Product Manual

Plant RT-PCRmer™ (Housekeeping Genes)

Plant Genemer™ (Housekeeping Genes)

Catalog No.: 40-1800-XX Store at –20°C

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

RT-PCRmer™

These RT-PCRmer™ will also amplify fragments from genomic DNA. It is essential that RNA be treated with RNase free DNase before cDNA preparation to exclude any possibility of genomic fragment amplification. In some cases different fragment size amplification from cDNA and genomic DNA is obtained.

Genemer™

The same primer pair are termed as Genemer™ when the template used is genomic DNA to amplify gene specific fragments.

Elongation Factor 1α (ef1α; ef1A) RT-PCRmer™ & Genemer™

Cyclophilin RT-PCRmer™ & Genemer™

Tomato LeGAI & LeBECI RT-PCRmer™ & Genemer™

Gene Link™

Material Supplied

Plant RT-PCRmer™ & Genemer™

Elongation Factor 1α (ef1α)
Cyclophilin
Tomato LeGAI & LeBECI RT-PCRmer™ & Genemer™

Storage Instructions: Store at –20°C upon receipt.

	Catalog No.	Primer Set	Product	Unit Size
❑	40-1800-11	ef1α F1/R1	Plant ef1α F1/R1 RT-PCRmer™ amplification primer pair	2 nmols
❑	40-1800-12	ef1α F1/R2	Plant ef1α F1/R2 RT-PCRmer™ amplification primer pair	2 nmols
❑	40-1800-13	Cyc F1/Cyc R1	Plant Cyclophilin F1/R1 RT-PCRmer™ amplification primer pair	2 nmols
❑	40-1800-14	Cyc F1/Cyc R2	Plant Cyclophilin F1/R2 RT-PCRmer™ amplification primer pair	2 nmols
❑	40-1802-11	LeGAI F1/R1	Tomato LeGAI F1/R1 RT-PCRmer™ amplification primer pair	2 nmols
❑	40-1802-12	LeBECI F1/R1	Tomato LeBECI F1/R1 RT-PCRmer™ amplification primer pair	2 nmols

*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.

Plant RT-PCRmer™

Description

RT-PCRmer™ are primer pairs for specific amplification of cDNA. Elongation factor 1α (ef1α: ef1A) transcripts has been shown to be consistently present under different stress conditions in plant and serves as a good representative housekeeping transcript as control (1). Actin, cyclophilin, β-tubulin, 18S rRNA and others are also used as controls for housekeeping genes. These are generally used as controls for measuring cDNA synthesis efficiency by reverse transcription and as controls for mRNA (cDNA) quantitative expression studies. The enclosed RT-PCRmer™ is supplied as a lyophilized powder in aliquots of 2 nmols. The 2 nmols of primer when dissolved in 200 µl sterile water or TE will give a solution of 10 µMolar i.e. 10 pmols/µl. The quantity supplied is sufficient for at least 100 regular 20 µl PCR reaction for ethidium bromide stained visualization. These RT-PCRmer™ have been tested with potato and tomato cDNA.
The product is supplied as a lyophilized powder, after reconstitution store at -20°C. Oligo purity is greater than 98% as determined by denaturing polyacrylamide gel electrophoresis.

Catalog No.	Primers	Fragment Size	Gene Product Name	GenBank Accession
40-1800-11	EF-1A F1/R1	101 bp	Elongation Factor 1α	AB061263
40-1800-12	EF-1A F1/R2	318 bp	Elongation Factor 1α	AB061263
40-1800-13	Cyc F1/Cyc R1	121 bp	Cyclophilin	AF126551
40-1800-14	Cyc F1/Cyc R2	382 bp	Cyclophilin	AF126551

Tomato Specific Genemer™				
Catalog No.	**Primers**	**Fragment Size**	**Gene Product Name**	**GenBank Accession**
40-1802-11	LeGAI F1/R1	820 bp	Lycopersicon esculentum GAI-like protein	AY269087
40-1802-12	LeBECI F1/R2	565 bp	Lycopersicon esculentum beclin 1 protein	AW223712

Procedure

RT-PCRmer™ Reconstitution

Primer Mix: Dissolve the supplied lyophilized RT-PCRmer™ in 200 µl sterile TE. The 2 nmols of primers when dissolved in 200 µl will give a solution of 10 µM i.e. 10 pmols/µl.

