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 CTG Repeat Genotyping GScan™ Kit V2

Myotonic Dystrophy CTG triple repeat amplification kit for fluorescent genotyping

Catalog No. 40-2026-15FM

Size: 100 X 25 µL Reactions

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.

20 years
results you can rely on
Gene Link™

Material Supplied

Myotonic Dystrophy CTG Repeat Genotyping GScan™ Kit V2

Note: This kit is Version 2. Components of this version are not compatible with components of previous versions.

Myotonic Dystrophy CTG Repeat Genotyping GScan™ V2 Kits			
Kit	**Catalog No.**	**Description**	**Size**
□	40-2026-15FM	DM CTG Repeat Genotyping GScan™ V2 6-Fam Kit. 100 X 25 µL rxns.	100 rxns
□	40-2026-15FMS	DM CTG Repeat Genotyping GScan™ V2 6-Fam Kit. 20 X 25 µL rxns.	20 rxns

❑ Myotonic Dystrophy CTG Repeat Genotyping GScan™ Kit Components 40-2026-15FM 100 Reactions			
Content	**Catalog No.**	**Description**	**Size**
❑	40-2026-15FFM	DM GScan™ V2 6-Fam Component FFM	650 µL
❑	40-2026-15E	PCR Component E; PCR additive	800 µL
❑	40-2026-15D	PCR Component D; PCR additive	200 µL
❑	40-2026-15M	PCR Component M; PCR additive	450 µL
❑	40-2026-15VS	DM GScan™ V2 Fam amplified control ~13/140 CTG Repeats	10 µL

❑ Myotonic Dystrophy CTG Repeat Genotyping GScan Kit Components 40-2026-15FMS 20 Reactions			
Content	**Catalog No.**	**Product**	**Size**
❑	40-2026-15FFMS	DM GScan™ V2 6-Fam Component FFMS	130 µL
❑	40-2026-15ES	PCR Component E; PCR additive	200 µL
❑	40-2026-15DS	PCR Component D; PCR additive	40 µL
❑	40-2026-15MS	PCR Component M; PCR additive	90 µL
❑	40-2026-15VSS	DM GScan™ V2 Fam amplified control ~13/140 CTG Repeats	5 µL

Storage Condition: -20°C

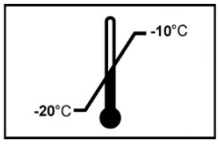
Certificate of Analysis & Product Specifications

The Myotonic Dystrophy GScan™ V2 kit contains components and Genemer™ DM-F1/R1 primer pair that has been validated to amplify the CTG triple repeat spanning region in the *DMPK* gene of human genomic DNA. 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

REF	Catalog No.	Description	Size
	40-2026-15FM	DM CTG Repeat Genotyping GScan™ V2 6-Fam Kit. 100 X 25 µL rxns.	100 rxns
	40-2026-15FMS	DM CTG Repeat Genotyping GScan™ V2 6-Fam Kit. 20 X 25 µL rxns.	20 rxns

RUO Research Use Only		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™ 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™ 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™ 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 V2 for fluorescent detection; 100 reactions kit	1 kit	40-2026-15XX
Myotonic Dystrophy GScan™ Kit V2 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.

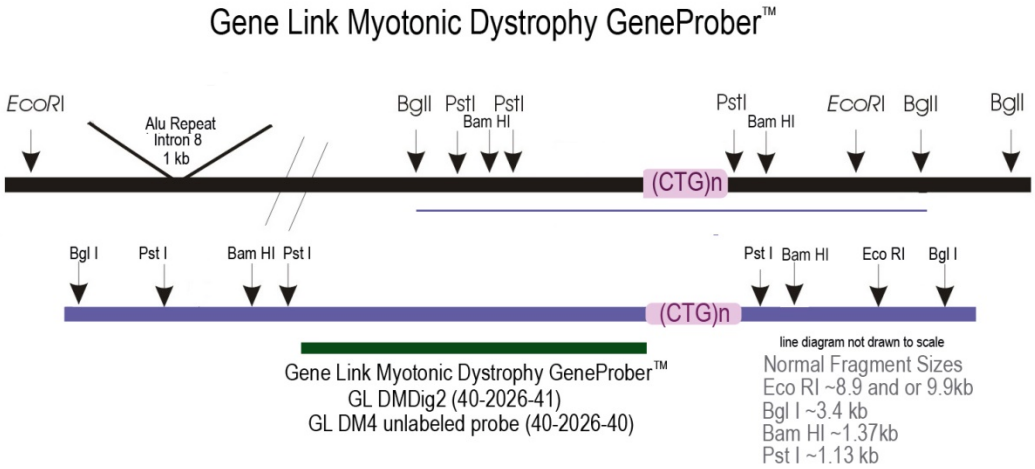
The underlying mutations of DM are expansions of the CTG repeats located in the 3' untranslated region (UTR) of the myotonic dystrophy protein kinase (*DMPK*) gene on chromosome 19q. Severity of the disease is correlated with the length of the repeat expansion. Normal individuals have from 5 to 30 repeat 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 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.

