Certificate of Analysis & Product Manual

Triple Repeat Disorders Genotyping
Fragile X, Myotonic Dystrophy, Friedreich's Ataxia, Huntington's disease
Fluorescent Probes, siRNA, Hybridization and Detection Reagents

Huntington's Disease CAG Repeat Genotyping GeneProber™ GLHDDig2X

Huntington Disease CAG triple repeat chemiluminescent Southern blot genotyping

Catalog No. 40-2025-41

Storage Condition: -20°C

For Research Use Only. Not for use in diagnostic procedures for clinical purposes

Important Information

All Gene Link products are for research use only.
Not for use in diagnostic procedures for clinical purposes.
Product to be used by experienced researchers appropriately trained in performing molecular biology techniques following established safety procedures. Additional qualification and certification is required for interpretation of results.

Material Supplied

Huntington's Disease CAG Repeat Genotyping GeneProber™ GLHDDig2X

REF	Catalog No.	Description	Size
	40-2025-41	Huntington Disease GLHDDig2X GeneProber™ Digoxigenin labeled	110 µL

Certificate of Analysis & Product Specifications

One tube containing 110 µL of Huntington Disease GeneProber™ GLHDDig2X probe at a concentration of ~40ng/uL. This probe is digoxigenin labeled for non-radioactive detection. The quantity supplied is sufficient for at least 5 20x20 cm blots using 20µL for each blot as probe.

The Huntington Disease GeneProber™ GLHDDig2X probe supplied has been validated to hybridize to the CAG triple repeat spanning region in the first exon of the *IT15* gene of Pst I digested human genomic DNA.

Appropriate nuclease free handling, dispensing and storage conditions required.

Product Label Information

RUO Research Use Only	-10°C -20°C	LOT
Research Use Only	**Storage** Store at -20°C to -10°C	**Lot Number** Stated on product tube and packing slip
Expiry One year from Date of Shipment	**Instructions** Consult product manual	**QR Code** Visit Gene Link website for product details

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 ^{32}P labeled southern blots. **Unlabeled GeneProber™ probes are also available for radio labeling and radioactive based detection.**

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
FRAXE/FMR2/AFF2 GeneProber™ AFF2-AJ31Dig1	110 µL	40-2054-41
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
Mitochondrial DNA deletion GeneProber™ GL557 Digoxigenin labeled	110 µL	40-2055-41

GScan™ Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data for routine triple-repeat genotyping of greater than 100 repeats of all triple repeat disorders listed.

Product	Unit Size	Catalog No.
Fragile X GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2004-15XX
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2054-15FM
Huntington's Disease GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15XX
Myotonic Dystrophy GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2026-15XX
Friedreich's Ataxia GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2027-15XX

Genemer™ Kits Product Ordering Information

Gene Link's Genemer™ kits contain optimized PCR amplification components for convenient agarose gel genotyping of triple repeat disorders and other genetic disorders. These are safe, convenient and sensitive, and afford rapid screening of samples of greater than 100 repeats of all triple repeat disorders listed.

Product	Unit Size	Catalog No.
Fragile X Genemer™ V2 Kit for gel based detection; 100 reactions kit	1 kit	40-2004-11
FRAXE/FMR2/AFF2 Genemer ™ Kit for gel based detection; 100 reactions kit	1 kit	40-2054-11
Huntington's Disease Genemer ™ V2 Kit for gel based detection; 100 reactions kit kit	1 kit	40-2025-11
Myotonic Dystrophy Genemer ™ Kit for for gel based detection; 100 reactions kit	1 kit	40-2026-11
Friedreich's Ataxia Genemer ™ Kit for gel based detection; 100 reactions kit	1 kit	40-2027-11

All Gene Link products are for research use only **Current pricing are posted at http://www.genelink.com/**

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 ITI5 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, $\leq$ 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, $\geq$ 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.

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.

