

Date 3/15-/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/ 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

<i>Reaction Composition:</i>		No Solvt Rxns	1M NMP	1M For. Mor	0.5M TMSO	1M TMSO
Component	Final Conc/amt	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	1 X
MgCl2	1.5 mM	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	0.4 mM
FP2	0.4 uM	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	0.4 uM
gDNA NA20230	100 ng	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	2.5 U
Water						
NMP		1 M				
For. Mor			1 M			
TMSO				0.5 M		1 M
Betaine						
total	25ul	25ul	25ul	25ul	25ul	25ul
<i>Cycling Conditions:</i> denat temp	91.7	76.4	82.9	79.1	76.4	
anng temp	51.3/70	68.8	68.8	70.0	70	

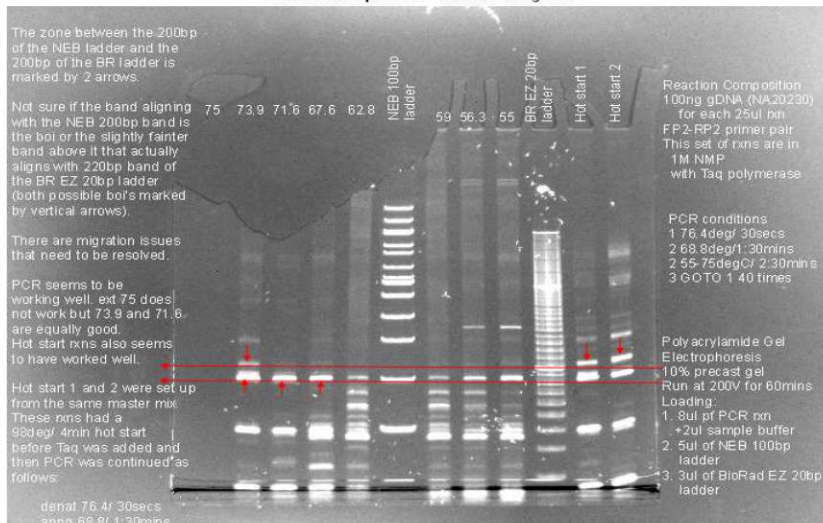
- 1 76.4degC/ 30secs
- 2 70degC/ 1:30mins
- 3 55-75degC/ 2:30mins
- 4 GOTO 2 40 times

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)

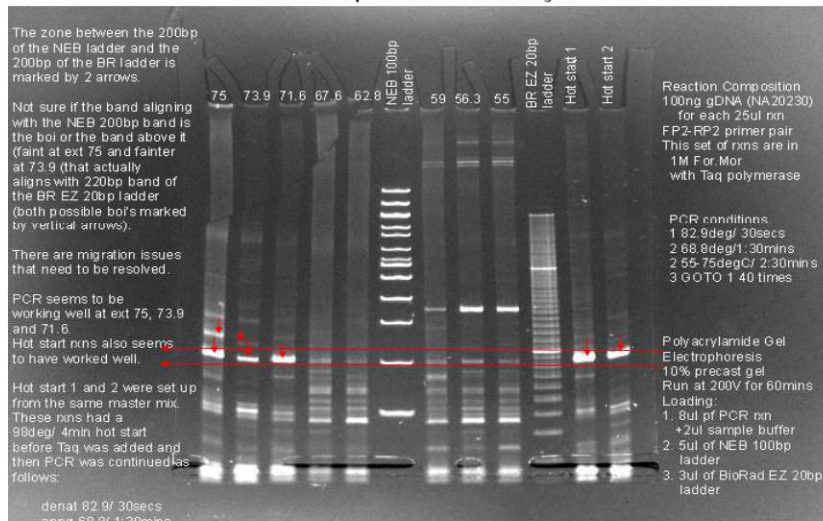
Gel Pictures Lanes are marked according to the annealing temp of the particular rxn.
 Expected product size is 224bp
FP-RP-Taq-NMP anng 68.8deg 20sec exp

2013-03-22 exp 20secs +1M NMP anng 68.8

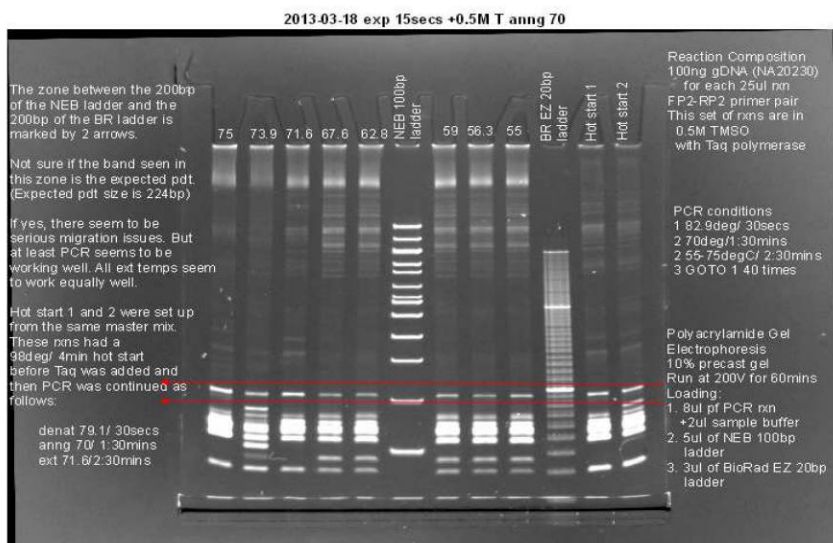


FP-RP-Taq-For.Mor 20sec exp

2013-03-25 exp 20secs +1M For.Mor anng 68.8



FP-RP-Taq-0.5M TMSO denat 79.1deg- anng 70deg 15sec exp



FP-RP-Taq-1M TMSO anng 70deg 20sec exp



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 from ext grad are as follows:

	denat temp	anng temp	ext temp		
			1 st	2 nd	3 rd
1 Taq + 0.5M T	79.1	70.0	75.0	73.9	71.6
2 Taq + 1M T	76.4	70.0	72.0		
3 Taq + For. Mor	82.9	68.8	75.0	73.9	71.6
4 Taq + NMP	76.4	68.8	73.9	71.6	
5 Taq - No solvt	91.7	70/ 51.3	no data yet*		

*: the PCRs did not work, presumably, Taq expired.

Both PCRs need to be rpt'd with new Taq.