Date 8/22/2013

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 modified as:
  - 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 0.1X, 0.02X, 0.01X

Primers: FT for and FT rev

Polymerase: Deep Vent (NEB)

Solvents: No Solvt, Rxns in 0.5M NMP, Rxns in 2.2M Betaine

Reaction Composition:

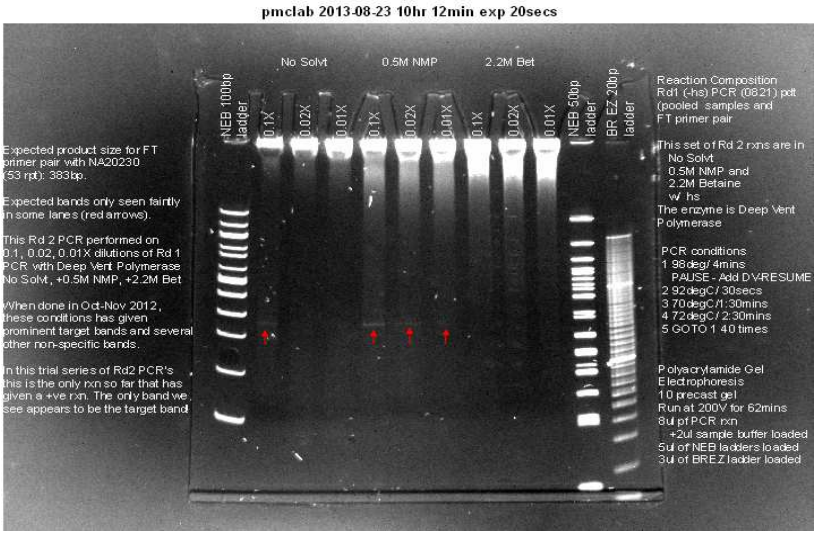
Component	**No Solvt** Final Conc/	**0.5M NMP** Final Conc/	**2.2M Betaine** Final Conc/
ThermoPol Buffer	1 X	1 X	1 X
dNTPs	0.4 mM	0.4 mM	0.4 mM
FT-For	0.4 uM	0.4 uM	0.4 uM
FT-Rev	0.4 uM	0.4 uM	0.4 uM
Rd 1 PCR pdt*	0.1 X	0.1 X	0.1 X
DV Polymerase	0.25 U	0.25 U	0.25 U
Water			
NMP		0.5 M	
Betaine			2.2 M
total	25.00	25.00	25.00

Cycling Conditions:

1 98deg/ 4mins
PAUSE-Add DV-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 - No Solvt-NMP-Betaine-w/ hot start (20sec exposure)**

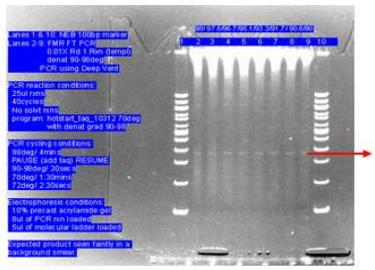


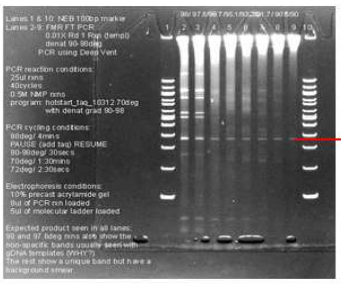
Comments Rd 2 PCR has worked better previously with 0.01X Rd 1 Pdt (using DV) in NMP
or betaine
See power point file: FMR-FT PCR Amplification Oct-Nov 2012 121012 (slide 18)
It has not worked as well in the current expt, however, the only band seen in the target band.
This needs to be repeated to confirm.

Round 2 PCR (NA20230): Use of Deep Vent

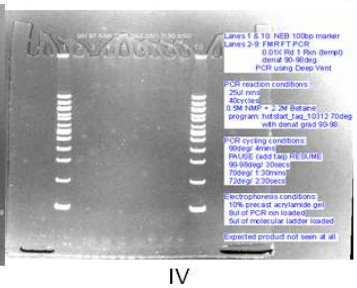
- denaturation temperature gradient 90°-98°C with annealing set at 70°C and extension set at 72°C
- testing different solvent combinations: 0.5M NMP, 2.2M Betaine, 0.5M NMP +2.2M Betaine,

I II





III IV



PCR cycling conditions:

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

Gradient Temperatures:

90°, 90.6°, 91.7°, 93.3°, 95.1°, 96.7°, 97.6°, 98°C

GelI: No Solvt (exp time 25secs)
GelII: 0.5M NMP (exp time 20secs)
GelIII: 2.2M Betaine (exp time 20secs)
GelIV: 0.5M NMP + 2.2M Betaine (exp time 25secs)

- DV rxns in NMP + Betaine do not work at all.
- The following rxns give a single band of expected product:
  - No solvt rxns denat 90-98°C
  - 0.5M NMP at denat 96.7-90°C
  - 2.2M Betaine denat 90.6°C
- Even though there are non-specific products DV rxns in NMP denat 98°-97.6°C and in Betaine denat 91.7°-98°C will be pursued because the rxn seems robust.

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