Date 8/20/2013

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 70deg/ 1:30mins
  - Extension temp. set to 72degC/ 2:30mins

Description

Template used: Rd 1 PCR pdt vol: 2.5ul, 5ul, 7ul for a ttl rxn vol of 25ul

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	2.5, 5, 7ul
Taq Polymerase	2.5 U
Water	
NMP	0.5 M
Betaine	2.2 M
total	**25ul**

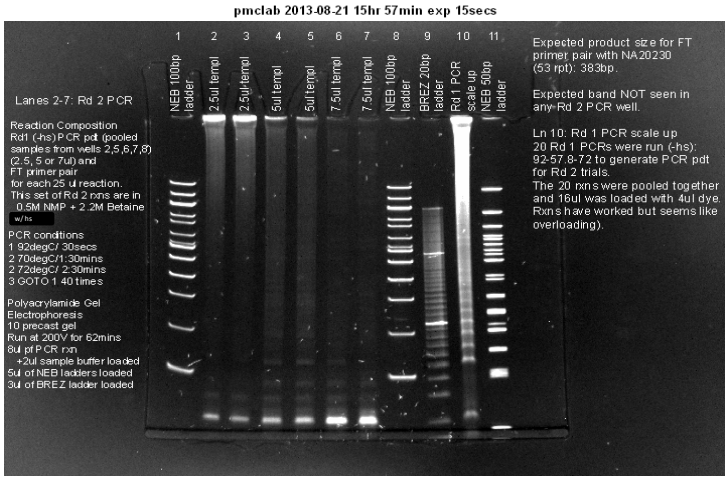
Cycling Conditions:

1 98deg/ 4mins
PAUSE-Add Taq-RESUME
2 92degC/ 30secs
3 70degC/ 1:30mins
4 72degC/ 2:30mins
5 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-w/ hot start (15sec exposure)**

Lane 10 is irrelevant to this expt. Please ignore



Comments None of the reactions have worked.

The possibility exists that the Rd 1 PCR had not been robust and so even a 0.1X dilution may be too much dilution for the Rd 2 PCR to work successfully.

Thus higher volumes of Rd 1 PCR rxn (than recommended) were tried, namely:

2.5ul	0.1X
5.0ul	0.2X
7.0ul	0.28X

However, even when 7ul (~0.3X dilution) of the Rd 1 PCR pdt was taken, Rd 2 has not worked.