Agarose Gels – key

Loraine_Sheared DNA

Lane 1	Lane2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
PCR Ladder (2ul)	Phas. Var 4, sheared	Phas. Var 4, intact	PCR Ladder – 4ul	Glycine, var 4, sheared	Glycine, var 4, intact	PCR ladder – 2ul	empty

Most of the sheared DNA is between markers 5, 6 which are the 150-300bp markers. The Phaseolus 'intact' DNA seems somewhat degraded already (much more smearing in the lane). This is the group 4 varieties.

WYBB_ShearedDNA

Lane 1	Lane2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
PCR Ladder (2ul)	Glycine, Var 3, sheared	Phas. Var 3, sheared	PCR Ladder – 5ul	Blank	Phaseolus, var 2, sheared	Glycine, var 2, sheared	empty

As with the above gel, most of the sheared DNA is between markers 5,6. Note that WY is Williams, Yen and their varieties were labeled as belonging to group 3, and that BB is Baxter and Brown, and their varieties were labeled as belonging to group 2. Both sets of sheared DNA appear in approximately the same intensities, so likely the loadings were similar.

SS_ST_SHearedDNA

Lane 1	Lane2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
Phaseolus, Var 1, sheared	Glycine, Var 1, sheared	PCR Ladder – 2ul	Glycine, var 5, sheared	Phaseolus, Var 5 rep1, sheared	Phaseolus, var 5 rep2, sheared	PCR Ladder – 5ul	empty

Although the ladder lanes show up, the only sheared DNA that appears is in Lane 1. This likely indicates that the DNA was too dilute to show up on this gel. We should repeat these 4 samples, after making sure that the dilutions are correct.

Acrylamide Gels - 8%, for testing the PCR products. We tested Actin and tubulin in Glycine varieties. ND is 'no DNA', the negative control, CD is 'control DNA' the DNA we supplied that we showed worked. G.a. means 'Glycine – actin' product, while G.T. means 'Glycine – tubulin' product. G.t.CD-64 means the Glycine tubulin reaction on Control DNA was carried out at 64C. In all cases 3ul of the PCR product was used per lane.

Xiao-Kyle (group 1)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Ladder	G.t. CD-64	G.t. CD-64	G.t. Var1-57	G.a. CD-57	G.a. CD-57	G.a. Var1-57	Ladder	G.a. CD-64	G.a. CD-64	G.a. Var1-64	-	-	-	-	-	-	-

Note that I only see the ladders on this gel, and the second ladder seems to be in lane 9 not lane 8 (perhaps one lane was skipped).

Group2_GlycinePCRtest (Baxter-Brown)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
-	-	-	-	-	-	-	-	-	Ladder	G.a. Var2	G.a.ND	G.a. CD	Ladder	G.t. Var 2	G.t. ND	G.t. CD	-

The ladder appears to be in lane 9. For the actin gene, there does appear to be some product from the sample DNA and a lot from the control DNA, and no contamination in the negative control. There appear to be two bands in both, which was unexpected, unless this is the tubulin at 57C. Then there is ladder again. Then there seems to be PCR product in the tubulin test of the variety, but nothing from the control DNA – note a couple of higher mw faint bands above the main product in the groups DNA lane – since the gel is slanted it is difficult to size the product precisely.

Group 3 _GlycinePCR test (Yen-Williams)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Ladder	G.t. Var 3 - 64	G.t. CD-64	G.t. ND-64	G.a.Var 3-57	G.a. CD-57	G.a. ND-57	-	G.t.Var 3 - 64	G.t. CD - 64	G.t. nd - 64	Ladder	Ladder	-	-	-	-	-

Unfortunately this gel did not run very well – it seems overloaded. However, clearly the PCR products were all produced, and none appears where it should not so the DNA is fine – personally I would probably run another gel for my notebook, though. It is really hard to

conclude anything about the size or number of bands at a given temperatire base on these results.

Group4_GlycinePCR test (Loraine)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

No key provided.

Group5_GlycinePCRtest (Turner/Smith) 8% polyacrylamide

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Ladder	G.t.Var 5	G.t.CD	G.t.ND	G.a. ND	G.a. CD	G.a. Var 5	Ladder	-	-	-	-	-	-	-	-	-	-

The order of loading seems off – there may be some primer-dimer ladder formation in tubulin –no DNA control. There does not appear to be any product from the tubulin primers in either the sample or control lanes, but the actin primers in both sample and control gave product. That is, the DNA is a good PCR template.

Overall conclusions on Glycine DNA:

For Group 1 DNA, since the Control DNA failed to give PCR product for either primer pair we cannot conclude anything about the quality of the DNA.

For Group 2 the sample and control gave actin product and the sample gave tubulin product so their DNA can be used in PCR.

For group 3, despite the really horrible gel it is clear that both the actin and tubulin reactions were successful on both the sample and control DNA, so we can proceed.

For group 4, in the absence of a key it is not possible to absolutely state that both the sample and control DNA gave successful actin and tubulin amplification, but something gave products of the correct size.

For group 5, the actin primers yielded product for both the control and the sample DNA, so this group has sample DNA that can be used for the next set of experiments.