

# Spring 2009

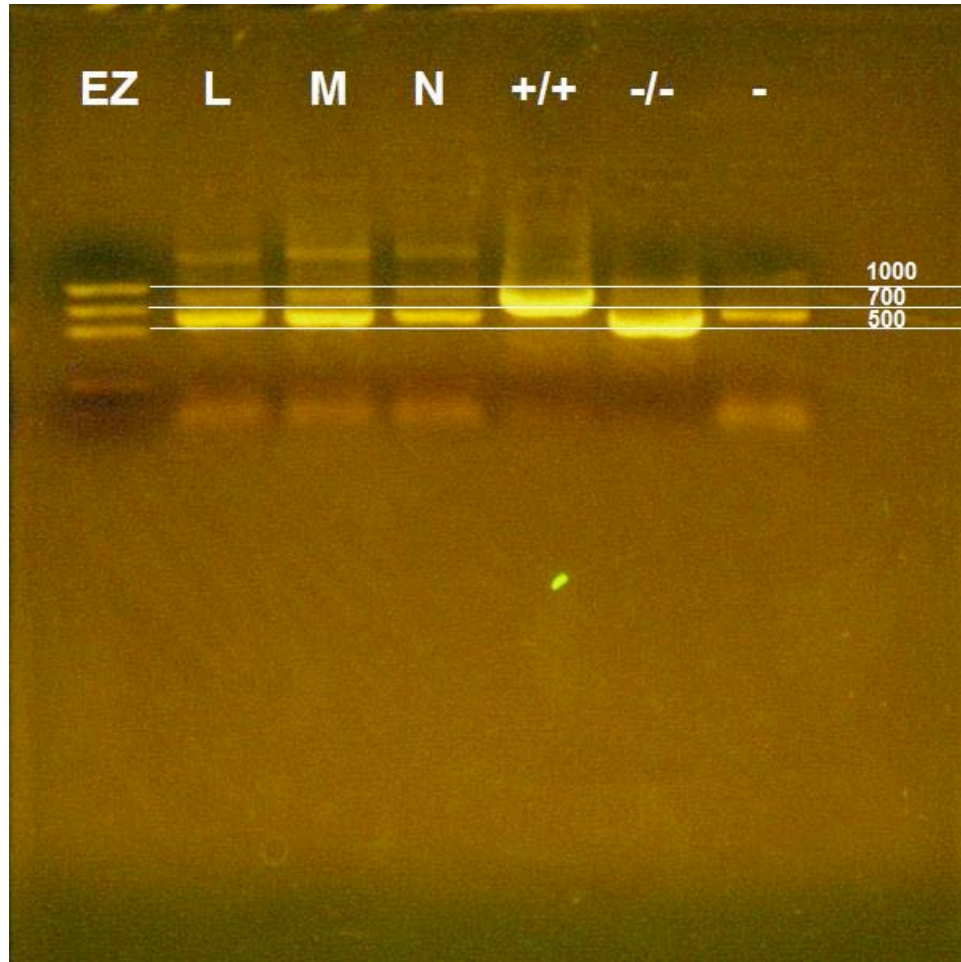
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NISHA PARIKH

## [PROJECT: PV 92]

REPORT FOR THE FIRST 5 RUNS WHICH WERE DONE IN APRIL 2009

EZ	L	M	N	+/+	-/-	-
20 $\mu$ L EZ Marker	25 $\mu$ L Linda's DNA	25 $\mu$ L Minh's DNA	25 $\mu$ L Nisha's DNA	25 $\mu$ L +/+ sample	25 $\mu$ L -/- sample	25 $\mu$ L negative control

1st experiment



**Protocol used:** Old machine, 40 cycles. 94°C (1mn) – 60°C (1mn) – 72°C (2mn). No hot start.

**Result:** This was the best result that we got: Each DNA sample gave 3 clear bands on its corresponding lane (lane L, M and N). Those bands tell us the PV92 genotypes of 3 of us are the same and are heterozygous (+/-).

