



Product Specification

Huntington Disease GLHD14 GeneProber™ unlabeled probe

Catalog Number: 40-2025-40

Huntington Disease Genotyping by Southern blot Analysis

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

Caution

Product to be used by experienced researchers properly trained in performing molecular biology techniques following established safety procedures; and qualified and certified for research using radioactive materials.

*Important information

Gene Link strongly recommends the use of non-radioactive gene detection systems. Consider switching to Gene Link's GLHD PCRProber™ non-radioactive detection system (Catalog Number 40-2025-32), GScan™ fluorescent based genotyping (Catalog Number 40-2025-15) and GLHDDig1 GeneProber™ Southern blot gene detection system (Catalog Number 40-2025-41).

Storage instructions

Upon receipt immediately store product -20°C

Material supplied

Huntington Disease GeneProber™ Unlabeled

Huntington disease CAG triple repeat spanning region unlabeled probe for radioactive labeling and Southern blot detection of Pst I digested DNA. Suitable for random primer labeling

Storage Instructions: Upon receipt immediately store components at -20°C

Catalog No.	Description	Size
40-2025-40	Huntington Disease unlabeled GeneProber™ GLHD14 Probe unlabeled	500 ng

Suggested Probe Reconstitution

The probe is supplied lyophilized. Add 100 µl sterile water to the tube to dissolve the probe. The probe concentration will be 5 ng/µl. Store at -20°C after reconstitution.

Huntington Disease Genotyping

Background

Huntington disease (HD) is an autosomal dominant, progressive neurodegenerative disorder with a prevalence rate of about 5-10 affected persons per 100,000 in most western populations. The disorder presents with motor impairment, cognitive deterioration, and psychiatric symptoms.

HD is caused by a CAG trinucleotide expansion within the first exon of the IT15 gene on chromosome 4p16. The expanded CAG repeats are translated into a polyglutamine tract in the Huntington protein, which is believed to cause a dominant gain of function, leading to neuronal dysfunction and neurodegeneration.

The number of CAG repeats correlates inversely with the age of onset of symptoms. The American College of Medical Genetics/American Society of Human Genetics/ Huntington Disease Genetics Testing Working Group divided genotype/phenotype correlation in the following four categories for CAG repeat lengths:

- Normal allele, ≤ 26 CAG repeats, generating a normal phenotype;
- Intermediate allele, 27-35 CAG repeats, mutable normal allele generating a normal phenotype;
- HD allele with reduced penetrance, 36-39 CAG repeats, generating a normal or HD phenotype;
- HD allele, ≥ 40 CAG repeats, generating a HD phenotype.

The CAG trinucleotide expansion is unstable and can lengthen during transmission from parents to offspring. Thus, the stage of onset can decrease from one generation to the next, a phenomenon known as anticipation. HD anticipation is more intense in paternal transmission.

