Product Manual

Taq DNA Polymerase and PCR Kits

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

Gene Link™

Material Supplied

❑	40-5200-40	Taq DNA Polymerase; 400 units; 5 µ/µl
❑	40-5211-01	Taq PCR Kit; 200 reactions
❑	40-5212-01	Taq PCR Kit with controls; 200 reactions
Taq DNA Polymerase is a thermostable DNA polymerase that possesses a 5´> 3´ polymerase activity and a double-strand specific 5´> 3´ exonuclease activity. It is the enzyme most widely used in PCR.		

Storage Instructions: Store at –20ºC upon receipt.

❑	**40-5200-40**	**Taq DNA Polymerase 400 units; 5 µ/µl; 80 µl**

❑	**40-5211-01**	**Taq PCR Kit; 200 reactions**

	Catalog No.	Unit Size	Product Description
❑	40-5200-30	300 units	Taq DNA Polymerase 300 units; 5 µ/µl; 60 µl
❑	40-3060-16	1.6 ml	PCR Buffer Standard (10 X)
❑	40-3061-16	1.6 ml	PCR Buffer Mg Free (10 X)
❑	40-3021-11	1.1 ml	dNTP 2mM (10X)
❑	40-3022-16	1.6 ml	$MgCl_2$; 25 mM
❑	40-3001-16	1.6 ml	Nuclease Free Water
❑	40-3005-01	100 µl	Omni-Marker™ Universal Unlabeled

❑	**40-5212-01**	**Taq PCR Kit with controls; 200 reactions**

	Catalog No.	Unit Size	Product Description
❑	40-5200-30	300 units	Taq DNA Polymerase 300 units; 5 µ/µl; 60 µl
❑	40-3060-16	1.6 ml	PCR Buffer Standard (10 X)
❑	40-3061-16	1.6 ml	PCR Buffer Mg Free (10 X)
❑	40-3021-11	1.1 ml	dNTP 2mM (10X)
❑	40-3022-16	1.6 ml	$MgCl_2$; 25 mM
❑	40-3001-16	1.6 ml	Nuclease Free Water
❑	40-3005-01	100 µl	Omni-Marker™ Universal Unlabeled
❑	40-2026-60PT	100 µl	Primer and Template Mix; 500bp; 40 reactions

Taq DNA Polymerase Unit Definition
One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 75°C.

Polymerase Chain Reaction (PCR)

Background

The polymerase chain reaction (PCR) is the single most commonly used procedure in molecular genetics. PCR was developed in 1985[7] and, due to its ability to amplify specific regions of DNA several million-fold, has since become the major contributing factor in the rapid pace of research in molecular genetics.[8,9]

PCR is based on the enzymatic amplification of a fragment of DNA that is flanked by two complementary, short, oligonucleotide primers whose sequences are known. These primers are designed such that each corresponds to one of the strands; the distance between these primers limits the amplification fragment size. PCR is also based on the property that in hybridization the primer, under specific conditions, will bind (hybridize, anneal) *only* to its cognate sequence in the DNA sample. This process is termed *annealing.* The annealing of the primer to target DNA is achieved by denaturing the target DNA through exposure to high temperature. Heat breaks the base-pair hydrogen bonds and results in a separation of the two DNA strands. Reducing the temperature favors base pairing/hybridization, which is equally competed for by the primers, present in excess.

Once the primers are correctly annealed, DNA polymerase will elongate the primer by copying the template sequence. The two primers are both extended beyond the binding site, independent of each other, resulting in the synthesis of variable-size fragments complementary to the template DNA. The steps of denaturation, annealing, and elongation constitute one cycle. In one cycle, a copy of the target sequence is achieved. The second cycle of amplification yields four copies, and so forth. The amplification process occurs exponentially and can result in the amplification of a target sequence several million-fold. A routine PCR process consists of 20 to 30 cycles, requiring 2 to 3 hours and using an automated thermal cycling instrument. Under proper conditions, a unique gene sequence from the genome can be routinely amplified from 50 to 100 ng of target DNA. PCR has been used to amplify sequences from one cell (approximately 7 pg DNA) and a single hair (300 ng).

Procedure

Material Supplied

Please refer to page 2 of this manual for materials supplied for the particular product ordered.

A. Primers: Reconstitution Protocol

Stock Primer Mix: Dissolve the primers in TE or sterile water to obtain a solution of 100 μM i.e. 100 pmols/μl. The stock solution concentration varies from laboratory to laboratory. Some laboratory prefer a 500 μM i.e. 500 pmols/μl stock solution.