Recipe

TE Buffer pH 7.5 Composition
1 X TE Buffer pH 7.5
10 mM Tris-HCl pH 7.5
1 mM EDTA

cDNA Amplification and Detection [can also use genomic DNA]

Set up the following amplification files on a thermal cycler. Please refer to the instrument manufacturer's manual for setting up of the program.

Program thermal cycler instrument with an amplification profile prior to starting the amplification protocol.
Consult appropriate instrument manufacturer's manual.

Hot Start		
Step	Time & Temperature	Cycles
Initial Denaturation	95°C for 5 minutes	1
Annealing	55°C Hold Infinity	Hold
Comments: Add Taq premix while on hold.		

Amplification Profile		
Step	**Temperature & Time**	**Cycles**
Denaturation	94°C for 30 seconds	30
Annealing	55°C for 30 seconds	
Extension	72°C for 60 seconds	
Fill up	72 °C for 7 minutes	1
Hold	4 °C hold for infinity	Hold

PCR*

1. **PCR Premix Preparation (PP). Label tube "PP"**

PCR Premix Preparation (PP)		
Component	1 X 20 µl Rxn.	10 X 20 µl Rxns.*
Sterile Water	14 µl	140 µl
10 X PCR Buffer	2 µl	20 µl
2.0 mM dNTP	2 µl	20 µl
10 pmol/µl Primer Mix	1 µl	10 µl
Template cDNA (~100 ng)	1-2 µl	Add cDNA to each tube
Total Volume	18 µl	
After adding template start hot start PCR File		

*Dispense 18 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 55 °C add 2 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 20 µl Rxn.	10 X 20 µl Rxns.
Sterile Water	1 µl	10 µl
10 X PCR Buffer	0.5 µl	2 µl
Taq Polymerase	0.5 µl	2.5 µl
Add 2 µl to each reaction when holding after hot start		

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

D. Agarose Electrophoresis

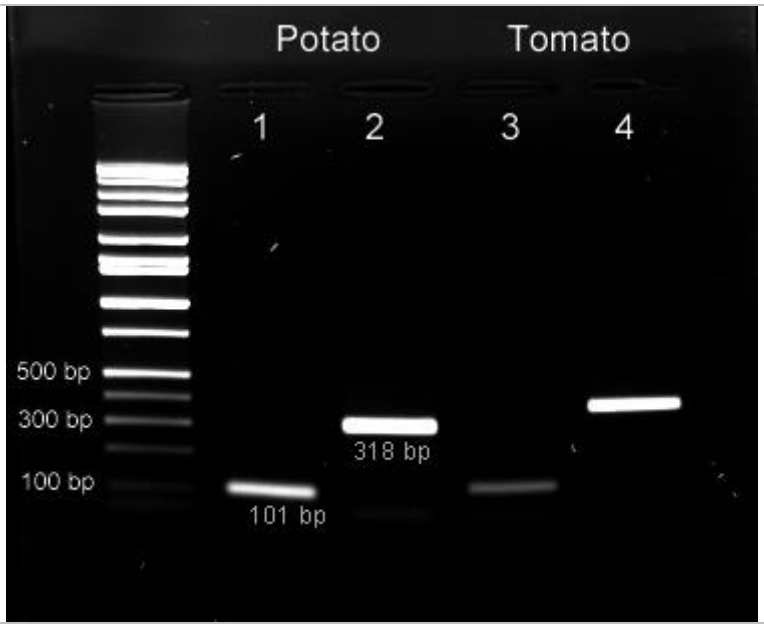
Load 5 to 10 µl samples to 1% agarose gel. Run at 90 mAmps. Confirm correct amplification fragment size.

Results and Interpretation

RT-PCR™ primer sets are specific for amplification of different size fragments of cDNA. See below for the expected fragment size for various RT-PCRmer™ .

