Date 3/4-5/2013

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: 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
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

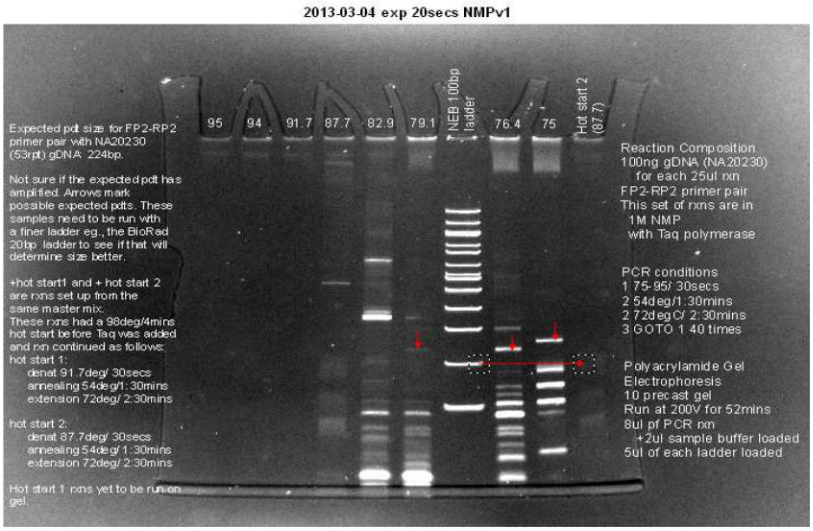
Gradient steps: 95, 94, 91.7, 87.7, 82.9, 79.1, 76.4, 75degC

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)

