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Devyner® Fragile X (FMR1 Gene) Carrier Screen Kit

Protocol Guide

(For research use only)

PCR master mix set up and thermal cycling:

Thaw ready to use mastermix on ice. After completely thawing the tube, vortex to ensure mixing.
Briefly spin tubes to make sure reaction mix is in the bottom of microtube .
Dispense 14 µL mastermix to each well or tube. Use a Repeater pipettor, if available.
Add 2.0 µL of the appropriate DNA (40-60 ng/µl) sample to each well. Pipette up/down at least twice to ensure adequate mixing.
Gently vortex the tube.
Centrifuge the tubes to remove bubbles (1 min at 1600 rcf).
Transfer the PCR tubes to a preprogrammed thermal cycler and run the appropriate cycling protocol:

PCR PROTOCOL	
Description	Duration
1 hold	95°C for 5 min
10 Cycles	97°C for 35 sec
	62°C for 35 sec
	68°C for 4 min
20 Cycles	97°C for 35 sec
	62°C for 35 sec
	68°C for 4 min + 20s/cycle*
1 hold	72°C for 10 min
1 hold	4°C forever

***Follow the instruction manual of the thermal cycler to add 20 seconds extension time per cycle for this step**.

Transfer PCR products for CE analysis or store at -15 to -30 ^{0}C until analyzed. PCR product stability at -15 to -30 ^{0}C has been verified for up to 10 days storage.

Capillary Electrophoresis POP-7:

Thaw the formamide and GK-500 Size Ladder (Keysar Size Standard)at room temperature.
Thoroughly vortex (15 seconds) and spin tubes before use.
Prepare a master mix solution by adding components in the order listed:

Hi-Di Formamide	**11 µL**
GK-500 Size Ladder	2 µL
Total Volume per well	13 µL

Mix all added reagents (by pulse vortexing 3-5 times), and spin down briefly to collect.
Aliquot 13.0 µL of Formamide/LIZ solution to each well of a new CE analysis plate.
Transfer 2 µL of PCR products to the CE plate, pipetting up and down 2 to 3 times to mix. A multi-channel pipette is recommended for transfer.
Seal the plate, vortex, centrifuge to remove bubbles and transfer to a thermal cycler.
Denature for 2 min at 95°C followed by 4 °C until ready for injection on the CE instrument.
Alternatively, the products may be stored on ice and protected from light after the denaturation step.
Note: Samples may be run up to 24 hours after denaturation.

The instrument must be calibrated for the detection of both FAM and LIZ fluorescent dyes.
Use the factory installed Fragment Analysis Protocol for POP-7 polymer and capillary length for your instrument as a base protocol.
Adjust the injection conditions and run time according to the particular instrument configuration and capillary length. Recommended starting values are listed in Table below.

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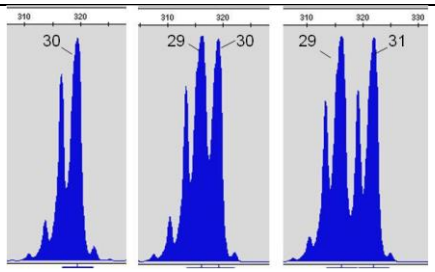
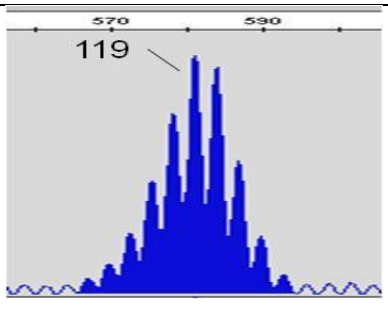
Run Time	Injection	Capillary Length	Instrument
2400 s	2.5 kV, 20 s	36 cm	3130,3130xl
4200 s	2.5 kV, 20 s	50 cm	3730, 3730xL
2400 s	2.5 kV, 20 s	50 cm	3500, 3500xL

Fragment Sizing Analysis:

Import data and process
a. Import the FSA files into GeneMarker®.
b. Process files according to the methods, panels (Keysar Fragile X Panel) and size standard settings established for *FMR1* PCR product analysis.
2. Qualify the run
b. Screen 600 or 500 LIZ dye Size Standard Peaks. Review Size Matches and Size Calling Curve of the Size Standard for all samples. Identify any irregularities in the fit or any missing peaks for the Ladder.
Critical! Samples without a properly called ladder must be excluded from further analysis.
Manufacturer default signal intensity cutoffs and low peak ranges for different CE instrument configurations.

