Date 8/19/2013

Objective Rd 2 PCR amplification of triplet repeat region of FMR1 using the Rd 1 PCR pdt as template:
- 1:10 and 1:50 dilutions of Rd 1 PCR pdt advised
- Cycling conditions of Rd 1 modified as:
  - Three step amplification process
  - Denat temp set to 92degC/ 30secs
  - Annealing temp grad* 50-70deg/ 1:30mins
  - Extension temp. set to 72degC/ 2:30mins

***: PCR conditions as follows - 90/70/72 has worked before (Oct-Nov 2012) with taq for rd 2 PCR**
In fact ann'g at temps 70, 68, 66 and 62 have all worked.

Description

Template used: Rd 1 PCR pdt to a final conc of 0.1X or 0.02X.
Primers: FT for and FT rev
Polymerase: Native Taq Polymerase (Life Technologies)
Solvents: Rxns in 0.5M NMP + 2.2M Betaine

Reaction Composition:

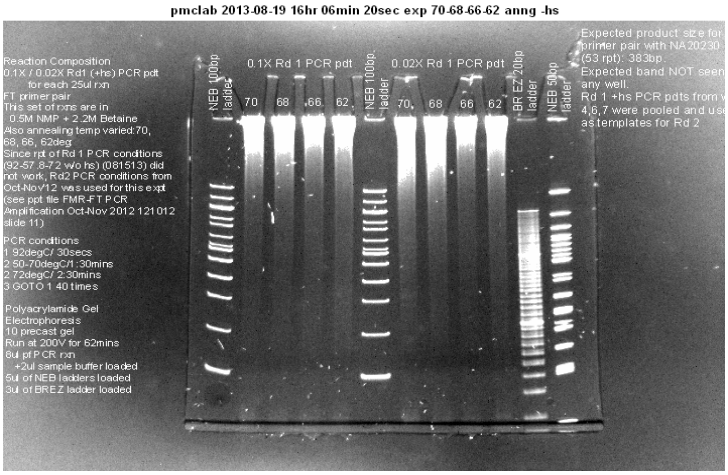
Component	**0.5M NMP + 2.2M Betaine rxns** Final Conc/ amt
Taq Buffer	1 X
MgCl2	1.5 mM
dNTPs	0.4 mM
FT-For/ SFS-for	0.4 uM
FT-Rev/ SFS-rev	0.4 uM
Rd 1 PCR rxn	0.1X or 0.02X
Taq Polymerase	2.5 U
Water	
NMP	0.5 M
Betaine	2.2 M
total	**25ul**

Cycling Conditions:

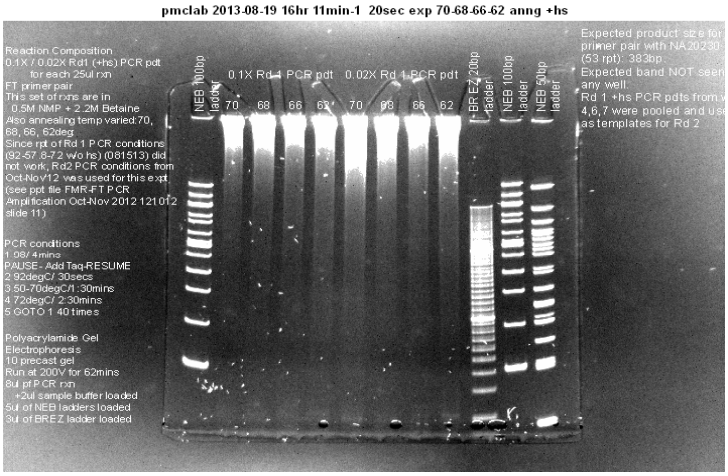
1 92degC/ 30secs
2 50-70degC/ 1:30mins
3 72degC/ 2:30mins
4 GOTO 2 40 times

Gel Electrophoresis: 10% precast polyacrylamide gels (Life Technologies)
8ul of PCR rxn + 2ul of (5X) sample buffer loaded
Molecular Ladders:
100bp ladder (NEB) 5ul
50bp ladder (NEB) 5ul
20bp ladder (BioRad) 3ul

Gel Pictures **FT primers - NMP + Betaine-no hot start(20sec exposure)**

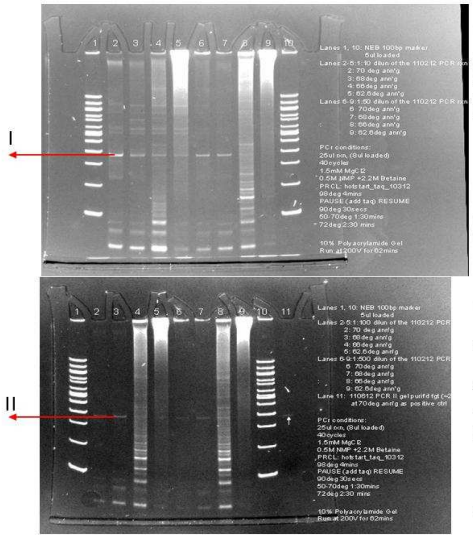


Gel Pictures **FT primers - NMP + Betaine-with hot start (20sec exposure)**



Comments None of the reactions have worked.
When attempting to reproducs Ram's work in Oct-Nov 2012, Rd 2 PCR using 0.1X, 0.02X, 0.01X of Rd 1 PCR with Taq with 90-70-72 has worked to produce a PCR pdt.
See power point file: FMR-FT PCR Amplification Oct-Nov 2012 121012 (slide 11)
Hence these conditions were tested again. However, the PCR did not work.

Round 2 PCR: NA20230 0.1-0.002X Round 1 reaction mix with native Taq Polymerase in 0.5M NMP +2.2M Betaine



PCR cycling conditions:

1. 98°C/ 4mins
   PAUSE- add Taq- RESUME
2. 90°C/ 30secs
3. 50°- 70°C/ 1:30mins
4. 72°C/ 2:30mins
5. GOTO 2 40 times

Gradient Temperatures:
50°, 51.3°, 53.9°, 57.8°, 62.6°, 66.6°, 68.8°, 70°C

Gel I: 0.1X and 0.02X
Gel II: 0.01X and 0.002X

Exposure time for these pics: 20secs

1. 0.1X rxns show several non-specific bands besides expected band.
2. At 70° and 68.8°C annealing, the 0.02X-0.002X rxns show only the expected band.
3. The bands <100bp are probably primer-dimers
4. The expected bands in the 0.002X rxns are quite faint.
5. 0.02X and 0.01X dilutions of the Rd1 gDNA PCR can be reamplified to give a specific expected band of 383bp by native taq polymerase in the presence of 0.5M NMP and 2.2M Betaine under denat 90°/ annealing 70°/ extension 72°C.
6. The PCR of the 0.02 and 0.01X dilutions as described in (5) has been performed 2X with identical results.

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