Date 3/8-13/2013

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: Native Taq Polymerase (Life Technologies)

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	No Solvt Rxns	1M NMP	1M For. Mor	0.5M TMSO	1M TMSO
	Final Conc/ amt	Final Conc/ amt	Final Conc/ amt	Final Conc/ amt	Final Conc/ amt
Taq Buffer	1 X	1 X	1 X	1 X	1 X
MgCl2	1.5 mM	1.5 mM	1.5 mM	1.5 mM	1.5 mM
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
Taq Polymerase	2.5 U	2.5 U	2.5 U	2.5 U	2.5 U
Water					
NMP		1 M			
For. Mor			1 M		
TMSO				0.5 M	1 M
Betaine					
total	**25ul**	**25ul**	**25ul**	**25ul**	**25ul**
denat temp	92	76	83	79	76

Cycling Conditions:

1 76.4degC/ 30secs
2 50-70degC/ 1:30mins
3 72degC/ 2:30mins
4 GOTO 2 40 times

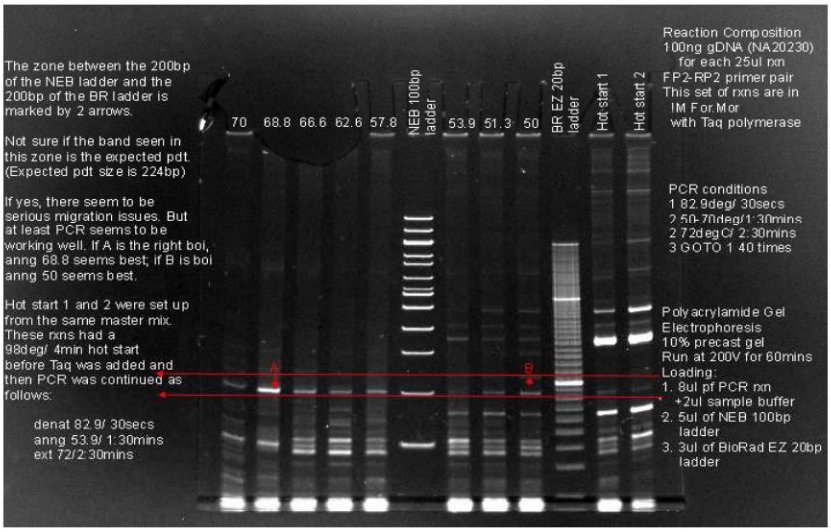
Gradient steps: 70, 68.8, 66.6, 62.6, 57.8, 53.9, 51.3, 50degC

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)

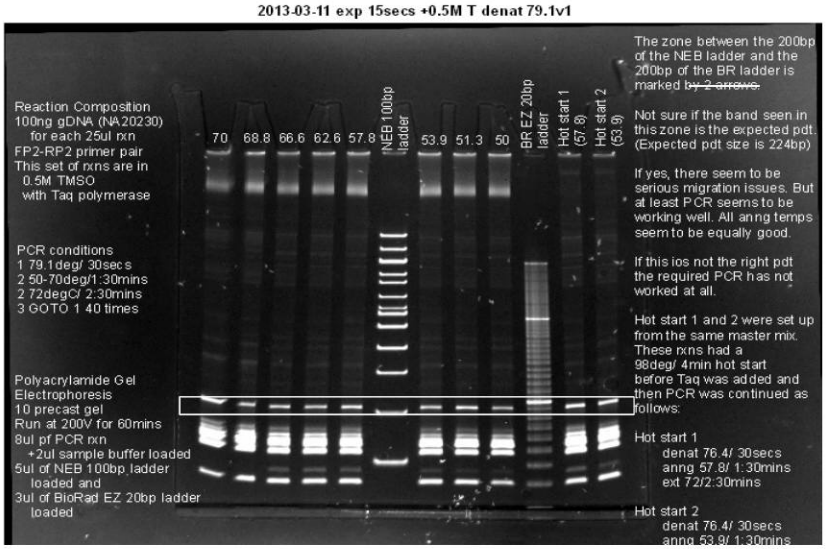
Gel Pictures Lanes are marked according to the annealing temp of the particular rxn.
Expected product size is 224bp