With larger expansions, Southern blot analysis of restriction fragments can be used for an accurate measure of the repeat size. Genomic DNA is digested with Bam HI or Pst I. The DNA blot is then hybridized with either GLDM1 or GLDM2 CTG repeat specific DNA probe.



Molecular Analysis

The direct analysis of CTG repeats in the *DMPK* gene (chromosomal locus 19q13) is clinically available. An increased number of CTG repeats is identified in essentially 100% of patients with DM. The number of CTG repeats ranges from 5 to 37 in normal alleles. GTG repeat lengths in the range from about 38 to 49 are considered "premutations." Persons with CTG expansions in the premutation range have not been reported as having developed symptoms, but their children are at risk of inheriting a larger repeat size. Persons with CTG repeat length greater than 50 are frequently symptomatic.

Myotonic Dystrophy genotyping can be done by direct PCR amplification of the CTG trinucleotide repeats region or by Southern analysis. In most cases both methods are used to complement the results. Congenital mutations usually cannot be identified by PCR and southern analysis is the preferred method to distinguish full mutations.

The size of the CTG repeats can be determined by PCR analysis and sizing preferably on a sequencing gel. 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 either Bam HI or Pst I enzyme This method detects the size of CTG repeats region by hybridization of probe GLDM1 or GLDM2 to DNA that has been digested with the appropriate restriction enzyme and blotted onto a membrane. The CTG repeat in the normal range yields a ~1377 bp with Bam HI and a ~1136 bp with Pst I digested DNA.

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 detection of the specific triple repeat fragment size and routinely detects greater than 120 CGG repeats.

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.	

PCR Analysis

The following table lists the size of PCR fragment in base pairs (bp) that can be expected when using the CTG primer mix DMF1/R1 that has been provided in the Genemer™ PCRProber™ and GScan™ kits. The formula for determining PCR fragment size is 113 + 3n, where n= the number of CTG repeats.

Myotonic Dystrophy CTG Repeats and Fragment Size*			
$CTG_{(n)}$	PCR Fragment (bp)	$CTG_{(n)}$	PCR Fragment (bp)
5	128	45	248
6	131	50	263
7	134	55	278
8	137	60	293
9	140	65	308
10	143	70	323
11	146	75	338
12	149	80	353
13	152	85	368
14	155	90	383
15	158	95	398
16	161	100	413
17	164	105	428
18	167	110	443
19	170	115	458
20	173	120	473
21	176	125	498
22	179	130	503
23	182	135	518
24	185	140	533
25	188	145	548
26	191	150	563
27	194	155	578
28	197	160	593
29	200	165	608
30	203	170	623
31	206	175	638
32	209	180	653
33	212	185	668
34	215	190	683
35	218	195	698
40	233	200	713
*PCR fragment in base pairs (bp) that can be expected when using the CTG primer mix DMF1/R1 that has been provided in the Genemer™ PCRProber™ and GScan™ kits. The formula for determining PCR fragment size is 113 + 3n, where n= the number of CTG repeats.			

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

Important Information

Genotyping using this kit requires use of the appropriate fluorescent genetic analyzer instrument(s) and software capable of detection of fluorescently labeled fragments of varying lengths. This kit has been optimized for an ABI310 genetic analyzer.