Low Peak Range (rfu)	Cutoff (rfu)	Instrument
10-49	50	3130, 3130xl
50-174	175	3730, 3730xl
50-174	175	3500, 3500xl

Peak selection guidelines based on size range and features.

Peak size	Features	Follow these guidelines for peaks exceeding the signal cutoff for the instrument	**Examples**
245-400 bp	Normal and Intermediate alleles	Select the highest peak, generally the right-most peak in this size range. There may be multiple peaks in the normal range. Confirm selection of all peaks (e.g. 30 or 29,30 or 29,31 CGG).	
~400-820 bp	Premutation alleles with a single peak population less than 8 peaks from center to end.	Select the highest peak, generally the center peak for multi-peak alleles or peaks in this size range (e.g. 119 CGG).	

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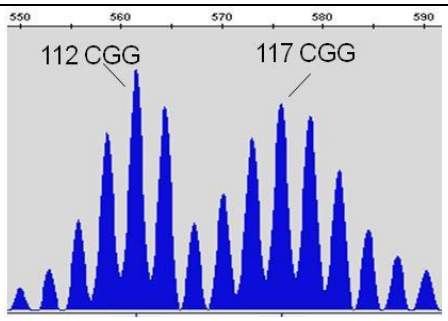
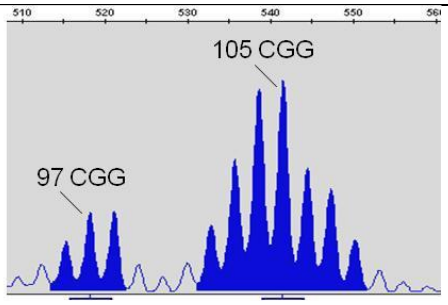
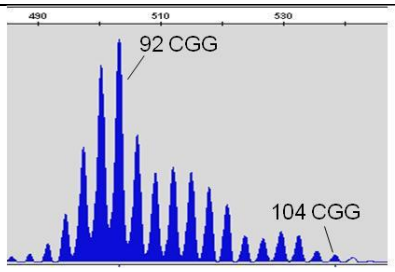
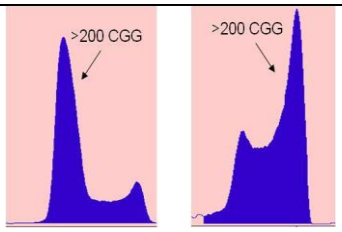
	Premutation alleles with complex distributions of peaks.	Select center peaks of two allele groups. If the peaks between the alleles exceed the signal cutoff, identify both groups separated by a dash "-" (e.g. 112-117 CGG).	
		If the peaks between two allele groups are less than 50 rfu (or other cutoff), the alleles can be identified with a comma as distinct alleles (e.g. 97, 105 CGG).	
		Select center-peak and the last-peak >50 rfu (or other cutoff) for alleles with more than 8 peaks from center to end (e.g. 92-104 CGG).	
> 820 bp	Full mutation alleles less than approximately 1000 bp that may be resolved from larger full mutation peaks and full mutation alleles.	Select only the component of the peak group containing the highest peak. Deselect other peaks within that group. Identify peak as >200 CGG.	

Convert peak size to CGG repeat length.

