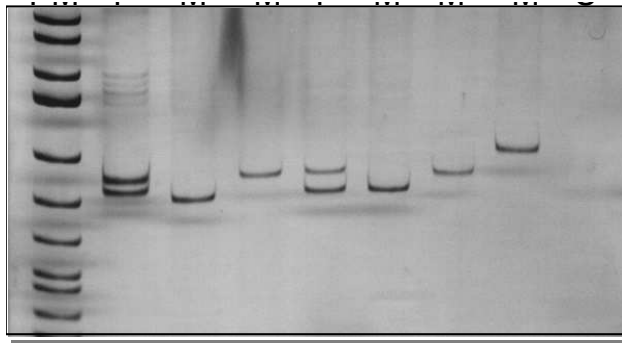


 ATGen SISTEMAS MOLECULARES	Code: KIT-001	Ver: 1
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Pre-FRAXA

Identification system of the number of CGG repeats in the FMR1 gene



Reg. MSP 2287

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The purchase of this product does not provide a license to carry out patented applications.

Kit Utility

The test allows identifying the number of CGG repeats in the region 5' UTR of the FMR1 gene, within the normal range of copies (6-60). The number of repeats is estimated from the size of the amplification product in the electrophoresis in acrylamide gel. As a control of the FMR1 amplification, a 5' fragment of the repeated region is also amplified.

Principle of the test

The Pre-FRAXA kit is based on the PCR amplification of a fragment of the FMR1 gene's 5' UTR region, which contains the CGG repeats region.

X Fragile Syndrome

The X Fragile Syndrome is the most frequent cause of inherited mental retardation, with an average incidence of 1 in 1,500 boys and 1 in 2,000 girls. It is associated with mental retardation both in men (severe) and women (less severe).

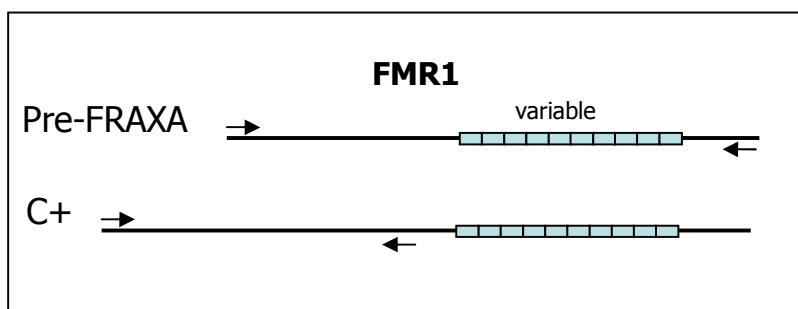
The FMR1 gene is located in the Xq27.3 region of the X-Chromosome. In the gene's 5' untranslated region (UTR) there exists a polymorphic CGG repetition that due to expansion generates most of the mutations associated with the X Fragile Syndrome. This expansion overlaps with a fragile region of the X-Chromosome known by cytogeneticists as FRAXA. These CGG repeats are classified into normal (6 to 40 repeats), intermediate (41 to 60 repeats), pre-mutation (61 to 200 repeats) and complete mutation (with more than 230 repeats). In normal individuals this region varies between 5 and 50 repeats, while in the X Fragile Syndrome there are more than 200 repeats.

Validity of the test

Within the 6-60 repeats range, the test has 100% sensitivity when performed in males and heterozygote women (with different number of CGG repeats in each allele). In women it is not possible to discern among a normal homozygote genotype (with the same number of repeats in both alleles) and a woman with a normal allele and an X-Fragile allele. The test does not allow the diagnosis of X-Fragile (detection of more than 200 CGG repeats), but the assertion of normal cases.

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Experimental Strategy



Kit Presentation

The Pre-FRAXA Kit includes:

- 1 Pre-FRAXA reaction mix tube, containing KCl, Tris-HCl, Triton, MgSO₄, dNTPs, and oligonucleotides.
- 1 Pre-FRAXA C+ reaction mix tube, containing KCl, Tris-HCl, Triton, MgCl₂, dNTPs and oligonucleotides.
- 1 Pre-FRAXA positive control DNA, containing a solution of heterozygote DNA in TE (Tris-HCl, EDTA). Once defrosted it is recommended to keep it at 4 °C.
- 1 Pre-FRAXA Taq DNA polymerase tube.
- 1 Pre-FRAXA Taq DNA polymerase C+ tube.

The kit must be kept at -20 °C.

Our kits are available in two sizes: 10 and 20 reactions.

Necessary materials not provided with the test

- DNA-free PCR tubes
- Suitable Pipettes
- Filtered pipette tips
- Gloves.
- Thermocycler
- Cuvette for vertical electrophoresis
- Acrylamide, electrophoresis buffer and loading buffer
- Gel coloring system with silver nitrate.
- Vortex
- Bio-hazard disposable container

Precautions

1. Only for in-vitro use.
2. All samples, reagents and controls should be considered as potentially infectious.
3. Do not use after the expiration date indicated on the package.



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Storage and Stability

The kit must be stored at -20°C in order to assure its optimal performance through the expiration date indicated on the package.

