Date 8/16/2013

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 conditionsof Rd 1 repeated ie:
  - Three step amplification process
  - Denat temp set to 92degC/ 30secs
  - Annealing temp set to 57.8deg/ 1:30mins
  - Extension temp. set to 72degC/ 2:30mins

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:

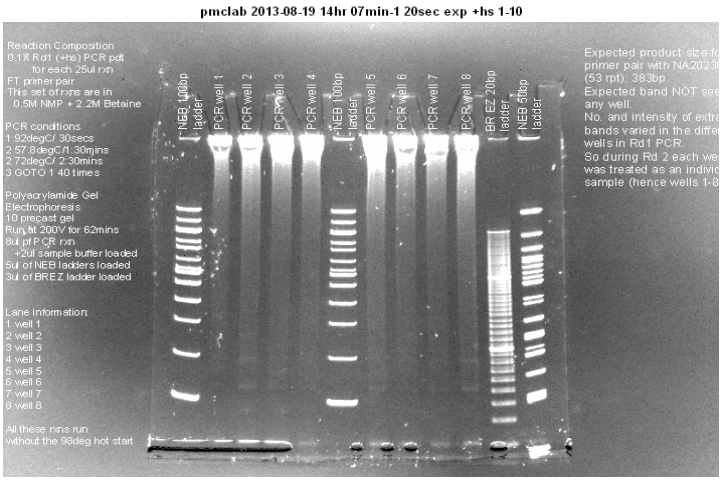
Component	**0.5M NMP + 2.2M Betaine rxns** Final Conc/ amt
Taq Buffer	1 X
MgCl2	1.5 mM
dNTPs	0.4 mM
FT-For/ SFS-for	0.4 uM
FT-Rev/ SFS-rev	0.4 uM
Rd 1 PCR rxn	0.1X or 0.02X
Taq Polymerase	2.5 U
Water	
NMP	0.5 M
Betaine	2.2 M
total	**25ul**

Cycling Conditions:

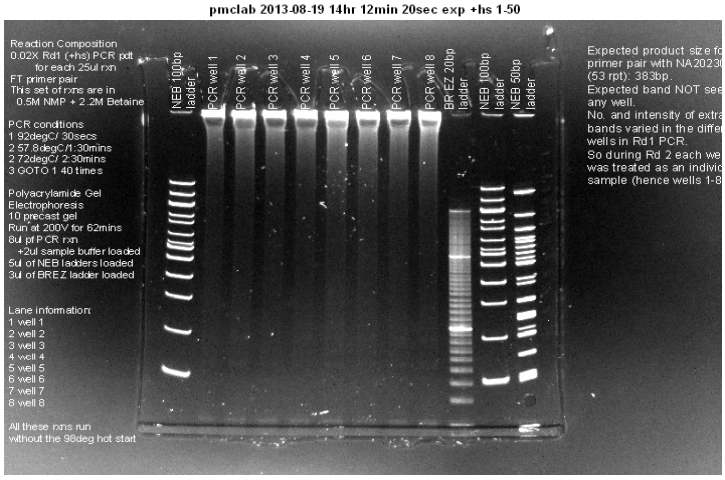
1 92degC/ 30secs
2 57.8degC/ 1:30mins
3 72degC/ 2:30mins
4 GOTO 2 40 times

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

FT primers - NMP + Betaine-with hot start-1:10 dilution (20sec exposure)

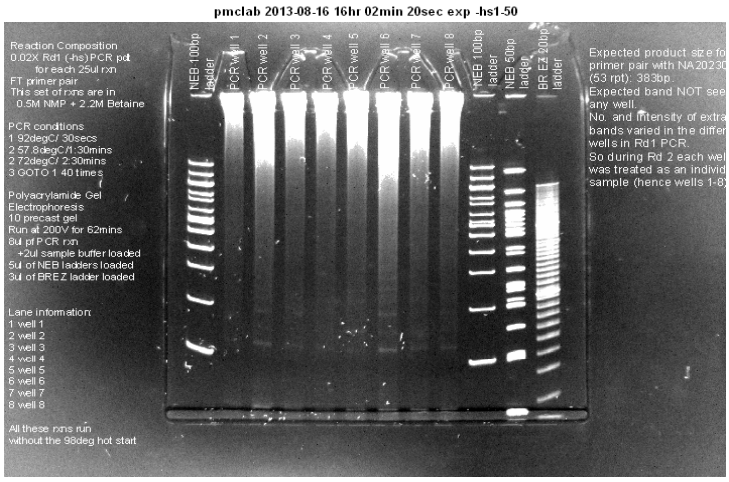


FT primers - NMP + Betaine-with hot start-1:50 dilution (20sec exposure)



Gel Pictures **FT primers - NMP + Betaine-no hot start- 1:10 dilution (20sec exposure)**

Gel Pictures **FT primers - NMP + Betaine-no hot start-1:50 dilution (20sec exposure)**



Comments None of the reactions have worked.