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Myotonic Dystrophy Confirmatory

INSTRUCTIONS FOR USE

Rv. 04 / 2019-05-27

Kit for the determination by fluorescent fragment analysis of the presence of healthy, premutated and mutated alleles of the DMPK gene of Myotonic Dystrophy type 1

CE

Catalog No. AD-MD-C-16

Store: Box 1 from -30ºC to -18ºC

Box 2 from 20ºC to 25ºC

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Intended Use

Adellgene® Myotonic Dystrophy Confirmatory is a kit designed for use in clinical laboratories which detects the number of repetitions of CTG of 3´UTR region of the DMPK gene located in chromosome 19 resulting in Myotonic Dystrophy disease. It aims to aid clinical diagnosis associated with clinical findings in myotonic dystrophy type 1 (DM1) that span from mild to severe symptoms.

The use of this kit is the confirmation of homozygous and detection of false homozygous for the occurrence of a higher range allele obtainable with Adellgene® Myotonic Dystrophy Screening kit. The technology is based on the polymerase chain reaction (PCR) of genomic DNA extracted and purified from peripheral blood followed by fluorescence analysis of the PCR fragments obtained in a genetic analyzer.

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Summary and explanation

Myotonic dystrophy type 1 or Steinert's disease is currently the most common form of muscular dystrophy in adults. It was first clinically recognized by Steinert (1) and Batten and Gibb (2) in 1909. Based on clinical ascertainment, worldwide prevalence is estimated to be 12.5/100000 (3), but it can be higher as many patients in older generation remain undiagnosed. Inheritance of this multisystem disease is autosomal dominant, and phenotypic expression is highly variable due to an unstable expansion CTG trinucleotide repeats in the 3´ untranslated region (3´UTR) of the dystrophia myotonica protein kinase gene (DMPK, MIM 605377) (4-6) located in the long arm of chromosome 19 (19q.21.3). The expanded DMPK gene produces a toxic RNA transcript that does not exit the nucleus (7).

Reference ranges for allele sizes were established by the Second International Myotonic Dystrophy Consortium (IDMC) in 1999 (8-11) for technical standards and guidelines for testing. Normal alleles: 5-34 CTG repeats; Mutable normal (premutation) alleles: 35-49 CTG repeats. Individuals with CTG expansions in the premutation range have not been reported to have symptoms, but their children are at increased risk of inheriting a larger repeat size and thus having symptoms (12). Full penetrance alleles >50 CTG repeats are associated with disease manifestations.

Clinical findings in myotonic dystrophy type 1 (DM1) span a continuum from mild to severe (13) and provide an excellent overview of all aspects of DM1. The clinical findings have been categorized into three somewhat overlapping phenotypes, mild, classic, and congenital, that generally correlate with CTG repeat size (Table 1) (8, 9, 14-17).

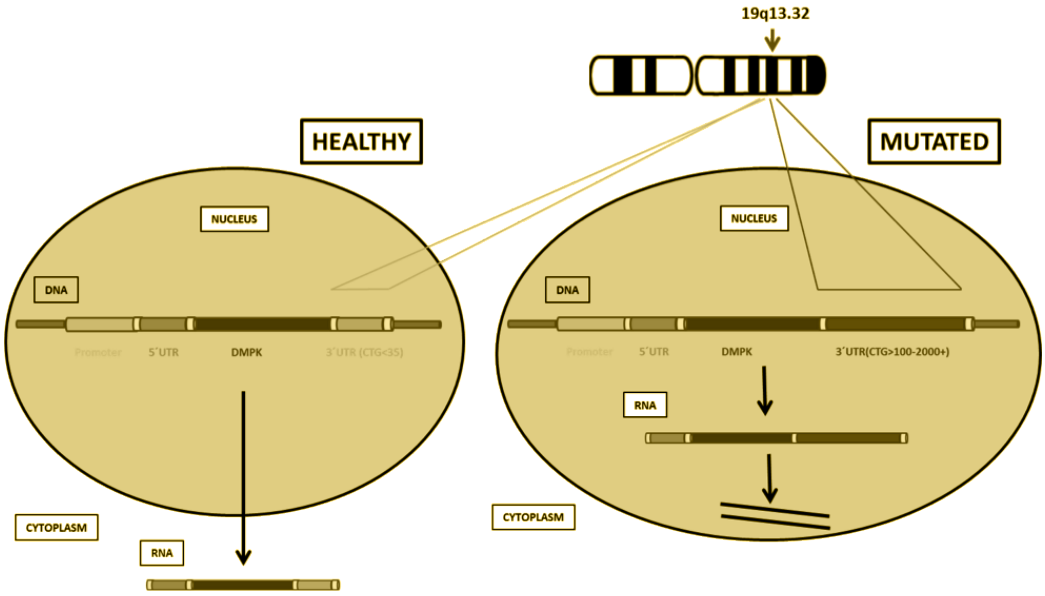

Figure 1. Representation of the DMPK gene types and their production depending on the degree of mutation

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Mild DM1 (50-150 CTG repeats) may have only cataract, mild myotonia, or diabetes mellitus. They may have fully active lives and a normal or minimally shortened life span (18).

