $\alpha$ -<sup>32</sup>P]dCTP to produce an end-labeled DNA. If possible, set up control reactions using two DNA samples known to contain alleles that differ in sequence by one or more base pairs and that are known to resolve on SSCP gels. In addition, set up a contamination control in which no template DNA is added to the reaction.
3. While the thermal cycler program is running, prepare a 20% polyacrylamide gel containing 10% (v/v) glycerol in 1x TBE gel buffer.
4. Dilute either 1.5  $\mu$ l of the original PCR or 5  $\mu$ l of the restriction-enzyme-digested PCR into 20  $\mu$ l of sucrose gel-loading buffer. Dilute similar aliquots into 20  $\mu$ l of formamide dye mix.
5. Boil the formamide-containing samples for 6 minutes, and then plunge the tubes directly into ice.
6. Use a Pasteur pipette or a Hamilton syringe to wash out the wells of the polyacrylamide gel with 1x TBE gel buffer. With a drawn-out glass capillary tube or a micropipettor equipped with a gel-loading tip, load 2  $\mu$ l of each sample on the polyacrylamide gel.
7. Apply 6-7 V/cm (approx. 250 V [and 15 mA] for a 40 x 40-cm gel) to the gel for approx. 14 hours.
8. At the completion of electrophoresis, separate the glass plates, and transfer the gel to a sheet of Whatman 3MM filter paper. Dry the gel on a vacuum dryer for 30-60 minutes.
9. Subject the dried gel to autoradiography for 4-16 hours at room temperature without an intensifying screen.

## REFERENCES

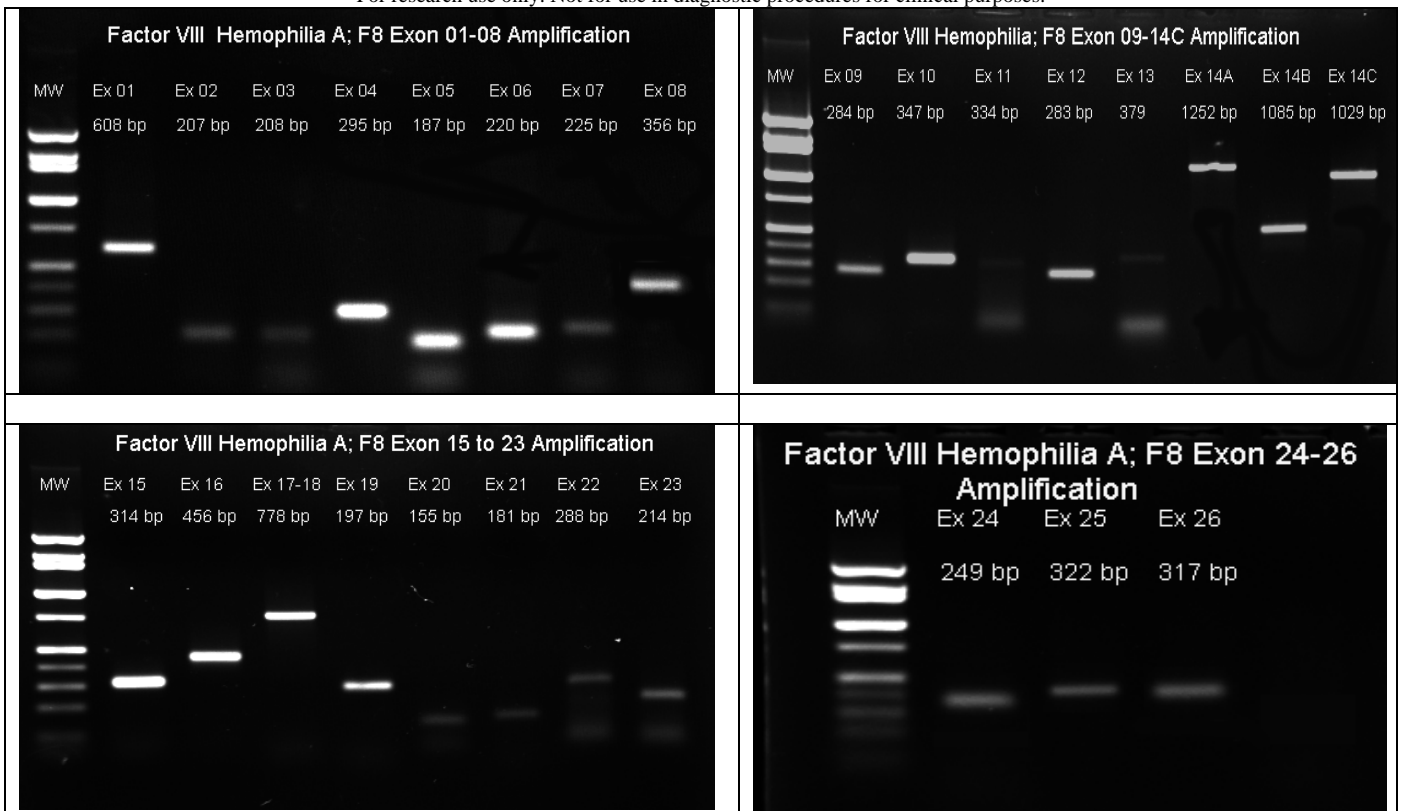
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2. Orita M., Suzuki Y., Sekiya T., and Hayashi K. (1989). Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 5:874-879.