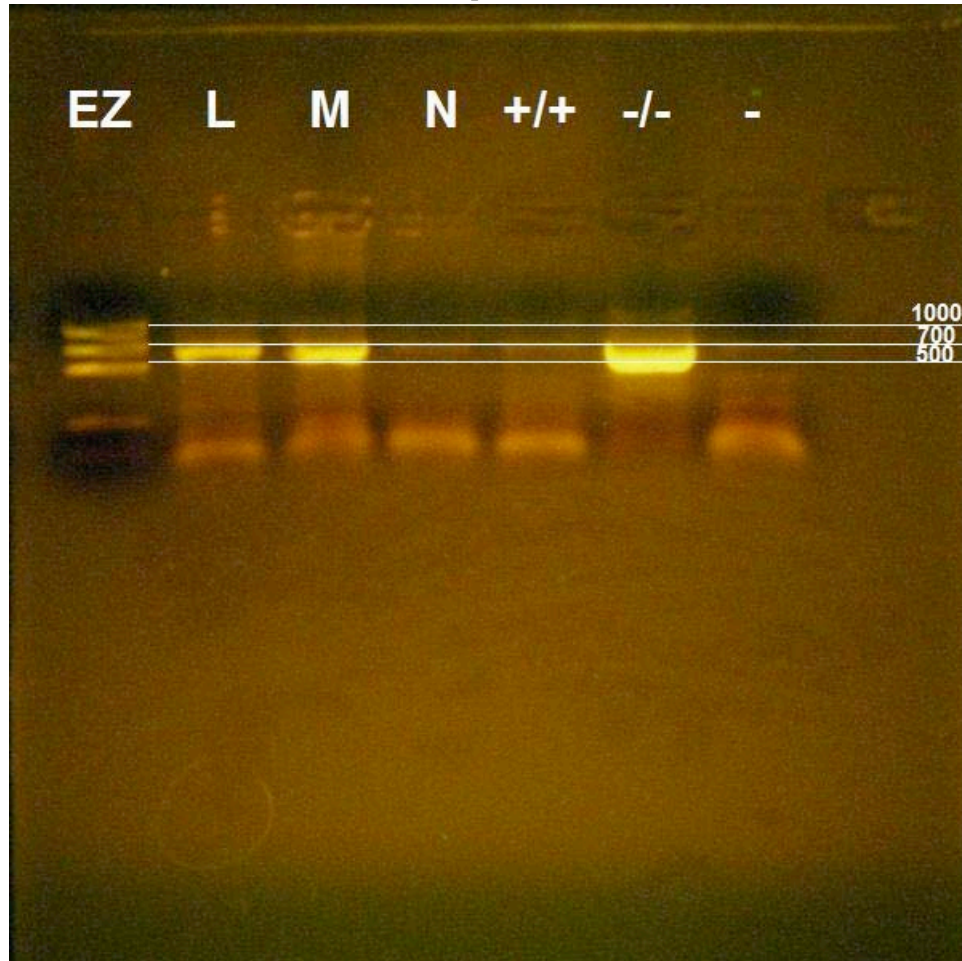
The band located in the 700-1000bp range is the band of the long fragment, which is 941bp long. The band located in the 500-700 range is the band of the short fragment, which is 641bp long. The extra band at the top of each lane is the result of the binding between the positive strand and the negative strand at some points, forming hairpins or loops, which take more space than the other two normal forms and make it move slowly on the electrophoresis gel.

However, we cannot conclude this protocol worked well with 100% confidence because on the negative control lane, we can see a faint band in the 500-700bp range. This happened because the PCR cocktail mix might get contaminated.

The faint bands at the bottom of all the lanes are the primer dimer bands, resulting from the annealing of the primers.