Within Classic DM1 (100-1000 CTG repeats), only a rough correlation with severity of symptoms exists. Individuals with these CTG repeat sizes usually develop classic DM1 with muscle weakness and wasting, myotonia, cataracts, and often cardiac conduction abnormalities. While the age of onset for classic DM1 is typically in the 20s and 30s (and less commonly after age 40 years), classic DM1 may be evident in childhood, when subtle signs such as myotonic facies and myotonia are observed. In the case of Congenital DM1 (>1000 CTG repeats), a transmission ratio distortion at conception favors transmission of larger CTG repeats than those present in the parent (19). Presence of a large repeat may lead to earlier onset and more severe disease, known as congenital

Phenotype	Clinical Signs	CTG Repeat Size	Age of Onset
Mutable normal (premutation)	None	35 to 49	Non defined
Mild	Cataracts		
	Mild myotonia	50 to ~150	20 to 70 yrs
Classic	Weakness		
	Myotonia		
	Cataracts		
	Balding		
	Cardiac arrhythmia		
	Others	~100 to ~1000	10 to 30 yrs
Congenital	Infantile hypotonia		
	Respiratory deficits		
	Intellectual disability		
	Classic signs present in adults	>1000	Birth to 10 yrs

Table 1. Correlation of Phenotype and CTG Repeat Length in Myotonic Dystrophy Type 1

DM1 (20, 21). Congenital DM1 often presents before birth as polyhydramnios and reduced fetal movement. After delivery, the main features are severe generalized weakness, hypotonia, and respiratory compromise. Mortality from respiratory failure is common. Surviving infants experience gradual improvement in motor function. Affected children are usually able to walk; however, a progressive myopathy occurs eventually, as in the classic form (15). These individuals may develop any of the typical features of DM1 including weakness, myotonia, cataracts, and cardiac problems. Intellectual disability is present in 50%-60% of individuals with congenital DM1. Autism spectrum disorder, common mood/anxiety disorders, impaired attention, and abnormal visual-spatial abilities have been reported (22, 23). Children with DM1 may have low visual acuity, hyperopia, or astigmatism (24).

The Primer Mix Confirmatory (PM2) is used for the confirmation of healthy and premutated (5-49), mild (50-150), partial classic (100-200) or (classic/congenital (>200) patients obtained with Adellgene® Myotonic Dystrophy Screening kit. The technology is based on the triplet primed polymerase chain reaction (TP-PCR) amplification of genomic DNA extracted and purified from peripheral blood followed by fluorescence analysis of the size of the PCR fragments obtained by genetic analyzer.

Principles of the procedure

The detection method employed by the Adellgene® Myotonic Dystrophy Confirmatory kit is based on the specific amplification of genomic DNA from purified fragment containing 3´UTR region of the DMPK gene, which containing the CTG repeats. PM2 includes a set of primers to do a triplet primed repeat PCR to test all the repeats. The use of ROX1000™ size marker is recommended.

Kit contents

Reference AD-MD-C-16 (16 tests)

Box 1 of 2

- AD-MD-PM2: Primer Mix Confirmatory (PM2). 1 vial x 135 µl
- AD-MD-POM: Polymerase Mix (POM). 1 vial x 344 µL

Box 2 of 2:

- AD-PUR-16: including all the reagents and plastics for purifying the amplicons of 16 samples:
    - Capture buffer: 9,8 mL
    - Wash buffer: 2.0 mL (see how to prepare in section Procedure / D)
    - Elution buffer: 300 µl
    - Micro - columns: 17 units
    - Collection tubes: 17 units

Kit storage

- Box 1 should be stored from -30ºC to -18ºC. Box 2 should be stored from 20ºC to 25ºC. Reagents are stable at these temperatures until their expiration date as indicated on the package.
- Allow the reagents (except AD-MD-POM) to reach room temperature before use. Shake gently all reagents except AD-MD-POM after thawing.
- Before to open the reagents tubes, perform a gentle centrifugation of each component so that the reagents are deposited on the bottom of the tube and no loose by the walls thereof.
- The test should be done keeping reagents on ice or cold block.
- Do not make more than 3 cycles of freeze / thawing the vials of Primer Mix (AD-MD-PM2), and Polymerase mix (AD-MD-POM) as this may reduce the sensitivity of the test and alter the results. It is recommendable to establish aliquots of these reagents, at first thawing cycle, depending of the use of the kit.
- Due to the photo-sensitive nature of the reagent AD-MD-PM2, avoid continued exposure to light.

