Certificate of Analysis & Product Manual

OligoProber™ cDNA Detection Probes

Specific for RT-PCRmer™ Primer Sets

Store at –20°C

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

Unlabeled and 5'-Biotinylated Probes for Hybridization of Amplified Fragments

Material Supplied

OligoProber™ cDNA Amplification Detection Probes

Specific for RT-PCRmer™ Primer Sets

Storage Instructions:
1. Shipped lyophilized at room temperature.
2. Store at –20 °C upon receipt.
3. Store at –20 °C after reconstitution.

Content	Catalog No.	Product Description	Size
❑	40-1101-02	OligoProber™ Human beta-actin; 2 nmols	2 nmols
❑	40-1111-02	OligoProber™ Biotinylated Human beta-actin; 2 nmols	2 nmols
OligoProber™ Human beta-actin is specifically designed to be used with Gene Link product 40-1001-10; beta actin A Human RT-PCRmer™ (F1/R1); Exon 3; 10 nmols			
❑	40-1102-02	OligoProber™ Rat beta-actin; 2 nmols	2 nmols
❑	40-1112-02	OligoProber™ Biotinylated Rat beta-actin; 2 nmols	2 nmols
OligoProber™ Rat beta-actin is specifically designed to be used with Gene Link product 40-1002-10; beta actin Rat RT-PCRmer™ (F1/R1); Exon 3; 10 nmols			
❑	40-1103-02	OligoProber™ Mouse beta-actin; 2 nmols	2 nmols
❑	40-1113-02	OligoProber™ Biotinylated Mouse beta-actin; 2 nmols	2 nmols
OligoProber™ Mouse beta-actin is specifically designed to be used with Gene Link product 40-1003-10; beta actin A Mouse RT-PCRmer™ (F1/R1) 3' Region; 10 nmols			
❑	40-1105-02	OligoProber™ GAPDH H/M/R; 2 nmols	2 nmols
❑	40-1115-02	OligoProber™ Biotinylated GAPDH H/M/R; 2 nmols	2 nmols
OligoProber™ GAPDH H/M/R is specifically designed to be used with Gene Link product 40-1005-10; GAPDH A Human/Mouse/RatRT-PCRmer™ (F1/R1) RT-PCRmer; 10 nmols			

Each tube supplied contains the lyophilized OligoProber™. Please refer to label on the specific tube. Each tube contains 2 nmols. The quantity supplied is sufficient for 20 to 40 hybridization assays using 20X20 cm membrane.

Certificate of Analysis & Product Specifications

OligoProber™ supplied has been validated to be specific for the corresponding amplified fragments using specific Gene Link RT-PCRmer™. Oligo probe purity is greater than 98% as determined by denaturing polyacrylamide gel electrophoresis.

Appropriate nuclease free handling, dispensing and storage conditions required.

Manufacturing lot numbers are stated on the label of each product and accompanying packing slip.

OligoProber™ cDNA Amplification Detection Probes

Specific for RT-PCRmer™ Primer Sets

IMPORTANT NOTE

The OligoProber™ probes are specifically designed to be used for detection of fragments amplified using Gene Link specific RT-PCRmer™ to amplify cDNA fragments. The probes can also be used directly for Northern blots.

Product Description

OligoProber™

OligoProber™ are specific oligonucleotide probes for hybridization to its cognate species. These are especially suited for use in conjunction with RT-PCRmer™ as the complementary sequence lies in the amplified sequence. The OligoProber™ can also be used for all northern blots. OligoProber™ are available for use as hybridization probes with either 5'OH for ^{32}P labeling or with 5' biotin for non-radioactive detection. The OligoProber™ is supplied as a lyophilized powder in aliquots of 2 nmoles. The 2 nmoles of primer when dissolved in 100µL sterile water or TE will give a solution of 20 µMolar i.e. 20 pmoles/µL.

