Date 2/13/2013

Objective PCR amplification of triplet repeat region of FMR1 with a modified program (as below):
- Without hot start
- Three step amplification process
- Denat temp set to 92degC/ 30secs
- Annealing temp set to 62.6deg/ 1:30mins
- Extension temp. grad (55-75deg); time set to 2:30mins

Description

Template used: 100ng genomic DNA NA20230, (53/54 rpt sample from Coriell)

Primers: FT for and FT rev

Polymerase: Native Taq Polymerase (Life Technologies)

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

Reaction Composition:

Component	**0.5M NMP + 2.2M Betaine rxns** Final Conc/ amt	
Taq Buffer	1	X
MgCl2	1.5	mM
dNTPs	0.4	mM
FT-For/ SFS-for	0.4	uM
FT-Rev/ SFS-rev	0.4	uM
gDNA NA203230	100	ng
Taq Polymerase	2.5	U
Water		
NMP	0.5	M
Betaine	2.2	M
total	**25ul**	

Cycling Conditions:

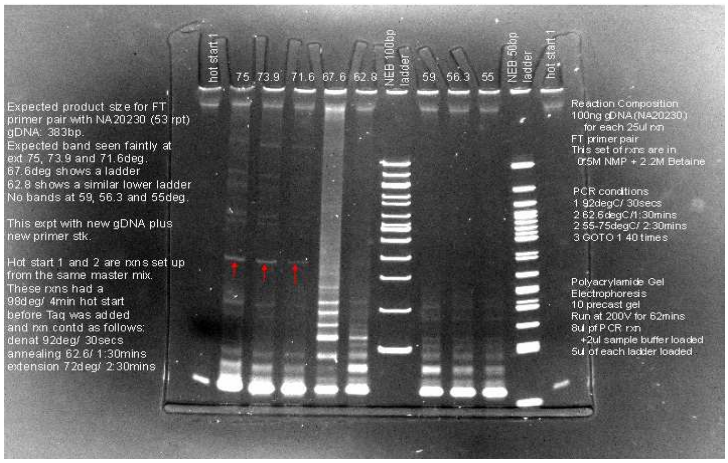
1 92degC/ 30secs
2 62.6degC/ 1:30mins
3 55-75degC/ 2:30mins
4 GOTO 2 40 times

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: 5ul loaded
100bp ladder (NEB)
50bp ladder (NEB)

Gel Pictures Lanes are marked according to the ext temp of the particular rxn

FT primers - NMP + Betaine (20sec exposure)



Comments Expected bands are seen at 75, 73.9 and 71.6deg ext, not very bright (pic is a 20secs exp)
Also, other bands are also seen.

The same ext grad (55-75) will also be tried with anng 57.8 to determine which gives better results.