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Materials required but not supplied

Isolation and Purification Reagents

- Reagents for DNA isolation and purification are not included. The DNA can be extracted by any method validated in the laboratory to ensure high quality and that the DNA is intact.
- Ethanol absolute

Capillary Electrophoresis Reagents (recommended)

- Genetic Analyzer for polymer POP-7 (ABI: 3130, 3730 or 3500).
- POP-7 polymer (ABI, Ref: 4363785 or equivalent)
- High purity formamide (Hi-Di Formamide; ABI, Ref.: 4311320 or equivalent)
- Calibrators for fluorophores FAM y ROX (ABI, Ref.:4345827, 4345829 or equivalent)
- Size standard marker ROX ™ 1000 (ABI, Ref.: 401098; Eurogentec, Ref.: MW-0195-80ROX)

Supplies and equipment

- General equipment of a laboratory dedicated exclusively to performing the PCR.
- Thermo cycler (ABI 9700, Veriti or equivalent)
- Centrifuge for 96-well plates
- Shaker (Vortex)
- Micro-centrifuge
- Pipettes (P1000, P200, P20 and P2) and filter tips specific pipette.
- PCR plates of 96 wells (optional)
- PCR Plate Sealer (optional)

Positive Control

- The recommended WHO standard for Fragile-X syndrome or any cell line whose DNA corresponds with a validated sample

Sample collection and preparation

- All biological and blood samples should be treated as potentially infectious. When handling, basic or 'universal' precautions must be observed. Any sample handling should be done with appropriate personal protection such as goggles, gloves and appropriate clothing.
- This test should only be performed with whole blood samples treated with EDTA or ACD anticoagulants. Heparin can interfere with PCR and should not be used in this procedure. No hyperlipemic, hemolyzed, jaundiced or proteinemia blood samples should be used
- DNAses contamination can produce DNA degradation, so should be used with filter pipette tips and DNAse-free tubes. To take care with handling, pipetting and working systems to avoid PCR failure.
- Do not use components from different lots. Do not use reagents beyond the expiration date.
- Before to use the kit, to ensure that the equipment (thermal cycler, genetic analyzer, ...) has been calibrated according to the manufacturers´ instructions.

Caution

Kit toxicological properties have not been studied in depth so it is recommended to avoid contact with skin and mucous membranes. Do not ingest. Manual Safety Data Sheets (MSDS) are available to the customer.

Procedure

A. DNA extraction

Extraction of genomic DNA by standard methods from whole blood collected with EDTA is compatible with the use of this kit. It is recommended to perform the evaluation of the quality and quantity of DNA obtained by measurements of absorbance at 260nm (OD260; concentration) and the ratio of the measurements at 260 and 280 nm (OD; purity). The DNA obtained can be stored at -20°C until use. The appropriate amount of DNA in each PCR reaction is about 50 ng (e.g.: 1 µl of DNA of 50 ng/µl).

B. Preparation and conditions of the PCR reaction

Precautions

- Thaw all kit components before starting the test, mix and centrifuge.
- Working on ice or in a cold block.
- The PCR should be mounted in the pre-PCR with all precautions discussed above.
- Use only filter tips and autoclaved 1.5 mL tubes.
- Always wear gloves and lab coat.

1. Mix preparation of the Primer Mix and Polymerase Mix for n+2 samples:

Reagent	Vol. for each sample (ul)
AD-MD-PM2	7,3
AD-MD-POM	18,7
Total volume for each reactions	**26**

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- To shake the sample gently 3-5 times before distribution in PCR tubes ensuring of the complete homogenization
- To perform a pulse centrifugation to ensure that there is no sample in the tube walls or bubbles.

NOTE: Excess AD-MD-PM2 could inhibit the PCR reaction

2. To pipette 26 µl of this mixture on an optical plate or sterile tube and add DNA volume subsequently needed to get 45-50 ng (p.e.: 1 µl of DNA to 45-50 ng/µl) or negative control in the case of contamination control well. In the case of low DNA concentration, a higher volume of DNA could be added independently of the increase of the total volume of the PCR reaction.

3. To seal the plate with coverslips (including with plates) or the tube and perform a pulse centrifugation to ensure mixing and there are no bubbles.

4. To place the plate in the thermo cycler and perform the PCR reaction.

	Number of cycles	Temperature (ºC)	Time (mm:ss)
DENATURATION	1	98	05:00
CYCLES	30	97	00:35
		64	00:35
		68	04:00+ 00:20SEG/CY
FINAL EXTENSION	1	72	30:00
COOLING	1	4	∞

C. Confirmation of the amplification products

Confirmation of the amplification products could be made through an appropriate system as the horizontal electrophoresis in agarose gels. To prepare an agarose gel at a concentration of 1-1.2% w/v validated according to the lab protocol and analyze 2ul of each amplified to certify that the PCR was successful.