HD L34202 digested with: BamHI, PstI

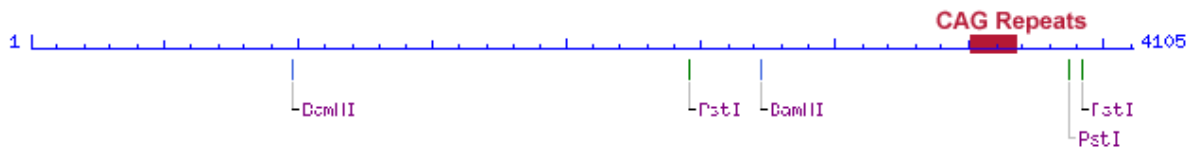


Table 1. Trinucleotide Repeats in Human Genetic Disease

Disease	Repeat ^a	Normal Length ^b	Intermediate Length (Premutation) ^{a,b}	Full Disease Length ^b
Fragile XA (FRAXA)	(CGG) _n	6-52	59-230	230-2,000
Fragile XE (FRAXE)	(CCG) _n	4-39	? (31-61)	200-900
Fragile XF (FRAXF)	(CGG) _n	7-40	?	306-1,008
FRA16A	(CCG) _n	16-49	?	1,000-1,900
Jacobsen Syndrome (FRA11B)	(CGC) _n	11	80	100-1,000
Kennedy Syndrome (SMBA)	(CAG) _n	14-32	?	40-55
Myotonic Dystrophy (DM)	(CTG) _n	5-37	50-80	80-1,000; congenital, 2,000-3,000
Huntington disease (HD)	(CAG) _n	10-34	36-39	40-121
Spinocerebellar ataxia 1 (SCA1)	(CAG) _n	6-39	None Reported	40-81
Spinocerebellar ataxia 2 (SCA2)	(CAG) _n	14-31	None Reported	34-59
Spinocerebellar ataxia 3 (SCA3)/Machado Joseph disease (MJD)	(CAG) _n	13-44	None Reported	60-84
Spinocerebellar ataxia 6 (SCA6)	(CAG) _n	4-18	None Reported	21-28
Spinocerebellar ataxia 7 (SCA7)	(CAG) _n	7-17	28-35	38-130
Haw River syndrome (HRS; also DRPLA))	(CAG) _n	7-25	?	49-75
Friedreich ataxia (FRDA)	(GAA) _n	6-29	? (>34-40)	200-900

^a Typically, repeats tracts contain sequence interruptions. See Pearson and Sinden (1998a) for a discussion of the sequence interruptions.

^b No. of triplet repeats.

^c A question mark (?) indicates potential mutagenic intermediate length, and an ellipsis (...) indicates none. Not all diseases are associated with a premutation length repeats tract or premutation disease condition.

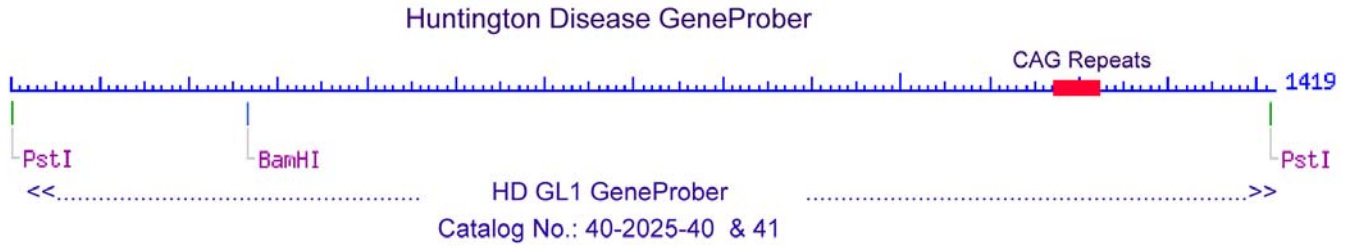
Molecular Analysis

The detection of expansion of a region of DNA sequence can be detected by PCR and Southern blotting procedures. These methods can be used for all disorders involving increase in size of a region of DNA. DNA analysis for direct detection of CAG expansion in Huntington Disease is based on enzymatic amplification of a fragment containing the CAG repeat sequence in exon I of the HD gene. This test detects the CAG expansion by the size of the amplified product; an increase in size is correlated with the corresponding number of CAG repeats and a calculated risk factor. Normal individuals have repeat numbers of up to 30, while individuals with a high probability of developing HD carry more than 37 repeats. Individuals with 30-37 repeats have a high probability of passing on repeats in the pathological size range.

Polymerase Chain Reaction (PCR) based methods are fundamentally similar. The two primers are constructed such that they span the region of the CAG trinucleotide repeat region. PCR is the most common method used to estimate the number of CAG repeats. Since the CAG repeats in the HD gene are immediately 5' of a CCG repeat which is also polymorphic in length, the PCR product of this primer pair excludes the known adjacent polymorphic CCG repeat that can contribute to an inaccurate determination of HD gene CAG repeat sizes in individuals who may have an HD gene CAG repeat allele close to the normal/affected boundary. Refer to HD GScan™ fluorescent PCR genotyping system [catalog No.: 40-2025-15FM]. Full mutations and PCR generated results that signify large expansions should be confirmed by Southern blot analysis.

Southern blot analysis for HD genotyping is performed by digestion of genomic DNA by Pst I followed by hybridization to a probe cognate to the Pst I fragment region that contains the amplified CAG repeats.

This manual details the Southern blot HD genotyping procedure using the HDGL1 GeneProber™.



Procedure

Caution

Product to be used by experienced researchers properly trained in performing molecular biology techniques following established safety procedures. End user must be qualified and certified for research using radioactive materials.

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

*Important information

Gene Link strongly recommends the use of non-radioactive gene detection systems. Consider switching to Gene Link's GLHD1 PCRProber™ non-radioactive detection system (Catalog Number 40-2025-32) and GLHDDig1 GeneProber™ Southern blot gene detection system (Catalog Number 40-2025-41).

