Date 2/7/2013

Objective PCR amplification of triplet repeat region of FMR1 with a modified program (as below):
- Without hot start
- Three step amplification process
- Denat temp grad 75-95deg
- 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)
This is the newly ordered (Jan 2013) DNA: all old DNA is over

Primers: FT for and FT rev

Polymerase: Herculase Fusion II (Agilent)

Solvents: No Solvt rxns
Rxns in 0.5M NMP + 2.2M Betaine
Rxns in 2.2M Betaine

Reaction Composition:

Component	**No solvt rxns** Final Conc/ amt	**0.5M NMP + 2.2M Betaine rxns** Final Conc/ amt	**2.2M Betaine rxns** Final Conc/ amt
Herc Reac Buffer	1 X	1 X	1 X
dNTPs	0.4 mM	0.4 mM	0.4 mM
FT-For/ SFS-for	0.4 uM	0.4 uM	0.4 uM
FT-Rev/ SFS-rev	0.4 uM	0.4 uM	0.4 uM
gDNA NA203230	100 ng	100 ng	100 ng
Taq Polymerase	0.25 ul	0.25 ul	0.25 ul
Water			
NMP		0.5 M	
Betaine		2.2 M	2.2 M
total	**25ul**	**25ul**	**25ul**

Cycling Conditions:

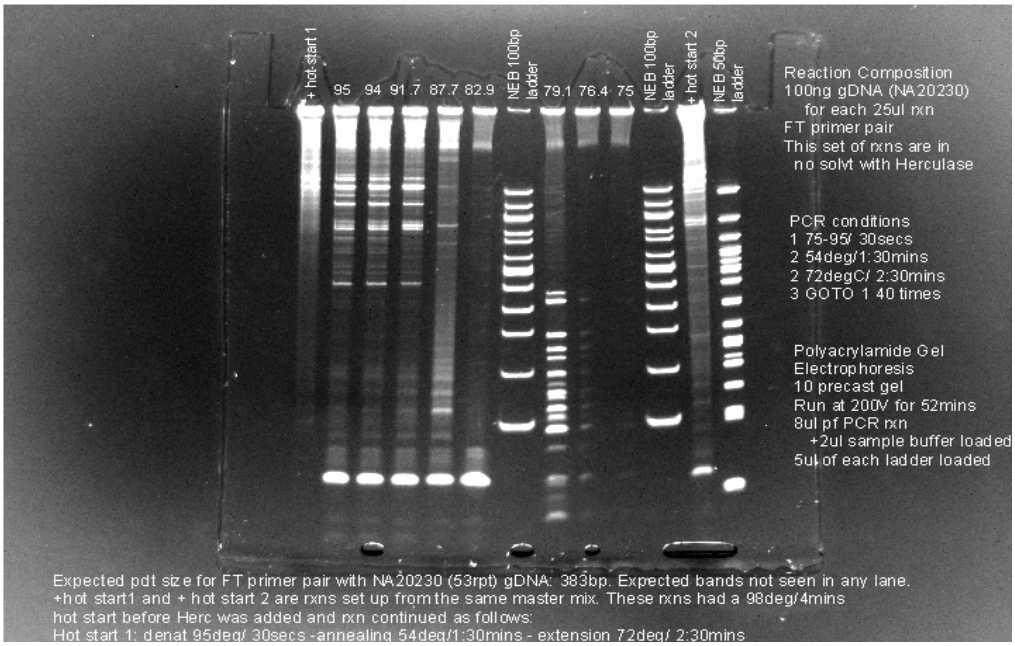
1 75-95degC/ 30secs
2 54degC/ 1:30mins
3 72degC/ 2:30mins
4 GOTO 2 40 times