D. Preparation of the samples for capillary electrophoresis

1. Purification of the PCR reaction

- Use the Box 2 of 2 included with the kit. The protocol is:

The first time that Box 2 is used, it is necessary to add 8mL of absolute ethanol to the Wash Buffer vial. Mark the corresponding box on the label of that vial to indicate that the addition has been made.

a. Add 500 ul Capture buffer to the sample (to use 17 ul of PCR volume)

b. Mix thoroughly.

c. Check that the capture buffer plus sample is yellow or pale orange in color

d. For each purification that is to be performed, place one micro-column into one collection tube

e. Centrifuge capture buffer with the sample briefly to collect the liquid in the bottom of the tube

f. Load the capture buffer with the sample onto the assembled micro-column and collection tube.

g. Spin the assembled column and collection tube at 16000 g for 30 seconds.

h. Discard the flow through by emptying the collection tube. Place the micro-column back inside the collection tube.

i. Add 500 ul Wash buffer to the micro-column.

j. Spin the assembled column and collection tube at 16000 g for 30 seconds.

k. Discard the collection tube and transfer the micro-column to a fresh DNase-free 1,5 ml microcentrifuge tube.

l. Add 10 ul Elution buffer to the center of the membrane in the assembled micro-column and sample collection tube.

m. Incubate the assembled micro-column and sample collection tube at room temperature for 1 minute.

n. Spin the assembled column and sample collection tube at 16000 g for 1 minute to recover the purified DNA.

o. Proceed to the general protocol.

2. Preparation of Samples for DNA analyzer

The following reaction mixture is performed with the PCR product. For amplicons of PM2, it is recommended to use from 1 ul to 2ul for the 3130/3130XL and from ½ to 1ul for the 3730xl DNA analyzer or to check the optimal volume between 1/2 or 2 ul to charge in the DNA analyzer.

Reagent	Vol. for each sample (µl)
PCR purified product	1 **
Hi-Di-Formamide *	10
LIZ500™ Maker *	0.3
Total volume for each reactions	**11.3**

** These items are not supplied with the kit*

*** See recommended volume for the PCR product of PM2 depending on the analyzer.*

- To perform a pulse of centrifugation (3-5 times) to ensure mixing of the reagents and transfer 2 µl the mixture to the corresponding plate for capillary electrophoresis
- To seal the plate, shake and centrifuge to remove bubbles and transfer to the thermal cycler.
- To denature 2 minutes at 95°C and transfer to ice protecting the sample from light until injection into the genetic analyzer.

It is recommended to use a positive control with a control sample which the number of repetitions is known (see section 6).

3. Module of DNA Analyzer

The following module of work is recommended for the corresponding DNA analyzer:

	DNA ANALYZERS	
PARAMETERS	3130/3130xl	3730xl
Oven Temperature	60 ºC	63 ºC
Poly Fill Vol.	7300 steps	6500 steps
Current Stability	5.0 µAmps	5.0 µAmps
Pre-Run Voltage	15.0 KVolts	15.0 KVolts
Pre-Run Time	180 sec	180 sec
Injection voltage	3.0 KVolts	1.6 KVolts
Injection time	15 sec	30 sec
Voltage Number of steps	20 nK	20 nK
Voltage Step Interval	15 sec	15 sec
Data Delay Time	60 sec	60 sec
Run voltage	15.0 kVolts	15.0 kVolts
Run time	3000 sec	2200 sec

NOTE: In both cases is recommended the use of polymer POP7

Results and interpretation

Adellgene® Myotonic Dystrophy Confirmatory is a complement technique for confirming the number of CTG triplet repeats obtained from Adellgene® Myotonic Dystrophy Screening. In the case of one sample had only one peak from Screening PM we could assure or discard the homozygosis of this sample using PM2 of the present kit. If the sample has two clear peaks, this component would confirm the presence of these peaks.

The resulting electropherogram of the Confirmatory kit includes the full length PCR products generated from the CTG repeat primed peaks. These peaks are separated by 3 bp, as expected and set up as a decreasing peak´s saw. The profile of peaks provides confirmatory information about a sample as previously mentioned.

Figure 2 represents examples of Screening kit and the corresponding Confirmatory patterns for homozygosis or heterozygosis of several samples (samples 1-3).

