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

Myotonic Dystrophy GeneProber™

GLDM4 unlabeled Probe

Myotonic Dystrophy CTG triple repeat Southern blot genotyping

Catalog No.: 40-2026-40

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

Myotonic Dystrophy GeneProber™ Unlabeled Probe

Content	Catalog No.	Description	Size
□	40-2026-40	Myotonic Dystrophy GeneProber™ GLDM4 unlabeled probe Myotonic dystrophy CTG triple repeat spanning region unlabeled probe for radioactive or chemiluminescent labeling and Southern blot detection of **Bam HI, PstI, BglI and EcoRI** digested DNA. Suitable for random primer labeling.	500 ng

Store at -20°C

Certificate of Analysis & Product Specifications

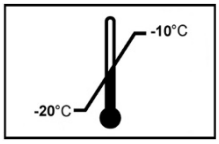
One tube containing 500 ng of lyophilized Myotonic Dystrophy GeneProber™ GLDM4 probe. This probe is unlabeled and is suitable for random primer labeling.

The Myotonic Dystrophy GeneProber™ GLDM1 or GLDM2 probe supplied has been validated to hybridize to the CTG triple repeat spanning region in the *DMPK* gene.

Appropriate nuclease free handling, dispensing and storage conditions required.

Lot Number: Manufacturing lot number is stated on the label of product and accompanying packing slip.

Product Label Information

		
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™ Related 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. No more decayed probes and radioactive exposure. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, 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
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™ GLDM4 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™ Related Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders. The kits contain optimized PCR amplification reagents and a wide array of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument. 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.

.Product	Unit Size	Catalog No.
Fragile X GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2004-15XX
Fragile X GScan™ V2 Kit for fluorescent detection; 20 reactions kit	1 kit	40-2004-15FMS
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 100 reactions kit	1 kit	40-2054-15FM
FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 20 reactions kit	1 kit	40-2054-15FMS
Huntington's Disease GScan™ V2 Kit for fluorescent detection; 100 reactions kit	1 kit	40-2025-15XX
Huntington's Disease GScan™ V2 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/**

Myotonic Dystrophy Genotyping

Background

Myotonic dystrophy (**Dystrophia Myotonica, DM**) is the most common form of adult onset muscular dystrophy. It is an autosomal dominant disorder with a prevalence of about 1 in 8000. The incidence varies from 1 in 475 in a region of Quebec to about 1 in 25,000 in European populations and is extremely rare in African populations. Clinical expression is highly variable and is related to age of onset. Onset of this disorder commonly occurs during young adulthood. However, it can occur at any age and is extremely variable in degree of severity. Myotonic dystrophy affects skeletal muscle and smooth muscle, as well as the eye, heart, endocrine system, and central nervous system. People with the mildest form of DM often go undiagnosed and usually cataracts and minimal muscle involvement are the only visible sign of the condition. The classical form of DM usually develops in early adult life and is characterized by progressive muscle stiffness and weakness.

Congenital DM (CDM) is the most severe form of the disease and is almost always inherited from affected mothers. It presents in newborn babies who suffer from respiratory distress, hypotonia, motor and mental retardation and facial diplegia. Diagnosis can be difficult if the family history is not known because muscle wasting may not be apparent and cataracts and myotonia are absent. CDM patients who survive the neonatal period eventually learn to walk but 60-70% are mentally retarded. By the age of 10 they develop myotonia and in adulthood they develop the additional complications associated with adult onset disease.

Identification of the mutation in DM

The myotonic dystrophy gene locus and the underlying mutation were identified in 1992 (1-3). An expressed sequence called cDNA25 was shown to detect a two-allele *Eco*RI polymorphism (8.6kb and 9.8kb) on Southern blots of normal individuals. It also detects a larger variable fragment in DM patients, which can be up to 5kb longer than the larger, normal allele. When this fragment is transmitted from an affected parent, it often increases in size, correlating well with the severity of the disease in the affected child. The variable band can also show somatic heterogeneity in lymphocyte DNA that is seen as a diffuse smear on a Southern blot. The *Eco*RI polymorphism is due to the insertion or deletion of consecutive Alu repeats 5 kb distal to the unstable region – the 8.6kb allele contains two Alu repeats and the 9.8kb normal allele and the enlarged DM alleles are associated with five Alu repeats. The discovery of unstable DNA at the DM locus provided an explanation for the phenomenon of anticipation seen in DM. Sequence analysis of genomic clones spanning the expanded region revealed that the mutation causing the instability is a trinucleotide repeat (CTG) which is highly polymorphic in the normal population and which increases dramatically in length in DM patients.