Primer Mix:

1. **From 100 μM i.e. 100 pmols/μl stock solution.** Prepare a 10 pmols/μl Primer Mix solution by a ten fold dilution of the 100 μM i.e. 100 pmols/μl stock primer mix. Example: Add 180 μl sterile TE or sterile water to a new tube, to this tube add 20 μl of primer stock solution. Label this tube as Primer Mix 10 pmols/μl.

2. **From 500 μM i.e. 500 pmols/μl stock solution.** Prepare a 10 pmols/μl Primer Mix solution by a fifty fold dilution of the 500 μM i.e. 500 pmols/μl stock primer mix. Example: Add 192 μl sterile TE or sterile water to a new tube, to this tube add 4 μl of primer stock solution. Label this tube as Primer Mix 10 pmols/μl.

B. Template DNA

The amount of template DNA added for amplification varies. Too high concentration will lead to non-specific fragment amplification as well. A guideline is given below.

Genomic DNA: 10 ng to 100 ng.
Plasmid DNA: 1 ng to 10 ng.

The PCR Kit with Controls contains the following component:

40-2026-60PT	100 µl	Primer and Template Mix; 500 bp; 40 reactions

This contains premixed control primers and template that will amplify a 500 bp fragment. The recommended amount to use is 2.5 µl for 50 µl reaction volume.

C. Thermal Cycler Files: Amplification Profile

The following amplification profile is given as an example. It can be used for amplification of the supplied control primer and template mix. Cycles can be modified as required.

All protocol given below as an example is for 50 µl total volume reactions. It is highly recommended to perform 25 µl total volume reaction when experienced. There is considerable savings in reagents.

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

Amplification File			
Step	Temperature	Time	Cycles
Denaturation	94 °C	30 sec.	30
Annealing	55 °C	30 sec.	
Elongation	72 °C	60 sec.	
Fill in Extension	72 °C	7 minutes	1
Hold	4 °C	Infinity	Hold

D. 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		

NOTE: If setting up for the control PCR, the control primer and template mix already contains template. There is no need to add template separately. Add 2.5 µl per reaction.

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 60 °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		

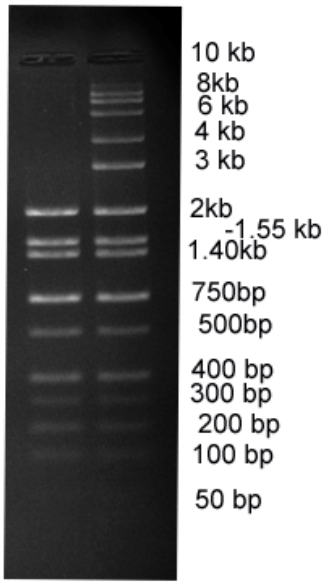
E. Omni-Marker™ Universal unlabeled [40-3005-01]

The supplied Omni-Marker™ Universal unlabeled pattern is given below. Normal recommended loading per lane is 5 µl. To visualize smaller fragment load 10 µl per lane.

Omni-Marker™ Universal and Low are unlabeled DNA markers containing a blend of fragments ranging from 50 base pairs to 10 kb. The universal contains fragments of the following sizes; 10 kb, 8 kb, 6 kb, 4 kb, 3 kb,2 kb, 1.55, 1.4 kb, 1 kb, 750 bp, 500 bp, 400 bp kb, all the bands and "low" version is also available containing fragments up to 2 kb size.

The gel picture shows the fragments obtained by electrophoresing in 1.5% agarose gel. The low and universal Omni-Markers are provided premixed with dye. The marker contains 0.02% sodium azide.

Omni-Marker™ Universal unlabeled	
Fragment Size	Approx. conc.
10 kb	30 ng
8 kb	30 ng
6 kb	45 ng
4 kb	60 ng
3 kb	85 ng
2 kb	150 ng
1.55 kb	100 ng
1.40 kb	100 ng
1.00 kb	120 ng
750 bp	30 ng
500 bp	60 ng
400 bp	20 ng
300 bp	40 ng
200 bp	30 ng
100 bp	20 ng
50 bp	15 ng



F. Agarose Electrophoresis

Load 5 to 10 µl samples to a 0.8% agarose gel. Run at 90 mAmps. Confirm correct amplification fragment size.

