Digest test - final

Tuesday, October 08, 2013 13:14

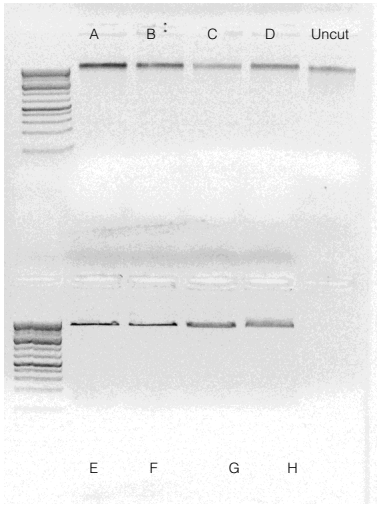
Pooled sunflower DNA

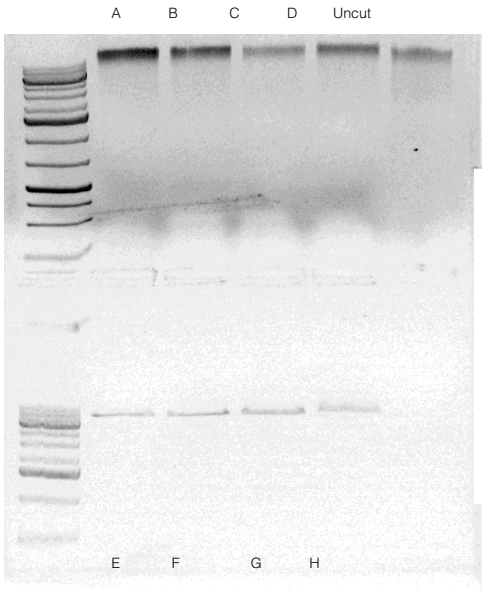
Sample A	Buffer H	Invitrogen	15U/uL	2.7 uL	For 40 U
Sample B	Buffer O	Thermoscientific	10U/uL	4 uL	For 40 U
Sample C	Cutsmart	NEB	20U/uL	2	For 40U
Sample D	Cutsmart	NEB-HF	20U/uL	2	For 40U

pAD2 - contains single unmethylated PstI site.

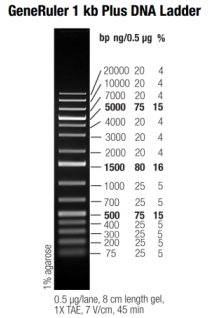
Sample E	Buffer H	Invitrogen	15U/uL	2.7 uL	For 40 U
Sample F	Buffer O	Thermoscientific	10U/uL	4 uL	For 40 U
Sample G	Cutsmart	NEB	20U/uL	2	For 40U
Sample H	Cutsmart	NEB-HF	20U/uL	2	For 40U

Electrophoresis after 70 minutes - 40 uL of 200 uL loaded for each sample. Top lanes - A -D + uncut DNA. Bottom lanes - pAD2



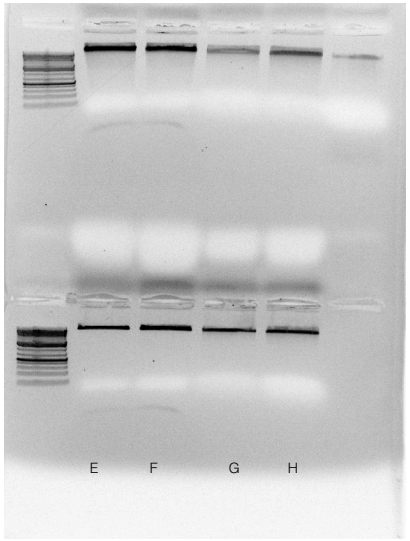


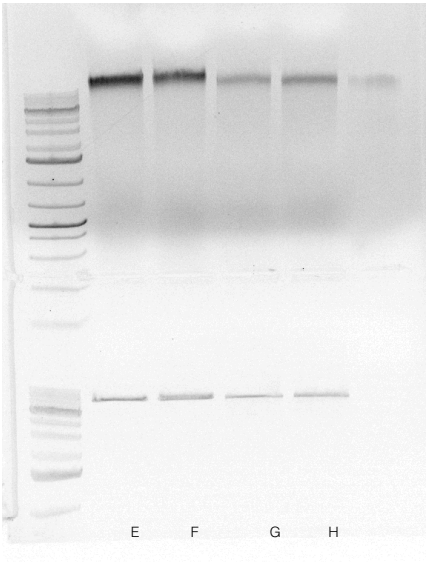
Lowest intense ladder marker Is 500 bp. Second lowest intense marker is 1500 bp.



Ladder = SM1331 - Fermentas/Thermofisher

Electrophoresis after18 hour digest - 60 uL of 160 uL loaded for each sample. Top lanes - A -D + uncut DNA. Bottom lanes - pAD2





Results - incomplete digestion after 70 minutes. 70 minute digestion is slightly greater than GBS protocol, prescribed, 3hrs with 10 U PstI. Partial digestion observed overnight. None of the enzymes exhibit appreciable start activity at 40 U enzyme for 200 uL digestion.

Reaction setup from October 7, 2013

Testing PstI NEB
PstI HF NEB
PstI Thermoscientific/Fermentas
PstI Invitrogen

Pool DNA from Dan B and Kate O
DB209
DB183
DB238
JA52
DB320

Measured pooled DNA with nanodrop - 260/280; 260/230/ ng/uL

1.66	25.67	49.7

G5D
MON014

Measured pooledDNA with nanodrop - 260/280; 260/230/ ng/uL

1.69	1.30	83.3

Digested 50 uL of DNA and 25 uL of DNA
Used volumes shown in column 4 to make 40 U of enzyme for each digest

Sample A	Buffer H	Invitrogen	15U/uL	2.7 uL	For 40 U
Sample B	Buffer O	Thermoscientific	10U/uL	4 uL	For 40 U
Sample C	Cutsmart	NEB	20U/uL	2	For 40U
Sample D	Cutsmart	NEB-HF	20U/uL	2	For 40U

Lot 0411106
Lot 0011010

200 uL digest
127.3 water Sample A
126 water B
128 water C
128 water D
20 buffer (see column 2 above)
PstI (see column 4 above) 2.7 A
4.0 B
2 C
2 D
DNA Samples A and B received 50 uL, C and D received 25 uL each