Number of CTG repeats	Clinical Condition	Symptoms
5-27 repeats	unaffected	
50-100 repeats	mild:	cataracts, slight muscle problems later on in life
100-1000 repeats	classical:	myotonia, muscle wasting, premature balding, gonadal atrophy, cardiac conduction defects
1000-4000	congenital:	hypotonia, mental retardation, facial diplegia

There are no definite repeat size boundaries for the three clinical groups and there are overlaps between the groups. A trimodal distribution is observed in European populations, with $(CTG)_5$ being the most frequently occurring allele, alleles of 11,12,13 and 14 make up the second mode and the final mode represents alleles of 19 and above.

Meiotic instability

The meiotic instability of the DM mutation has been shown to be dependent on the size of the parental repeat. For $(CTG)_n$ repeats of <0.5kb a positive correlation between the size of the repeat and inter-generational enlargement was found equally in male and female meioses but with CTG sequences of more than 0.5 kb observed that intergenerational variation was greater through female meioses (4). The tendency for a repeat to undergo contraction was observed almost exclusively in male meioses. It was found that the length of the CTG repeat expansion in DM patients was greater in DNA isolated from muscle than in lymphocyte DNA (5). Rare cases have been reported where expansion of the CTG repeats is not seen in individuals where the clinical symptoms are unequivocal and this may due to a deletion or point mutation as seen in some of the other triplet repeat disorders such as fragile X syndrome.

The underlying mutations of DM are expansions of the CTG repeats located in the 3' untranslated region (UTR) of the myotonic dystrophy protein kinase (*DMPK*) gene on chromosome 19q. Severity of the disease is correlated with the length of the repeat expansion. Normal individuals have from 5 to 27 repeat copies; mildly affected persons have at least 50 repeats, while more severely affected patients have expansion of the repeat-containing segment up to several kilobase pairs.

Expansion is frequently observed in parent-to-child transmission, but extreme expansions are not transmitted through the male line. This explains: 1.) the occurrence of the severe congenital form is almost exclusively in the offspring of affected women; 2.) anticipation is commonly observed in affected families, that is, the disease demonstrates earlier onset and greater severity in each successive generation. The overall risk of having a congenitally affected child for any carrier woman is about 10%. If the woman has clinical signs of the condition, the risk of congenital myotonic dystrophy in offspring is 40% and this rises to 50% in subsequent pregnancies if an affected child has previously has been born.