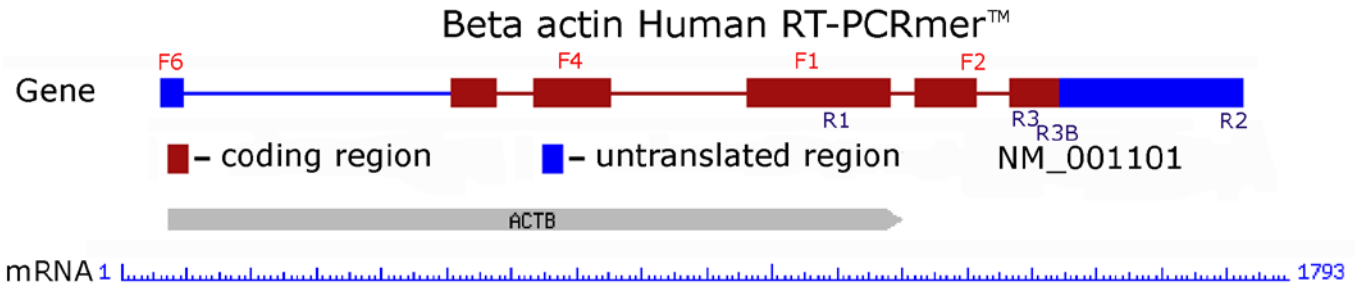
RT-PCRmer™

RT-PCRmer™ are primer pairs for specific amplification of cDNA. ß-actin is ubiquitously expressed and serves as a positive control for northern and other expression studies. These are generally used as controls for measuring cDNA synthesis efficiency by reverse transcription and as controls for mRNA (cDNA) quantitative expression studies. The RT-PCRmer™ are supplied as a lyophilized powder in aliquots of 10 nmols. The 10 nmols of primer when dissolved in 500 µL sterile water or TE will give a solution of 20 µMolar i.e. 20 pmols/µL. The quantity supplied is sufficient for at least 400 regular 25 µL PCR reaction* for ethidium bromide stained visualization. The amplified product may also be detected by hybridization using Gene Link OligoProber™ that are specific probes directed to the amplified fragment. These are available with either a free 5' OH for ^{32}P labeling or 5' biotinylated probe for non-radioactive detection.

OligoProber™ Human beta-actin

Content	Catalog No.	Product Description	Size
❑	40-1101-02	OligoProber™ Human beta-actin; 2 nmols	2 nmols
❑	40-1111-02	OligoProber™ Biotinylated Human beta-actin; 2 nmols	2 nmols

OligoProber™ Human beta-actin is specifically designed to be used with
Gene Link product 40-1001-10; beta actin A Human RT-PCRmer™ (F1/R1); Exon 3; 10 nmols



Catalog No.	OligoProber™	Accession #	Probe Position	Probe Size	Tm
40-1101-02 40-1111-02	OligoProber™ Human beta-actin OligoProber™ Biotinylated Human beta-actin	NM_001101	Exon 3; 705-729	25mer	55 to 60°C

Catalog No.	RT-PCRmer™	Accession #	Primer Position	cDNA Fragment Size	Genomic Fragment Size
40-1001-10	Human beta actin A (F1/R1)	NM_001101	Exon 3	289 bp	289 bp

OligoProber™ Rat beta-actin

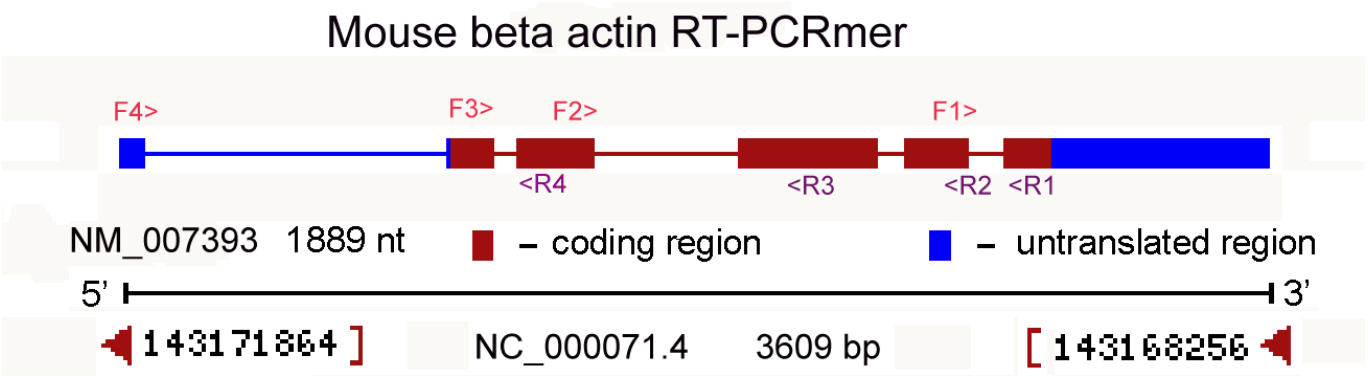
Content	Catalog No.	Product Description	Size
❑	40-1102-02	OligoProber™ Rat beta-actin; 2 nmols	2 nmols
❑	40-1112-02	OligoProber™ Biotinylated Rat beta-actin; 2 nmols	2 nmols
OligoProber™ Rat beta-actin is specifically designed to be used with Gene Link product 40-1002-10; beta actin Rat RT-PCRmer™ (F1/R1); Exon 3; 10 nmols			

