

Date 3/6-7/2013

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

- Without hot start
- Denaturation temp gradient 75-95degC
- Three step amplification process
- Extension and annealing temp.s and times (that have worked previously
ie 54deg/ 1:30mins annealing-72deg/ 2:30mins extension)

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:

	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
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			1 M		
TMSO				0.5 M	1 M
total	25ul	25ul	25ul	25ul	25ul

Cycling Conditions:

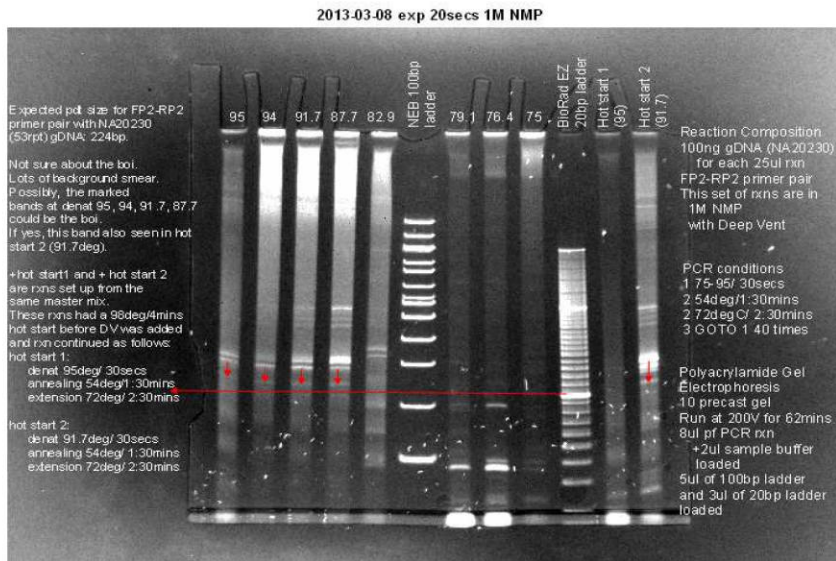
- 1 75-95degC/ 30secs
- 2 54degC/ 1:30mins
- 3 72degC/ 2:30mins
- 4 GOTO 2 40 times

Gradient steps: 95, 94, 91.7, 87.7, 82.9, 79.1, 76.4, 75degC

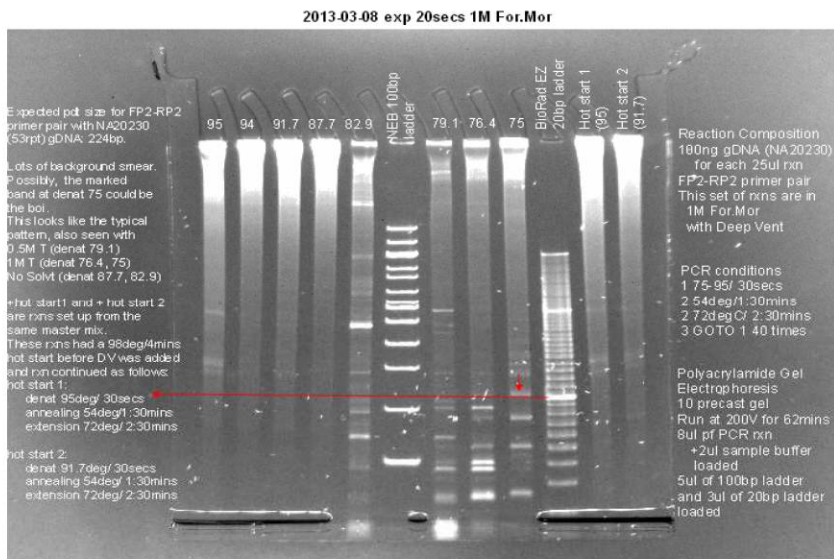
Gel Electrophoresis: 10% precast polyacrylamide gels (Life Technologies)
8ul of PCR rxn + 2ul of (5X) sample buffer loaded
Molecular Ladders:
NEB 100bp ladder (5ul)
BioRad EZ 20bp ladder (3ul)

Gel Pictures

Lanes are marked according to the denaturation temp of the particular rxn.
FP-RP-DV-NMP 20sec exp