Brief Product Protocol

The protocol given below can be substituted by your laboratory's established protocol for Southern blot analysis using random prime labeled probes

Material Supplied

One tube containing 500 ng of lyophilized GLHD1 GeneProber™ probe. The DNA probe is stable in dried state for an extended period at room temperature. Upon reconstitution it should be stored at -20 °C. The quantity supplied is sufficient for at least 5 random prime labeling reactions using 100ng for each reaction. Gene Link recommends using 25ng for each labeling reaction sufficient for 20 labeling reactions.

Probe reconstitution

Add 100 µl sterile water to the tube to dissolve the probe. The probe concentration is 5 ng/µl

Southern Protocol

A. Genomic DNA Digestion

Important Note

-Digest genomic DNA with Pst I when using GLHD14 GeneProber™ as labeled probe.

Restriction Digestion	
Component	Volume Quantity
Genomic DNA	5 to 10µg
10x Pst I Buffer	10 µl
Pst I (~40 u/µl)	4 µl
H₂O to	100 µl
Overnight digestion at 37°C	

Ethanol precipitate the digests, dissolve the pellets in 10 µl of 1x Loading buffer.

B. Electrophoresis and Transfer

1. Load samples to a 1.2% agarose gel. Electrophorese over night at 45mA for 20-24 hours. (~1 kb fragment on the bottom of the gel).
2. Depurinate with 0.25N HCl (add 10 ml HCl to 500 ml H₂O) for 10 minutes.
3. Denature the DNA with 0.4N NaOH/0.6M NaCl for 30 min. at room temperature (RT).
4. Neutralize with 1.5M NaCl/0.5M Tris (pH 7.5) for 30 min. at RT.
5. Transfer overnight by Southern blot procedure to positively charged nylon membrane using 10xSSC.
6. Wash the membrane with 2x SSC and then bake at 80°C for 2 hours.

C. Probe Labeling

It is convenient to perform the labeling reaction while performing prehybridization. See section D below.

Follow directions for probe labeling if using a commercial random prime labeling kit.

An example is given below using any random primer DNA labeling kit.

Random Prime Labeling	
GLHD14 GeneProber™	25 -100 ng
H ₂ O	up to 9 µl
Boil 5 minutes, and put on ice. Then add	
Reaction mix	2 µl
dNTP w/o dCTP	3 µl
α ³² PdCTP (3000 Ci/mmol)	5 µl (50 µCi)
Klenow (2 U/µl)	1 µl
Total	20 µl

Incubate at 37°C for 30 minutes. Add 500 µl of 5 x SSC to the reaction tube, boil 5 minutes, then add to the 50 ml Falcon tube containing the membrane and Lumisol I hybridization solution, mix well, incubate in shaking water bath at 50°C overnight.

D. Hybridization

1. Perform pre-hybridization at 50°C for 3 hours in 10 ml of Lumisol I hybridization buffer (Gene Link) or any other hybridization buffer.

It is convenient to perform random prime labeling reaction at this stage. See procedure in Section C above.

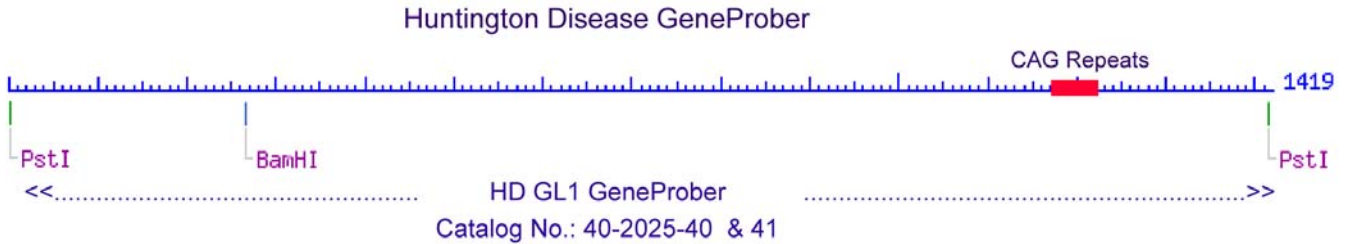
2. Replace hybridization solution with fresh 10 of hybridization solution. Add the labeled probe as detailed in section C above. Hybridize overnight.
3. Wash the membrane in 2 x SSC/ 0.1% SDS at RT twice (5 min per wash), then wash with 0.1 x SSC/ 0.1% SDS at 65°C twice (30 min. per wash). Wrap the membrane and put X-ray film on it, expose at -80°C over night. Develop the film next morning.