After capillary electrophoresis, the size of the target amplicon is derived from comparison to a co-injected size standard, e.g. GK-500 Size Ladder. The size of each peak may be converted to repeat length according to Equation 1 below.

$$CGG_i = \frac{Peak_i - c_0}{m_0}$$

Configuration	c_0	m_0
3130, 3130*xl* 36 cm	229.4	2.965
3730, 3730xL 50 cm	231.9	2.937
3500, 3500xL, 50 cm	232.6	2.962

Interpreting data

Alleles are reported as whole-integer repeats associated with a specific genotype category: normal, intermediate premutation and full mutation and full mutation mosaic. The reportable range is 5-200 repeats; above 200 repeats all alleles are identified as >200 CGG.

Example CGG RP PCR Results:

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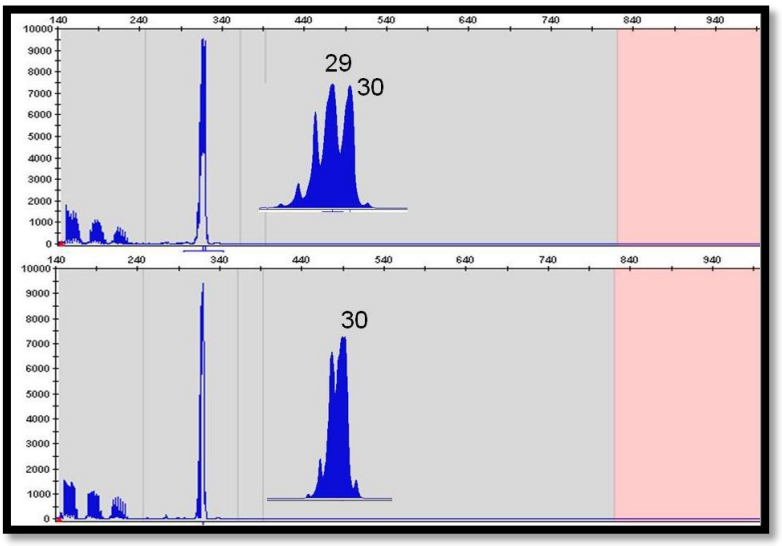

Figure 1: Normal 29,30 alleles

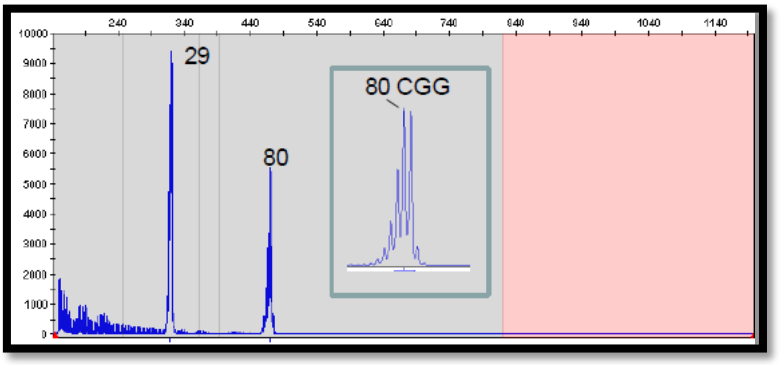

Figure 2: premutation allele **representing 80 CGG allele.**

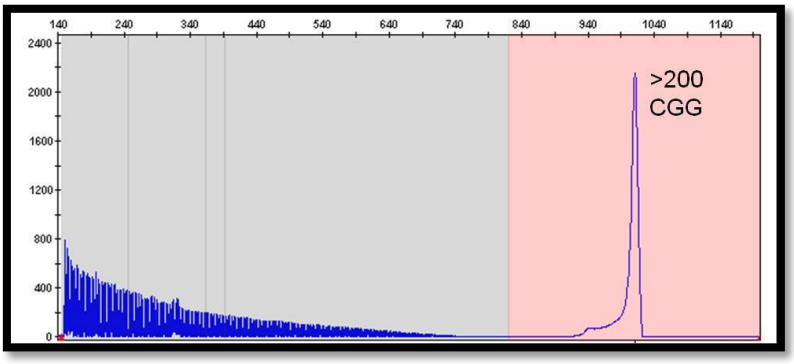

Figure 3: full mutation allele; male sample. The full length product peak exceeds 200 CGG and is identified as >200 CGG.