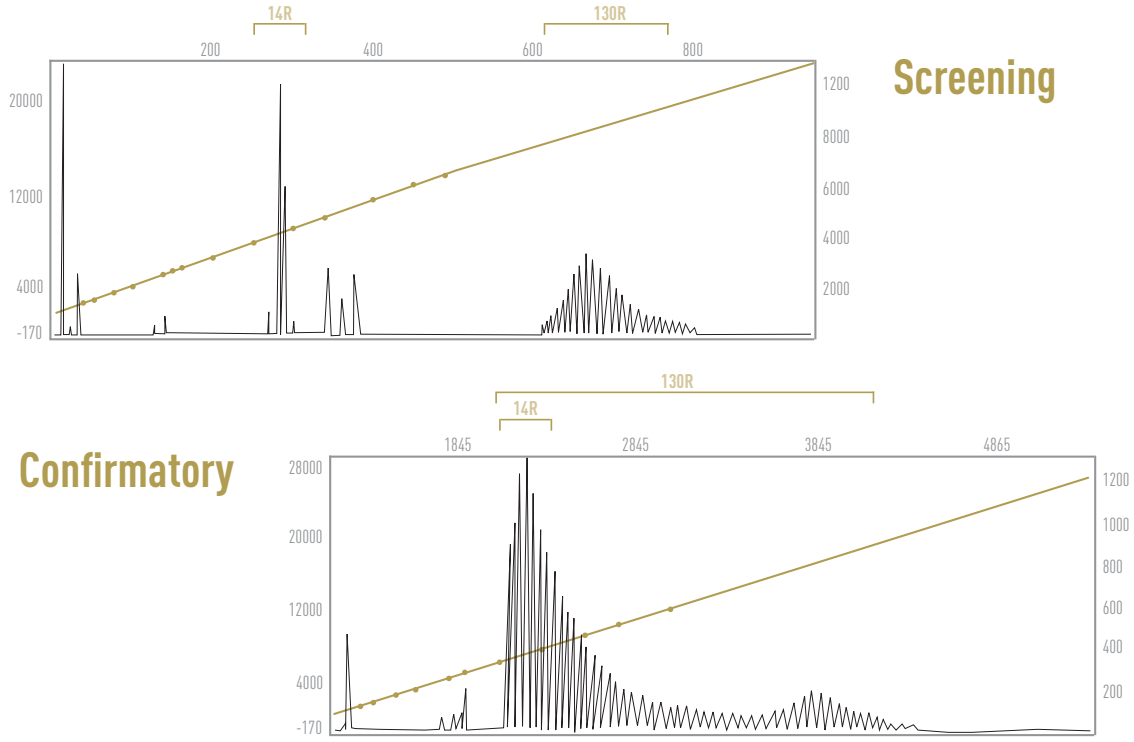

Figure 2. Fragment analysis of Myotonic Dystrophy Screening and Confirmatory. Sample 1

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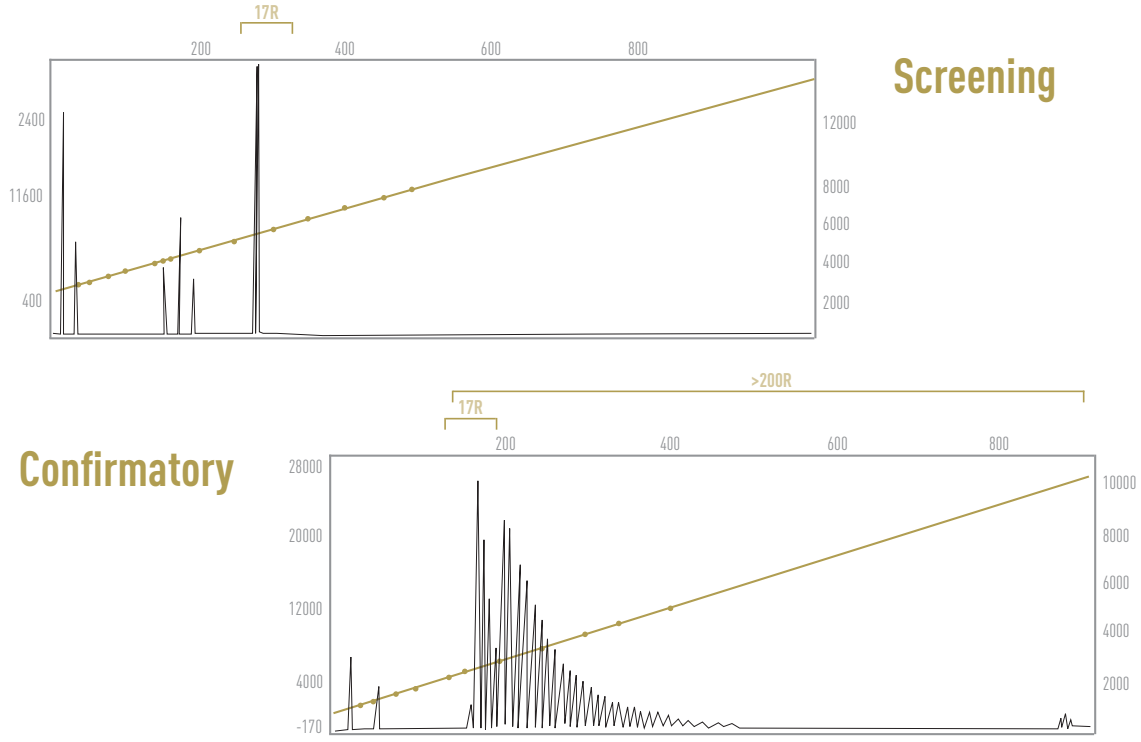

Figure 2. Fragment analysis of Myotonic Dystrophy Screening and Confirmatory. Sample 2

