Date 1/29/2013

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: SFS for and SFS rev
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	No Solvt Rxns Final Conc/ amt	0.5M NMP + 2.2M Betaine rxns Final Conc/ amt
Taq Buffer	1 X	1 X
MgCl2	1.5 mM	1.5 mM
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
Taq Polymerase	2.5 U	2.5 U
Water		
NMP		0.5 M
Betaine		2.2 M
total	**25ul**	**25ul**

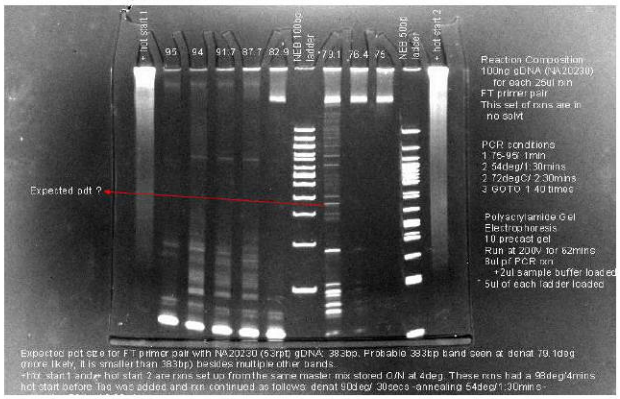
Cycling Conditions:
1 75-95degC/ 30secs
2 54degC/ 1:30mins
3 72degC/ 2:30mins
4 GOTO 2 40 times

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

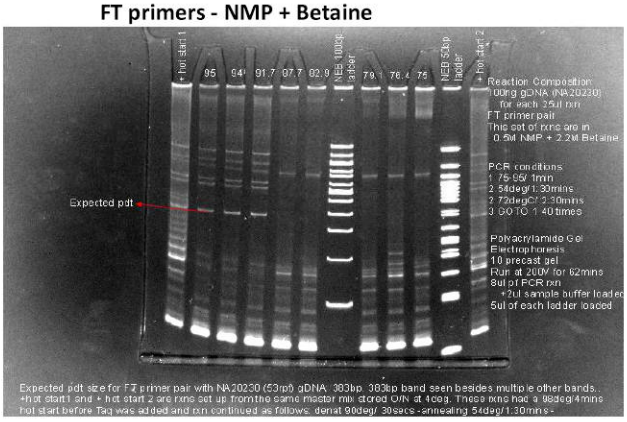
Gel Electrophoresis: 10% precast polyacrylamide gels (Life Technologies)
8ul of PCR rxn + 2ul of (5X) sample buffer loaded
Molecular Ladders: 5ul loaded
100bp ladder (NEB)
50bp ladder (NEB)

Gel Pictures All pictures are 20sec exposures
Expected product size is 383 bp
Lanes are marked according to the denaturation temp of the particular rxn.
See ***012913 NA20230 rePCRd FT-SFS PMC Program 2 denat 75-95***
for the gel pics of SFS PCRs
This expt originally done on 1/29/13 has been put here again (only FT primer data)
for purposes of completeness

FT primers - No solvt



FT primers - NMP + Betaine



Comments **Only rxns with FT primers in 0.5M NMP + 2.2M Betaine show expected bands:**

- Reactions without hot start work at a denat temp of 95, 94, 91.7degC
  giving the expected pdt of 383bp.
- Lower denat temps do not work.
- However, even in the positive rxns, there are multiple other bands
  (of both smaller and higher sizes).
- Hotstart 1 and Hotstart 2 were set up from the same mastermix stored O/N
  (2.5U taq in N+B solvt O/N) at 4degC.
  However, in these rxns more taq was added after the hot start
  and before cycling
- These also show the 383bp band but show many more extra bands.
- The hot start rxns were PCR'd as follows:
  denat 90deg/ 30secs
  annealing 54deg/ 1:30mins
  extension 72deg/ 2:30mins
  40 cycles

All other test conditions do not produce any bands of expected size.
Even for these corresponding rxns were set up with hot start (as described above).
However, even the hot start rxns do not produce any bands of expected size.
The 79.1deg denat rxn with FT primers (no solvt) shows a band in the expected region but is more likely smaller than 383bp. Not sure.

Conclusion 92degC/ 30secs is going to be the preferred denat temp (without hot start)