

Date 3/11-20/2013

Objective PCR amplification of triplet repeat region of FMR1 with a modified program (as below):

- Without hot start
- Denaturation temp set according to denat temp grad expt
(see report denat 75-95 comments)
- Three step amplification process
- annealing temp grad 50-70deg/ 1:30mins
- Extension temp. and time (that has worked previously
ie 72deg/ 2:30mins)

Description *Template used:* 100ng genomic DNA NA20230, (53/54 rpt sample from Coriell)
Primers: FP2-RP2
Polymerase: Deep Vent (NEB)
Solvents: No Solvt rxns
 Rxns in 1M NMP
 Rxns in 1M Formyl Morpholine
 Rxns in 0.5M TMSO
 Rxns in 1M TMSO

Reaction Composition:

| | 0.5M TMSO | 1M TMSO | 1M For.Mor | No Solvt | 1M NMP |
|---------------------------------------|----------------|----------------|----------------|----------------|----------------|
| Component | Final Conc/amt | Final Conc/amt | Final Conc/amt | Final Conc/amt | Final Conc/amt |
| ThermoPol Buffer | 1 X | 1 X | 1 X | 1 X | 1 X |
| dNTPs | 0.4 mM | 0.4 mM | 0.4 mM | 0.4 mM | 0.4 mM |
| FP2 | 0.4 uM | 0.4 uM | 0.4 uM | 0.4 uM | 0.4 uM |
| RP2 | 0.4 uM | 0.4 uM | 0.4 uM | 0.4 uM | 0.4 uM |
| gDNA NA203230 | 100 ng | 100 ng | 100 ng | 100 ng | 100 ng |
| DV polymerase | 0.3 U | 0.3 U | 0.3 U | 0.3 U | 0.3 U |
| Water | | | | | |
| NMP | | | | | 1 M |
| For. Mor | | | | | |
| TMSO | 0.5 M | 1.00 M | | | |
| For. Mor | | | 1.00 M | | |
| total | 25ul | 25ul | 25ul | 25ul | 25ul |
| <i>Cycling Conditions:</i> denat temp | 79 | 76 | 75 | 83 | 88 |

- 1 79.1/ 30secs
- 2 50-70degC/ 1:30mins
- 3 72degC/ 2:30mins
- 4 GOTO 2 40 times

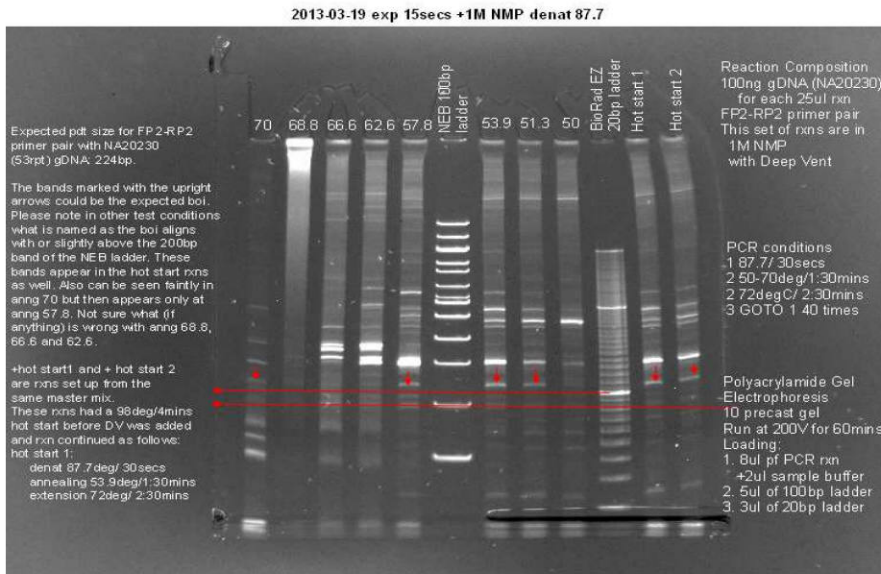
Gradient steps: 70, 68.8, 66.6, 62.6, 57.8, 53.9, 51.3, 50degC