Genotyping

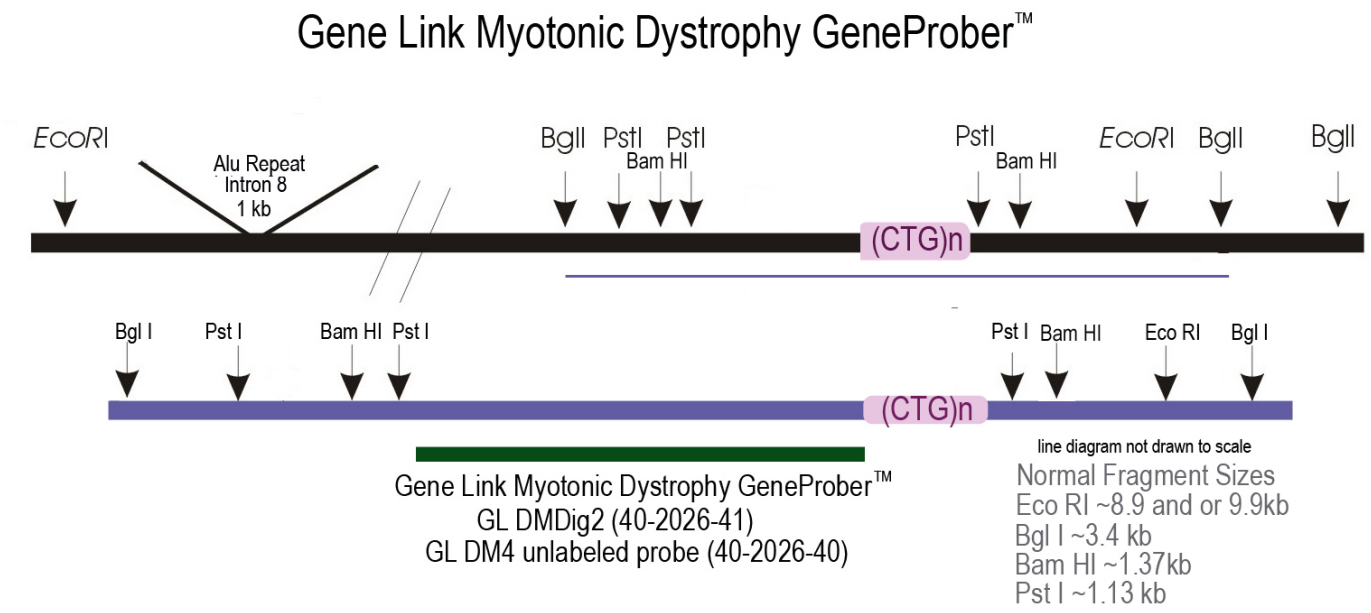
Molecular Analysis

The direct analysis of CTG repeats in the *DMPK* gene (chromosomal locus 19q13) is available. Molecular diagnosis of myotonic dystrophy involves a combination of direct PCR analysis and Southern blotting tests to determine the CTG-repeat number within the DMPK gene. PCR can identify CTG expansions between 5-200 CTG repeats. Congenital mutations usually cannot be identified by PCR and southern analysis is the preferred method to distinguish full mutations.

With larger expansions, Southern blot analysis of restriction fragments can be used to determine the range of the large repeat size. Genomic DNA is digested with BglI I, BamHI or PstI or/and EcoRI to determine the presence or absence of the ~1 kb Alu repeat. The DNA blot is then hybridized with either Gene Link's GLDMDig2 Digoxigenin labelled probe for non-radioactive chemiluminescent detection or for radioactive Southern Blot by using random prime labelled Gene Link's GLDM4 probe. Both of these probes are specifically designed to exclude the CTG repeat region to yield very low background specific hybridization signals to the fragments containing the CTG repeat.

The size of the CTG repeats can be determined by PCR analysis and sizing preferably on a sequencing gel or fluorescent fragment analyzers. The PCR products can be either labeled with ^{35}S or ^{32}P followed by autoradiography. Another attractive alternate is to run a cold PCR reaction followed by blotting and hybridization with an alkaline phosphatase conjugated probe for non-radioactive detection.

Southern blot analysis for Myotonic Dystrophy mutation detection involves the cleavage of DNA with BglI, BamHI or PstI enzyme combined with EcoRI to determine the 1 Kb Alu repeat genotype. This method detects the size of CTG repeats region by hybridization of Gene Link's probe GLDM1, GLDM2, GLDM3 or GLDM4 to DNA that has been digested with the appropriate restriction enzyme and blotted onto a membrane.



DMPK Gene Normal Southern Blot Fragment Analysis Pattern Restriction Digestion*	
Restriction Enzyme	Normal Fragment Size
EcoRI	~8.9 or/and 9.9 kb
BglI	~3.4 kb
BamHI	1.37 kb
PstI	1.13 kb
*Probed with Gene Link DMPK CTG repeat region specific probes GL DMDig2, DM3 and DM4.	

Gene Link offers safe and reliable chemiluminescent detection methods as an alternate to radioactive based detection methods. Genemer™, PCR-Prober™, GScan™ and GeneProber™ line of products replaces radioactive based methods. Gene Link's GScan™ and Genemer™ kits are for PCR amplification followed by agarose gel electrophoresis or fluorescent fragment length detection of the specific triple repeat fragment size and routinely detects greater than 120 CGG repeats for fragile X.