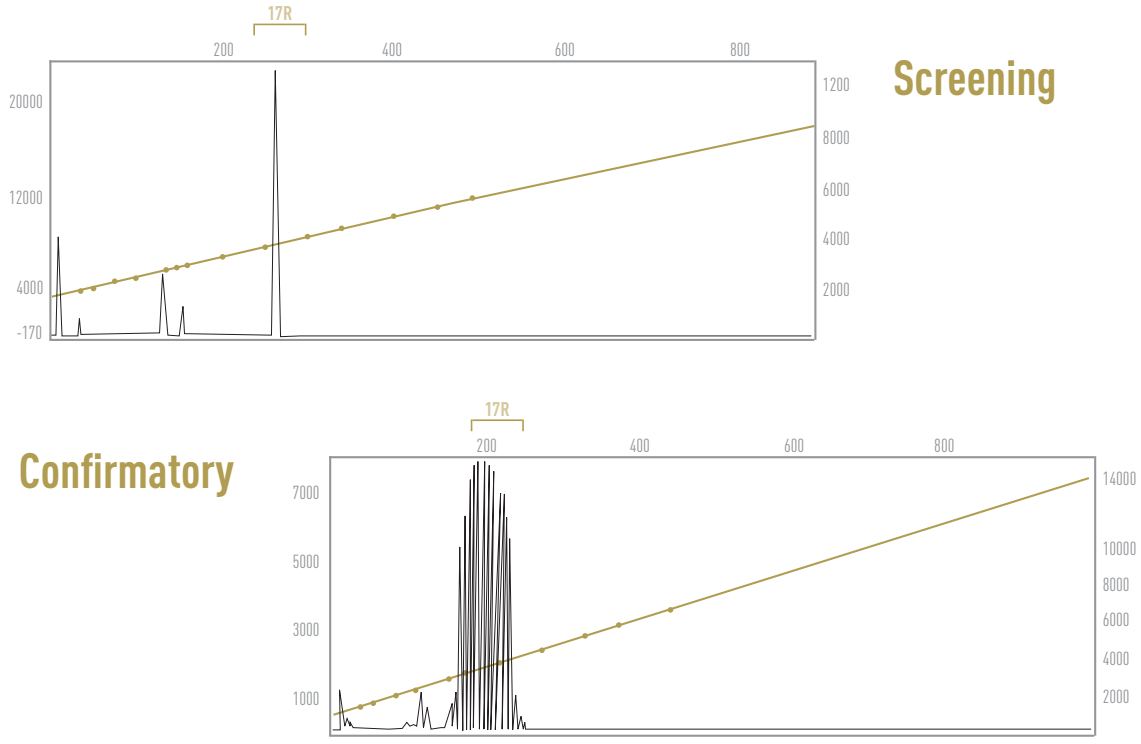

Figure 2. Fragment analysis of Myotonic Dystrophy Screening and Confirmatory. Sample 3

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Quality control

Due to the quantitative nature of this test, it is necessary to perform calibration of the FAM and LIZ fluorophores in the genetic analyzer.

Furthermore, as described in the procedure, the addition of the marker fragment analysis LIZ 500™ for determining the size of the DNA fragments is required.

A contamination control should carry out by replacing the DNA for a negative control and a positive control of a known size (see section Materials required but not supplied).

The user should consider all precautions named in section Sample collection and preparation and the limitations in section Procedure limitations of this procedure.

The test shall be carried out as recommended by this protocol, as well as with other quality control procedures that comply with local, state, federal and / or certifying agencies.

Specific data of performance

1. Analytical specificity and sensitivity

The primers of this kit are specific of the human Myotonic Dystrophy gene DMPK and comprise the CTG repeat region within of 3´UTR of the gene. The specific amplification of this region was verified by DNA sequencing and by assaying samples of healthy individuals and patients well characterized. There is no reported case of cross-reactivity with another gene from genomic DNA.

2. Diagnostic specificity

Adellgene® Myotonic Dystrophy T1 kit component 1 is 3´UTR region DMPK specific assay to detect the number of CTG repeats. If only one peak is found, the use of Component 2 of the kit or other alternative technique is necessary to confirm the presence or not of unhealthy alleles (>200 CTG repeats). Mutations (point mutations, insertions, deletions) at amplification primer sites are possible and may result of the lack of allele definition. Other technologies could be necessaries to resolve the typing. Homozygous results must be confirmed by alternative procedures.

3. Range of

- DNA concentration

  For the best range of amount of DNA in each assay tests were performed between 10 ng to 200 ng with a sample heterozygous female. The assignment of peaks obtained size was independent of the amount of DNA used. The recommended working range is between 45-50 ng of DNA by PCR.

- Results of the kit

  The method of this kit can assign a number of CTG triplet repeats of between 5 and 200 repeat alleles. The use of this kit is the determination of both healthy individuals (5 to 49) repetitions and patients affected with 50 repetitions or more with different penetrance (see section 2).

4. Accuracy

- Assignment of fragment size

  The assignment of the peaks is given by the size of the amplicon obtained and peak morphology. If there are several peaks, it will be considered the highest and the central peak. The accuracy of the number of

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the repeats was determined comparing sequenced samples with the sizes obtained with the present kit comprising a variability of No. of repeats ± 1 for healthy alleles and ±3 for 50-200 repetitions alleles.