## Results and Interpretation

### Agarose Gel Electrophoresis

All amplification products should be analyzed by agarose gel electrophoresis to ascertain correct amplification. The gel images below represent the amplification of all the F8 exons using the supplied Genemer™. The amplification reactions were performed at 50  $\mu$ l final volume and 10  $\mu$ l aliquots of the PCR reaction were directly loaded on a 1.6% agarose gel. Ethidium bromide was included in the gel and running buffer.

NOTE: On occasion it has been observed that certain Exons are not amplified or amplified at a very low yield using old DNA which has gone through numerous freeze and thaw cycles. It is recommended that these 'exon dropout' amplifications be repeated with more template DNA and/or with fresh DNA. The cause for this is possibly due to the large gene size and fragmentation of genomic DNA.



**Restriction Digestion**

Amplified products should be processed for restriction digestion as listed on Table 3. Other restriction enzymes can also be used. The purpose is to generate shorter fragments for SSCP analysis.

**SSCP Analysis**

Appendix section of this manual contains protocols for DNA fragment purification. It is recommended that before SSCP analysis it is confirmed that the fragment analyzed is not contaminated with non specific amplified products. Samples yielding positive results in SSCP analysis indicate the presence of polymorphism/mutation. These fragments should be subjected to sequencing to determine the exact sequence. Refer to Appendix for methods of purification of amplified fragments.

## Appendix

### 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  
Rapid DNA Purification Protocol for 300 µl Whole Blood

Catalog Number: 40-4010-01

#### A. Initial Preparation

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

#### B. Cell Lysis

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

#### C. Protein Precipitation

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

#### D. DNA Precipitation

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

#### E. DNA Reconstitution & Use

1. Add 100 µl of GD-4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4°C. For long-term storage, place sample at -20°C or -80°C.
3. Average yield of 10 µg is expected from 300 µl blood DNA. The range is between 5 µg to 15 µg.
4. The 100 µl of purified DNA obtained will have an average concentration of ~ 100 ng/µl.
5. For PCR amplification use 1-2µl.
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 Components and Analysis

### Buffer Condition

PCR buffer conditions vary and it is imperative to optimize buffer conditions for each amplification reaction. At Gene Link most amplification reactions have been optimized to work with the following standard buffer condition, unless indicated.

Standard Gene Link PCR Buffer Composition	
10 X PCR buffer	1 X PCR buffer
100 mM Tris-HCl pH 8.3	10 mM
500 mM KCl	50 mM
15 mM MgCl <sub>2</sub>	1.5 mM
0.01% Gelatin	0.001%

### dNTP Concentration

Standard dNTP concentration of 0.2mM of each base is used. See section on PCR additives when dNTP concentration is changed.

