Date 2/12/2013

Objective PCR amplification of triplet repeat region of FMR1 with a modified program (as belov

- Without hot start
- Three step amplification process
- Denat temp set to 92degC/ 30secs
- Annealing temp grad 50-70deg/ 1:30mins
- Extension temp. and time (that has worked previously
  ie 72deg/ 2:30mins extension)

Expt first tried on 2/5/13 with old gDNA and the (old) 1:50 diluted Ram's FT primer stock.
This expt (2/12/13) with new gDNA and also a new (fresh) aliquot of SM's FT primer stock
SM's FT primer stock has not been used previously.
SM's FT primer stock is already 20uM and does not need dilun.

Description

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

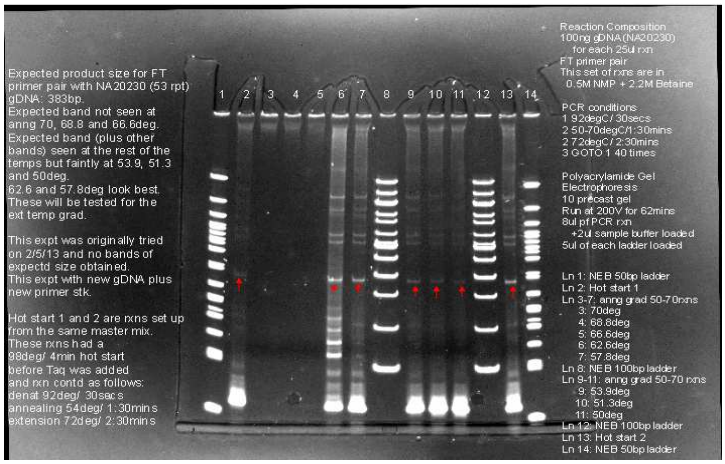
1 92degC/ 30secs
2 50-70degC/ 1:30mins
3 72degC/ 2:30mins
4 GOTO 2 40 times

Gradient steps: 70, 68.8, 66.6, 62.6, 57.8, 53.9, 51.3, 50degC

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

See gel pic for lane information
Expected product size is 383bp.
FT primers - NMP + Betaine (20sec exposure)



Comments

Annealing temps 62.6 to 50deg show expected band (as expected)
Also, as expected, 70, 68.8 and 66.6deg ann'g do not show bands
Both 62.6 and 57.8deg anng will be tested with extension gradient.