Date 3/11-26/2013

Objective PCR amplification of triplet repeat region of FMR1 with a modified program (as below):
- Without hot start
- Three step amplification process
- Denaturation temp set according to denat temp grad expt (see report denat 75-95 comments)
- Annealing temp set according to anng temp grad expt (see report anng 50-70 comments)
- Extension temp. grad (55-75deg)

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:

Component	0.5M TMSO	1M TMSO	1M For.Mor	No Solvt	1M NMP
	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**

Cycling Conditions:

denat temp	79	76.4/ 82.9	79	83	88
anng temp	70.0		57.8/ 70	70.0	57.8

1 79.1/ 30secs
2 57.8degC/ 1:30mins
3 55-75degC/ 2:30mins
4 GOTO 2 40 times

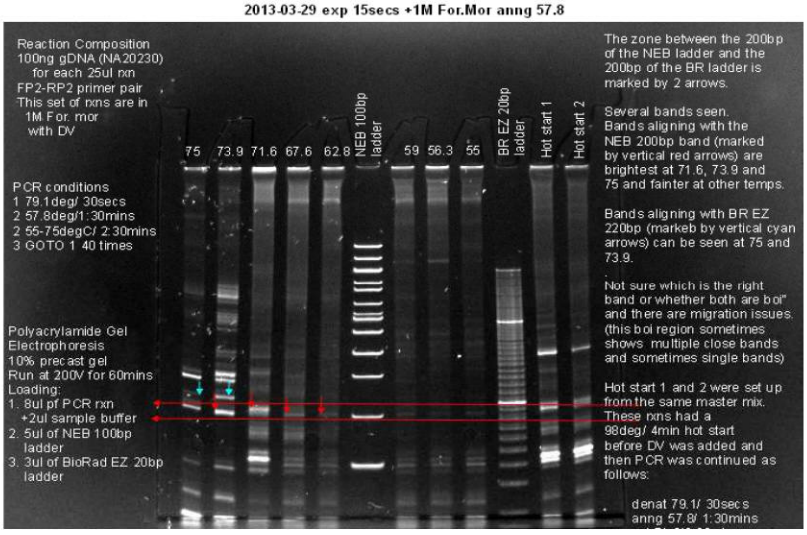
Gradient steps: 75, 73.9, 71.6, 67.6, 62.8, 59, 56.3, 55degC

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)

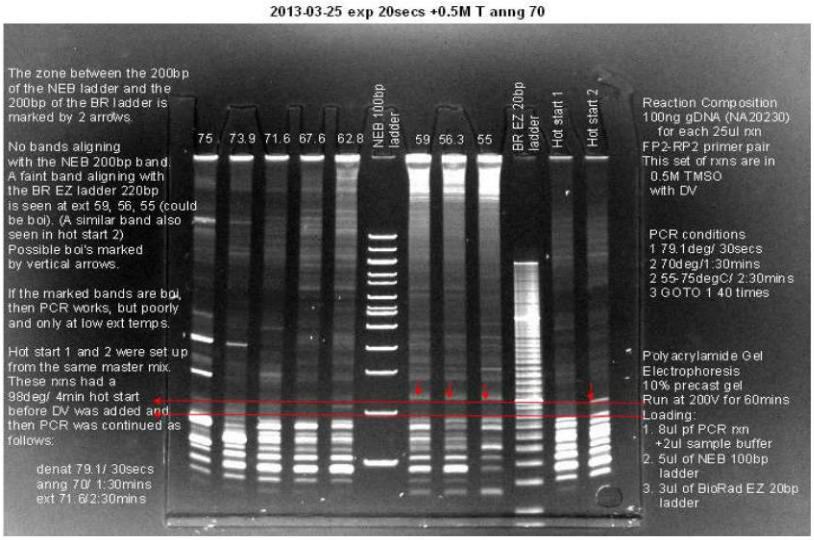
Gel Pictures Lanes are marked according to the annealing temp of the particular rxn.

FP-RP-DV-NMP 20sec exp

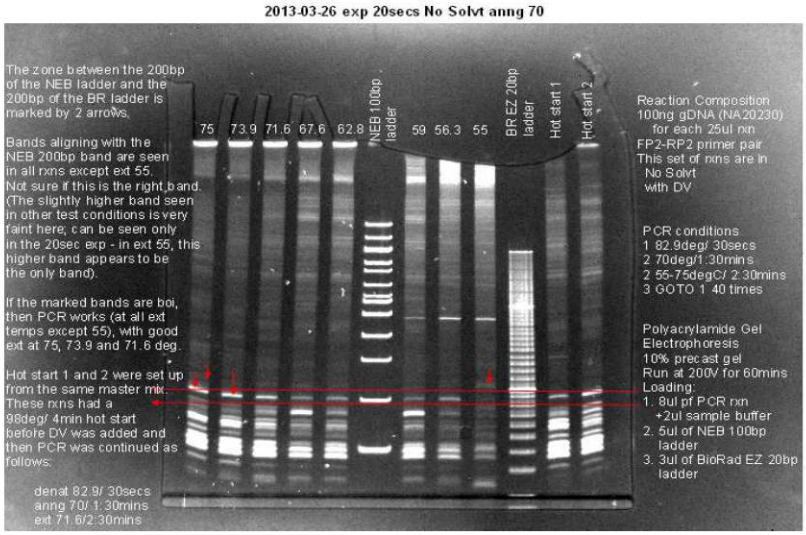
FP-RP-DV-For.Mor 15sec exp



FP-RP-DV-0.5M TMSO 20sec exp



FP-RP-DV-No solvt exp 20secs



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 from ext grad are as follows:

	denat temp	anng temp	ext temp 1st	2nd	3rd
1 DV + 0.5M T	79.1	70.0	59	56.3	55.0
2 DV + 1M T*	no data				
3 DV + For. Mor*	79.1	57.8	73.9	75.0	71.6
		70.0			
4 DV + NMP	87.7	57.8	71.6	67.6	
5 DV - No solvt	82.9	70.0	72	55.0	

*: 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 and this has not been pursued.
The second choice denat temp for For. Mor (79.1) did work.
Two possible anng temps were selected (see report anng 50-70)
Anng 57.8 ext grad has been performed and the gel pic is available here.
Anng 70 ext grad has not yet been performed.