Recipe	
2.0 mM dNTP Stock Solution Preparation*	
100 mM dGTP	100 µl
100 mM dATP	100 µl
100 mM dTTP	100 µl
100 mM dCTP	100 µl
Water	4.6 ml
<b>Total Volume</b>	<b>5 ml</b>
*Aliquot and freeze	

### MgCl<sub>2</sub> Concentration

The concentration of Mg<sup>++</sup> will vary from 1-5 mM, depending upon primers and substrate. Since Mg<sup>2+</sup> ions form complexes with dNTPs, primers and DNA templates, the optimal concentration of MgCl<sub>2</sub> has to be selected for each experiment. Low Mg<sup>2+</sup> ions result in a low yield of PCR product, and high concentrations increase the yield of non-specific products and promote mis-incorporation. Lower Mg<sup>2+</sup> concentrations are desirable when fidelity of DNA synthesis is critical. The recommended range of MgCl<sub>2</sub> concentration is 1-4 mM, under the standard reaction conditions specified. At Gene Link, using the standard PCR buffer with KCl, a final dNTP concentration of 0.2 mM, a MgCl<sub>2</sub> concentration of 1.5 is used in most cases. If the DNA samples contain EDTA or other chelators, the MgCl<sub>2</sub> concentration in the reaction mixture should be raised proportionally. Given below is an MgCl<sub>2</sub> concentration calculation and addition table using a stock solution of 25 mM MgCl<sub>2</sub>.

MgCl <sub>2</sub> Concentration & Addition Table								
Final concentration of MgCl <sub>2</sub> in 50µl reaction mix, (mM)	1.0	1.25	1.5	1.75	2.0	2.5	3.0	4.0
Volume of 25mM MgCl <sub>2</sub> , µl	2	2.5	3	3.5	4	5	6	8

### Primer Concentration

The final concentration of primers in a PCR reaction is usually 0.5 to 1 µM (micromolar). This is equivalent to 0.5 to 1 pmol/µl. For a 100 µl reaction you would add 50 to 100 pmols. At Gene Link we use 0.5 pmol/µl in the final PCR.

### Genemer™ Reconstitution

**Stock Primer Mix:** Dissolve the supplied 10 nmols of lyophilized Genemer™ in 100 µl sterile TE. The 10 nmols of primers when dissolved in 100 µl will give a solution of 100 µM i.e. 100 pmols/µl.

**Primer Mix:** Prepare a 10 pmols/μl Primer Mix solution by a ten fold dilution of the stock primer mix.  
 Example: Add 180 μl sterile TE to a new tube, to this tube add 20 μl of primer stock solution. Label this tube as Primer Mix 10 pmols/μl.

### Amplification Thermal Cycling

**Hot Start:** It is essential to have a 'Hot Start' profile for amplification of any fragment from a complex template like human genomic DNA. Taq polymerase has low activity at room temperature and it is essential to minimize any mis-priming in the first cycle of amplification. A typical hot start profile is given below. Various enzyme preparations are available which are activated by heat in the first cycle. A simple hot start protocol is given below that can be used with regular Taq polymerase. See the section on PCR additives for amplification of products from high GC content templates.

Hot Start		
Step	Time & Temperature	Cycles
Initial Denaturation	95 °C for 5 minutes	1
Annealing	60 °C Hold Infinity	Hold
Comments: Add Taq premix while on hold.		

### Amplification File

The initial denaturation step at 94 °C for 30 seconds is sufficient for all templates. The number of cycles is usually set to 30 and is sufficient to amplify 1-10 μg of product depending on the initial concentration of template. A higher number of cycles from 35-45 cycles may be used, but internal priming on the product and over amplification of unwanted bands often result from over-cycling. Generally, it is better to focus on optimizing reaction conditions than to go beyond 35 cycles.