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

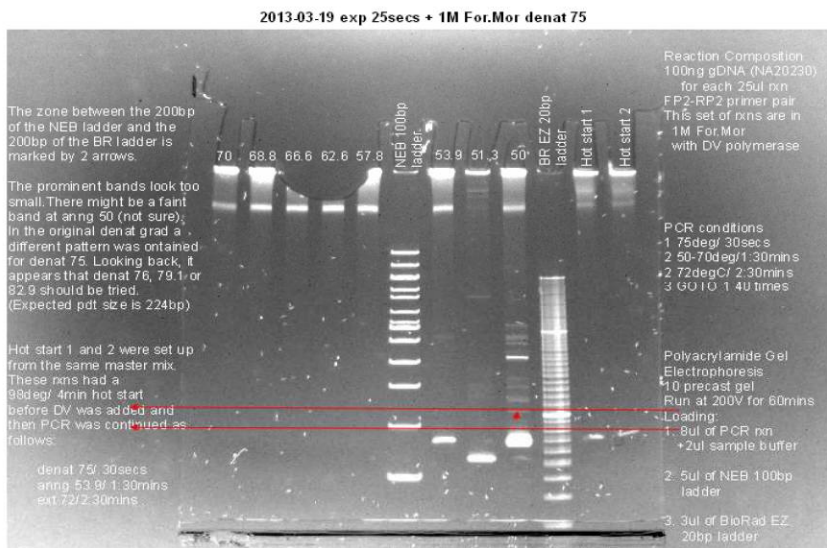
Gel Pictures

Lanes are marked according to the annealing temp of the particular rxn.