HD L34202 digested with: BamHI, PstI

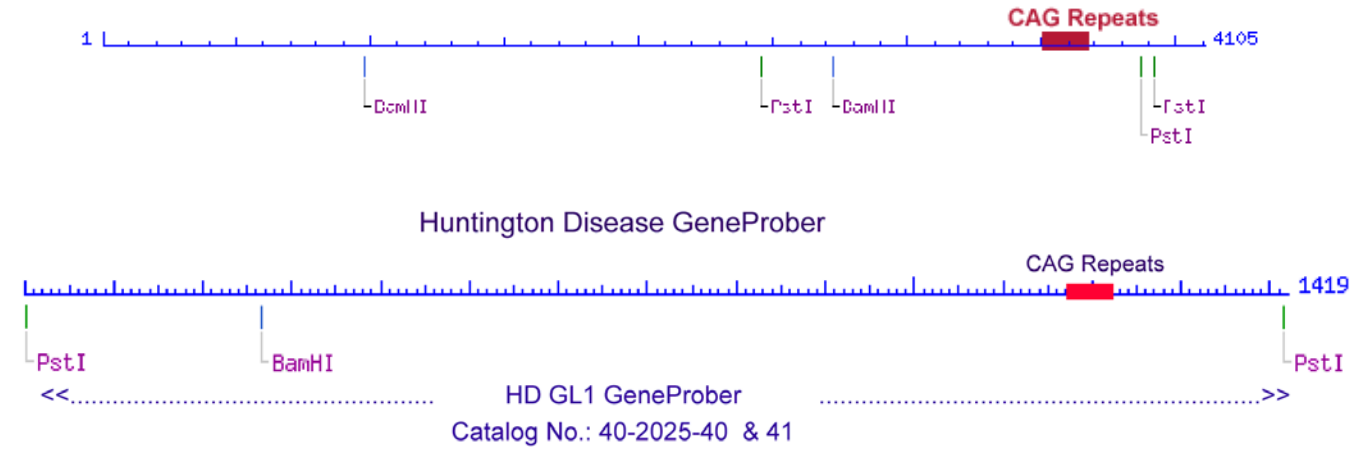


Table 1. Trinucleotide Repeats in Human Genetic Disease

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 (1998*a*) for a discussion of the sequence interruptions.