Catalog No.	OligoProber™	Accession #	Probe Position	Probe Size	Tm
40-1101-02 40-1111-02	OligoProber™ Rat beta-actin OligoProber™ Biotinylated Rat beta-actin	NM_007393	Exon 3	27mer	55 to 60^{o}C

Catalog No.	RT-PCRmer™	Accession #	Primer Position	cDNA Fragment Size	Genomic Fragment Size
40-1002-10	Rat beta actin A (F1/R1)	NM_007393	Exon 3	289	289
Note: This primer set will amplify a fragment of 289 bp from human and rat cDNA.					
The fragments can be distinguished from rat or human source by digestion with Pvu II; the rat amplified 289 bp fragment is digested to give a 132 and 157 fragments whereas the human amplified fragment is not digested due to the absence of the Pvu II.					

OligoProber™ Mouse beta-actin

Content	Catalog No.	Product Description	Size
❑	40-1103-02	OligoProber™ Mouse beta-actin; 2 nmols	2 nmols
❑	40-1113-02	OligoProber™ Biotinylated Mouse beta-actin; 2 nmols	2 nmols
OligoProber™ Mouse beta-actin is specifically designed to be used with Gene Link product 40-1003-10; beta actin A Mouse RT-PCRmer™ (F1/R1) 3' Region; 10 nmols			

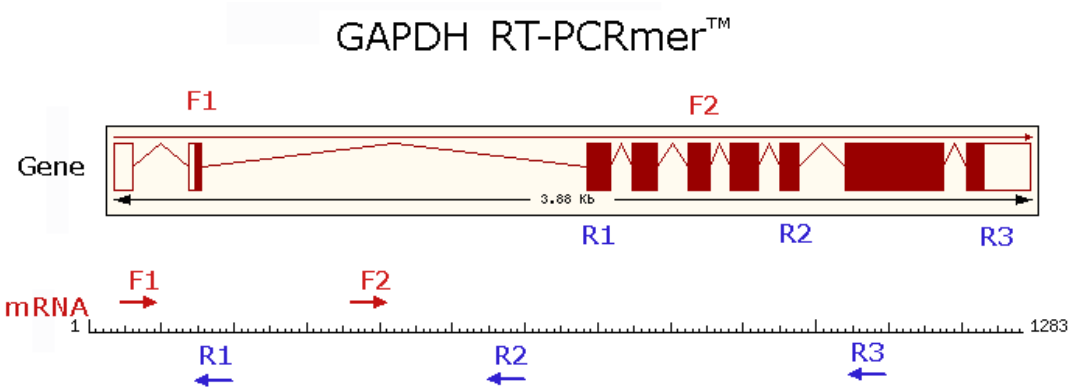


Catalog No.	OligoProber™	Accession #	Probe Position	Probe Size	Tm
40-1103-02 40-1113-02	OligoProber™ Mouse beta-actin OligoProber™ Biotinylated Mouse beta-actin	NM_007393	3' region	30mer	55 to 60°C

Catalog No.	RT-PCRmer™	Accession #	Primer Position	cDNA Fragment Size	Genomic Fragment Size
40-1003-10	Mouse beta actin A (F1/R1)	NM_007393	3' region	339 bp	474 bp

OligoProber™ GAPDH Human/Mouse/Rat

Content	Catalog No.	Product Description	Size
❑	40-1105-02	OligoProber™ GAPDH H/M/R; 2 nmols	2 nmols
❑	40-1115-02	OligoProber™ Biotinylated GAPDH H/M/R; 2 nmols	2 nmols
OligoProber™ GAPDH H/M/R is specifically designed to be used with Gene Link product 40-1005-10; GAPDH A Human/Mouse/RatRT-PCRmer™ (F1/R1) RT-PCRmer; 10 nmols			



Catalog No.	OligoProber™	Accession #	Probe Position	Probe Size	Tm
40-1105-02 40-1115-02	OligoProber™ GAPDH H/M/R OligoProber™ Biotinylated GAPDH H/M/R	NM_002046	Exon 2	27mer	55 to 60°C

Catalog No.	RT-PCRmer™	Accession #	Primer Position	cDNA Fragment Size	Genomic Fragment Size
40-1005-10	GAPDH A (F1/R1)	NM_002046	Exon 2 & 3	109 bp	1741 bp

Procedure

Reconstitution

Recommended reconstitution is at a concentration of 20 µM (20 pmol/ µL) in RNase-free DEPC treated water or RNase-free TE pH 7.0.