Myotonic Dystrophy CTG Repeats Analysis by PCR

PCR Premix Preparation

Thaw individual components. *Promptly store at -20°C after use*. Prepare ***fresh*** before use enough PCR premix for the number of reactions to be performed. Prepare 10% more for pipetting allowance. Prepare premix following the volumes given below. Follow the same ratio for preparing other final volumes.

Material Supplied: Please see page 2 of this manual.

PCR Thermal Cycler Files

Prepare the following PCR thermal cycler files

Hot Start File	
Step	**Time and Temperature**
Denaturation	5 minutes at 98°C
Hold	62°C

DM Amplification File		
Step	**Time and Temperature**	**Cycles**
Denaturation	30 seconds at 94°C	30 Cycles
Annealing	30 seconds at 60°C	
Extension	3 minute at 72°C	
Fillup	7 minutes at 72°C	1 Cycle
Hold	Hold for infinity at 4°C	Hold for infinity

Protocol:

PCR Amplification

A. PCR premix preparation

Given below is a protocol for preparing a PCR premix for 25 µL reactions. This can be scaled up as required.

PCR Premix Preparation			
Component	**1 x 25 µL rxn.**	**10 x 25 µL rxns.**	**Worksheet**
DM GScan™ Component F	6 µL	60 µL	
PCR Component E	7.5 µL	75 µL	
PCR Component D	1.5 µL	15 µL	
PCR Component M	4.0 µL	40 µL	
Sterile water	5.0 µL	50 µL	
Total	**24 µL**	**240 µL**	

B. Enzyme premix preparation

Enzyme Mix Preparation			
Component	**1 x 25 µL rxn.**	**10 x 25 µL rxns.**	**Worksheet**
PCR premix (above)	2.0 µL	20 µL	
Taq Polymerase	0.5 µL	5 µL	
Total	**2.5 µL**	**25 µL**	

C. PCR reaction

'Hot Start' PCR

For each sample add the following

Hot Start PCR	
Component	**Quantity**
PCR premix (above)	22 µL
DNA Template (~100ng chromosomal DNA)	1 µL
Total	**23 µL**

Start "Hot Start" file.

After initial denaturation while thermal cycler is 'holding' at 60°C

Add 2 µL of Enzyme premix to each tube and start DM amplification PCR file.

D. Post PCR Processing

1. Process sample for Omni-Clean purification of amplified products. This purification method is preferred over Sephadex G-50 spin column chromatography or ethanol precipitation as it is the most effective way to reduce the fluorescent primers and shorter primer dimers if present. Omni-Clean Kit is available from Gene Link, catalog number 40-4130-10 or 40-4140-10.

2. Process sample for Sephadex G-50 spin column chromatography. G-50 columns should be equilibrated with sterile water.

3. Ethanol precipitate and dissolve pellet in 20 µL sterile water. OR

4. Collected sample is ready for genetic analysis.

E. GScan™ Analysis

Follow genetic analyzer manufacturer's instructions. The amplified fragments are labelled with 6-Fam as the fluorophore. Use appropriate filter set for detection of 6-Fam emission. Molecular weight markers preferable should be 500 Liz or other compatible dye set for particular instrument type.

Run two aliquots of sample at different concentrations. A brief protocol is given below for ABI3100 instrument.

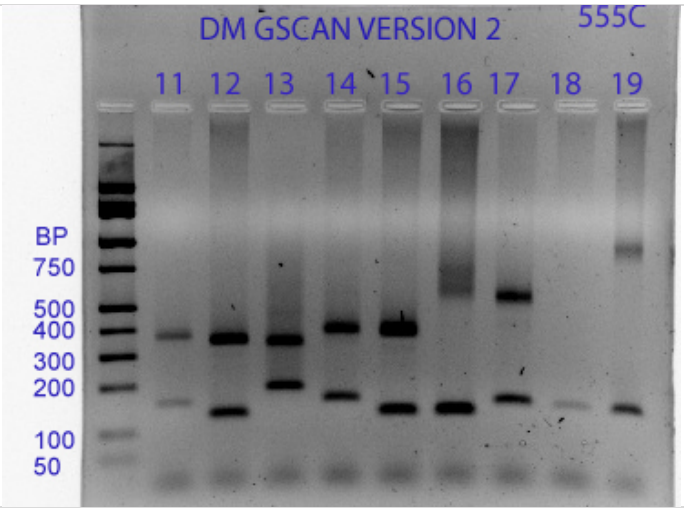
1. Add 0.5 µL of appropriate size standards.
2. Add 2 & 4µL of sample in duplicate tubes.
3. Add 12 µL of formamide loading buffer.
4. Mix and heat denature at 95 °C for 5 minutes.
5. Transfer to ice.
6. Samples ready to load on instrument.