D. Stripping & Rehybridization

If required, strip the membrane by incubating in 0.5 N NaOH for 1 hour at room temperature with constant agitation. Change the solution and incubate overnight if necessary. Rinse the membrane with 2x SSC, air dry.

E. Results & Interpretation

1. Normal hybridization pattern is ~1419 bp fragment with genomic DNA digested with Pst I / using GLHD14 GeneProber™ as labeled probe.
2. Larger fragment size is attributable to expanded CAG repeat region. See HD probe region figure below.



Southern Blot Analysis of Genomic DNA using GLHD14 ³²P labeled probe



GLHD14 probe was labeled with ³²P and hybridized with human genomic DNA of known CAG repeats (lane 1-5) and unknown CAG repeat samples (lane 6-7). The CAG repeats were based on PCR analysis. PCR based CAG repeat and Southern blot results using GLHD14 probe are confirmatory.

Appendix: Protocols

Genomic DNA Purification

Genomic DNA is usually extracted from blood. A simple procedure is given below that purifies ~10 µg DNA from 300 µl blood using a 30 minute procedure.

Omni-Pure™ Genomic DNA Purification System Catalog Number: 40-4010-01
Rapid DNA Purification Protocol for 300 µl Whole Blood

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 900 µl GD-1 solution (RBC Lysis Solution) to one tube for each sample.
3. Add 300 µl Isopropanol (2-propanol) to one tube for each sample. Cap the tubes.

B. Cell Lysis

1. To the tube containing 900 µl GD-1 solution (RBC Lysis Solution) using a filter tip pipet transfer 300 µl whole blood. Cap and gently mix by inversion. Incubate for 1-3 minutes at room temperature. Mix by inversion a few times during this incubation period. Incubate longer for fresh blood cells as they are intact and not lysed already.
2. Centrifuge at 3 K rpm for 20 seconds to pellet the white blood cells. A reddish white pellet should be clearly visible. Decant and discard supernatant leaving behind the last few droplets. Do not totally remove the supernatant.
3. Completely resuspend the white blood cell pellet by vigorously vortexing the tube. Ensure that the pellet is completely resuspended.
4. To the resuspended cells add 300 µl GD-2 solution (Cell Lysis Solution). Mix by gentle vortexing. You will notice release of DNA by the thickening of the liquid in the sample. Samples may be stored at this stage for processing later. It has been shown that the samples are stable in Cell Lysis Solution for at least 2 years at room temperature.

C. Protein Precipitation

1. Add 100 µl GD-3 solution (Protein Precipitation Solution) to the sample in cell lysis solution.
2. Vortex vigorously for 20 seconds. Small particles of brown color will appear and be visible at this stage.
3. Centrifuge at 5 K rpm for 1 minute to pellet the precipitated proteins. A clearly visible brown pellet containing proteins should be collected at the bottom of the tube.

D. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube (see initial preparation above) containing 300 µl 100% Isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Mixing by inversion 30-40 is usually sufficient.
3. Centrifuge at 6 K rpm for 1 minute to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place tube inverted on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 6 K rpm for 1 minute to collect the DNA as a pellet. Gently take out the tubes so that the pellet is not dislodged. While holding the tube, rotate tube so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place tube inverted on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

E. DNA Reconstitution & Use

1. Add 100 µl of GD-4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4 °C. For long-term storage, place sample at -20 °C or -80 °C.
3. Average yield of 10 µg is expected from 300 µl blood DNA. The range is between 5 µg to 15 µg.
4. The 100 µl of purified DNA obtained will have an average concentration of ~ 100 ng/µl.
5. For PCR amplification use 1-2 µl.
6. Use 100 µl for restriction digestion followed by Southern blot analysis.
7. It is convenient to perform multiple 300 µl blood DNA purification instead of scaling up the procedure.

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.

PAGE 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.

