Product Sheet

Myotonic Dystrophy Genemer™

Non-radioactive Myotonic Dystrophy CTG repeat genotyping

Catalog No. 40-2026-10 10 nmole

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

Myotonic Dystrophy Genemer™ Primer pair for amplification of CTG triple repeat spanning region.
The quantity supplied is sufficient for 400 regular 50 µl PCR reactions.

Background

Myotonic dystrophy (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. 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.

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 restriction enzyme digested with Bam HI or Pst I .The DNA blot is then hybridized with a DM CTG repeat specific DNA probe. For more information, refer to **GLDM GeneProber™**.

Material supplied

One tube containing 10 nmole lyophilized primers. The quantity supplied is sufficient for 400 regular 50 µl PCR reactions.

Reconstitution

1. **Stock Primer solution**: Add 50 µl sterile water to the tube containing the primers. The 10 nmole of primer when dissolved in 50 µl water will give a solution of 200 µM, i.e. 200 pmole/µl.
2. **Primer Mix**: Prepare a 10 pmole/µl Primer Mix solution. Example: Transfer 10µl of stock primer solution to a new tube. Add 190 µl sterile water to this tube. Label this tube as **Primer Mix 10 pmole/µl**.

PCR* reaction

PCR profile

Denaturation	94ºC	30 sec.
Annealing	63ºC	30 sec.
Elongation	72ºC	1 min.

30 cycles, followed by 7 min at 72ºC, and hold at 4ºC.

Electrophoresis

Load samples in a 1.5% agarose gel. Run gel with appropriate voltage and time according to the electrophoresis device in your lab.

Results and interpretation

Normal individuals have from 5 to 30 CTG repeats. For an individual with 10 CTG repeats, a 144-bp PCR product would be expected from the PCR reaction. A general formula, 114 + 3n bp, can be applied to calculate other sizes of CTG repeats, where n represents the number of CTG repeats.

References:

1. The International Myotonic Dystrophy Consortium (2000) Neurology 54: 1218-1221.
2. Steinbach, P. et al. (1998) Am. J. Hum. Genet. 62: 278-285.

Procedure

Important Information

Components required in the procedure below require components not supplied with this product. Catalog Number 40-2026-11 contains the additional components.

Triple repeats size analysis by PCR and detection by agarose gel electrophoresis yields gross fragment size amplification results. Exact repeat size should be determined by sequencing, fragment size analysis by fluorescent genetic analyzer using GScan™ kits or by PCRProber™ using appropriate size standards. For Southern blot analysis use GeneProber™ probes.

PCR Premix Preparation

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

Material Supplied: One tube containing 10 nmole lyophilized primers. The quantity supplied is sufficient for 400 regular 50 µl PCR reactions.

PCR Thermal Cycler Files

Prepare the following PCR thermal cycler files

Hot Start File	
Step	**Time and Temperature**
Denaturation	5 minutes at 94°C
Hold	60°C

DM CTG Repeat 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 20 µl reactions. This can be scaled up as required.

PCR Premix Preparation		
Component	**1 x 20 µl rxn.**	**10 x 20 µl rxns.**
DM Genemer™ Component A	14.0 µl	140 µl
PCR Component M	3.0 µl	30 µl
PCR Component N	2.0 µl	20 µl
Total	**19 µl**	**190 µl**

B. Enzyme premix preparation

Enzyme Mix Preparation		
Component	**1 x 20 µl rxn.**	**10 x 20 µl rxns.**
PCR premix (above)	3.0 µl	30 µl
Taq. Polymerase	0.5 µl	5 µl
Total	**3.5 µl**	**35 µl**

C. PCR reaction (20 µl)

'Hot Start' PCR

For each sample add the following

Hot Start PCR	
Component	**Quantity**
PCR premix (above)	16 µl
DNA Template (~100ng chromosomal DNA)	1 µl
Total	**17 µl**

Start "Hot Start" file.

After initial denaturation while thermal cycler is 'holding' at 60^{o}C

Add 3 µl of Enzyme premix to each tube and start DM amplification PCR file.