- General testing

Region 3'UTR/DMPK	Samples Tested
Analyzed samples with 2 normal alleles (5-34 rep.)	17
Analyzed samples with 1 premutated allele (35-49 rep.)	1
Analyzed samples with 1 mutated allele (>50 rep.)	38
Total number of analyzed samples	**56**

- Interferences

A number of substances which may be present in the peripheral blood that may potentially interfere with the PCR-based methodology, inhibiting polymerase activity have been described in the literature. Therefore it is necessary that the DNA obtained has the purity required to avoid the interference. Most DNA extraction standard methods eliminate these substances and therefore it is recommended that the DNA extraction method used in the laboratory for this point is validated.

Procedure limitations

- The method detects alleles from 5 repeats and stablish a pattern of more than 200 CTG repeats (see section Results and interpretation).
- The conditions described for the PCR should be accurately controlled. Deviations from these parameters can lead to poor outcome.
- All work from Adellegene® Myotonic Dystrophy kit must be made in accordance with good laboratory practices and in compliance with local regulations, such as the international standard.
- The thermo cycler must be calibrated according to manufacturer's recommendations and used within the limits specified by them.
- The DNA analyzer must be calibrated according to the manufacturer's recommendations for the fluorophores used in compliance with the limits specified by them.
- Do not mix components from other kits and lots.
- Do not use the kit after the expiry date exceeded.
- Do not use the kit in case of suspected loss of reactivity, contamination, deterioration of the container or any other incident that may affect performance.
- The interpretation of the data and typing results should be reviewed by qualified personnel.
- Remove expired reagents following applicable regulations.

Troubleshooting guide

PCR negative control is positive

- **Primer mix contamination**
  - Repeat the experiment with new aliquots of Primer mix / Negative Control
  - Handle kit components provided as commonly accepted practices to avoid contamination.
  - Check storage and handling conditions.
  - Discard contaminated reagents
- **Contamination in the Pre-PCR area**
  - Confirm that the necessary precautions have been followed in the PCR area.
  - Check possible contamination problems in other PCR techniques
  - Confirm the suitability of the consumables used (tubes, pipette tips)
  - Confirm that the Taq is not contaminated
- **Pipetting error**
  - Verify that the sample added always corresponds to the assigned worksheet

Weak or absent signal in the PCR product

- **Poor quality of DNA samples**
  - Repeat the extraction of DNA
- **Samples with very low concentration of DNA**
  - Check the DNA concentration
- **DNA samples with high concentration**
  - Make a preliminary assessment of the extraction system by testing sample dilutions
- **Presence of PCR inhibitors in genomic DNA**
  - Avoid the use of whole blood containing heparin. Re-extract DNA and repeat PCR when possible
- **DNA polymerase not added to master mix or insufficient mixing of PCR mix**
  - Repeat PCR ensuring all components are added and mixed sufficiently

- Thermal cycling problems
  - Check the thermal cycling run parameters and ensure thermal cycler is operating according to manufacturer´s specifications and maintenance
- No Ethidium bromide (or other alternative DNA colorant) is added
  - Ensure Ethidium bromide is added to gel and electrophoresis buffer

Incorrect band size or number of bands

- Incorrect kit used
  - Check the appropriate kit is used
- Incorrect thermal cycling program used
  - Check thermal cycler parameters
- PCR contamination
  - Check the negative control. Proceed with descontamination protocols and repeat PCR to identify the origin of contamination

Weak signal of electropherograms

- Degradation of the kit
  - Confirm proper storage of the kit
  - Avoid over 3 cycles of freeze / thaw of reagents
  - Perform aliquot fractions reagents as necessary.
  - Repeat with fresh reagents batch
- Taq polymerase activity lost
  - Confirm the activity of Taq polymerase
  - Repeat with a new Taq polymerase
- Weak PCR product
  - Check gel image and proceed accordingly
- Insufficient reaction products applied to DNA analyzer
  - Check analyzer parameters
- Wrong purification process
  - Take extreme care in purification process

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Negative control simple yields a positive result

- Cross-contamination
  - Handle always the kit components according to commonly accepted practice to avoid contamination.
- Pipetting error
  - Verify that the sample added in a given position always corresponds to the assigned worksheet

High fluorescence intensity

- Too much PCR product
  - Check gel image. Dilute PCR product
- Too much product applied to the DNA analyzer
  - Check instrument parameters
- Pipetting error
  - Verify that the volume added in each well is correct

High backgroud (noisy baseline)

- PCR product contaminated
  - See above
- Poor PCR purification
  - Ensure purification process was perfomed following mentioned instrucions

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References

1. H. Steinert, "Uber das klinishe und anatomische bild des muskelschwundes der myotoniker," Deutsche Zeitschrift f¨ur Nervenheilkunde, vol. 37, p. 38, 1909.

2. F. E. Batten andH. P.Gibb, "Myotonia atrophica," Brain, vol. 32, no. 2, pp. 187–205, 1909.

3. P. S. Harper, Myotonic Dystrophy, WB Saunders, London, UK, 3rd edition, 2001.

4. J. D. Brook, M. E. McCurrach, H. G. Harley et al. et al., "Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member," Cell, vol. 68, no. 2, pp. 799–808, 1992.