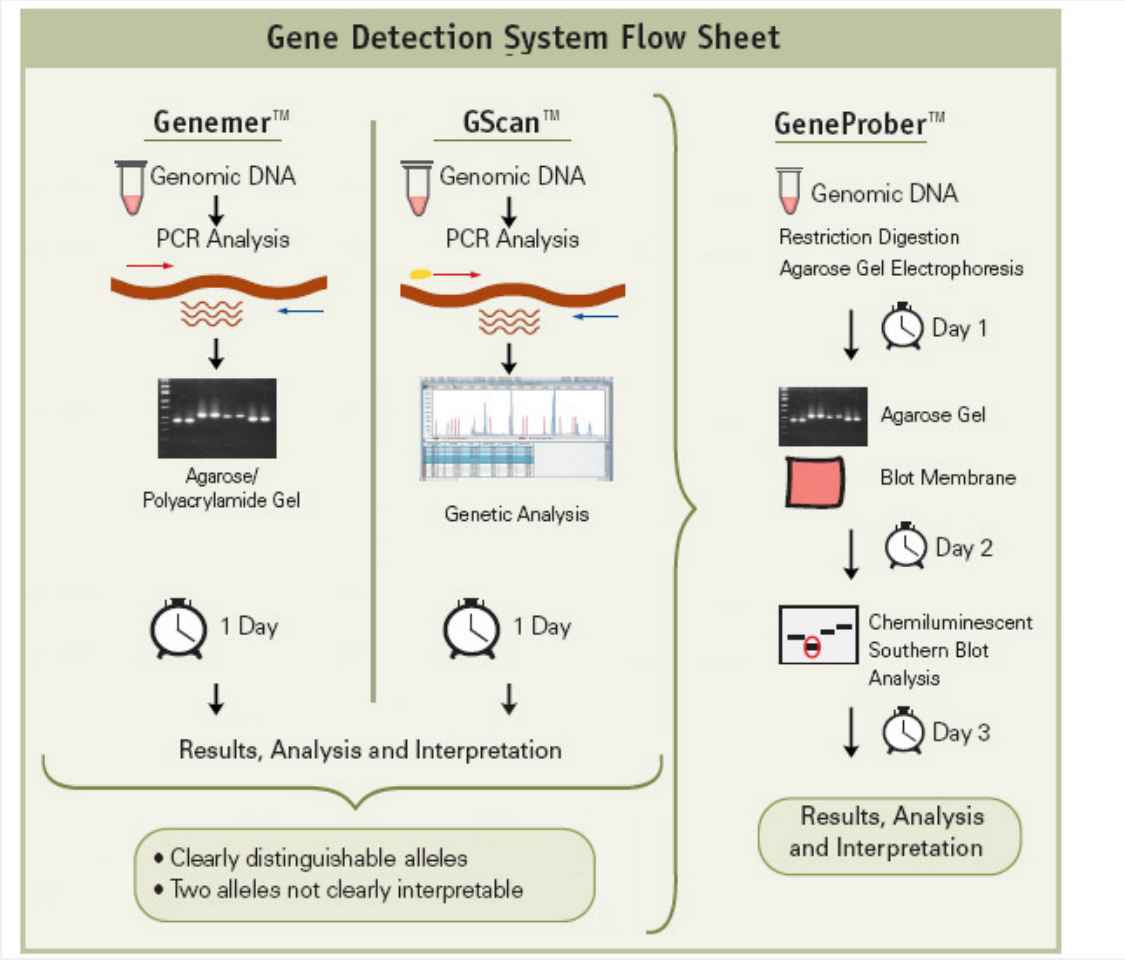
[b] No. of triplet repeats.

[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.

Genemer™ Kit Agarose Gel Analysis
Genemer™ kit contains optimized components for PCR amplification of greater than 100 triple repeats using standard Taq polymerase. Amplified samples are resolved by agarose gel electrophoresis. This Genemer™ method or GScan™ fluorescent detection is recommended for initial screening of all samples.

GScan™ Kit
GScan™ kit contains optimized components for PCR amplification of greater than 100 triple repeats using standard Taq polymerase. Amplified samples are resolved by genetic analyzers capable of fluorescent detection or agarose gel electrophoresis. This Genemer™ Kit or GScan™ kit for fluorescent detection is recommended for initial screening of all samples.

GeneProber™ Probes for Southern Blot Analysis
Digoxigenin labelled probes for chemiluminescent Southern blot detection method or unlabeled probe for end user to perform radioactive label. Gene Link offers safe and reliable chemiluminescent detection methods as an alternate to radioactive based detection methods.



Procedure: Chemiluminescent Southern Protocol

Material Supplied

One tube containing 110 µL of GeneProber™ GLHDDig2X probe at a concentration of ~40ng/µL. This probe is digoxigenin labeled for non-radioactive detection. The quantity supplied is sufficient for at least five 20x20 cm blots using 20 µL for each blot as probe. Experienced users can save and reuse the hybridization solution 2-3 times.

A. Genomic DNA Digestion

Important Note

-Digest genomic DNA with Pst I when using GLHDDig2X 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
H_2O to	**100 µL**
Overnight digestion at 37^{O}C	

Ethanol precipitate the digests; dissolve the pellets in 10 µL of 1x Loading buffer.

B. Electrophoresis and Transfer

1. Load samples to a 0.8% agarose gel. Electrophorese over night at 45mA for 20-24 hours. (1.6 kb fragment on the bottom of the gel).
2. Depurinate with 0.25N HCl (add 10 mL HCl to 500 ml H_2O) 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^{O}C for 2 hours.