F. Pre-run CTG Control DNA Sample Analysis

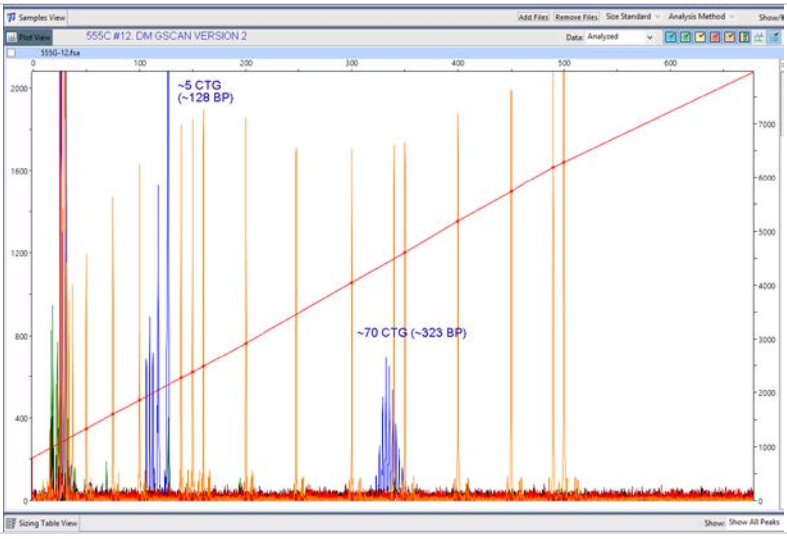
Due to their high GC content and the increased probability of secondary structure, fragments containing the CTG expansion tend to migrate at different rates during gel electrophoresis than corresponding molecular weight standards that usually have random GC contents. Furthermore, electrophoretic mobility increases with the size of the CTG expansion. To correct for the anomalous migration rate of the CTG fragments, an amplified GLDM Control DNA is provided with the kit. Refer to page 2 of material supplied list to determine the exact CTG repeat size amplified control DNA provided in this lot. We recommend running 1 µL of the control DNA standard along with your molecular weight standards. Follow genetic analyzer manufacturer's instructions relating to running samples, data recovery, and interpretation.