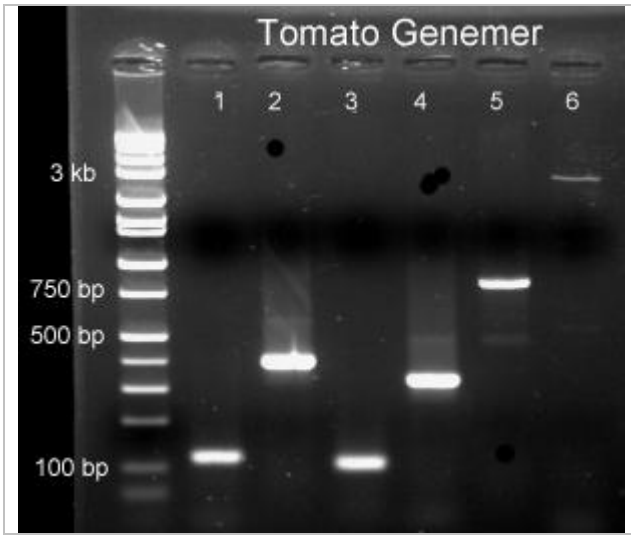
Catalog No.	Primers	Fragment Size	Gene Product Name	GenBank Accession
40-1800-11	EF-1A F1/R1	101 bp	Elongation Factor 1α	AB061263
40-1800-12	EF-1A F1/R2	318 bp	Elongation Factor 1α	AB061263
40-1800-13	Cyc F1/Cyc R1	121 bp	Cyclophilin	AF126551
40-1800-14	Cyc F1/Cyc R2	382 bp	Cyclophilin	AF126551

Elongation Factor 1α RT-PCRmer™ Amplification Pattern.



PCR was performed using 50 ng potato or tomato Omni-cDNA™ as the template. The protocol for amplification was followed as given in this manual. 10 µl of amplified fragment was loaded on a 1% agarose gel. The amplified fragment size obtained 101 bp for EF-1A F1/R1 and 318 bp for EF-1A F1/R2 with the cDNA used as specified on the gel image

Catalog No.	Primers	Fragment Size	Gene Product Name	GenBank Accession
40-1800-11	EF-1A F1/R1	101 bp	Elongation Factor 1α	AB061263
40-1800-12	EF-1A F1/R2	318 bp	Elongation Factor 1α	AB061263
40-1800-13	Cyc F1/Cyc R1	121 bp	Cyclophilin	AF126551
40-1800-14	Cyc F1/Cyc R2	382 bp	Cyclophilin	AF126551
Tomato Specific RT-PCRmer™ & Genemer™				
40-1802-11	LeGAI F1/R1	820 bp cDNA & 900 bp Genomic	Lycopersicon esculentum GAI-like protein	AY269087
40-1802-12	LeBEC F1/R1	565 bp cDNA & 3.5 kb Genomic	Lycopersicon esculentum beclin 1 protein	AW223712



Total genomic DNA

Tomato Genomic DNA amplification. Molecular weight followed by Lane 1– Cyclophilin F1/R1= 121 bp; 2- Cyclophilin F1/R2= 382 bp; 3- E1FA- F1/R1= 101 bp; 4-E1FA-F1/R2= 318 bp; 5- LeGAI F1/R1= ~900 bp; 6- LeBEC F1/R1= 3.5 kb

References

1. Nicot,N., Jean-Hausman, J-F., Hoffmann, L. and Evers, D. (2005)Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. Journal of Experimental Botany, 56(421) 2907–2914.

Appendix

PCR Components and Analysis
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 otherwise indicated.

Recipe

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

Recipe

2.0 mM dNTP Stock Solution Preparation*	
Component	**Volume**
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	

Buffer Condition

$MgCl_2$ Concentration
The concentration of Mg^{++} will vary from 1-5 mM, depending upon primers and substrate. Since Mg^{2+} ions form complexes with dNTPs, primers and DNA templates, the optimal concentration of $MgCl_2$ has to be selected for each experiment. Low Mg^{2+} 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^{2+} concentrations are desirable when fidelity of DNA synthesis is critical. The recommended range of $MgCl_2$ 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 $MgCl_2$ concentration of 1.5 is used in most cases. If the DNA samples contain EDTA or other chelators, the $MgCl_2$ concentration in the reaction mixture should be raised proportionally. Given below is an $MgCl_2$ concentration calculation and addition table using a stock solution of 25 mM $MgCl_2$.

$MgCl_2$ Concentration & Addition Table								
Final concentration of $MgCl_2$ in 50 µl reaction mix, (mM)	1.0	1.25	1.5	1.75	2.0	2.5	3.0	4.0
Volume of 25 mM $MgCl_2$, 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.

Always use filter barrier pipette tips to prevent cross contamination

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.

Recipe

TE Buffer pH 7.5 Composition
1 X TE Buffer pH 7.5
10 mM Tris-HCl pH 7.5
1 mM EDTA

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

• Program your thermal cycler instrument with an amplification profile prior to beginning the amplification protocol.
Consult your appropriate instrument manufacturer's manual.