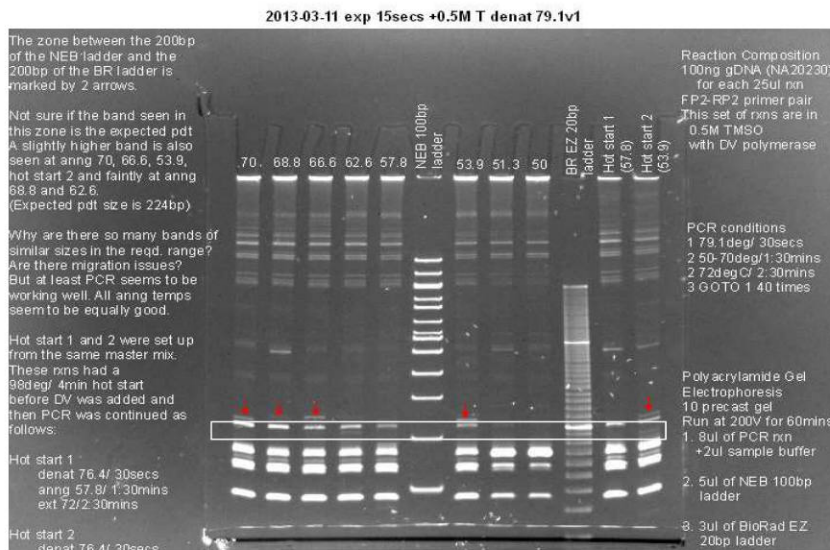
FP-RP-DV-NMP 15sec exp



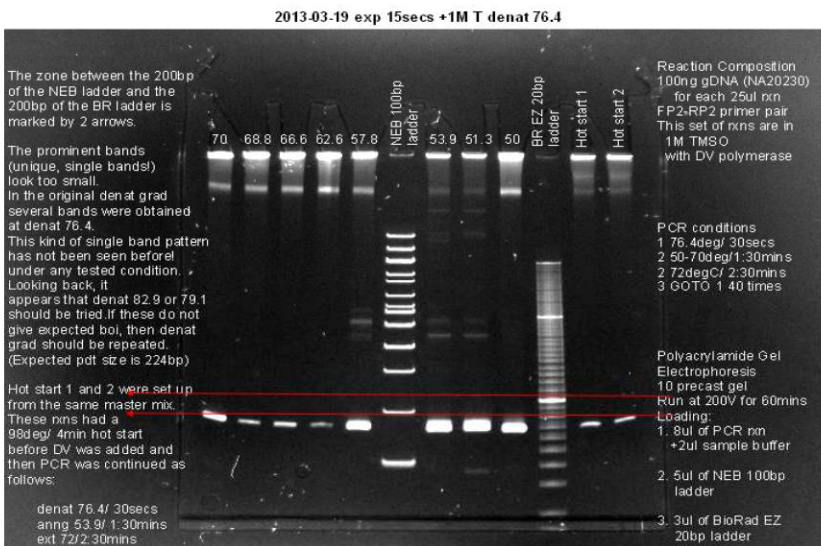
FP-RP-DV-For.Mor 25sec exp



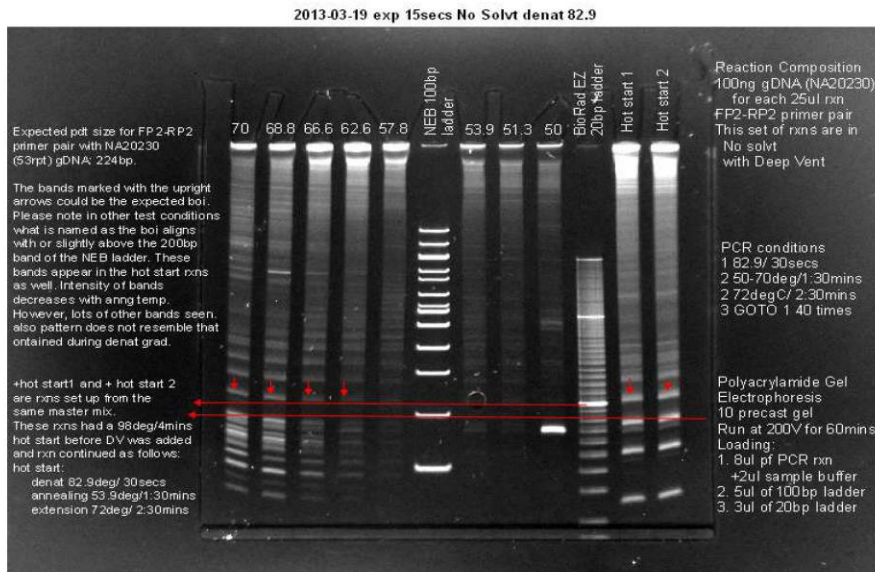
FP-RP-DV-0.5M TMSO 15sec exp



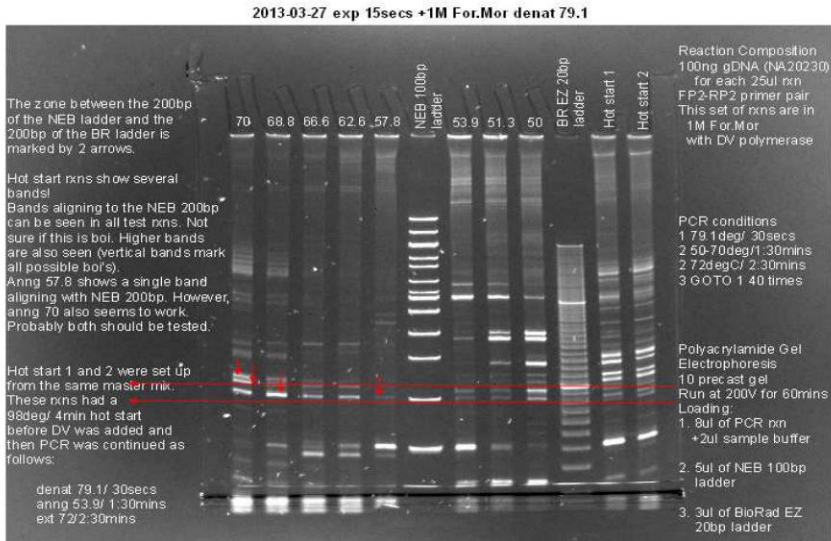
FP-RP-DV-1M TMSO 15sec exp



FP-RP-DV-No solvt exp 15secs



FP-RP-DV-For.Mor 15sec exp anng grad rpt @ denat 79.1



FP-RP-DV-1M TMSO 20sec exp anng grad rpt @ denat 82.9



Comments

As mentioned previously, even tho' the 1000bp band migrate similarly for the NEB 100bp ladder and the BioRad EZ 20bp ladder, there is a marked difference in the 200bp band for both ladders

Because of this and the slight differences in the sizes of bands obtained in the 200bp range, horizontal arrows mark the 200bp band of both the two ladders as boundaries.

It is possible that for the test rxns, the bands inside this box are the expected band. In some conditions, a slightly higher band is also seen (see gel pic for details).

If yes, there seem to be serious migrations differences for the same expected pdt under different conditions.

If all these ARE the expected band, then the PCR seems to be working quite well.

Conditions selected for ext grad are as follows:

| | denat | anng temp | | |
|------------------|-----------|--|-----------------|------------------------|
| | temp | 1 st | 2 nd | 3 rd choice |
| 1 DV + 0.5M T | 79.1 | 70.0 | 53.9 | |
| 2 DV + 1M T* | 76.4 82.9 | no data for either denat temp (see gel pics) | | |
| 3 DV + For. Mor* | 79.1 | 57.8 | 70.0 | |
| 4 DV + NMP | 87.7 | 57.8 | 70.0 | |
| 5 DV - No solvt | 82.9 | 70.0 | | |

*: The original denat temp selected from the denat grad expts (see report denat 75-95) did not seem to work for 1M TMSO and wored very faintly for 1M For. Mor. The second choice denat temp (82.9 and 79.1) were tried. This second choice also did not work for 1M TMSO. At this point DV + 1M TMSo has not been pursued any further.