



Product Manual

Factor V Leiden (G1691A; R506Q) Genemer™ **Primer pair for genotyping of the Factor V Leiden (G1691A) mutation**

Catalog No.: 40-2035-10

Store at -20°C

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

Factor V Leiden (G1691A, R506Q) Genemer™

Material Supplied

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"/>	40-2035-10	Factor V Leiden (G1691A) Genemer™	10 nmols
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*The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.

Factor V Leiden (G1691A) Genemer™

The term "factor V Leiden" refers to the specific G-to-A substitution at nucleotide 1691 in the gene for factor V that predicts a single amino acid replacement (R506Q) at one of three APC cleavage sites in the factor Va molecule.

The gene for factor V is termed as F5 and the protein made by the F5 gene is termed as coagulation factor V

Factor V Leiden is the most common inherited form of thrombophilia. Between 3 percent and 8 percent of the Caucasian (white) population in the United States and Europe carry one copy of the factor V Leiden mutation in each cell, and about 1 in 5,000 people have two copies of the mutation. The mutation is less common in other populations.

Summary Gene Reviews

<http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=factor-v-leiden>

<http://ghr.nlm.nih.gov/condition=factorvleidenthrombophilia>

Background

The hemostatic regulation is an orchestrated balance of prothrombotic and antithrombotic factors in the vasculature. Activated Factor V (Va) serves as an essential protein cofactor in the prothrombinase complex for the conversion of prothrombin to thrombin by activated factor X (Xa). Activated protein C regulates the functionality of the complex by proteolytic degradation of factor Va at amino acid Arg506, Arg306, and Arg679.

When factor Va is resistant to degradation by activated protein C, the anticoagulation pathway will not operate properly, and patients have an increased risk for thrombosis. Individuals with activated protein C resistance have a mutated factor V. More than 95% of cases are due to a point mutation, known as the factor V Leiden mutation. The factor V Leiden mutation is a single G-to-A nucleotide transition in exon 10 of the factor V gene. This alteration in the gene eliminates an *Mnl* I restriction site and also leads to a substitution of arginine with glutamine at amino acid residue number 506.

The factor V Leiden mutation is present in 3% to 8% of the general white population in heterozygous form. It is less common in other races and ethnic groups, such as those of African or Asian ancestry. The Factor V Leiden mutation is responsible for increased risk of venous thrombosis in heterozygotes as well as homozygotes. Heterozygotes for factor V Leiden have an approximately eightfold increased relative risk for the development of venous thrombosis, and homozygotes are estimated to have an approximately 90-fold increased relative risk.

Diagnosis/testing

Factor V Leiden thrombophilia is suspected in individuals with a history of venous thromboembolism (VTE) manifest as deep vein thrombosis (DVT) or pulmonary embolism, especially in women with a history of VTE during pregnancy or in association with oral contraceptive use, and in individuals with a personal or family history of recurrent thrombosis. The diagnosis of factor V Leiden thrombophilia is made either using a coagulation screening test or by DNA analysis of the *F5* gene, which encodes the factor V protein. The term "factor V Leiden" refers to the specific G-to-A substitution at nucleotide 1691 in the gene for factor V that predicts a single amino acid replacement (R506Q) at one of three APC cleavage sites in the factor Va molecule.



Material Supplied

A single tube containing 10 nmols of lyophilized forward and reverse primers. The quantity supplied is sufficient for 400 regular 50 μ l PCR reaction.

Procedure

A. Genemer™ Reconstitution

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

Primer Mix: Prepare a 10 pmols/ μ l Primer Mix solution by a ten fold dilution of the stock primer mix. Example: Add 180 μ l sterile TE to a new tube, to this tube add 20 μ l of primer stock solution. Label this tube as **F5 Primer Mix 10 pmole/ μ l**.

B. Thermal Cycler Files

Protocol for F8 Exon Fragment Amplification

The following amplification profile has been optimized for F5 exon specific product amplification using the supplied Genemer™.

Program the following thermal cycler files.

1. Hot Start

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.		

2. Amplification File

F8 Exon Amplification File			
Step	Temperature	Time	Cycles
Denaturation	94 °C	30 sec.	30
Annealing	55 °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		

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

Process amplified PCR product for Mnl I digestion as described below.

Mnl I digestion of F5 Exon 10 amplified fragments	
Component	/25 µl Rxn
PCR amplified fragment	22 µl
10 X PCR Buffer	2.5 µl
Mnl I (5 unit/ µl)	1 µl
Incubate overnight at 37°C	

Load 10µl of undigested and 15 µl of Mnl I digested samples to a 1.6% agarose gel. Run at 60 mAmps till bromophenol dye is at bottom of gel.