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

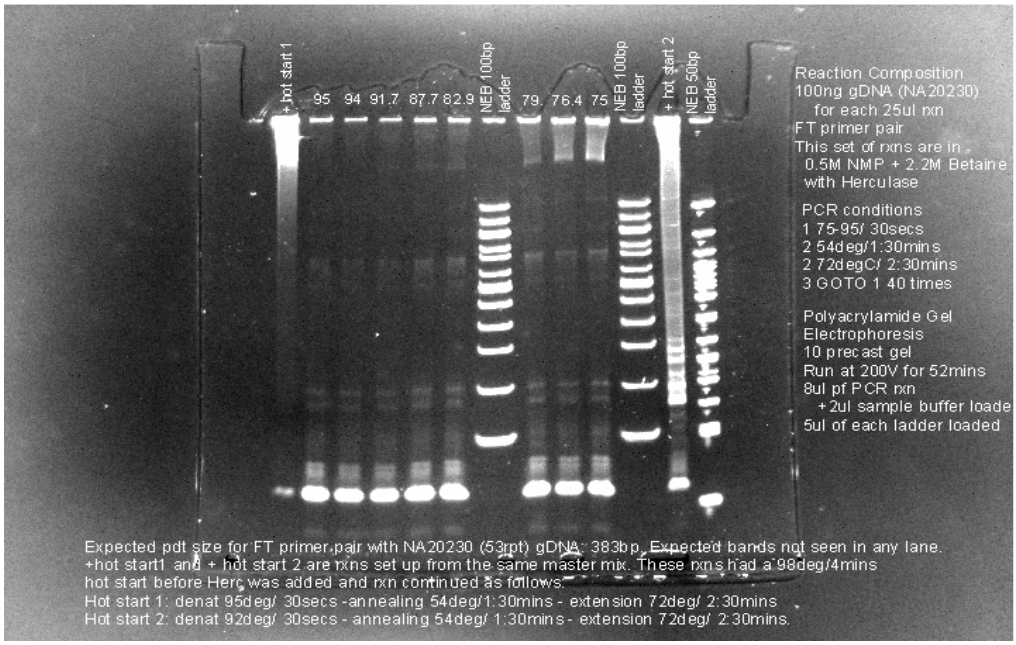
Gel Electrophoresis: 10% precast polyacrylamide gels (Life Technologies)
8ul of PCR rxn + 2ul of (5X) sample buffer loaded
Molecular Ladders: 5ul loaded
100bp ladder (NEB)
50bp ladder (NEB)

Gel Pictures Lanes are marked according to the Denat temp of the particular rxn
Expected product size is 383bp.

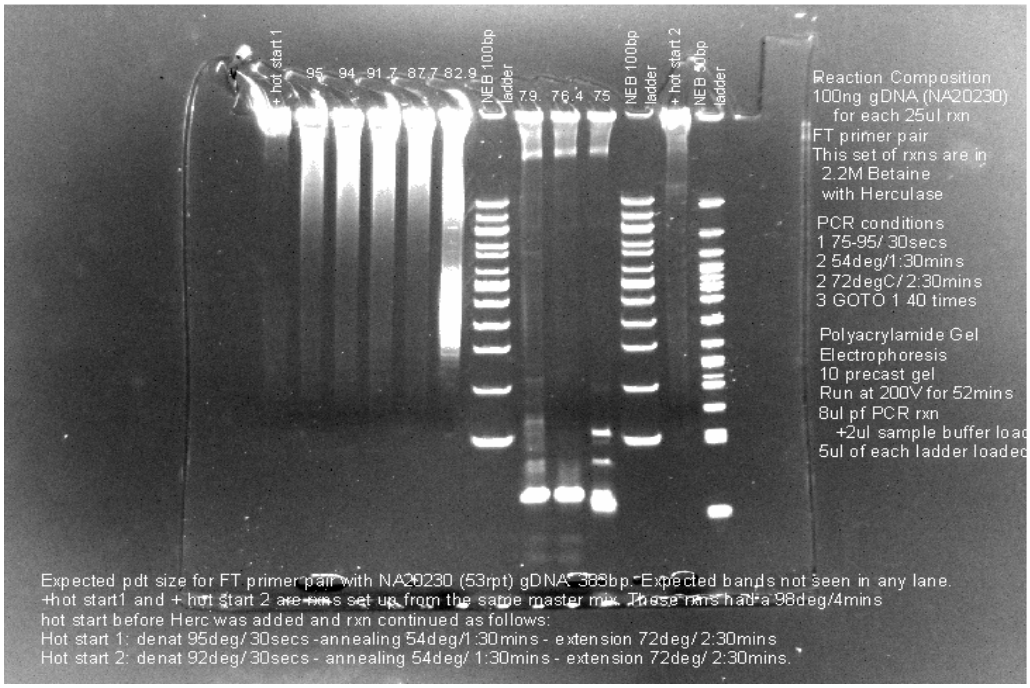
FT-No Solvt 20sec exposure



FT-Herc-0.5M NMP +2.2M Betaine 20sec exposure



FT-Herc + 2.2M Betaine 20sec exposure



Comments Expected bands not seen in any condition.
Even hot start rxns do not seem to have worked.
All Herc rxns were carried out with the new batch of gDNA.
(This expt was with the old (Ram's primer stks)