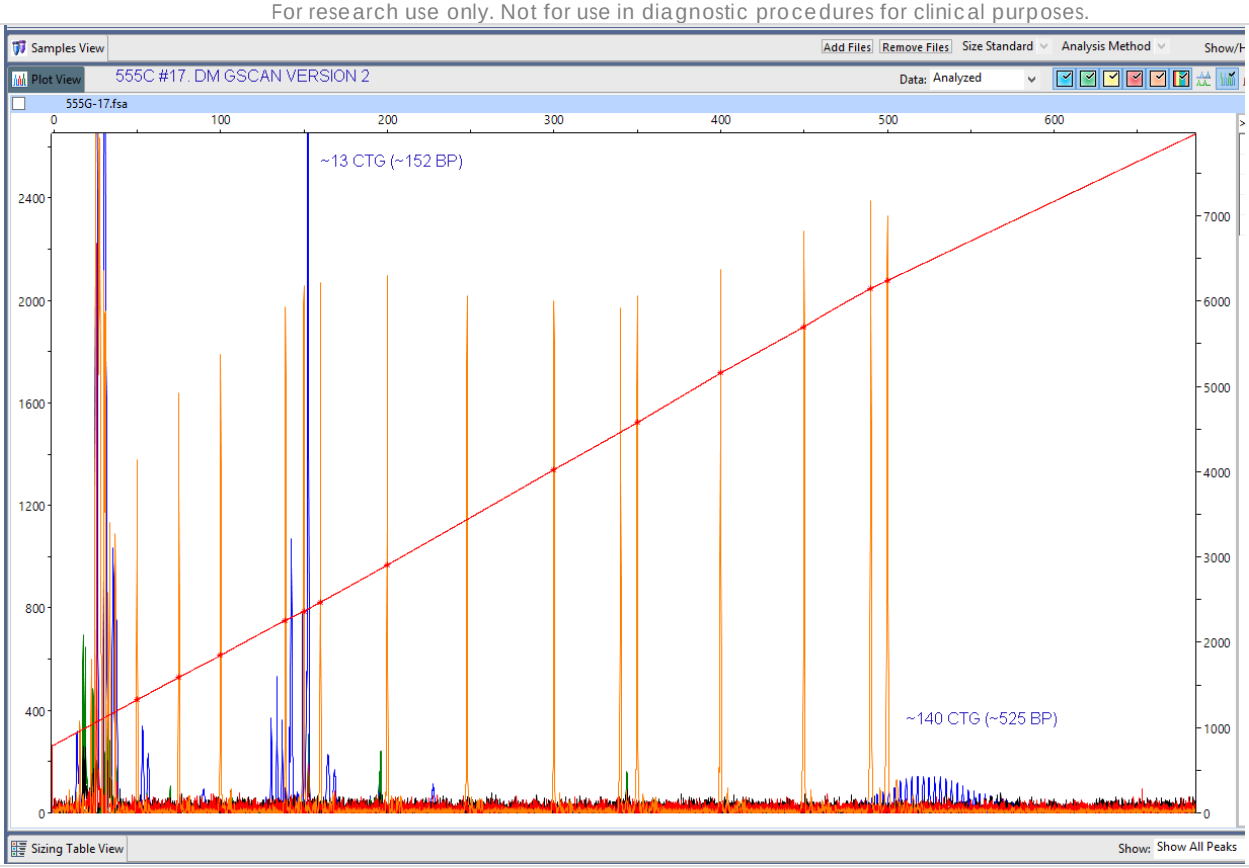
Results and Interpretation

The results obtained from the genetic analyzer will approximately show the fragment size amplified, based on these results an interpretation can be made about the genotype of the sample. It is known that there is an overlap between the normal and DM allele sizes. The repeat sizes obtained falling in the overlap region should be preferably repeated and possibly run with more samples from other family members. Refer to the table 2 for determining the CTG repeats and fragment size expected. The formula for determining PCR fragment size is 113 + 3n, where n= the number of CTG repeats.



DM Gscan V2 reactions performed on various samples of known large repeats of human genomic DNA. 10 µL aliquots were electrophoresed on a 2% agarose gel and stained with ethidium bromide. Approximate CTG repeats of samples from lane 11 to lane 19 are as follows; 11/75; 5/60; 24/80; 15/150; 5/160; 5/185;13/200; 10/?? and 10/380.




Samples View
Add Files
Remove Files
Size Standard
Analysis Method
Plot View
555C #17. DM GSCAN VERSION 2
Data: Analyzed
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~13 CTG (~152 BP)
~140 CTG (~525 BP)
Sizing Table View
Show: Show All Peaks

Results and Interpretation

The results obtained from agarose gel electrophoretic pattern will approximately show the fragment size amplified, based on these results an interpretation can be made about the genotype of the sample. It is known that there is an overlap between the normal and DM allele sizes. The repeat sizes obtained falling in the overlap region should be preferably repeated and possibly run with more samples from other family members. Refer to the table 2 for determining the CTG repeats and fragment size expected. The formula for determining PCR fragment size is 113 + 3n, where n= the number of CTG repeats.

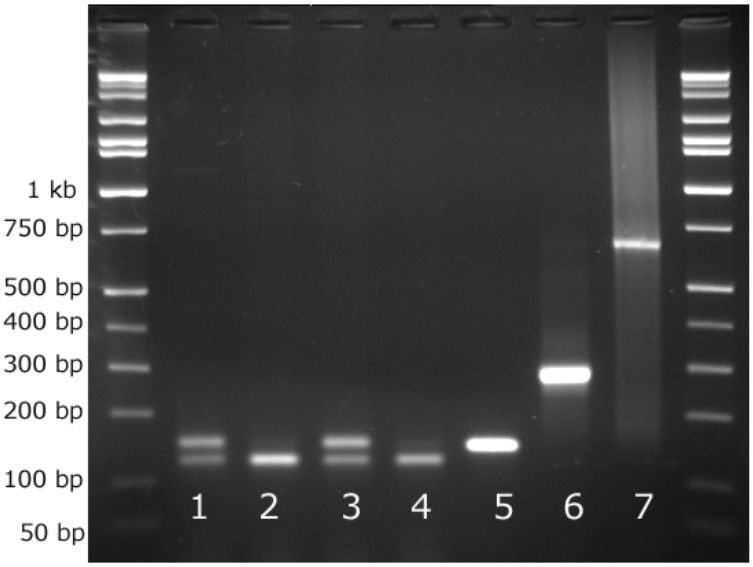
Myotonic Dystrophy Molecular Analysis & Results Interpretation of CTG Repeats