References

1. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH (1995) High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). *Blood* 85:1504–1508.
2. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM (1996) A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 8:698–703.
3. Grody, WW, et al. (2001) *Genetics in Medicine* 3(2): 139-148.
4. Van Cott, EM, et al. (2002) *Arch Pathol Lab Med* 126: 577-582

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



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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 ₂	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
Total Volume	5 ml
*Aliquot and freeze	

MgCl₂ Concentration

The concentration of Mg²⁺ will vary from 1-5 mM, depending upon primers and substrate. Since Mg²⁺ ions form complexes with dNTPs, primers and DNA templates, the optimal concentration of MgCl₂ has to be selected for each experiment. Low Mg²⁺ ions result in a low yield of PCR product, and high concentrations increase the yield of non-specific products and promote mis-incorporation. Lower Mg²⁺ concentrations are desirable when fidelity of DNA synthesis is critical. The recommended range of MgCl₂ 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 MgCl₂ concentration of 1.5 is used in most cases. If the DNA samples contain EDTA or other chelators, the MgCl₂ concentration in the reaction mixture should be raised proportionally. Given below is an MgCl₂ concentration calculation and addition table using a stock solution of 25 mM MgCl₂.

MgCl ₂ Concentration & Addition Table								
Final concentration of MgCl ₂ 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 ₂ , 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 _m 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 ₂ O	37.5 µl
Total Volume	50 µl

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

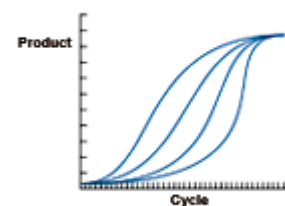
Taq Premix Preparation

Taq Premix (/50µl)	
Component	Volume
PCR Premix	6 µl
Taq polymerase (5 u/µl)	0.25µl
Add 5µl/50 µl rxn. After initial denaturation	
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 10⁶ 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**

- 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.



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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 H₂O.
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]trimethyl ammonium)	Reduces T _m 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 0.01µg/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 _m 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 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.



Genemer™ (Selected List) Primer pair for gene or mutation specific amplification. Special optimized conditions may be required for certain amplifications

Product	Size	Catalog No.	Price, \$
Fragile X (spanning CGG triple repeat region)	10 nmols	40-2004-10	100.00
Huntington Disease (spanning CAG triple repeat region)	10 nmols	40-2025-10	100.00
Myotonic Dystrophy (spanning CTG triple repeat region)	10 nmols	40-2026-10	100.00
Friedreich's Ataxia (spanning GAA triple repeat region)	10 nmols	40-2027-10	100.00
Factor V	10 nmols	40-2035-10	100.00
Factor VIII (Hemophilia) Genemer™ Pack	10 nmols	40-2036-10	650.00
STS (Steroid Sulfatase)	10 nmols	40-2023-10	100.00
HGH (Human Growth Hormone)	10 nmols	40-2024-10	100.00
Sickle Cell	10 nmols	40-2001-10	100.00
RhD (Rh D gene exon 10 specific)	10 nmols	40-2002-10	100.00
Rh EeCc (Rh Ee and Cc exon 7 specific)	10 nmols	40-2003-10	100.00
Gaucher (various mutations)	10 nmols	40-2047-XX	100.00
Cystic Fibrosis (various mutations)	10 nmols	40-2029-XX	100.00
SRY (sex determining region on Y)	10 nmols	40-2020-10	100.00
X alphoid repeat	10 nmols	40-2021-10	100.00
Y alphoid repeat	10 nmols	40-2022-10	100.00

*Please visit www.genelink.com for other Genemer™ not listed here

GScan™ Kits Product Ordering Information

Kits 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.

Product	Size	Catalog No.	Price, \$
Fragile X GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2004-15XX	650.00
Fragile X GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2004-15FMS	150.00
Huntington's Disease GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15XX	650.00
Huntington's Disease GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2025-15FMS	150.00
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2026-15XX	650.00
Myotonic Dystrophy GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2026-15FMS	150.00
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2027-15XX	650.00
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2027-15FMS	150.00
Kennedy Disease GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2032-15XX	650.00
Kennedy Disease GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2032-15FMS	150.00

**The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.

Prices subject to change without notice

All Gene Link products are for research use only