Typical Amplification File			
Step	Temperature	Time	Cycles
Denaturation	94 °C	30 sec.	30
Annealing	*	30 sec.	
Elongation	72 °C	30 sec.	
Fill in Extension	72 °C	7 minutes	1
Hold	4 °C	Infinity	Hold
Based on the T <sub>m</sub> of the primers. Usually varies from 50°C to 65°C			

**Typical Reaction Premix**

Typical PCR Premix (/50µl)	
Component	Volume
10 x PCR Buffer	5 µl
2.0 mM dNTP mix (each)	5 µl
Primer Mix (10 pmol/µl each) or 2.5µl of 10 pmol/µl of individual primer (final 25 pmol of each primer/50µl)	2.5 µl
H <sub>2</sub> O	37.5 µl
<b>Total Volume</b>	<b>50 µl</b>

**Typical PCR Reaction Mix**

PCR reaction (/50µl)	
Component	Volume
PCR premix	45 µl
100ng/µl diluted DNA	1 µl
Hot start and then add	
Taq premix	5 µl

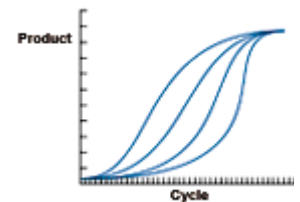
**Taq Premix Preparation**

Taq Premix (/50µl)	
Component	Volume
PCR Premix	6 µl
Taq polymerase (5 u/µl)	0.25µl
<b>Add 5µl/50 µl rxn. After initial denaturation</b>	
Use 2.5 units of Taq for 100 µl reactions. Taq is usually supplied at a concentration of 5 units/µl	

**Yield and Kinetics**

The target will be amplified by up to 10<sup>6</sup> fold in a successful reaction, but the amplification will usually plateau at 1-10µg. Thus, 1 pg of target sequence in the reaction is a good place to begin.

PCR reactions produce product in a nonlinear pattern. Amplification follows a typical exponential curve until some saturation point is reached. Generally products will not be further amplified once 1-5 µg has been generated. Saturation by one product of a reaction does not always prevent further amplification of other generally unwanted products. Over-cycling may decrease the quality of an otherwise good reaction. When first optimizing a reaction, it is advisable to take samples every 5 or 10 cycles to determine the number of cycles actually needed.



## Gel Electrophoresis of PCR Products

Gel electrophoresis of PCR products is the standard method for analyzing reaction quality and yield. PCR products can range up to 10kb in length, but the majority of amplifications are at 1kb and below. Agarose electrophoresis is the classical method to analyze amplification products from 150 bp to greater than 10kb. Polyacrylamide gel electrophoresis should be used for resolution of short fragments in the range of 100 bp to 500 bp when discrimination of 10 bp difference is required.

PAGE gels for PCR products formulated with the amount of cross-linker chosen to give pore sizes optimal for the size of DNA fragment desired. Gels are most often stained in ethidium bromide, even though the fluorescence of this stain is quenched by polyacrylamide, which decreases sensitivity 2-5 fold. This decrease in sensitivity generally does not present a problem, because most PCR reactions yield product levels in the microgram range, and Ethidium will detect as little as 1/10 of this amount. Polyacrylamide gels can be stained by silver staining for more sensitive detection.

## Purification of PCR Product

Various purification methods are available for the purification of PCR products. The selection of a particular method over other 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 application the amplified product should be purified from the primers and any other minor amplification products.

The preferred method of purification of an amplified fragment is the excision of the fragment band after agarose gel electrophoresis. This method yields the purification of a single fragment; as such care should be taken to excise a gel piece containing a single electrophoretically resolved fragment. Omni-Clean™ Purification System available from Gene Link can be used for this purpose. **Catalog No. 40-4110-10 for bead based system; 40-4120-10 for spin column based system and 40-4130-10 for DNA concentration. Please refer to product insert for detailed protocol or visit [www.genelink.com](http://www.genelink.com)**

- A. **Purification of DNA from gel slices using glass beads. Provides purified single fragment.**  
[Omni-Clean™ Gel DNA Beads Purification System; Catalog No. 40-4110-10]