- Spin the tube briefly to bring down the contents of the tube that may have lodged in the cap during shipment. Pellet may be very small and not visible.
- Dissolve the supplied 2 nmols lyophilized OligoProber™ in 100 µL sterile TE pH 7.5. The 2 nmols of probe when dissolved in 100 µL will give a solution of 20 µM i.e. 20 pmols/µL.
- Store at -20^{o}C or below after reconstitution.

Probe Use

The unlabeled probe can be labeled with ^{32}P using polynucleotide kinase and γ^{32}P ATP. The biotinylated probe is used for non-radioactive using streptavidin HRP or Alkaline phosphatase.

Radioactive Labeling & Detection

Follow established laboratory method. An example of a typical labelling reaction is given below.

Radioactive Labeling with T4 Polynucleotide Kinase (PNK)	
OligoProber™ Unlabeled	1 µL (20 pmol, up to 50 pmol can be used)
10X T4PNK Reaction Buffer	5 µL
[γ-32P] ATP	5 µL of 50 pmol of [γ-32P] ATP/µL
T4 PNK	2 µL (20 units)
Water	up to 50 µL
Total Volume	50 µL
Incubate at 37°C for 30 minutes. Heat inactivate by incubating at 65°C for 20 minutes. Note: [^{33}P] ATP may be substituted for [^{32}P] ATP	

Chemiluminescent Detection

Follow established laboratory methods.

The reconstituted biotinylated OligoProber is at 20 µM i.e. 20 pmols/mL solution. A recommended usage is 2 to 5 µL (40 -100 pmols) for hybridization.

We suggest to use streptavidin-Alkaline Phosphatase (AP) instead of streptavidin-Horse Radish Peroxidase (HRP) as AP gives better signal and can be used with chemiluminescent substrates.

This product detail does not contain detailed information about hybridization and detection protocol. Alternate protocol sources or existing laboratory methods should be followed for the following steps.

1. Northern or Southern blot hybridization if you are planning to perform blot hybridization.
2. In situ hybridization protocol is using in situ hybridization.
3. Streptavidin-Enzyme conjugate binding and washing.
4. Chemiluminescent detection protocol.

Lumisol™ II & III Hybridization Solutions

Specifically formulated for chemiluminescent detection

Product Description

Lumisol™ II & III are ready-to-use hybridization solutions specifically formulated for non-radioactive chemiluminescent hybridization.

Lumisol™ II: Catalog No. 40-5023-20

Lumisol™ II is for use with digoxigenin or biotin labeled DNA or RNA probes in Southern & Northern blot protocols. It can also be used with radioactive labeled probes.

Lumisol™ III: Catalog No. 40-5024-20

Lumisol™ III is specifically formulated for use with oligonucleotide probes labeled with biotin, digoxigenin, alkaline phosphatase or other detection ligand or enzymes.

Applications

Lumisol™ II hybridization solution can be used for all stringent types of nucleic acid blot hybridization conditions, particularly using non-radioactive digoxigenin labeled probes. Lumisol™ II hybridization solution can be used for prehybridization and hybridization. The hybridization temperature should be calculated for proper results. Duration of hybridization can be reduced to 6 hours and overnight hybridization can be used for high sensitivity requirements and for convenience.

Lumisol™ III hybridization solution is specifically formulated for oligonucleotide probes that require only 30 minute pre-hybridization and 30 minute hybridization. Longer hybridization is not recommended. The hybridization temperature should be calculated for proper results and should not be more than 55°C for alkaline phosphatase labeled oligo probes.

Hybridization Temperature for Lumisol™ II

Hybridization temperature is an essential criterion for obtaining reliable hybridization results and should preferably be calculated. A rule of the thumb hybridization temperature of 50°C is satisfactory for perfectly homologous probes greater than 100 bp; probes of this and larger fragment length are usually achieved by random prime labeling method.