PCR Control Primer Results

The kit contains primer and template mix to be amplified as controls. The control primer sequences are derived from human myotonic dystrophy gene and the template contains the corresponding cloned fragment. A 500 bp fragment will be amplified using the supplied control primer and template mix [40-2026-60PT].

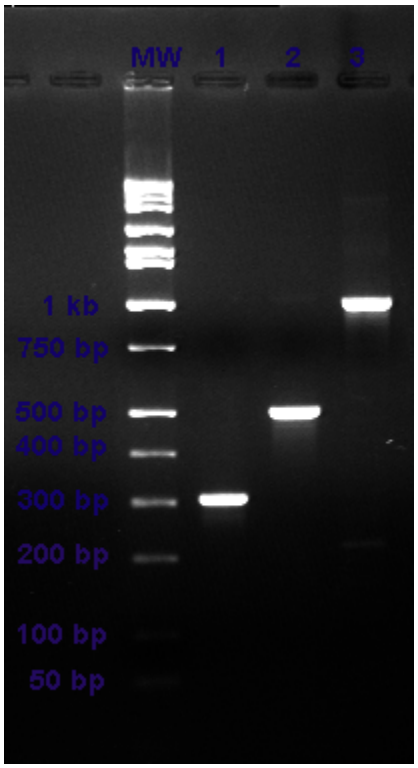


Figure 1. Typical amplification products electrophoresed in a 0.8% agarose gel using 0.5X TAE. Lane marked as MW is molecular weight markers. Lane 1-3 are amplified fragments. Lane 2 is the 500 bp fragment that will be amplified using the supplied control primer and template mix [40-2026-60PT].

References:

1. Saiki RK, Scharf SJ, Faloona F, Mullis KB, et aI. Enzymatic amplification *of* beta-globin sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 1985;230:1350-1354.
2. Mullis KB The unusual origin of the polymerase chain reaction. Sci *Am* 1990;262:56-65.
3. Innis MA, Gelfand DH, Sninsky JJ.,White TJ. eds *PCR Protocols: A Guide* to *Methods and Applications.* New York, NY: Academic Press; 1990.
4. Saiki RK. Gelfand DH. Stoffel S. ScharfSJ. et al Primer directed enzymatic amplification *of* DNA with a thermostable DNA polymerase *Science* 1988;239487-491.
5. Chien A, Edgar DB, Trela JM Deoxyribonucleic acid polymerase from the extreme thermophile Thermus, *aquaticus. J Bacterio 1976;* 1271 550-1 557.

Appendix

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	
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.

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 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

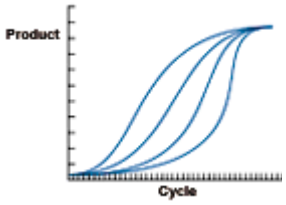
PCR reaction (/50µl)	
Component	**Volume**
PCR premix	45 µl
100ng/µl diluted DNA	1 µl
Hot start and then add	
Taq premix	5 µl

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.



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.

Gel Electrophoresis of DNA

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.
Polyacrylamide gels for PCR products can be 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.

TAE vs. TBE

Agarose gel electrophoretic resolution of DNA depends on the concentration of agarose and the ionic strength of electrode buffer. There is a choice of buffers; TAE and TBE (Tris-borate EDTA). TAE is the most commonly used electrophoresis buffer for routine molecular biology work.

The resolution of supercoiled DNAs and large DNA is better in TAE than TBE. The buffering capacity of TAE is lower than TBE and is progressively depleted during successive electrophoresis. In contrast, TBE has a more stable and higher buffering capacity. Double stranded linear DNA fragments longer than ~500 bp migrate approximately 10 % faster in TAE than in TBE.

In summary, use TAE buffer for regular resolution of DNA fragments longer than ~500 bp but use TBE buffer for clear and higher resolution of smaller DNA fragments on agarose gels. Critical DNA sizes and gel concentrations for a clear separation were about 2-kb for the 0.8% agarose and 300-bp for the 2.0% agarose. DNA fragments larger than the critical size (>2-kb on 0.8% agarose gel) migrate faster in TAE, and the smaller fragments (<300-bp on 2% agarose gel) migrate faster in TBE showing better resolution.

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.
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_20.

6. Run an aliquot on an agarose gel to confirm the presence of the correct amplified product. The purified DNA is sequence grade and can be used directly for sequencing.

