Product Specifications

Electrophoresis Reagents, Polymerase Chain Reaction
Custom Primers and Probes
Hybridization and Detection Reagents

PCR Additives

Store at -20°C

	Catalog Number	Description	Size
❑	40-3020-05	dNTP 10mM	0.5 ml
❑	40-3021-11	dNTP 2mM (10X)	1.1 ml
❑	40-3022-16	$MgCl_2$; 25 mM	1.6 ml
❑	40-3023-16	$MgCl_2$; 50 mM	1.6 ml
❑	40-3031-10	DMSO	1 ml
❑	40-3053-10	TMAC (Tetramethyl ammonium chloride) 100 mM	1 ml
❑	40-3059-10	KCl 300 mM	1 ml
❑	40-3032-10	Betaine; 5M	1 ml

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 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 0.01µg/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 *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 15-100mM to eliminate non-specific priming.

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 indicated.

Standard Gene Link PCR Buffer	
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	

$MgCl_2$ 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 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.

Primer 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 Tm 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_2O	37.5 µl
Total Volume	50 µl

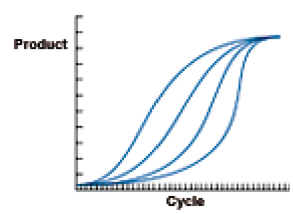
Typical PCR Reaction Mix

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 10^6 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.

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.

Ordering Information

Product	Catalog No.	Size	Price $
Taq DNA Polymerase; 400 units; 5 µ/µl; 80 µl	40-5200-40	400 units	$75.00
Taq PCR Kit; 200 reactions	40-5211-01	200 reactions	$110.00
Taq PCR Kit with controls; 200 reactions	40-5212-01	200 reactions	$125.00
PCR Master Mix (2X); 100 reactions (2 x 1.3 ml)	40-5213-01	100 reactions	$70.00
PCR Master Mix (2X); 200 reactions (4 x 1.3 ml)	40-5213-02	200 reactions	$120.00

Related Products Ordering Information

PCR Reagents			
Product	Catalog No.	Size	Price $
Taq DNA Polymerase 300 units; 5 µ/µl; 60 µl	40-5200-30	300 units	$60.00
PCR Buffer Standard (10 X)	40-3060-16	1.6 ml	$8.00
PCR Buffer Mg Free (10 X)	40-3061-16	1.6 ml	$8.00
Taq Polymerase Dilution Buffer; 1 ml	40-3070-10	1 ml	$8.00
dNTP 10 mM	40-3020-05	0.5 ml	$75.00
dNTP 2 mM (10X)	40-3021-11	1.1 ml	$35.00
$MgCl_2$; 25 mM; 1.6 mL	40-3022-16	1.6 ml	$8.00
$MgCl_2$; 50 mM; 1.6 mL	40-3023-16	1.6 ml	$16.00
Omni-Marker™ Universal Unlabeled	40-3005-01	100 µl	$15.00
Primer and Template Mix; 500 bp; 40 reactions	40-2026-60PT	100 µl	$15.00
Nuclease Free Water	40-3001-16	1.6 ml	$5.00
DMSO	40-3031-10	1 ml	$8.00
TMAC (Tetramethyl ammonium chloride) 100 mM	40-3053-10	1 ml	$8.00
KCl 300 mM	40-3059-10	1 ml	$8.00
Betaine; 5M	40-3032-10	1 ml	$8.00

Omni-Marker™			
Product	Catalog No.	Size*	Price $
Omni-Marker™ Universal unlabeled	40-3005-01	100 µl	15.00
Omni- Marker™ Universal unlabeled	40-3005-05	500 µl	50.00
Omni-Marker™ Universal unlabeled	40-3005-10	1 ml	90.00
Omni- Marker™ Low unlabeled	40-3006-01	100 µl	15.00
Omni-Marker™ Low unlabeled	40-3006-05	500 µl	50.00
Omni- Marker™ Low unlabeled	40-3006-10	1 ml	90.00
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	40-3062-01	100 µl	75.00
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	40-3062-05	500 µl	325.00

Buffers & Reagents

Product	Catalog No.	Size	Price $
Agarose Tablets, 0.5 gm each	40-3011-10	100 tablets	100.00
Agarose LE Molecular Biology Grade; 100 gms	40-3010-10	100 gms	120.00
Agarose LE Molecular Biology Grade; 500 gms	40-3010-50	500 gms	410.00
Hybwash A, Hybridization Wash Solution	40-5020-20	200 ml	65.00
Hybwash B, Hybridization Wash Solution	40-5021-10	100 ml	50.00
TAE Buffer; 50 X Concentrate; 100 ml	40-3007-01	100 ml	32.00
TAE Buffer; 50 X Concentrate; 1000 ml	40-3007-10	1000 ml	128.00
TBE Buffer; 5 X Concentrate	40-3008-10	1000 ml	35.00
10x Washing buffer	40-5025-20	200 ml	125.00
10% Blocking solution	40-5026-10	100 ml	75.00
Seq. Loading buffer	40-5027-00	1 ml	10.00
10x AP Detection buffer (alkaline phosphatase detection buffer)	40-5031-10	100 ml	65.00
Lumisol™ I Hybridization Solution; contains formamide	40-5022-20	200 ml	75.00
Lumisol™ II Hybridization Solution; for non-toxic hybridizations	40-5023-20	200 ml	75.00
Lumisol™ III Hybridization Solution; for oligo probes	40-5024-20	200 ml	75.00

Loading Buffers

Product	Catalog No.	Size	Price $
Loading Buffer 5X BPB/XC non-denaturing	40-3002-01	100 µl	5.00
Loading Buffer 5X BPB/XC non-denaturing	40-3002-10	1 ml	10.00
Loading Buffer 5X Orange G/XC non-denaturing	40-3004-01	100 µl	5.00
Loading Buffer 5X Orange G/XC non-denaturing	40-3004-10	1 ml	10.00
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-01	100 µl	5.00
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-10	1 ml	10.00

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