C. Hybridization

1. Perform prehybridization at 55^{o}C for 3 hours in 10 mL of Easy Hyb buffer (Roche Biochemicals).
2. Boil 20µL GeneProber™ GLHDDig2X probe in 500µL of Easy Hyb for 10 minutes. Chill directly on ice.
3. Add the above probe to 10 mL of Easy Hyb.
4. Discard the prehybridization buffer and replace it with the hybridization buffer containing the boiled probe. Hybridize overnight at 55^{o}C.
5. Washing. Wash the membrane in 2xSSC/0.1% SDS at RT twice (5 min/wash), 0.5xSSC, 0.1%SDS twice at 65^{o}C to 70^{o}C (15 min/wash).
6. Warm the blocking reagent at this point. Prepare fresh 100 mL of Buffer MB by adding 10 mL of 10% blocking reagent [Gene Link Catalog no: 40-5026-10; Blocking solution for hybridization (10%)] and 10 mL of Maleic acid buffer 10X (Buffer M 10X) [Gene Link Catalog no: 40-5025-20; Maleic acid buffer 10X (Buffer M 10X)] to 80 mL of sterile water. Use 80 mL for blocking, the rest of 20 mL for making Anti-DIG-AP conjugate.

D. Anti-Dig Alkaline Phosphatase Binding

1. Equilibrate the membrane in 100 mL of 1x washing buffer M for 1 minute.
2. Incubate the membrane in 80 mL of Buffer MB (prepared in step 6 above) blocking solution at RT for 30 min.
3. Prepare 1:10000 Anti-DIG-AP conjugate at this point. Example, add 2 µl to 20 ml Buffer MB (prepared in step 6 above).
4. Incubate the membrane in 20 mL of Anti-DIG-AP conjugate solution at RT for 30 min.
5. Wash the membrane twice, 15 min/wash in 200 mL of 1x washing buffer M at RT.
6. Equilibrate the membrane in 50 mL of 1x Detection buffer for 2 min. Repeat 2 times. Total of three washes.

E. Detection

Detection with CDP star (Tropix) as substrate will yield reliable result by exposing to Kodak X-OMAT or XAR X-ray film for 1 hour to overnight at room temperature.

Transfer blot to a plastic sheet, (sheet protector cut from two sides to open up) and drain off excess buffer. Wipe off edges with paper towel. Blot should not be allowed to dry.

Spray CDP-star ready-to-use substrate evenly to cover the blot. DO NOT OVER SPRAY. Cover the blot with plastic sheet and wipe entire surface of the covered blot to expel any excess substrate and air bubbles. Expose the film at room temperature for 1 hr. or for shorter or longer time as required.

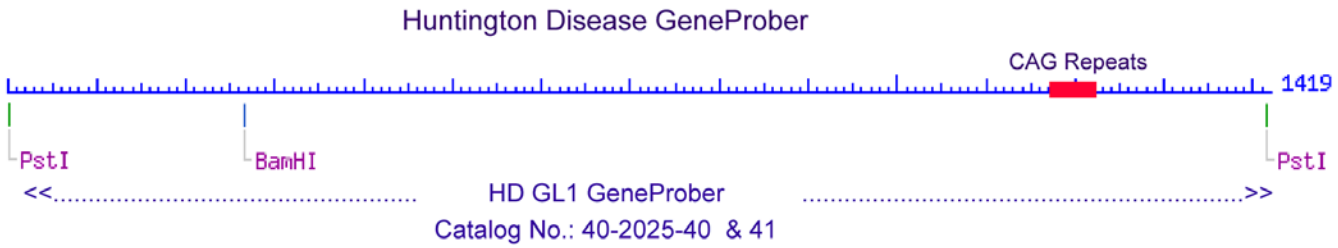
Luminescence continues for at least 24 hours and signal intensity remains almost constant during the first few hours. Multiple exposures can be taken to achieve the desired signal strength.