### Protocol

1. By weight, determine the volume of the excised DNA fragment.
2. Add 3 volumes of NaI solution and heat to 55°C. Visually determine the dissolution of gel pieces.
3. Add 1 µl of glass bead suspension per µg of DNA and vortex.
4. Centrifuge at 2K rpm for 20 seconds to pellet glass bead/DNA complex. Discard supernatant.
5. Re-suspend pellet in 400 µl Omni-Clean™ wash buffer. Centrifuge at 2K rpm for 20 seconds and discard wash buffer.
6. Pipet out any remaining buffer in the tube.
7. Add 25 µl water or TE; re-suspend pellet and centrifuge at 2K rpm for 20 seconds.
8. 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 gel slices using spin column. Provides purified single fragment.**  
[Omni-Clean™ Gel DNA Spin Column Purification System; Catalog No. 40-4120-50]

### Protocol

1. By weight, determine the volume of the excised DNA fragment.
2. Add 3 volumes of NaI solution and heat to 55°C. Visually determine the dissolution of gel pieces.
3. Add the above solution to the spin column assembled on a collection tube.
4. Let the solution flow by gravity or centrifuge at 2K rpm for 20 seconds. Discard flow through collected in the collection tube.
5. Add 400 µl Omni-Clean™ wash buffer to the spin column. Centrifuge at 2K rpm for 2 minutes and discard wash buffer collected in the collection tube.
6. Replace the collection tube with a new appropriately labeled eppendorf tube.
7. Add 25 µl water or TE to the spin column. Let sit for 3 minutes.
8. Centrifuge at 2K rpm for 2 minutes.
9. The collection tube contains the purified DNA.

- C. **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 3 volumes of NaI solution.
2. Add 1 µl of glass bead suspension per µg of DNA.
3. Centrifuge at 2K rpm for 20 seconds to pellet glass bead/DNA complex. Discard supernatant.
4. Re-suspend pellet in 400 µl Omni-Clean™ wash buffer.
5. Centrifuge at 2K rpm for 20 seconds and discard wash buffer.
6. Pipet out any remaining buffer in the tube.
7. Add 25 µl water or TE; re-suspend pellet and centrifuge at 2K rpm for 20 seconds.
8. The supernatant contains the purified DNA. Using a pipet, collect the supernatant and transfer to a new appropriately labeled tube.

**D. 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 3 volumes of NaI solution.
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™ 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 eppendorf 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 20µl of H<sub>2</sub>O.
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 700µl 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 eppendorf 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 eppendorf tube is free of salts and primers shorter than 35-40mer.

### PCR 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 use 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  $T_m$  and the complex secondary structure thus the duplex stability (1-5). Tetramethyl ammonium chloride (TMAC) actually increases the specificity of hybridization and increases the  $T_m$ . 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 T <sub>m</sub> 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 <b>not</b> 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 0.01µg/µl to 0.1µg/ 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 <i>Taq</i> 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 stabilise <i>Taq</i> 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 <i>Taq</i> 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 T <sub>m</sub> and minimizes mis-pairing.	TMAC is generally used at a final concentration of 15-100mM to eliminate non-specific priming.

**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 polymerase pauses 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.

## Factor VIII, Hemophilia A, F8 Product Ordering Information

### GeneProber™ Probe

Product	Size	Catalog No.
F8 GLF8-1 GeneProber™ Factor VIII, Probe unlabeled Factor VIII, Hemophilia A Intron 22 Inversion Genotyping by Southern Blot Analysis. Unlabeled probe for radioactive labeling and Southern blot detection. Suitable for random primer labeling.	500 ng	40-2036-40
F8 GLF8-Dig1 GeneProber™ Factor VIII, Probe Digoxigenin labeled Factor VIII, Hemophilia A Intron 22 Inversion Genotyping by Southern Blot Analysis. Digoxigenin labeled probe for non-radioactive labeling and Southern blot detection.	110 µL	40-2036-41

**Genemer™** Primer pair for gene or mutation specific amplification. Special optimized conditions may be required for certain amplifications.