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.

Myotonic Dystrophy Genemer™ Fragment Analysis

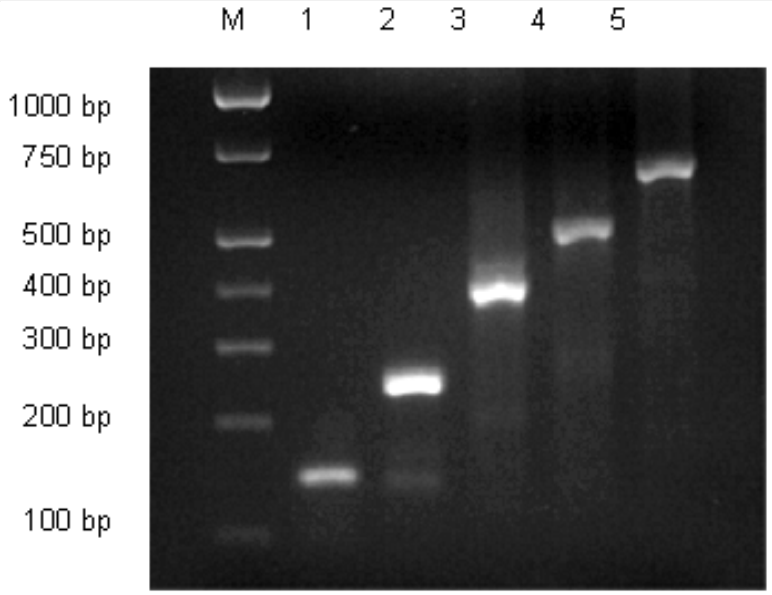
DM Genemer CTG Repeat Amplification



Lanes 1-4 are chromosomal DNA samples from a family and represent ~6 CTG repeats (~131 bp) and ~12 CTG repeats of ~149 bp fragment. Lanes 5-7 are Genemer Control DNA samples as follows.
Lane 5; DM Genemer Control DNA; 40-2026-01; ~12 CTG repeats
Lane 6; DM Genemer Control DNA; 40-2026-02; ~45 CTG repeats
Lane 5; DM Genemer Control DNA; 40-2026-05; ~182 CTG repeats

After PCR amplification 10 µl of the reaction was directly loaded to a 2% TAE agarose gel containing ethidium bromide. Electrophoresis was performed at 60 mAmps till a reasonable resolution was achieved. Usually a 1.5 hour run is sufficient.

Myotonic Dystrophy Genemer™ Control DNA Amplification



Lane M is molecular weight markers. Lanes 1 -5 represents PCR products from DM genomic clones that contain 12, 45, 93, 129 and 182 CTG repeats respectively.

PCR amplification of Myotonic Dystrophy Genemer Control DNA using GLDM GScan™ 6-Fam labeled kit. Samples electrophoretically resolved on a 2% agarose gel followed by ethidium bromide staining.

Trouble Shooting

1. No amplified fragment. The most common reason for not observing an amplification of a specific fragment from chromosomal DNA is the quality of DNA. Try using multiple DNA samples of known quality that have yielded good amplification of chromosomal DNA fragments.
2. Faint and low level of amplification. Try scaling up the reaction volume to 50 or 100 µl followed by ethanol precipitation of the PCR product. Load the total volume. The kit has been tested and works with the protocol in this manual. It should not be necessary to increase the reaction volume on a routine basis.

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
Mitochondrial DNA deletion GeneProber™ GL557 Digoxigenin labeled	110 µL	40-2055-41

GScan™ Products 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

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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