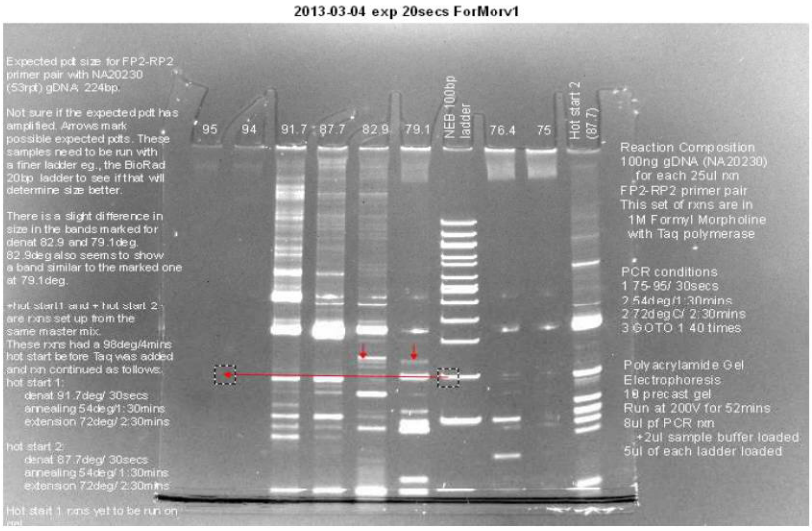
Gel Pictures

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

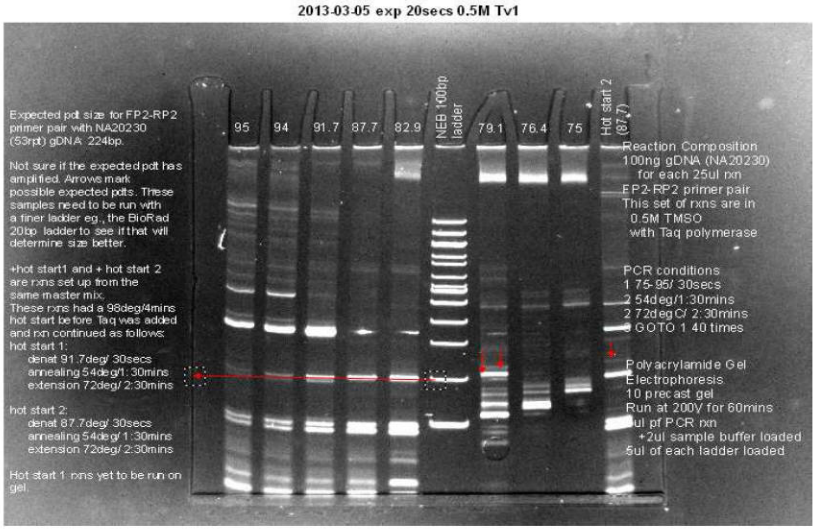
FP-RP-Taq-NMP 20sec exp



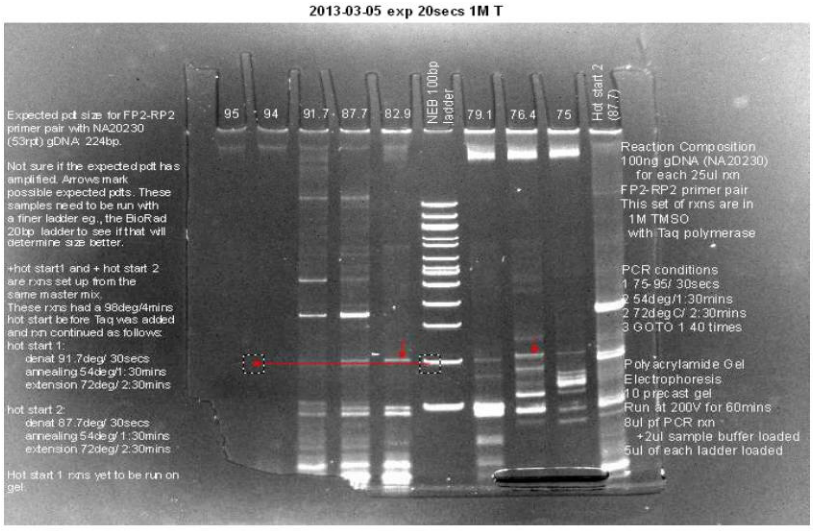
FP-RP-Taq-For.Mor 20sec exp



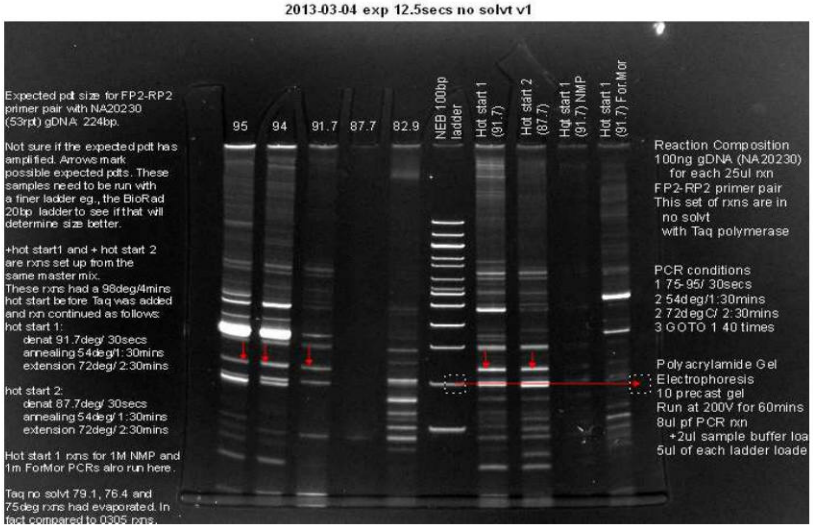
FP-RP-Taq-0.5M TMSO 20sec exp



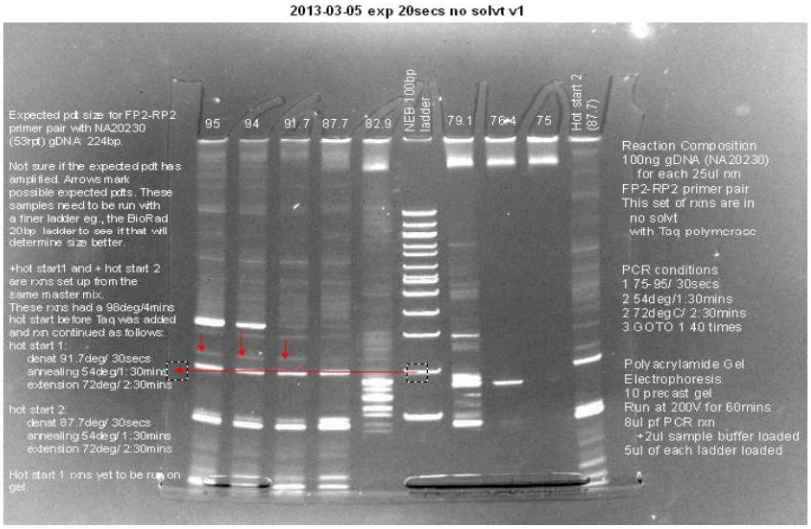
FP-RP-Taq-1M TMSO 20sec exp



FP-RP-Taq-No solvt 1 12.5sec exp

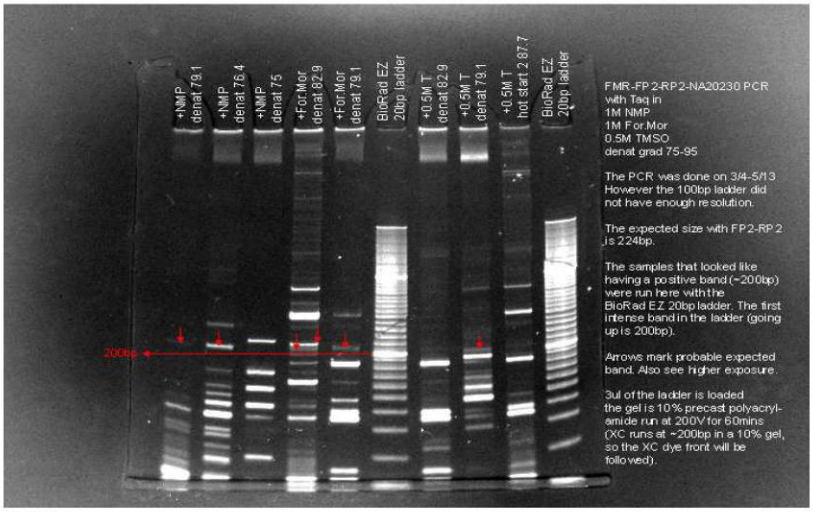


FP-RP-Taq-No solvt 2 20sec exp



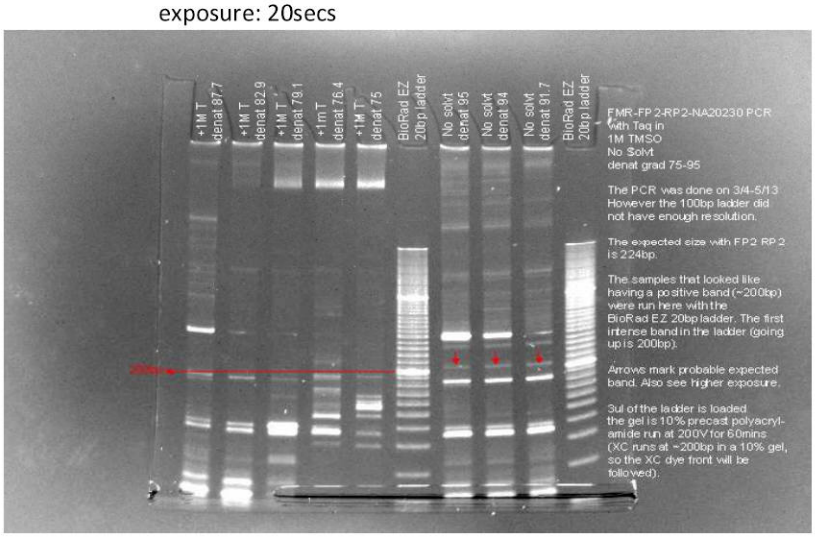
NMP-For.Mor-0.5M TMSO PCR rxns run with the BioRad EZ 20bp ladder

(as per comments section)
exposure: 20secs



1M TMSO-No Solvt rxns run with BioRad EZ 20bp ladder

(as per comments section)
exposure: 20secs



Comments

All the solvents tested seem to show bands between 200-300bp but the BioRad 20bp ladder is required to finely resolve the sizes of the bands obtained to see which one (if any) is closest to 224bp (expected size).

The following samples were run on a gel (with the BR EZ 20bp ladder) :

1	Taq + NMP denat 79.1, 76.4, 75deg	3
2	Taq + For. Mor denat 82.9, 79.1deg	2
3	Taq + 0.5M T denat 82.9, 79.1 and hot start (87.7)	3
4	Taq + 1M T denat 87.7- 75deg	5
5	Taq - no solvt denat 95, 94, 91.7	3

The no solvt rxns were rpt'd because of evaporation issues during the first trial.
The 3/4 and 3/5 rxns show a similar pattern.

The 3/4 rxns are brighter (can be explained by the rxns becoming concentrated due to evaporation.)

Conditions selected for anng grad (after running samples with the BioRad EZ 20bp ladder and discussion with PMC 3/7/13)

		denat temp		
		1st	2nd	3rd
1	Taq + NMP	76	75.0	
2	Taq + For.Mor	83	79.1	
3	Taq + 0.5M T	79		
4	Taq + 1M T	76		
5	Taq-No solvt	92	94.0	95.0