Product	Size	Catalog No.
F8-Exon 01 Genemer™	10 nmols	40-2036-51
F8-Exon 02 Genemer™	10 nmols	40-2036-52
F8-Exon 03 Genemer™	10 nmols	40-2036-53
F8-Exon 04 Genemer™	10 nmols	40-2036-54
F8-Exon 05 Genemer™	10 nmols	40-2036-55
F8-Exon 06 Genemer™	10 nmols	40-2036-56
F8-Exon 07 Genemer™	10 nmols	40-2036-57
F8-Exon 08 Genemer™	10 nmols	40-2036-58
F8-Exon 09 Genemer™	10 nmols	40-2036-59
F8-Exon 10 Genemer™	10 nmols	40-2036-60
F8-Exon 11 Genemer™	10 nmols	40-2036-61
F8-Exon 12 Genemer™	10 nmols	40-2036-62
F8-Exon 13 Genemer™	10 nmols	40-2036-63
F8-Exon 14A Genemer™	10 nmols	40-2036-64A
F8-Exon 14B Genemer™	10 nmols	40-2036-64B
F8-Exon 14C Genemer™	10 nmols	40-2036-64C
F8-Exon 15 Genemer™	10 nmols	40-2036-65
F8-Exon 16 Genemer™	10 nmols	40-2036-66
F8-Exon 17-18 Genemer™	10 nmols	40-2036-67
F8-Exon 19 Genemer™	10 nmols	40-2036-68
F8-Exon 20 Genemer™	10 nmols	40-2036-69
F8-Exon 21 Genemer™	10 nmols	40-2036-70
F8-Exon 22 Genemer™	10 nmols	40-2036-71
F8-Exon 23 Genemer™	10 nmols	40-2036-72
F8-Exon 24 Genemer™	10 nmols	40-2036-73
F8-Exon 25 Genemer™	10 nmols	40-2036-74
F8-Exon 26 Genemer™	10 nmols	40-2036-75
F8-Exon Genemer™ Pack, Contains 10 nmols each of all F8 Genemer™	10 nmols	40-2036-10

## Genemer™ Product Ordering Information

**Genemer™** Primer pair for gene or mutation specific amplification. Special optimized conditions may be required for certain amplifications

Product	Size	Catalog No.
Fragile X (spanning CGG triple repeat region) Genemer™; 10 nmols	10 nmols	40-2004-10
Huntington Disease (spanning CAG triple repeat region) Genemer™; 10 nmols	10 nmols	40-2025-10
Myotonic Dystrophy (spanning CTG triple repeat region) Genemer™; 10 nmols	10 nmols	40-2026-10
Friedreich's Ataxia (spanning GAA triple repeat region) Genemer™; 10 nmols	10 nmols	40-2027-10
Factor V Genemer™; 10 nmols	10 nmols	40-2035-10
Factor VIII (Hemophilia) Genemer™ Pack Genemer™; 10 nmols	10 nmols	40-2036-10
STS (Steroid Sulfatase) Genemer™; 10 nmols	10 nmols	40-2023-10
HGH (Human Growth Hormone) Genemer™; 10 nmols	10 nmols	40-2024-10
Sickle Cell Genemer™; 10 nmols	10 nmols	40-2001-10
RhD (Rh D gene exon 10 specific) Genemer™; 10 nmols	10 nmols	40-2002-10
Rh EeCc (Rh Ee and Cc exon 7 specific) Genemer™; 10 nmols	10 nmols	40-2003-10
Gaucher (various mutations) Genemer™; 10 nmols	10 nmols	40-2047-XX
Cystic Fibrosis (various mutations) Genemer™; 10 nmols	10 nmols	40-2029-XX
SRY (sex determining region on Y) Genemer™; 10 nmols	10 nmols	40-2020-10
X alphoid repeat Genemer™; 10 nmols	10 nmols	40-2021-10
Y alphoid repeat Genemer™; 10 nmols	10 nmols	40-2022-10

## Genemer™ Control DNA Product Ordering Information

**Genemer™ control DNA** is a cloned fragment of the mutation region of a particular gene. These control DNA are an ideal genotyping template for optimizing and performing control amplification with unknown DNA.