Genemer™ Kit Agarose Gel Analysis

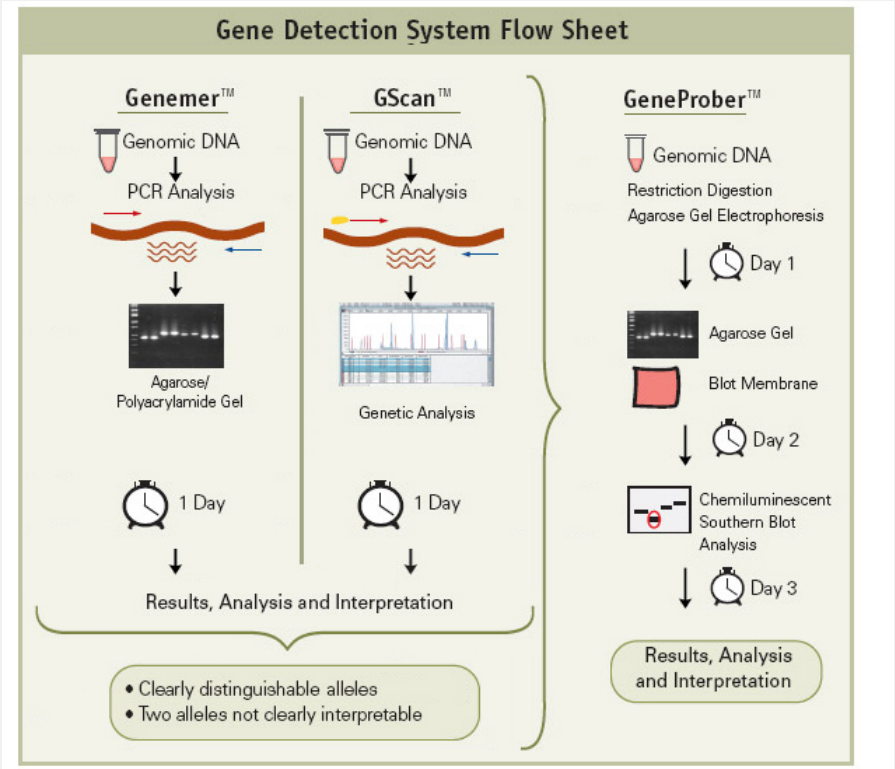
Optimized Genemer™ kit with components for PCR amplification of up to ~400 CGG repeats using standard Taq polymerase. Amplified samples are resolved by agarose gel electrophoresis. This Genemer™ method or GScan™ fluorescent detection is recommended for initial screening of all samples.

GScan™ Kit

Optimized GScan™ kit with components for PCR amplification of up to ~400 CGG repeats using standard Taq polymerase. Amplified samples are resolved by fragment 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.



References

1. Fu YH, Pizzuti A, Fenwick RG Jr, King J, Rajnarayan S, Dunne PW, Dubel J, Nasser GA, Ashizawa T, de Jong P, et al. (1992) An unstable triplet repeat in a gene related to myotonic muscular dystrophy. Science 255: 1256-1258.
2. Aslanidis et al. (1992) Cloning of the essential myotonic dystrophy region and mapping of the putative defect. Nature 355: 548-551.
3. Brook et al. (1992) Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3-prime end of a transcript encoding a protein kinase family member. Cell 68: 799-808.
4. Lavedan et al. (1993) Myotonic dystrophy: size- and sex-dependent dynamics of CTG meiotic instability, and somatic mosaicism. Am. J. Hum. Genet. 52: 875-883.
5. Anvret et al. ((1993) Larger expansions of the CTG repeat in muscle compared to lymphocytes from patients with myotonic dystrophy. Human Molecular Genetics 2:1397-1400.
6. Mathieu J, Allard P, Potvin L, Prevost C, Begin P (1999) A 10-year study of mortality in a cohort of patients with myotonic dystrophy. *Neurology* 52:1658-62
7. Redman JB, Fenwick RG Jr, Fu YH, Pizzuti A, Caskey CT (1993) Relationship between parental trinucleotide GCT repeat length and severity of myotonic dystrophy in offspring. *JAMA* 269:1960-5

Procedure: Southern Blot Hybridization Protocol

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.

Important Information

Gene Link strongly recommends the use of non-radioactive gene detection systems. Consider switching to Gene Link's GLDMDig2 GeneProber™ Southern blot gene detection system (Catalog Number 40-2026-41), GScan™ fluorescent detection system (Catalog Number 40-2026-15) and Genemer™ (Catalog Number 40-2026-11) agarose or polyacrylamide gel detection systems.