Agarose Gel Electrophoresis of DNA

Agarose gels are typically run at 20 to 150V. The upper voltage limit is the amount of heat produced. At room temperature about 5 Watts is correct for a minigel (Volts x Amps = Watts). At low voltages migration is linearly proportional to voltage, but long DNA molecules migrate relatively faster in stronger fields. Migration is inversely proportional to the log of the fragment length; a log function also governs migration rate and gel concentration (0.5 to 2% for most purposes). Furthermore, supercoiled / circular DNA molecules migrate at different rates from linear molecules; single-stranded DNA and RNA migrate at similar rates, but usually faster than double-stranded DNA of the same length. Salt in the samples increases conductivity and, hence, migration rate.

The buffers used for most neutral agarose gels (the gel itself and the solution in which it lies) is 1 x TAE or 1 x TBE. Agarose powder is added to the buffer at room temperature, heated in a microwave and boiled slowly until the powder has dissolved. Cast the gel on a horizontal surface once the agarose has been cooled to ca. 60° C (just cool enough to hold) and add 0.1 µg of ethidium bromide solution for each ml of gel volume. At times, during removal of the comb, it is possible to tear the bottom of the sample wells gels, which results in sample leakage upon loading. This can be avoided by removing the comb after the gel has been placed in the running buffer.

- Use TAE buffer for most molecular biology agarose gel electrophoresis.

Recipe

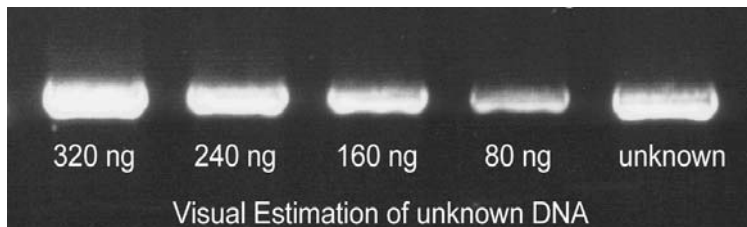
1 X TAE Buffer
Agarose Gel Electrophoresis Buffer
40 mM Tris-Acetate pH 7.8
1 mM EDTA

1 X TBE
Agarose and Polyacrylamide Gel Electrophoresis Buffer
0.089 M Tris
0.089 M Boric Acid
0.002 M EDTA


Spectrophotometric Determination of DNA Concentration & Estimation by Agarose Gel Electrophoresis


Measuring the optical density (OD) or absorbance at 260 nm (A_{260}) in a UV spectrophotometer is a relatively accurate method for calculating the concentration of DNA in an aqueous solution if a standard curve is meticulously prepared. An A_{260} of 1, using a 1 cm path length, corresponds to a DNA concentration of 50 $\mu\text{g/ml}$ for double stranded DNA, 40 $\mu\text{g/ml}$ for RNA and 33 $\mu\text{g/ml}$ for oligonucleotides. However, this method is not suitable for determining concentrations of dilute solutions of DNA, as the sensitivity of this method is not very high. For reliable readings, the concentration of double stranded DNA must be greater than 1 $\mu\text{g/ml}$. A simple, inexpensive method for the estimation of nanogram quantities of DNA is described in the following section. We recommend the use of agarose gel electrophoresis for routine approximate determination of DNA concentration.

The amount of DNA in a sample may be estimated by running the sample alongside standards containing known amounts of the same-sized DNA fragment. In the presence of ethidium bromide staining, the amount of sample DNA can be visually estimated by comparing the band intensity with that of the known standards.



An unknown amount of a 4 kb DNA fragment (unknown) was run alongside known quantities (indicated in nanograms) of the same DNA fragment. As estimated by visual comparison with the known standards, the unknown sample contained 240-320 ng of DNA.

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

-  Use 0.1 μg of ethidium bromide solution for each ml of gel volume.

Polymerase Chain Reaction

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.

dNTP Concentration

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

MgCl₂ Concentration

The concentration of Mg²⁺ will vary from 1-5 mM, depending upon primers and substrate. Since Mg²⁺ ions form complexes with dNTPs, primers and DNA templates, the optimal concentration of MgCl₂ has to be selected for each experiment. Low Mg²⁺ ions result in a low yield of PCR product, and high concentrations increase the yield of non-specific products and promote mis-incorporation. Lower Mg²⁺ concentrations are desirable when fidelity of DNA synthesis is critical. The recommended range of MgCl₂ concentration is 1-4 mM, under the standard reaction conditions specified. At Gene Link, using the standard PCR buffer with KCl, a final dNTP concentration of 0.2 mM, a MgCl₂ concentration of 1.5 is used in most cases. If the DNA samples contain EDTA or other chelators, the MgCl₂ concentration in the reaction mixture should be raised proportionally. Given below is an MgCl₂ concentration calculation and addition table using a stock solution of 25 mM MgCl₂.