The appropriate hybridization temperature is calculated according to GC content according to the following equation:
Tm = 49.82 + 0.41 (% G + C) - (600/l)
[l = length of hybrid in base pairs]
Topt. = Tm -(20 to 25°C)
(The given numbers of the equation are according to a standard equation for denaturing hybridization solutions)

Topt. can be regarded as a stringent hybridization temperature allowing up to 18 % mismatches between probe and target. When the degree of homology of the probe to template is less than 80%, the Topt should be lowered; approximately 1.4°C below Tm per 1 % mismatch. Likewise the stringent washing steps should be adjusted accordingly by increasing the SSC concentration and/or lowering the washing temperature.
Example For hybridization of human genomic DNA with a 100% homologous probe use 50°C to 55°C, depending on the GC content of the probe.

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.

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.

Recipe

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

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 **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		

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

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	

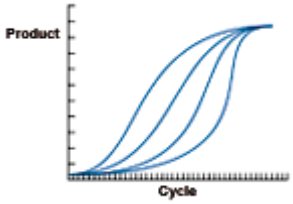
• The PCR premix preparation protocol is written considering that more than one amplification reaction will be performed at the same time. If only one reaction is planned then there is no need to prepare the Taq Enzyme Mix (EM).

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

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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

RT-PCRmer™	Catalog No.	Size
Human beta actin A RT-PCRmer™ (F1/R1); Exon 3	40-1001-10	10 nmols
Human beta actin B RT-PCRmer™ (F4/R3B); Exon 2-5	40-1021-10	10 nmols
Human beta actin C RT-PCRmer™ (F6/R1); 5'UTR-Exon 3	40-1022-10	10 nmols
Human beta actin D RT-PCRmer™ (F2/R2); Exon 4-3' UTR	40-1023-10	10 nmols
Human beta actin F RT-PCRmer™ (F6/R2); 5' UTR- 3' UTR	40-1024-10	10 nmols
Human beta actin G RT-PCRmer™ (F4/R1); Exon 2-3	40-1025-10	10 nmols
Human beta actin TM RT-PCRmer™ for Taqman assay (F2/R3); Exon 4-5	40-1026-10	10 nmols
Human beta actin TaqMan Probe (F2/R3); Exon 5	40-1027-02	2 nmols
Human beta actin MB RT-PCRmer™ for Molecular Beacon assay (F2/R3B); Exon 4-5	40-1028-10	10 nmols
Human beta actin Molecular Beacon Probe (F2/R3B); Exon 5	40-1029-02	2 nmols
Mouse beta actin A RT-PCRmer™ (F1/R1); Exon 2-3	40-1003-10	10 nmols
Mouse beta actin B RT-PCRmer™ (F2/R2); Exon 6-7	40-1014-10	10 nmols
Mouse beta actin C RT-PCRmer™ (F3/R3); Exon 2-7	40-1015-10	10 nmols
Mouse beta actin D RT-PCRmer™ (F4/R4); Exon 2-9	40-1016-10	10 nmols
GAPDH A RT-PCRmer™ (F1/R1); Exon 2-3	40-1005-10	10 nmols
GAPDH B RT-PCRmer™ (F2/R2); Exon 6-7	40-1006-10	10 nmols
GAPDH C RT-PCRmer™ (F1/R2); Exon 2-7	40-1007-10	10 nmols
GAPDH D RT-PCRmer™ (F1/R3); Exon 2-9	40-1008-10	10 nmols
rat beta actin RT-PCRmer;	40-1002-10	10 nmols
mouse beta actin RT-PCRmer;	40-1003-10	10 nmols
beta2 microglobulin RT-PCRmer;	40-1004-10	10 nmols
Beta actin control PCR mix (human & rat)	40-1002-00	200 uL

OligoProber™	Catalog No.	Size
OligoProber™ Human beta-actin; 2 nmols	40-1101-02	2 nmols
OligoProber™ Biotinylated Human beta-actin; 2 nmols	40-1111-02	2 nmols
OligoProber™ Rat beta-actin; 2 nmols	40-1102-02	2 nmols
OligoProber™ Biotinylated Rat beta-actin; 2 nmols	40-1112-02	2 nmols
OligoProber™ Mouse beta-actin; 2 nmols	40-1103-02	2 nmols
OligoProber™ Biotinylated Mouse beta-actin; 2 nmols	40-1113-02	2 nmols
OligoProber™ GAPDH H/M/R; 2 nmols	40-1105-02	2 nmols
OligoProber™ Biotinylated GAPDH H/M/R; 2 nmols	40-1115-02	2 nmols

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