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 GLDM4 *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.

A. Chromosomal DNA digestion

- Digest genomic DNA with Pst I, Bam HI, BglI or EcoR1.
- Given below is an example of a restriction digest setup. Follow your own routine laboratory protocol if required.

Restriction Digestion	
Component	Volume|Quantity
Genomic DNA	5 to 10µg
10x Buffer	10 µL
Restriction Enzyme (40-100 units)	4 µL
H_2O to	**100 µL**

♦ **Incubate over night at 37°C**

♦ **Ethanol Precipitate the digests**

-To 100 µL DNA add 10 µL of 3M NaAc
-Add 2 volumes (250 µL) of 100% ethanol
-Put in the freezer (-20 °C) for 20-30 minutes
-Spin at -10 °C for 5 minutes and discard the supernatant
-Add 100 µL of 70% ethanol, vortex.
-Spin again at -10 °C for 5 minutes
-Dry samples

♦ **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 40mA for ~14 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 and Random Primer Labeling

1. Perform pre-hybridization at 50^{o}C for 3 hours in 10 mL of Lumisol I buffer (Gene Link).
2. While prehybridizing label the probe as following: (Any Random Primer DNA Labeling Kit for radioactive or chemiluminescent labelling system).

Random Primer Labeling	
Component	Volume| Quantity
GLDM GeneProber™	25 -100 ng
H_2O	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^{O}C for 30 minutes	

3. Add 500 µL of 5 x SSC to the reaction tube, boil for 5 minutes, then add to the prehybridization solution with the membrane and Lumisol I solution, mix well, incubate in shaking water bath at 50^{O}C overnight.
4. 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 60^{O}C twice (30 min. per wash). Wrap the membrane and put X-ray film on it, expose at -80^{O}C overnight. Develop the film next morning.