MgCl ₂ Concentration & Addition Table								
Final concentration of MgCl ₂ in 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.

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

● 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%

● 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	



Always use filter barrier pipette tips to prevent cross contamination

● Recipe

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



• Program your thermal cycler instrument with an amplification profile

fragment from a complex template like human genomic DNA. Taq polymerase has low activity at room temperature and it is essential to minimize any mis-priming in the first cycle of amplification. A typical hot start profile is given below. Various enzyme preparations are available which are activated by heat in the first cycle. A simple hot start protocol is given below that can be used with regular Taq polymerase. See the section on PCR additives for amplification of products from high GC content templates.

Hot Start		
Step	Time & Temperature	Cycles
Initial Denaturation	95 °C for 5 minutes	1
Annealing	60 °C Hold Infinity	Hold
Comments: Add Taq premix while on hold.		

Amplification File

The initial denaturation step at 94 °C for 30 seconds is sufficient for all templates. The number of cycles is usually set to 30 and is sufficient to amplify 1-10 µg of product depending on the initial concentration of template. A higher number of cycles from 35-45 cycles may be used, but internal priming on the product and over amplification of unwanted bands often result from over-cycling. Generally, it is better to focus on optimizing reaction conditions than to go beyond 35 cycles.

Typical Amplification File			
Step	Temperature	Time	Cycles
Denaturation	94 °C	30 sec.	30
Annealing	*	30 sec.	
Elongation	72 °C	30 sec.	
Fill in Extension	72 °C	7 minutes	1
Hold	4 °C	Infinity	Hold
*Based on the T _m of the primers. Usually varies from 50 °C to 65 °C			

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 ₂ O	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 EM (/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	

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)	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		

i • 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).

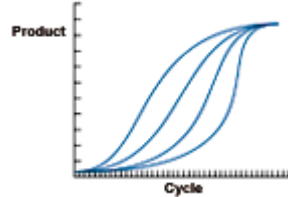
Recipe

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 10⁶ 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.



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 T_m and the complex secondary structure, thus the duplex stability (1-5). Tetramethyl ammonium chloride (TMAC) actually increases the specificity of hybridization and increases the T_m. The use of TMAC is recommended in PCR conditions using degenerate primers.

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

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



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 1.5ml 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 1.5ml 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 20 µl of H₂O.
6. Run an aliquot on an agarose gel to confirm the presence of the correct amplified product. The purified DNA is sequence grade and can be used directly for sequencing.

Gel Filtration

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

Protocol

1. Hydrate Sephadex G-50 ahead of time in sterile water or TE (10mM Tris pH 8, 1 mM EDTA). Take out from fridge if already stored hydrated. Bring to room temperature.
2. Assemble a spin column on a collection tube.
3. Add 700 µl 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 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 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.

GeneProber™ Product Ordering Information

The GeneProber™ product line is based on the chemiluminescent Southern blot detection method. Gene Link's non-radioactive detection systems for genotyping of triple repeat disorders are rapid, reliable and as sensitive as the ³²P labeled southern blots. No more decayed probes and radioactive exposure. Kits are available for reliable genotyping of the fragile X, myotonic dystrophy and other triple repeat mutation group disorders.

Unlabeled GeneProber™ probes are also available for radio labeling and radioactive based detection. Gene Link strongly recommends the use of non-radioactive gene detection systems. Consider switching to Gene Link's product line of non-radioactive detection systems

Product	Unit Size	Catalog No.
Fragile X GeneProber™ GLFX1 Probe unlabeled	500 ng	40-2004-40
Fragile X GeneProber™ GLFXDig1 Probe Digoxigenin labeled	110 µL	40-2004-41
Huntington's Disease GeneProber™ GLHD14 Probe unlabeled	500 ng	40-2025-40
Huntington's Disease GeneProber™ GLHDDig2X Probe Digoxigenin labeled	110 µL	40-2025-41
Myotonic Dystrophy GeneProber™ GLDM1 Probe unlabeled	500 ng	40-2026-40
Myotonic Dystrophy GeneProber™ GLDMDig2 Probe Digoxigenin labeled	110 µL	40-2026-41
Friedreich's Ataxia GeneProber™ GLFRDA21 Probe unlabeled	500 ng	40-2027-40
Friedreich's Ataxia GeneProber™ GLFRDADig21 Probe Digoxigenin labeled	110 µL	40-2027-41

GScan™ Products Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data. The kits contain optimized PCR amplification reagents and a wide array of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument(s). Included in these kits are ready-to-run control samples of various repeats of the triple repeat disorder kit. These control samples are for calibration with the molecular weight markers for accurate size determination of the amplified fragments.