Gel Filtration

Primers and salts are efficiently removed by gel filtration using Sephadex G-50. This method is recommended for downstream DNA sequencing application.

Protocol

1. Hydrate Sephadex G-50 ahead of time in sterile water or TE (10mM Tris pH 8, 1 mM EDTA). Take out from fridge if already stored hydrated. Bring to room temperature.
2. Assemble a spin column on a collection tube.
3. Add 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.

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.	Unit Size
Taq DNA Polymerase; 400 units; 5 µ/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 Reagents

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

Omni-Marker™

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

Buffers& Reagents

Product	Catalog No.	Unit Size
Agarose Tablets, 0.5 gm each	40-3011-10	100 tablets
Agarose LE Molecular Biology Grade; 100 gms	40-3010-10	100 g
Agarose LE Molecular Biology Grade; 500 gms	40-3010-50	500 g
Hybwash A, Hybridization Wash Solution	40-5020-20	200 ml
Hybwash B, Hybridization Wash Solution	40-5021-10	100 ml
TAE Buffer; 50X Concentrate; 100 ml	40-3007-01	100 ml
TAE Buffer; 50X Concentrate; 1000 ml	40-3007-10	1000 ml
TBE Buffer; 5X Concentrate	40-3008-10	1000 ml
10x Washing buffer	40-5025-20	200 ml
10% Blocking solution	40-5026-10	100 ml
Seq. Loading buffer	40-5027-00	1 ml
10x AP Detection buffer	40-5031-10	100 ml
Lumisol™ I Hybridization Solution; contains formamide	40-5022-20	200 ml
Lumisol™ II Hybridization Solution; for non-toxic hybridizations	40-5023-20	200 ml
Lumisol™ III Hybridization Solution; for oligo probes	40-5024-20	200 ml

Loading Buffers

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

Prices subject to change without notice All Gene Link products are for research use only

Gene Link Distributors

Argentina
Migliore Laclaustra S.R.L.
Buenos Aires 1055 Argentina
(11) 4372 9045
(11) 4373 8052
Email :
miglac@migliorelaclaustra.com.ar

Belgium
Gentaur
Kampenhout 1910 Belgium
0032 16 58 90 45
0032 16 50 90 45
Email : info@gentaur.com
Web : www.gentaur.com

Brazil
LGC do Brasil
Rio de Janeiro 20770-270 Brazil
Tel 21-592-6642
Toll Free 0800- 407047
Fax 21-2593-3232
Web : www.lgcscientific.com

China
Hangzhou AOWEI Bioengineering
Hangzhou China
Tel: 86-871-56773688 /
86-571-56773688
86-571-56773066
Email : liuwg@aowei21.com
Web : www.aowei21.com

Egypt
Lab Technology
P.O.Box 5959 Heliopolis West
Cairo 11351 Egypt
20-2-2622 6761
20-2-2622 6760
Email : subhi@labtechnology.net

India
Lab Equipments & Chemicals
Kolkata 700 001 India
Tel: 91-33-2235-1958/1806/0274
Fax: 91-33-2221-5682
Email : info@labquipindia.com
Web : www.labquipindia.com

Ireland
Isis Ltd.
Bray, Co Wicklow Ireland
Tel: 00353-1-2867777
Fax: 00353-1-2867766
Email : enquiries@isisco.ie
Web :
www.isisco.ie/isis/Main/Home.htm

Italy
DBA Italia S.R.L
Milano Italy
Tel: 39-02-26922300
Fax: 39-02-26923535
Email : info@dbaitalia.it
Web : www.dbaitalia.it

Japan
Funakoshi Co., Ltd.
Tokyo 113-0033 Japan
Tel: (3) 5684-1620
Fax: (3) 5684-1775
Email : reagent@funakoshi.co.jp
Web : www.funakoshi.co.jp

Jordan
LeanGene for Medical & Electronic Supplies
Amman 11110 Jordan
(962) 6 46 55 401
(962) 6 46 55 402
Email : mgr@leangene-jo.com

Jordan
Advanced Scientific Consulting Group Co.
City Terminal bldg. Office 303
[144753]
Amman Jordan
962 6 5812210
962 6 5812214
Email : asem@advanced.com.jo
Web : www.advanced.com.jo

Jordan
CGenomix
Amman Jordan
+ 962 (6) 55 22 365
+ 962 (6) 55 22 367
Email : info@cgenomix.com
Web : www.cgenomix.com