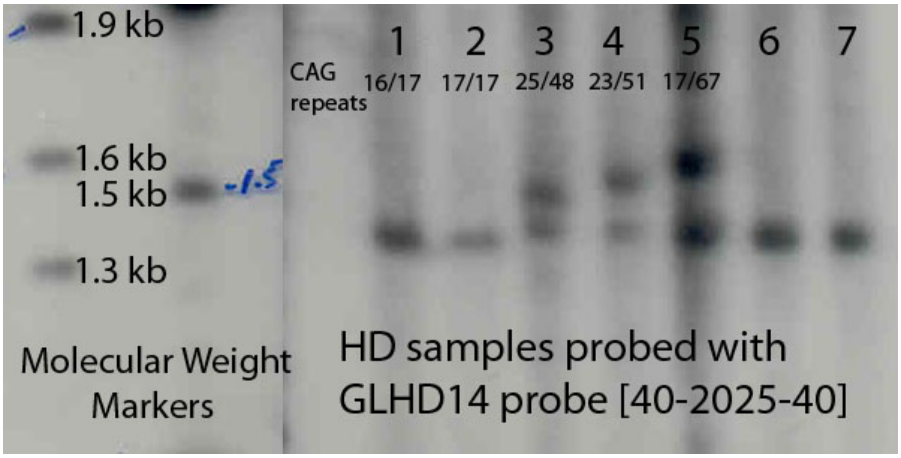
F. Stripping

Wash the membrane in water to remove the substrate. Then wash the membrane in 0.2N NaOH/0.1% SDS at 37^{o}C for 30 minutes. Rinse the membrane in 2XSSC. Air dry.

G. Results & Interpretation

1. Normal hybridization pattern is ~1419 bp fragment with genomic DNA digested with Pst *I* using GLHDDig2X GeneProber™ as labeled probe.
2. Larger fragment size is attributable to expanded CAG repeat region. See HD probe region figure below.





Required reagents with recommended suppliers

Roche Applied Science http://www.roche-applied-science.com

Product Description	Catalog Number
Nylon Membranes, positively charged ; 20 sheets 10 x 15 cm	11209272001
DNA Molecular Weight Marker III, DIG-labeled ; 500 µl 10 µg/ml 5 µg	11218603910
DIG Easy Hyb ; 500 ml	11603558001
DIG Wash and Block Buffer Set ; 1 set 30 blots	11585762001
Anti-Digoxigenin-AP, Fab fragments from sheep; 200 µL 150 U	11093274910
CDP Star Ready to use; 2X 50 mL	12041677001

Gene Link **http://www.genelink.com/geneprodsite/category.asp?c=44**

Non-radioactive Southern Blot Reagents

Product Description	Catalog No.	Unit Size
Agarose LE Molecular Biology Grade 100 gms	40-3010-10	100 gms
TAE Buffer 50 X Concentrate 1000 ml	40-3007-10	1 L
TBE Buffer 5 X Concentrate; 1L	40-3008-10	1 L
Loading buffer 10X BPB/XC non-denaturing; 1mL	40-3003-10	1 mL
Loading buffer 10X BPB/XC non-denaturing ; 15 mL	40-3003-15	15 mL
Lumisol II, Hybridization Solution; 200 mL	40-5023-20	200 mL
Depurination Solution (2X) for Southern Blotting; 1 L	40-5034-10	1 L
Denaturation Solution (2X) for Southern Blotting; 1L	40-5035-10	1 L
Neutralization Solution (2X) for Southern Blotting; 1L	40-5036-10	1 L
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC); 250 mL	40-5020-25	250 mL
Hybwash B, Hybridization Wash Solution (10% SDS) ; 100 mL	40-5021-10	100 mL
Maleic acid buffer 10X (Buffer M 10X); 200 mL	40-5025-20	200 mL
10% Blocking Reagent; 200 mL	40-5026-20	200 mL
Detection Buffer 10X; Alkaline Phosphatase detection buffer; 100 mL	40-5031-10	100 mL
CDP-Star® Substrate; Ready-to-Use 0.25 mM in spray bottle; 20 mL	40-5010-20	20 mL

Reagent Preparation

Most reagents with composition listed below are available in a molecular biology laboratory or these can be prepared in house. Gene Link catalog numbers are also listed if you like to purchase these common reagents.