The GScan™ kits are simple and robust for routine triple-repeat detection of greater than 100 repeats of all triple repeat disorders listed, except Fragile X. The CGG repeat in Fragile X can be detected up to ~50 repeats.

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

All Gene Link products are for research use only

Current pricing are posted at <http://www.genelink.com/>

Huntington's Disease Product Ordering Information

Product	Unit Size	Catalog No.
Huntington's Disease Genemer™ Primer pair Primers for amplification of CAG triple repeat spanning region. The quantity supplied is sufficient for 400 regular 50 µl PCR reactions.	10 nmols	40-2025-10
Huntington's Disease PCRProber™ AP labeled probe Alkaline phosphatase labeled probe	12 µL	40-2025-31
Huntington's Disease PCRProber™ Kit for chemiluminescent detection Kit for performing PCR amplification and chemiluminescent based detection.	5 blots [50 rxns]	40-2025-32
Huntington's Disease Genemer™ Kit for Radioactive Detection Kit for amplification and radioactive detection of Huntington's Disease CAG triple repeat region amplified PCR products using ³⁵ S or ³² P. 100 Reactions.	1 Kit [100 rxns]	40-2025-20
Huntington's Disease GScan Kit for fluorescent detection Kit for performing fluorescent PCR amplification based detection. Various dye kits. XX=FM for 6-Fam; HX for Hex; TT for Tet; C3 for Cy3 and C5 for Cy5.	1 Kit [100 rxns]	40-2025-15XX

Genemer™ GScan Control DNA Cloned fragment of the mutation region of a particular gene. These control DNAs are ideal genotyping templates for optimizing and performing control amplification with unknown DNA. The size of the triple repeats has been determined by sequencing and gel electrophoresis. The stability of size repeats upon cloning and amplification has NOT been determined. Thus, the size should be considered approximate and there is no claim for each fragment to contain the exact number of triple repeats. These control DNAs are sold with the express condition that these NOT be used for exact triple repeat size determination of DNA of unknown genotype. The control DNA should be used for determining the performance of specific Genemer™ and PCRProber™ Gene Link products.

Huntington's Disease 7 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-05HX
Huntington's Disease 18 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-01HX
Huntington's Disease 31 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-07HX
Huntington's Disease 34 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-02HX
Huntington's Disease 37 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-08HX
Huntington's Disease 44 ~CAG repeat GScan Genemer Control DNA	25 µL	40-2025-03HX
Huntington's Disease 49 ~CAG repeat GScan Genemer Control DNA	25 µL	40-2025-09HX
Huntington's Disease 89 ~CAG repeat GScan Genemer Control DNA	25 µL	40-2025-04HX
Huntington's Disease 116 ~CAG repeat GScan Genemer Control DNA	25 µL	40-2025-06HX
Huntington's Disease 134 ~CAG repeat GScan Genemer Control DNA	25 µL	40-2025-61HX
Huntington's Disease 182 ~CAG repeat GScan Genemer Control DNA	25 µL	40-2025-62HX

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Related Products Ordering Information