Specimen Characteristics

The sample must be a DNA solution with a 150-200 ng / µl concentration, apt for PCR amplification.

ATGen recommends obtaining the DNA from blood specimens by using ADN Facil kit.

Protocol

Defrost both reaction mixes (Pre-FRAXA and C+) and shake them vigorously by vortexing.

If possible, perform all manipulations in cold room.

Preparation of the amplification mix:

It should be done in the Pre-amplification area.

- 1. Add 18 µl of reaction mix per sample to be tested.
- 2. Add 0.3 µl of Pre-FRAXA Taq ADN polymerase to the Pre-FRAXA reaction mix per sample to be tested.
- 3. Add 0.5 µl of Pre-FRAXA Taq ADN polymerase C+ to the Pre-FRAXA C+ reaction mix per sample to be tested.
- 4. Homogenize by moderate vortexing or pipetting.

It is recommended to prepare a single amplification mix containing the necessary quantities of reaction mix and DNA Taq polymerase, according to the number of samples to be analyzed.

It is necessary to add two reactions, one for the positive control and one for the negative control.

Note: It is suggested to add an extra volume (approx. 10%) of each reactive to the amplification mix in order to compensate pipetting errors.

Amplification:

- 5. Aliquot the amplification mix, dispensing 18 µl in properly labeled PCR tubes.
- 6. Add 2 µl of sample to each tube.

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The samples should contain 150 - 200 ng of DNA (We recommend to use ATGen's ADN Facil Kit for the DNA extraction).

- 7. Add 2 µl of Pre-FRAXA control DNA into the positive control tube and 2 µl of the water previously used to dissolve the sample DNA in the negative control tube.
- 8. Run the programs for Pre-FRAXA and for C+.

Pre-FRAXA Program: 30 cycles at 95 °C/1:00', 65 °C/2:00'; an initial denaturing step: 10 minutes at 95 °C and a final extension step: 10 minutes at 65 °C.

C+ Program: 35 cycles at 94 °C/1:00', 68 °C/1:00', 72 °C/1:00'; an initial denaturing step for 7 minutes at 94 °C and a final extension step for 10 minutes at 72 °C

- 9. Place the tubes into the thermocycler when it reaches 94°C.

Note: For thermocyclers without hot lid, a drop of sterile mineral oil should be added.

Obtention of test results

- 1. Load 5 µl of each amplification product of both mixes (Pre-FRAXA and C+ Pre-FRAXA) and a molecular weight marker, in a 10% acrylamide-bis-acrylamide 29:1 gel. Each sample should be prepared with a suitable loading buffer quantity.
- 2. Migrate until bromophenol Blue reaches the end of the gel.
- 3. Stain with silver nitrate.

Interpretation of the test results

It is possible to determine the size of the amplified fragment from the electrophoretic pattern of the molecular weight marker.

The PCR product obtained with the C+ Pre FRAXA mix always contains 301 bp while the PCR product obtained with the Pre FRAXA mix varies according to the number of CGG repeats. A 221 bp band indicates the presence of 6 CGG repeats.

The following results may be obtained with male samples:

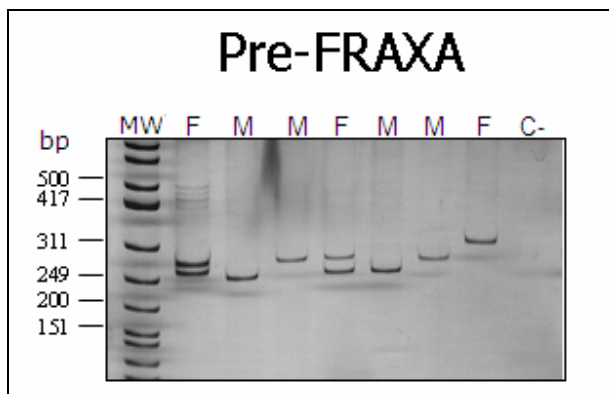
PCR product	Result
1 band between 221 and 400bp	Normal
1 band between 401 and 800 bp	Pre-mutated
There is no amplification	Uncertain

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- The following results may be obtained with female samples:

PCR product	Result
2 bands between 221 and 400bp	Normal
1 band between 221 and 400 + 1 band between 401 and 800	Pre-mutation carrier
2 bands between 401 and 800	Pre-mutated
1 band between 221 and 800	Uncertain
There is no amplification	Uncertain

Example:



10% acrylamide gel stained with silver nitrate, showing the variability of the number of CGG repeats in the FMR1 gene both in female (F) and male (M) DNA samples when using the ATGen's Pre-FRAXA kit.

The F samples showing 2 amplification bands are NORMAL.

The M samples showing 1 amplification band are NORMAL.

The F sample showing 1 amplification band is uncertain, there is one allele within the normality range but it cannot be concluded if the second allele has the same molecular weight or if it cannot be amplified because of its size.

Bibliography

- OMIM : 309550 FRAGILE SITE MENTAL RETARDATION 1 GENE; FMR1
- Crawford et al. FMR1 and the Fragile X Syndrome. *Genetics in Medicine* 2001. 3: 359-71. HuGe Reviews. HGEN. Centers for Disease Control and Prevention (CDC).