Korea
Genes Laboratories Inc.
Soojeong-gu, Seongnam-si
(461-701)
Gyeonggi-do Korea
82-(0)31-889-3085
82-(0)31-696-5453
Email : info@geneslabs.com
Web : www.geneslabs.com

Korea
GenomicBase Co. Ltd.
Dongdaemun-Gu [130-842]
Seoul Korea
Tel: 82. 502.335.4926
Fax: 82. 502.335.4927
Email : info@genomicbase.com
Web : www.genomicbase.com

Kuwait
Tri Alpha Co.
Al-Awadi Tower #3, 6th Floor
Kuwait City 72263 Kuwait
(00965) 22 43 13 44
(00965) 22 43 51 06
Email : walter@trialphaco.com

Latvia
Valdai
Latvia LV-1010 Latvia
Tel: + 371-7227620
Fax: + 371-7326091
Email : Valdai@apollo.lv

Lebanon
DPC/Lebanon SARL
PO BOX 55440
Beirut Lebanon
Tel: (961) 502 812
Fax: (961) 805 851
Email : dpcliban@dm.net.lb
Web : www.dpcleb.com

Malaysia
Nano Life Quest Sdn Bhd
47100 Puchong, Selangor D. E.
MALAYSIA
Tel : +603 8062 4678
Fax : +603 8062 4678
Email : info@nanolifequest.com
Web : www.nanolifequest.com

Norway
AME Bioscience A/S
Toroed 3135 Norway
47- 3361-0718
47-3340-1444
Email:aewing@amebioscience.com

Pakistan
Worldwide Scientific
30-Ferozepur Road
Lahore 54000 Punjab Pakistan
Tel: 92-42-755-2355
Fax: 92-42-755-3255
Email : wws@brain.net.pk

Saudi Arabia
Labs Care
Riyadh 11322 Saudi Arabia
Tel: (1) 465-9452
Fax: (1) 465-9452
Email : manager@labscare.com
Web : www.labscare.com

Singapore
Sciencewerke Pte Ltd
#04-21 S139550
Singapore
Tel: +65 777 1045
Fax: +65 777 3054
Email : enquiry@sciencewerke.com
Web : www.sciencewerke.com

Spain
bioNova científica, s.l.
28007
Madrid Spain
Tel: +34 91 551 54 03
Fax: +34 91 433 45 45
Email : info@bionova.es
Web : www.bionova.es

Spain
Attendbio Research SL
08018
Barcelona Spain
Tel: +34 934019842
Fax: +34 934019843
Email : attendbio@attendbio.com
Web : www.attendbio.com

Taiwan
Watson Biotechnology Co., Ltd.
Xinzhuang Dist.
New Taipei City 242 Taiwan
Tel: 886-2-89916881
Fax: 886-2-89936967
Email : tensci.gene@msa.hinet.net
Web : www.tenscigene.com.tw

Thailand
Qualitech Instruments Co.,Ltd
Ladprow
Bangkok 10230 Thailand
Tel: (662) 538-1843
Fax: (21) 2593-3232
Email : sales@qualitech-instruments.com
Web : www.qualitech-instruments.com

Turkey
Diagen Biyoteknolojik Sistemler
Halk Sokak 27/2 Sihhye
Ankara 06420 Turkey
Tel: (312) 432-0611
Fax: (312) 432-3595
Email : info@diagen.com.tr
Web : www.diagen.com.tr

UK & Switzerland
Molecular Solutions Europe Ltd.
London EC1V 4PY UK UK & Switzerland
Tel:+44-(0)870-1995067
Fax:+44-(0)207-2539040
Email : info@mseu.co.uk
Web : www.mseu.co.uk

United Arab Emirates
Al Nawras Medi-Lab Supplies
Industrial Area No. 11
Sharjah United Arab Emirates
Tel: (9716) 5344550
Email : alnawras@eim.ae

Gene Link, Inc.

190 Saw Mill River Road
Hawthorne, NY 10532
USA

Telephone:

1-800-GENE-LINK
(914) 769-1192

Facsimile:

1-888-GENE-LINK
(914) 769-1193

Email:

CUSTOMER SERVICE
cust_service@genelink.com

SALES
sales@genelink.com

CUSTOM OLIGO ORDERS
oligos@genelink.com

TECHNICAL SUPPORT
support@genelink.com

SEQUENCING/GENOTYPING
genotyping@genelink.com

ALL ORDERS
orders@genelink.com

Gene Link™