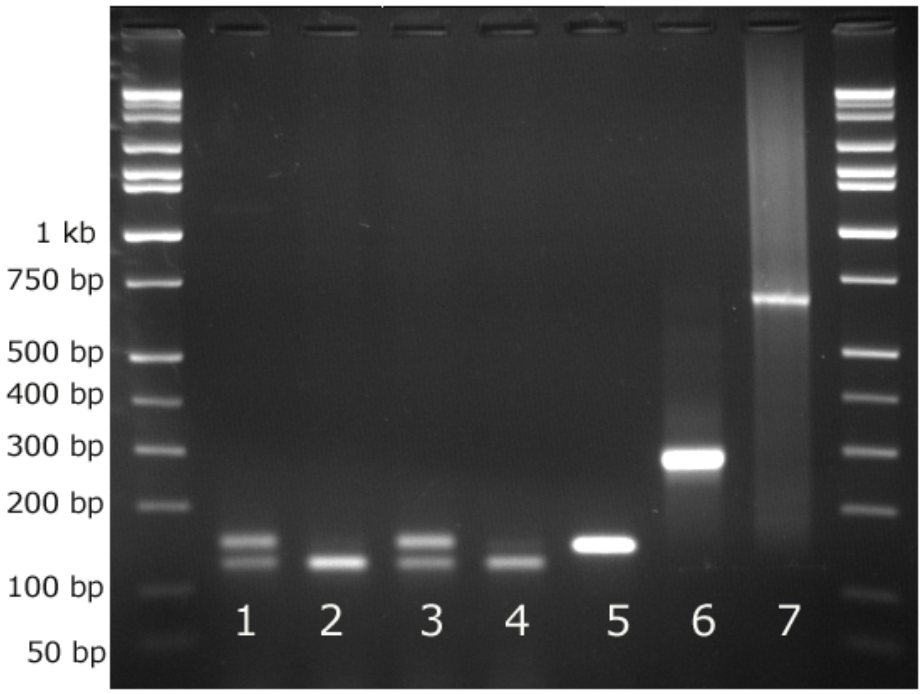
D. Gel Electrophoresis

1. Prepare a 2 % agarose gel with ethidium bromide. Follow appropriate safety procedures.
2. Load 10µl or more if required of the amplified product.
3. Follow established laboratory protocol for agarose electrophoresis.

Results and Interpretation

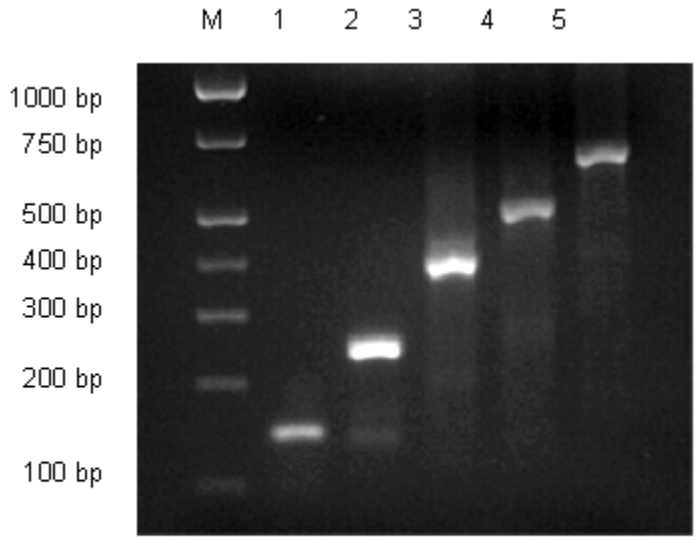
The results obtained from agarose gel electrophoretic pattern will approximately show the fragment size amplified, based on these results an interpretation can be made about the genotype of the sample. It is known that there is an overlap between the normal and DM allele sizes. The repeat sizes obtained 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.

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.



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.

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.

Myotonic Dystrophy Product Ordering Information

Product	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

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Genemer™ Product Ordering Information

Genemer™ Primer pair for gene or mutation specific amplification. Special optimized conditions may be required for certain amplifications

Product	Size	Catalog No.
Fragile X (spanning CGG triple repeat region) Genemer™; 10 nmols	10 nmols	40-2004-10
Huntington Disease (spanning CAG triple repeat region) Genemer™; 10 nmols	10 nmols	40-2025-10
Myotonic Dystrophy (spanning CTG triple repeat region) Genemer™; 10 nmols	10 nmols	40-2026-10
Friedreich's Ataxia (spanning GAA triple repeat region) Genemer™; 10 nmols	10 nmols	40-2027-10
Factor V Genemer™; 10 nmols	10 nmols	40-2035-10
Factor VIII (Hemophilia) Genemer™ Pack Genemer™; 10 nmols	10 nmols	40-2036-10
STS (Steroid Sulfatase) Genemer™; 10 nmols	10 nmols	40-2023-10
HGH (Human Growth Hormone) Genemer™; 10 nmols	10 nmols	40-2024-10
Sickle Cell Genemer™; 10 nmols	10 nmols	40-2001-10
RhD (Rh D gene exon 10 specific) Genemer™; 10 nmols	10 nmols	40-2002-10
Rh EeCc (Rh Ee and Cc exon 7 specific) Genemer™; 10 nmols	10 nmols	40-2003-10
Gaucher (various mutations) Genemer™; 10 nmols	10 nmols	40-2047-XX
Cystic Fibrosis (various mutations) Genemer™; 10 nmols	10 nmols	40-2029-XX
SRY (sex determining region on Y) Genemer™; 10 nmols	10 nmols	40-2020-10
X alphoid repeat Genemer™; 10 nmols	10 nmols	40-2021-10
Y alphoid repeat Genemer™; 10 nmols	10 nmols	40-2022-10