5. M. Mahadevan, C. Tsilfidis, L. Sabourin et al., "Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene," Science, vol. 255, no. 5049, pp. 1253–255, 1992.

6. Y. H. Fu, A. Pizzuti, R. G. Fenwick et al., "An unstable triplet repeat in a gene related to myotonic muscular dystrophy," Science, vol. 255, no. 5049, pp. 1256–1258, 1992.

7. Amin Haghighat Jahromi1,2, Masayoshi Honda3, Steven C. Zimmerman1,2,* and

Maria Spies3,* Single-molecule study of the CUG repeat–MBNL1 interaction and its inhibition by small molecules. Nucleic Acids Research, 2013, 1–11

8. International Myotonic Dystrophy Consortium. New nomenclature and DNA testing guidelines for myotonic dystrophy type 1 (DM1). Neurology 2000;54;1218-1221

9. Moxley RT, Meola G. The Myotonic Dystrophies. In: The Molecular and Genetic Basis of Neurologic and Psychiatric Disease. Rosenberg RN, DiMauro S, Paulson HL, Ptacek L, Nestler EJ, eds. Philadelphia, PA: Wolters Kluwer; 2008:532-41 .

10. Prior TW; American College of Medical Genetics (ACMG) Laboratory Quality Assurance Committee. Technical standards and guidelines for myotonic dystrophy type 1 testing. Available online. 2009. Accessed 5-8-13.

11. Kamsteeg EJ, Kress W, Catalli C, Hertz JM, Witsch-Baumgartner M, Buckley MF, van Engelen BG, Schwartz M, Scheffer H. Best practice guidelines and recommendations on the molecular diagnosis of myotonic dystrophy types 1 and 2. Eur J Hum Genet. 2012;20:1203–8.

12. Martorell L, Cobo AM, Baiget M, Naudó M, Poza JJ, Parra J. Prenatal diagnosis in myotonic dystrophy type 1. Thirteen years of experience: implications for reproductive counselling in DM1 families. Prenat Diagn. 2007;27:68–72.

13. Udd B, Krahe R. The myotonic dystrophies: molecular, clinical, and therapeutic challenges. Lancet Neurol. 2012;11:891–905.

14. Gharehbaghi-Schnell EB, Finsterer J, Korschineck I, Mamoli B, Binder BR. Genotype-phenotype correlation in myotonic dystrophy. Clin Genet. 1998;53:20–6.

15. Harper PS. Major Problems in Neurology: Myotonic Dystrophy. London, UK: WB Saunders.

16. de Die-Smulders CE, Howeler CJ, Thijs C, Mirandolle JF, Anten HB, Smeets HJ, Chandler KE, Geraedts JP. Age and causes of death in adult-onset myotonic dystrophy. Brain. 1998;121(Pt 8):1557–63.

17. Mathieu J, Allard P, Potvin L, Prevost C, Begin P. A 10-year study of mortality in a cohort of patients with myotonic dystrophy. Neurology. 1999;52:1658–62.

18. Arsenault ME, Prevost C, Lescault A, Laberge C, Puymirat J, Mathieu J. Clinical characteristics of myotonic dystrophy type 1 patients with small CTG expansions. Neurology. 2006;66:1248–50.

19. Dean NL, Loredo-Osti JC, Fujiwara TM, Morgan K, Tan SL, Naumova AK, Ao A. Transmission ratio distortion in the myotonic dystrophy locus in human preimplantation embryos. Eur J Hum Genet. 2006;14:299–306.

20. De Temmerman N, Sermon K, Seneca S, De Rycke M, Hilven P, Lissens W, Van Steirteghem A, Liebaers I. Intergenerational instability of the expanded CTG repeat in the DMPK gene: studies in human gametes and preimplantation embryos. Am J Hum Genet. 2004;75:325–9.

21. Rakocevic-Stojanovic V, Savic D, Pavlovic S, Lavrnic D, Stevic Z, Basta I, Romac S, Apostolski S. Intergenerational changes of CTG repeat depending on the sex of the transmitting parent in myotonic dystrophy type 1. Eur J Neurol. 2005;12:236–7.

22. Ekström AB, Hakenäs-Plate L, Samuelsson L, Tulinius M, Wentz E. Autism spectrum conditions in myotonic dystrophy type 1: a study on 57 individuals with congenital and childhood forms. Am J Med Genet B Neuropsychiatr Genet. 2008;147B:918–26.

23. Douniol M, Jacquette A, Cohen D, Bodeau N, Rachidi L, Angeard N, Cuisset JM, Vallée L, Eymard B, Plaza M, Héron D, Guilé JM. Psychiatric and cognitive phenotype of childhood myotonic dystrophy type 1. Dev Med Child Neurol. 2012;54:905–11.

24. Ekström AB, Tulinius M, Sjöström A, Aring E. Visual function in congenital and childhood myotonic dystrophy type 1. Ophthalmology. 2010;117:976–82.

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