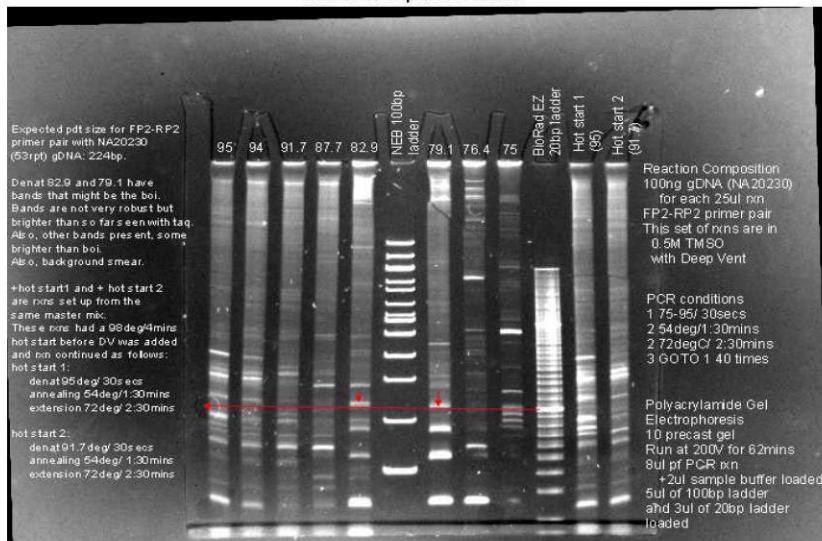


FP-RP-DV-For.Mor 20sec exp



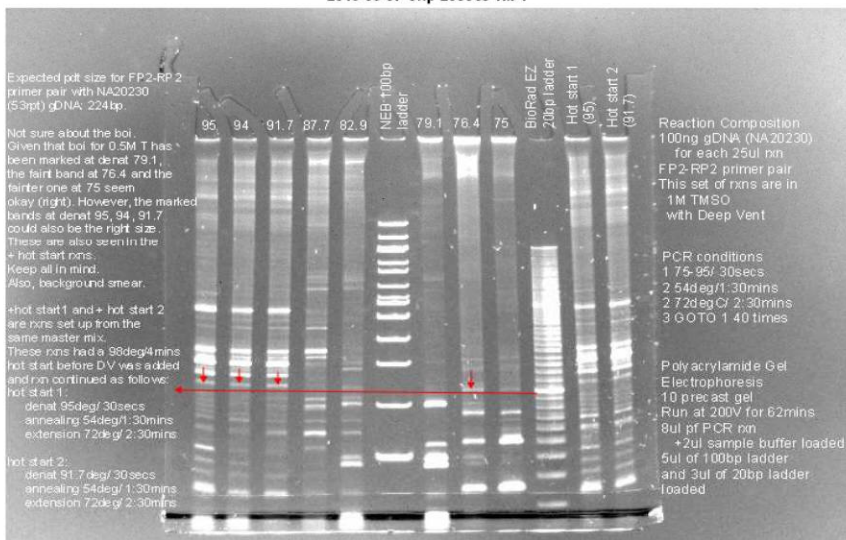
FP-RP-DV-0.5M TMSO 20sec exp

2013-03-07 exp 20secs 0.5M T

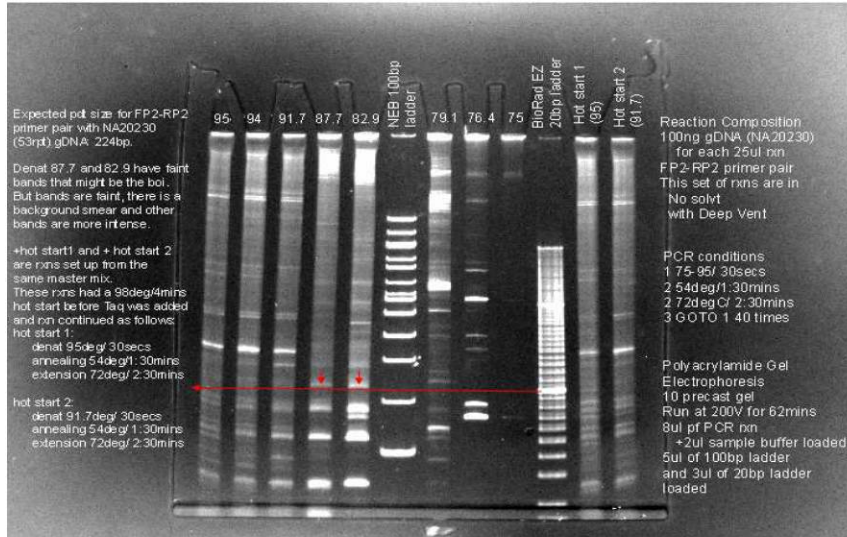


FP-RP-DV-1M TMSO 20sec exp

2013-03-07 exp 20secs 1M T



FP-RP-DV-No solvt exp 20secs



Comments

The NEB 100bp ladder and the BioRad EZ 20bp ladder have been simultaneously run with this set of rxns

The first intense band (going up) on the BioRad EZ 20bp ladder is 200bp (marked by the long red horizontal arrow in all the gels).

This band doesn't seem to coincide with the second band (going up) of the NEB 100bp ladder, which is also 200 bp.

However, the 1000bp marker for both coincide in migration (see last band going up of the BioRad EZ 20bp ladder).

Expected band (224bp) should be slightly higher than the 200bp band of the BioRad EZ 20bp ladder.

Arrows mark probable expected band. These are faint (brightest is 0.5M T, cleanest is 1M For. Mor) (also there is the typical background smear of DV).

Conditions selected for anng grad are as follows:

	denat temp		
	1 st	2 nd	3 rd
1 DV + 0.5M T	79.1	82.9	
2 DV + 1M T	76.4	82.9	75.0
3 DV + For. Mor	75.0	79.1	
4 DV + NMP	87.7	91.7	
5 DV - No solvt	82.9	87.7	