FP-RP-Taq-NMP denat76.4deg 20sec exp

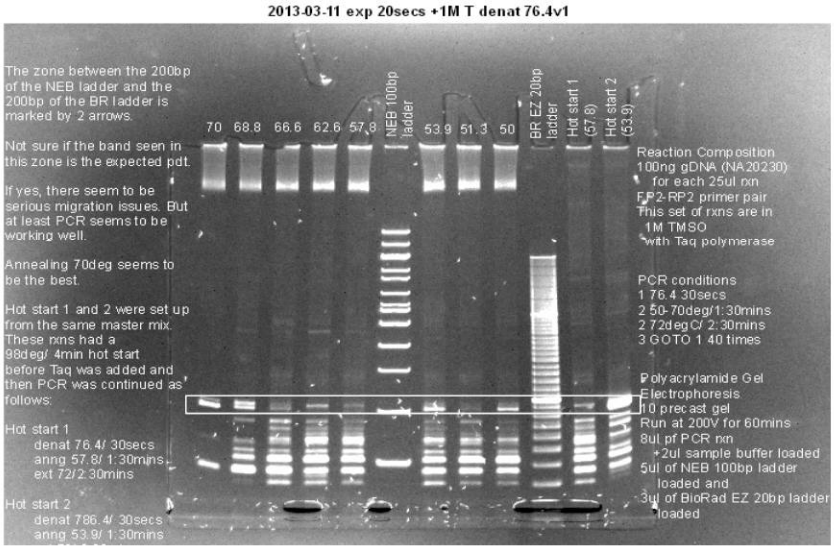
FP-RP-Taq-For.Mor 15sec exp



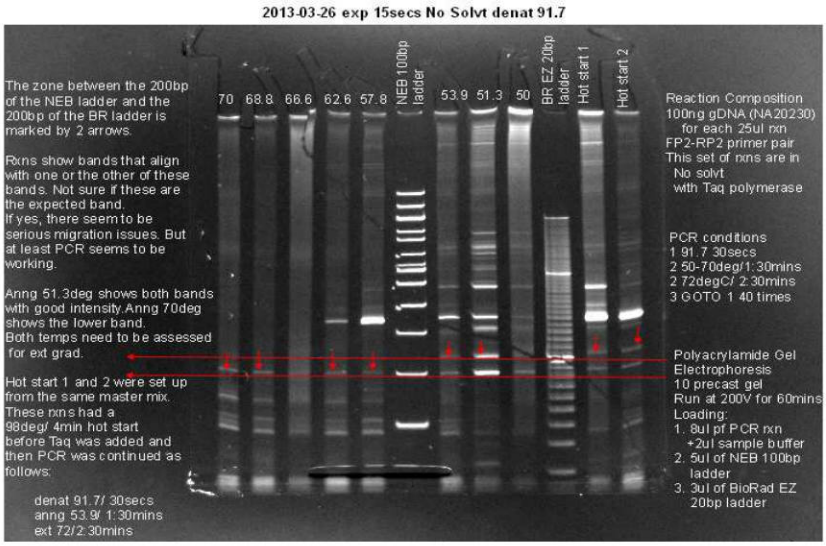
FP-RP-Taq-0.5M TMSO denat 79.1deg 15sec exp



FP-RP-Taq-1M TMSO denat 76.4deg 20sec exp



FP-RP-Taq-No solvt exp 15secs



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, a box has been drawn keeping the two ladders as boundaries.

It is possible that for the test rxns, the bands inside this box are the expected band.

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

		denat temp	anng temp 1st	2nd	3rd
1	Taq + NMP	76	68.8	51.3	
2	Taq + For.Mor	83	68.8/	50	
3	Taq + 0.5M T	79	70.0		
4	Taq + 1M T	76	70.0		
5	Taq-No solvt	92	70/	51.3	