Genemer™ Control DNA Product Ordering Information

Genemer™ control DNA is a cloned fragment of the mutation region of a particular gene. These control DNA are an ideal genotyping template for optimizing and performing control amplification with unknown DNA.

Product	Size	Catalog No.
Sickle Cell Genemer control DNA (HbA, S and C available)	500 ng	40-2001-0X
GLFX CGG Genemer Control DNA; Fragile X (16, 29, 40, 60 & 90 CGG repeats available)	500 ng	40-2004-0X
GLHD CAG Genemer Control DNA; Huntington Disease (18, 34, 44, 89 & 134 CAG repeats available)	500 ng	40-2025-0X
GLDM CTG Genemer Control DNA; Myotonic Dystrophy (12, 45, 93, 129 & 194 CTG repeats available)	500 ng	40-2026-0X

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Related Products Ordering Information

Taq Polymerase & Master Mix		
Product	**Catalog No.**	**Unit Size**
Taq DNA Polymerase; 400 units; 5 µ/µL; 80 µL	40-5200-40	400 units
Taq PCR Kit; 200 x 50 µL reactions	40-5211-01	200 reactions
Taq PCR Kit with controls; 200 reactions	40-5212-01	200 reactions
PCR Master Mix (2X); 100 x 50 µL reactions (2 tubes x 1.3 mL)	40-5213-01	100 reactions
PCR Master Mix (2X); 200 x 50 µL reactions (4 tubes x 1.3 mL)	40-5213-02	200 reactions

Related Products Ordering Information

PCR Additives & Reagents		
Product	**Catalog No.**	**Unit Size**
Taq DNA Polymerase 300 units; 5 µ/µL; 60 µL	40-5200-30	300 units
PCR Buffer Standard (10 X); 1.6 mL	40-3060-16	1.6 mL
PCR Buffer Mg Free (10 X) ; 1.6 mL	40-3061-16	1.6 mL
Taq Polymerase Dilution Buffer; 1 mL	40-3070-10	1 mL
dNTP 2mM (10X) ; 1.1 mL	40-3021-11	1.1 mL
$MgCl_2$; 25 mM; 1.6 mL	40-3022-16	1.6 mL
Omni-Marker™ Universal Unlabeled; 100 µL	40-3005-01	100 µL
Primer and Template Mix; 500 bp; 40 reactions; 100 µL	40-2026-60PT	100 µL
Nuclease Free Water; 1.6 mL	40-3001-16	1.6 mL
DMSO; 1 mL	40-3031-10	1 mL
TMAC (Tetramethyl ammonium chloride) 100 mM; ; 1 mL	40-3053-10	1 mL
KCl 300 mM; 1 mL	40-3059-10	1 mL
Betaine; 5M; 1 mL	40-3032-10	1 mL

Omni-Marker™		
Product	**Catalog No.**	**Unit Size***
Omni-Marker™ Universal unlabeled; 100 µL	40-3005-01	100 µL
Omni- Marker™ Universal unlabeled; 500 µL	40-3005-05	500 µL
Omni-Marker™ Universal unlabeled; 1 mL	40-3005-10	1 mL
Omni- Marker™ Low unlabeled;100 µL	40-3006-01	100 µL
Omni-Marker™ Low unlabeled; 500 µL	40-3006-05	500 µL
Omni- Marker™ Low unlabeled; 1 mL	40-3006-10	1 mL
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp; 100 µL	40-3062-01	100 µL
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp; 500 µL	40-3062-05	500 µL

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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; 200 mL	40-5020-20	200 mL
Hybwash B, Hybridization Wash Solution; 100 mL	40-5021-10	100 mL
TAE Buffer; 50X Concentrate; 100 mL	40-3007-01	100 mL
TAE Buffer; 50X Concentrate; 1000 mL	40-3007-10	1000 mL
TBE Buffer; 5X Concentrate; 1000 mL	40-3008-10	1000 mL
10x Washing buffer; 200 mL	40-5025-20	200 mL
10% Blocking solution; 100 mL	40-5026-10	100 mL
Seq. Loading buffer; 1 mL	40-5027-00	1 mL
10x AP 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

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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