Depurination Solution (0.25M HCl)		
Product Description	**Catalog No.**	**Volume**
Depurination Solution (2X) for Southern Blotting	40-5034-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

Denaturation Solution (0.5M NaOH, 1.5M NaCl)		
Product Description	**Catalog No.**	**Volume**
Denaturation Solution (2X) for Southern Blotting	40-5035-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

Neutralization Solution (0.5M Tris-HCl pH 7.5, 1.5M NaCl)		
Product Description	**Catalog No.**	**Volume**
Neutralization Solution (2X) for Southern Blotting	40-5036-10	150 mL
Sterile water		150 mL
Total Volume		**300 mL**

Hybwash I (2xSSC, 0.1% SDS)		
Product Description	**Catalog No.**	**Volume**
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC)	40-5020-25	35 mL
Sterile water		311 mL*
Hybwash B, Hybridization Wash Solution Concentrate (10% SDS)	40-5021-10	4 mL*
Total Volume		**350 mL**
*** Volumes adjusted to whole numbers**		

Hybwash II (0.5xSSC, 0.1%SDS)		
Product Description	**Catalog No.**	**Volume**
Hybwash A; Hybridization Wash Solution Concentrate (20X SSC)	40-5020-25	9 mL*
Sterile water		337 mL
Hybwash B, Hybridization Wash Solution Concentrate (10% SDS)	40-5021-10	4 mL*
Total Volume		**351 mL**
*** Volumes adjusted to whole numbers**		

1X Maleic Acid Buffer (Buffer M 1X) (100 mM Maleic acid, 150 mM NaCl pH7.5)		
Product Description	**Catalog No.**	**Volume**
Maleic acid buffer 10X (Buffer M 10X)	40-5025-20	10 mL
Sterile water		90 mL
Total Volume		**100 mL**

Buffer MB (1 x Maleic acid buffer (Buffer M) with Blocking Reagent) **Always prepare fresh!**		
Product Description	**Catalog No.**	**Volume**
Maleic acid buffer 10X (Buffer M 10X)	40-5025-20	10 mL
Sterile water		80 mL
10% Blocking Reagent*	40-5026-10	10 mL
Total Volume		**100 mL**
The prepared reagent will be turbid yellow in color * The 10% Blocking Reagent is turbid yellow in color and will form precipitates on storage. Warm to 50°C and shake well before aliquoting. DO NOT SHAKE VIGOROUSLY		

1X Detection Buffer, Alkaline phosphatase detection buffer (100mM Tris-HCl pH 9.5, 100mM NaCl)		
Product Description	**Catalog No.**	**Volume**
Detection Buffer 10X; Alkaline phosphatase detection buffer	40-5031-10	10 mL
Sterile water		90 mL
Total Volume		**100 mL**

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.

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 Genemer™ Kit Primers for amplification of CAG triple repeat spanning region. The quantity supplied is sufficient for 400 regular 50 µL PCR reactions.	100 rxns	40-2025-11
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
Huntington's Disease GeneProber™ GLHD14 Probe unlabeled Probe for radioactive labelling and Southern blot analysis	500 ng	40-2025-40
Huntington's Disease GeneProber™ GLHDDig2X Probe Digoxigenin labeled Probe for non-radioactive chemiluminescent Southern blot analysis	110 µL	40-2025-41

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; HEX labeled	25 µL	40-2025-03HX
Huntington's Disease 49 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-09HX
Huntington's Disease 89 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-04HX
Huntington's Disease 116 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-06HX
Huntington's Disease 134 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-61HX
Huntington's Disease 182 ~CAG repeat GScan Genemer Control DNA; HEX labeled	25 µL	40-2025-62HX

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

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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.		

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