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

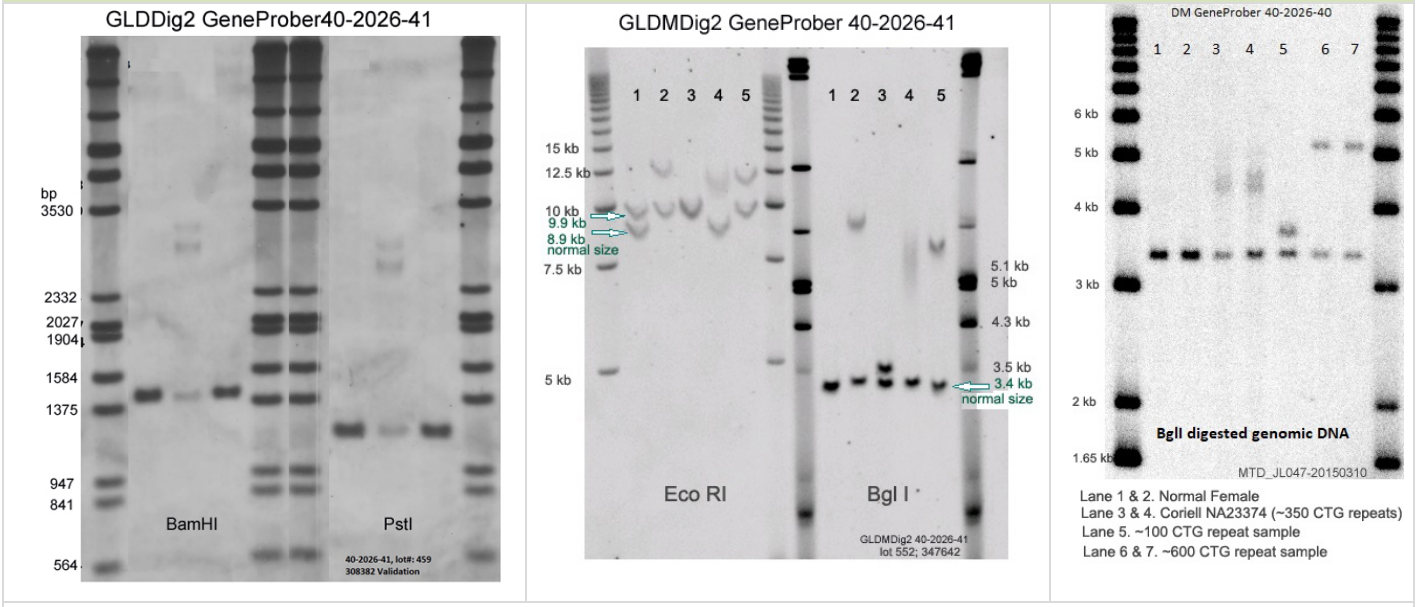
Results and Analysis

Gene Link's Myotonic Dystrophy CTG repeat Southern blot genotyping probes GLDMdig2, GLDM3 and GLDM4 are especially designed to exclude the CTG repeat region and thus yield cleaner blot with less background as compared to other probes including Gene Link's older GLDM1 and GLDM2 probes.

Traditionally Southern blot analysis for Myotonic Dystrophy CTG repeat genotyping involves the cleavage of DNA with restriction enzymes EcoRI and BglI. EcoRI digested DNA detects the presence or absence of the ~1kb Alu polymorphism, the detection of this polymorphism is not essential for genotyping the CTG repeat status except to have additional data for the Alu polymorphism status. EcoRI Southern blot analysis alone is not recommended as it may mask mid-range CTG expansion. If EcoRI Southern blot analysis is performed then it should always be in conjunction with an additional blot analysis with BglI, BamHI or PstI.

BglI, BamHI or PstI Southern blot analysis is the preferred method to supplement the PCR genotyping results. These restriction enzymes yield normal fragments that are between ~1.1 kb to 3.4 kb for easy visualization of larger fragments containing large CTG repeats.

Gene Link GeneProber™ DM Southern Blot Fragment Analysis

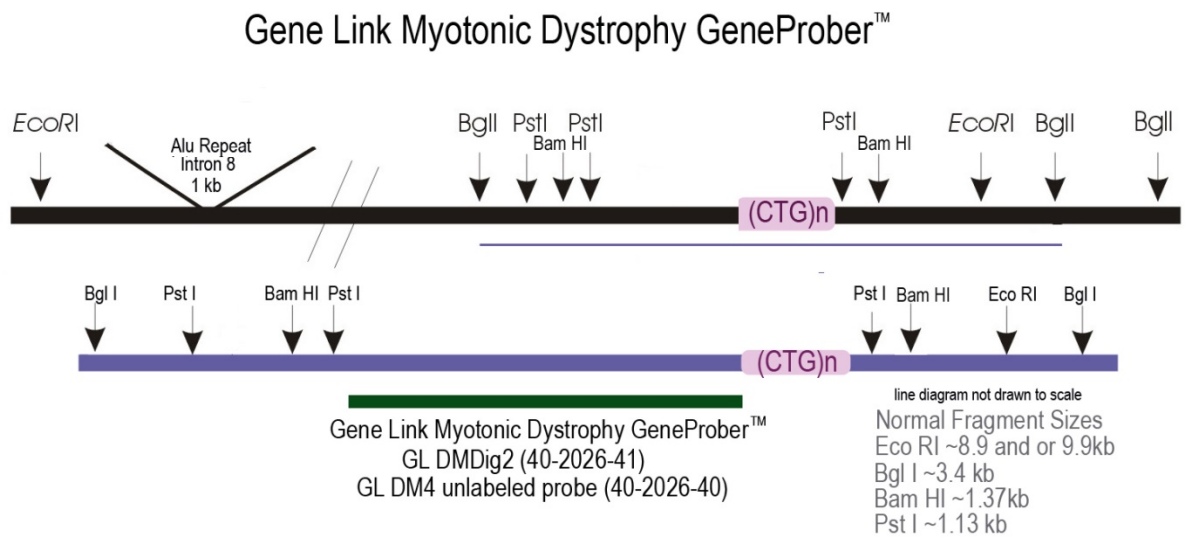


Human genomic DNA was digested with BamHI, PstI, BglI or EcoRI. After electrophoretic separation, the agarose gels were processed for non-radioactive Southern blot analysis using Gene Link's GLDMDig2 GeneProber™ or GLDM4 unlabeled probe labeled with radioactivity as described in the procedure section of this manual. Sizing of fragments compared to molecular weight markers and control fragment pattern assist in genotyping the approximate range of CTG repeats of the DMPK gene.