Omni-Pure™ DNA & RNA Purification Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Pure™ Blood DNA Purification System	40-4010-01	100
Omni-Pure™ Blood DNA Purification System	40-4010-05	500
Omni-Pure™ Blood DNA Purification System	40-4010-10	1000
Omni-Pure™ Tissue DNA Purification System	40-4050-01	100
Omni-Pure™ Tissue DNA Purification System	40-4050-05	500
Omni-Pure™ Tissue DNA Purification System	40-4050-10	1000
Omni-Pure™ Plant DNA Purification System	40-4060-01	100
Omni-Pure™ Plant DNA Purification System	40-4060-05	500
Omni-Pure™ Plant DNA Purification System	40-4060-10	1000
Omni-Pure™ Viral DNA Purification System	40-3720-01	100
Omni-Pure™ Viral DNA Purification System	40-3720-05	500
Omni-Pure™ Microbial DNA Purification System	40-3700-01	100
Omni-Pure™ Microbial DNA Purification System	40-3700-05	500
Omni-Pure™ Viral RNA Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Purification System	40-3650-05	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Clean™ Gel DNA Purification and Concentration Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Clean™ Gel DNA Beads Purification System	40-4110-10	100
Omni-Clean™ Gel DNA Beads Purification System	40-4110-50	500
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-10	100
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-50	500
Omni-Clean™ DNA Beads Concentration System	40-4130-10	100
Omni-Clean™ DNA Beads Concentration System	40-4130-50	500
Omni-Clean™ DNA Spin Column Concentration System	40-4140-10	100
Omni-Clean™ DNA Spin Column Concentration System	40-4140-50	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Pure™ Plasmid DNA Purification Systems

Product	Catalog No.	Unit Size*(Purifications)
Omni-Pure™ Plasmid DNA Purification System	40-4020-01	100
Omni-Pure™ Plasmid DNA Purification System	40-4020-05	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

All Gene Link products are for research use only

Current pricing are posted at <http://www.genelink.com/>

Southern Blot Buffers & Reagents

Product	Catalog No.	Unit Size
Agarose Tablets, 0.5 gm each; 100 tablets	40-3011-10	100 tablets
Agarose LE Molecular Biology Grade; 100 g	40-3010-10	100 g
Agarose LE Molecular Biology Grade; 500 g	40-3010-50	500 g
Hybwash A, Hybridization Wash Solution (20X SSC); 200 mL	40-5020-20	200 mL
Hybwash B, Hybridization Wash Solution (10% SDS); 100 mL	40-5021-10	100 mL
TAE Buffer; 50 X Concentrate; 100 mL	40-3007-01	100 mL
TAE Buffer; 50 X Concentrate; 1 L	40-3007-10	1 L
TBE Buffer; 5 X Concentrate; 1 L	40-3008-10	1 L
Buffer M 10X (Maleic Acid buffer); 100 mL	40-5025-10	100 mL
10% Blocking solution; 100 mL	40-5026-10	100 mL
Loading Buffer 2X BPB/XC Denaturing for Sequencing; 1 mL	40-5027-10	1 mL
10x AP Detection buffer (alkaline phosphatase detection buffer); 100 mL	40-5031-10	100 mL
Lumisol™ I Hybridization Solution; contains formamide; 200 mL	40-5022-20	200 mL
Lumisol™ II Hybridization Solution; for non-toxic hybridizations; 200 mL	40-5023-20	200 mL
Lumisol™ III Hybridization Solution; for oligo probes; 200 mL	40-5024-20	200 mL
CDP-Star® Substrate; Ready-to-Use 0.25 mM in spray bottle; 10 mL	40-5010-10	10 mL

Loading Buffers

Product	Catalog No.	Size
Gel Loading Buffer 5X BPB/XC non-denaturing; 1 mL	40-3002-10	1 mL
Gel Loading Buffer 5X BPB/XC non-denaturing; 15 mL	40-3002-15	15 mL
Gel Loading Buffer 10X BPB/XC non-denaturing; 1 mL	40-3003-10	1 mL
Gel Loading Buffer 10X BPB/XC non-denaturing; 15 mL	40-3003-15	15 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing; 1 mL	40-3004-10	1 mL
Gel Loading Buffer 5X Orange G/XC non-denaturing; 15 mL	40-3004-15	15 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing; 1 mL	40-5027-10	1 mL
Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing; 15 mL	40-5027-15	15 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer ; 1 mL	40-5028-10	1 mL
DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer; 15 mL	40-5028-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide; 1 mL	40-5029-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide; 15 mL	40-5029-15	15 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide ; 1 mL	40-5030-10	1 mL
RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide; 15 mL	40-5030-15	15 mL

Omni-Marker™

Product	Catalog No.	Size*
Omni-Marker™ Universal unlabeled; 1 mL	40-3005-10	1 mL
Omni-Marker™ Low unlabeled; 1 mL	40-3006-10	1 mL
Omni-Marker™ GScan™-2 Tamra labeled 50 bp - 600 bp; 500 µL	40-3062-05	500 µL

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