Product	Size	Catalog No.
Sickle Cell Genemer control DNA (HbA, S and C available)	500 ng	40-2001-0X
GLFX CGG Genemer Control DNA; Fragile X (16, 29, 40, 60 & 90 CGG repeats available)	500 ng	40-2004-0X
GLHD CAG Genemer Control DNA; Huntington Disease (18, 34, 44, 89 & 134 CAG repeats available)	500 ng	40-2025-0X
GLDM CTG Genemer Control DNA; Myotonic Dystrophy (12, 45, 93, 129 & 194 CTG repeats available)	500 ng	40-2026-0X

## 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 <sup>32</sup>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	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
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.

Product	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
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

All Gene Link products are for research use only

Current pricing are posted at <http://www.genelink.com/>

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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Current pricing are posted at <http://www.genelink.com/>

## Related Products Ordering Information

### Taq Polymerase & Master Mix

Product	Catalog No.	Unit Size
Taq DNA Polymerase; 400 units; 5 µL; 80 µL	40-5200-40	400 units
Taq PCR Kit; 200 x 50 µL reactions	40-5211-01	200 reactions
Taq PCR Kit with controls; 200 reactions	40-5212-01	200 reactions
PCR Master Mix (2X); 100 x 50 µL reactions (2 tubes x 1.3 mL)	40-5213-01	100 reactions
PCR Master Mix (2X); 200 x 50 µL reactions (4 tubes x 1.3 mL)	40-5213-02	200 reactions

## Related Products Ordering Information

### PCR Additives & Reagents

Product	Catalog No.	Unit Size
Taq DNA Polymerase 300 units; 5 µL; 60 µL	40-5200-30	300 units
PCR Buffer Standard (10 X); 1.6 mL	40-3060-16	1.6 mL
PCR Buffer Mg Free (10 X) ; 1.6 mL	40-3061-16	1.6 mL
Taq Polymerase Dilution Buffer; 1 mL	40-3070-10	1 mL
dNTP 2mM (10X) ; 1.1 mL	40-3021-11	1.1 mL
MgCl <sub>2</sub> ; 25 mM; 1.6 mL	40-3022-16	1.6 mL
Omni-Marker™ Universal Unlabeled; 100 µL	40-3005-01	100 µL
Primer and Template Mix; 500 bp; 40 reactions; 100 µL	40-2026-60PT	100 µL
Nuclease Free Water; 1.6 mL	40-3001-16	1.6 mL
DMSO; 1 mL	40-3031-10	1 mL
TMAC (Tetramethyl ammonium chloride) 100 mM; ; 1 mL	40-3053-10	1 mL
KCl 300 mM; 1 mL	40-3059-10	1 mL
Betaine; 5M; 1 mL	40-3032-10	1 mL

### Omni-Marker™

Product	Catalog No.	Unit Size*
Omni-Marker™ Universal unlabeled; 100 µL	40-3005-01	100 µL
Omni- Marker™ Universal unlabeled; 500 µL	40-3005-05	500 µL
Omni-Marker™ Universal unlabeled; 1 mL	40-3005-10	1 mL
Omni- Marker™ Low unlabeled; 100 µL	40-3006-01	100 µL
Omni-Marker™ Low unlabeled; 500 µL	40-3006-05	500 µL
Omni- Marker™ Low unlabeled; 1 mL	40-3006-10	1 mL
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp; 100 µL	40-3062-01	100 µL
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp; 500 µL	40-3062-05	500 µL

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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; 200 mL	40-5020-20	200 mL
Hybwash B, Hybridization Wash Solution; 100 mL	40-5021-10	100 mL
TAE Buffer; 50X Concentrate; 100 mL	40-3007-01	100 mL
TAE Buffer; 50X Concentrate; 1 L	40-3007-10	1 L
TBE Buffer; 5X Concentrate; 1 L	40-3008-10	1 L
10x Washing buffer; 200 mL	40-5025-20	200 mL
10% Blocking solution; 100 mL	40-5026-10	100 mL
Seq. Loading buffer; 1 mL	40-5027-00	1 mL
10x AP 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

### 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

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