Date 2/8/2013

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: Deep Vent (New England Biolabs)

Solvents: No Solvt rxns
Rxns in 1M TMSO

Reaction Composition:

Component	No solvt rxns Final Conc/ amt	1M TMSO rxns Final Conc/ amt
Thermopol Buffer	1 X	1 X
dNTPs	0.4 mM	0.4 mM
FT-For/ SFS-for	0.4 uM	0.4 uM
FT-Rev/ SFS-rev	0.4 uM	0.4 uM
gDNA NA203230	100 ng	100 ng
Deep Vent	0.25 U	0.25 U
Water		
TMSO		1 M
total	**25ul**	**25ul**

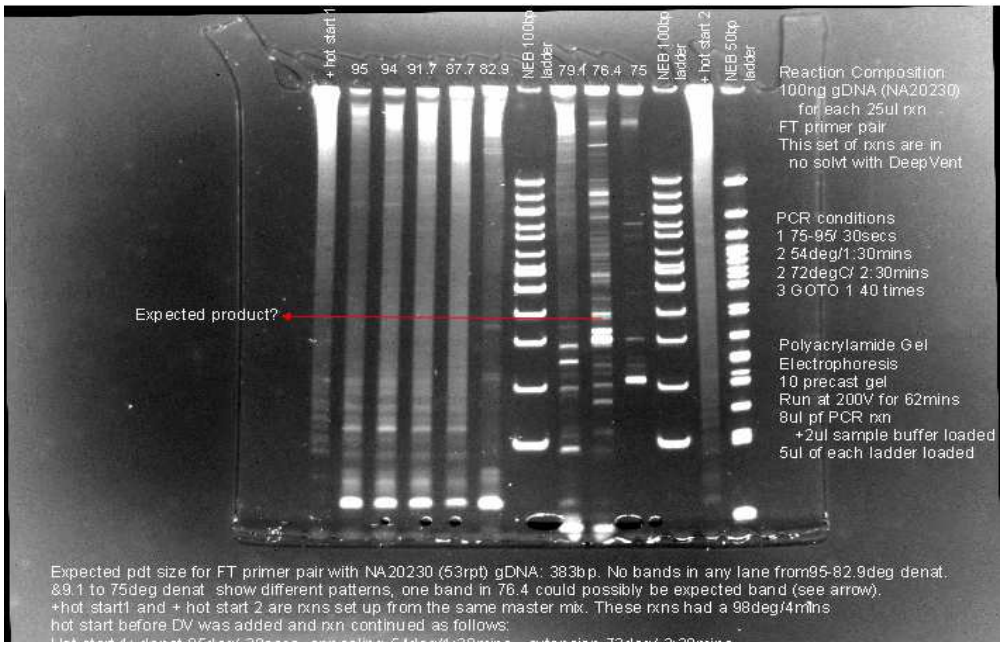
Cycling Conditions:
1 75-95degC/ 30secs
2 54degC/ 1:30mins
3 72degC/ 2:30mins
4 GOTO 2 40 times

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

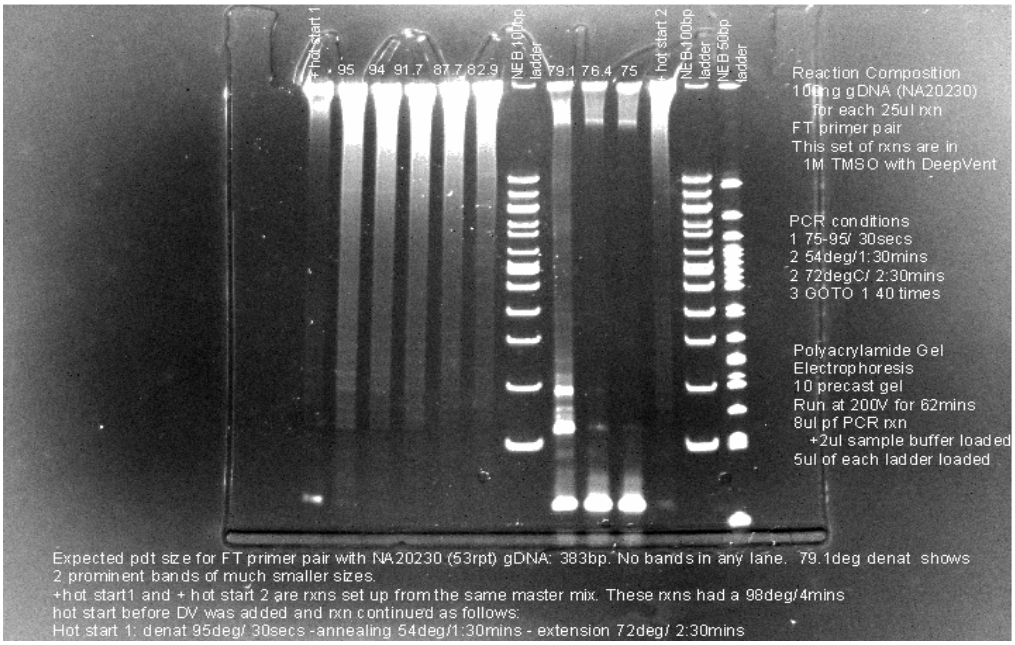
Gel Electrophoresis: 10% precast polyacrylamide gels (Life Technologies)
8ul of PCR rxn + 2ul of (5X) sample buffer loaded
Molecular Ladders: 5ul loaded
100bp ladder (NEB)
50bp ladder (NEB)

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

FT-DV- No Solvt 20sec exposure



FT-DV-1M TMSO 20sec exposure



Comments No bands of expected size.
Based on Taq results, expt will be repeated with new primer stks as well.