DMPK Gene Normal Southern Blot Fragment Analysis Pattern Restriction Digestion*

Restriction Enzyme	Normal Fragment Size
Eco RI	~8.9 or/and 9.9 kb
Bgl I	~3.4 kb
Bam HI	~1.37 kb
Pst I	~1.13 kb

*Probed with Gene Link DMPK CTG repeat region specific probes GL DMDig2, DM3 or DM4.



Myotonic Dystrophy Molecular Analysis & Results Interpretation of CTG Repeats Genotyping				
Clinical Category	**Normal (male/female)**	**Intermediate Allele Mild**	**DM Allele with Classical Condition**	**DM Allele with Congenital Condition and mental retardation**
Risk mutation will become full mutation in next generation	0%	moderate	significant	high
Number of CTG repeats	5-27	50-100	100-1000	>1000
Size of CTG repeats; bp	15-81	150-300	300-3000	>3000
Total Fragment Size; bp	128-194	267-413	428-1328	>1328
There are no definite repeat size boundaries for the three clinical groups and there are overlaps between the groups. A trimodal distribution is observed in European populations, with $(CTG)_5$ being the most frequently occurring allele, alleles of 11,12,13 and 14 make up the second mode and the final mode represents alleles of 19 and above.				

Required reagents with recommended suppliers

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 I, Hybridization Solution; 200 mL	40-5022-20	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

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**		

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.

Myotonic Dystrophy Product Ordering Information

Product	Unit Size	Catalog No.
Myotonic Dystrophy Genemer™ Primer pair Primers for amplification of CTG triple repeat spanning region. The quantity supplied is sufficient for 400 regular 50 µL PCR reactions.	10 nmols	40-2026-10
Myotonic Dystrophy GeneProber™ GLDM1 Probe unlabeled Myotonic dystrophy CTG triple repeat spanning region unlabeled probe for radioactive labeling and Southern blot detection of Bam HI digested DNA.	500 ng	40-2026-40
Myotonic Dystrophy GeneProber™ GLDM2 Probe unlabeled Myotonic dystrophy CTG triple repeat spanning region unlabeled probe for radioactive labeling and Southern blot detection of Pst I digested DNA.	500 ng	40-2026-39
Myotonic Dystrophy GeneProber™ GLDMDig1 Probe Digoxigenin labeled Myotonic Dystrophy CTG triple repeat spanning region digoxigenin labeled probe for non-radioactive Southern blot detection.	110 µL	40-2026-41
Myotonic Dystrophy PCRProber ™ AP labeled probe Alkaline phosphatase labeled probe	12 µL	40-2026-31
Myotonic Dystrophy PCRProber ™ Kit for chemiluminescent detection Kit for performing PCR amplification and chemiluminescent based detection.	5 blots [50 rxns]	40-2026-32
GLDM Genemer™ Kit for Radioactive Detection Kit for amplification and radioactive detection of Myotonic Dystrophy CTG triple repeat region amplified PCR products using ^{35}S or ^{32}P. 100 Reactions.	1 Kit [100 rxns]	40-2026-20
GLDM 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]	40-2026-15XX

Genemer™ GScan Control DNA Cloned fragment of the mutation region of a particular gene. These control DNA's 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 DNA's 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.

GLDM 12 ~CTG repeat Genemer™ Control DNA	500 ng	40-2026-01
GLDM 45 ~CTG repeat Genemer™ Control DNA	500 ng	40-2026-02
GLDM 93 ~CTG repeat Genemer™ Control DNA	500 ng	40-2026-03
GLDM 129 ~CTG repeat Genemer™ Control DNA	500 ng	40-2026-04
GLDM 194 ~CTG repeat Genemer™ Control DNA	500 ng	40-2026-05

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

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. 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
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

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/**

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

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.

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

Warranty and Liability

Information in this document is subject to change without notice. This document and all information presented in this document are written as a guide. Gene Link, Inc. does not warrant this document to be free of errors and assumes no responsibility for any errors that may appear in this document.
Gene Link disclaims all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. In no event shall Gene Link be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

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

Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact sales@genelink.com.

Gene Link, Inc.
190 Saw Mill River Road
Hawthorne,
NY 10532
USA

Tel: (914)769-1192
Email: support@genelink.com
www.genelink.com