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 **r**	Infinity	Hold
Based on the Tm of the primers. Usually varies from 50 °C to 65 °C			

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
Taq Enzyme Mix (EM) See below for preparation	5 µl	50 µl
Template DNA (~500 ng)	1-2 µl	Add 1-2 µl DNA to each tube
Total Volume	50 µl	
Keep on ice during set up. After adding template start PCR File		

Recipe

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_2O	37.5 µl
Total Volume	50 µl

Recipe

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

Recipe

Taq Premix (/50µl)	
Component	**Volume**
PCR Premix	6 µl
Taq polymerase (5 u/µl)	0.25µl
Add 5 µl/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	

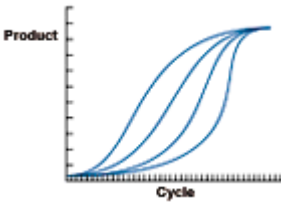
- The PCR premix preparation protocol is written considering that more than one amplification reaction will be performed at the same time. If only one reaction is planned then there is no need to prepare the Taq Enzyme Mix (EM).

Recipe

Gene Link PCR Buffer
1 X PCR Buffer
10 mM Tris-HCl pH 8.3
50 mM KCl
1.5 mM $MgCl_2$
0.001% Gelatin

Yield and Kinetics

The target will be amplified by up to 10^6 fold in a successful reaction, but the amplification will usually plateau at 1-10 µg. 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 10 kb in length, but the majority of amplifications are at 1 kb and below. Agarose electrophoresis is the classical method to analyze amplification products from 150 bp to greater than 10 kb. Polyacrylamide gel electrophoresis should be used for resolution of short fragments in the range of 100 bp to 500 bp when discrimination of as small as a 10 bp difference is required.

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

Purification of PCR Product

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

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. The 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.

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 used in PCR suffers the same fate and GC-rich DNA sequences often require laborious work to optimize the amplification assay. The GC-rich sequences possess high thermal and structural stability, presumably because the high duplex melting temperature that permits stable secondary structures to form, thus preventing completion of a faithful replication (2).

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

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

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

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.

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Ordering Information

Plant RT-PCRmer™ Primer pair for specific amplification of cDNA. Special optimized conditions may be required for certain amplifications

Product	Size	Catalog No.	Price, $
Plant EF-1A F1/R1 (elongation factor)	2 nmols	40-1800-11	100.00
Plant EF-1A F1/R2 (elongation factor)	2 nmols	40-1800-12	100.00
Plant Cyclophilin F1/Cyc R1	2 nmols	40-1800-13	100.00
Plant Cyclophilin F1/Cyc R2	2 nmols	40-1800-14	100.00
Tomato Specific RT-PCRmer™ & Genemer™			
Tomato LeGAI F1/R1	2 nmols	40-1802-11	100.00
Tomato LeBEC F1/R1	2 nmols	40-1802-12	100.00
*Please visit www.genelink.com for other RT-PCRmer™ not listed here			

Related Products Ordering Information

GAPDH RT-PCRmer™ Primer pair for specific amplification of cDNA. Special optimized conditions may be required for certain amplifications

Product	Size	Catalog No.	Price, $
GAPDH A (F1/R1); Exon 2-3	10 nmols	40-1005-10	100.00
GAPDH B (F2/R2); Exon 6-7	10 nmols	40-1006-10	100.00
GAPDH C (F1/R2); Exon 2-7	10 nmols	40-1007-10	100.00
GAPDH D (F1/R3); Exon 2-9	10 nmols	40-1008-10	100.00
*Please visit www.genelink.com for other RT-PCRmer™ not listed here			

RT-PCRmer™ Primer pair for specific amplification of cDNA. Special optimized conditions may be required for certain amplifications

Product	Size	Catalog No.	Price, $
RT-PCRmer; human beta actin	10 nmols	40-1001-10	100.00
RT-PCRmer; rat beta actin	10 nmols	40-1002-10	100.00
RT-PCRmer; mouse beta actin	10 nmols	40-1003-10	100.00
RT-PCRmer; beta2 microglobulin	10 nmols	40-1004-10	100.00
Beta actin control PCR mix (human & rat)	200 ul	40-1002-00	110.00
*Please visit www.genelink.com for other RT-PCRmer™ not listed here			

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

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	750.00
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

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