EZ	L	M	N	+/+	-/-	-
20 $\mu$ L EZ Marker	25 $\mu$ L Linda's DNA	25 $\mu$ L Minh's DNA	25 $\mu$ L Nisha's DNA	25 $\mu$ L +/+ sample	25 $\mu$ L -/- sample	25 $\mu$ L negative control

2<sup>nd</sup> experiment



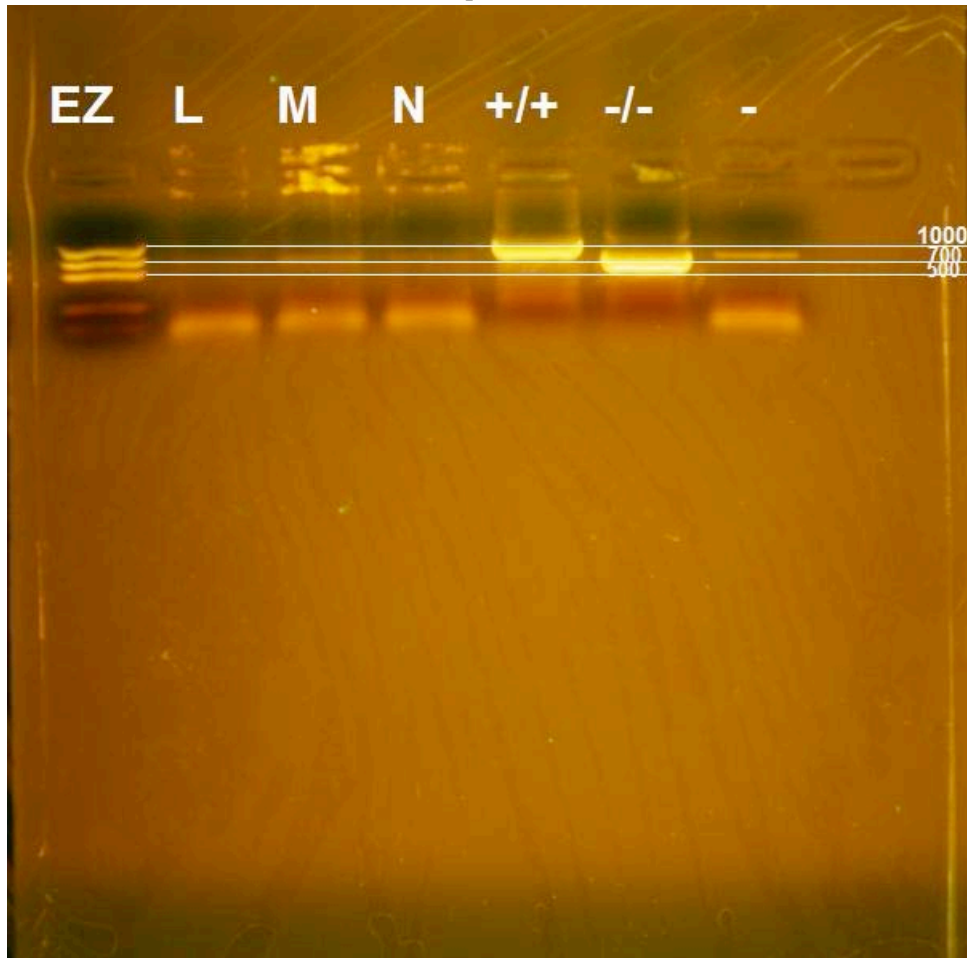
**Protocol used:** **New machine**, 40 cycles. 90°C (30sec) – 60°C (40sec) – 72°C (45sec). No hot start.

**Result:** This clearly did not work, as the negative control lane has bands (very faint), the +/+ positive control band does not have clear band and the -/- negative control band has more than one band (with one of them is located above 1000bp range). The band on the negative control lane might be caused by the contamination of the cocktail mix as mentioned in experiment 1 discussion.

**Note: In experiment 3 to 5, we made new DNA samples and put those samples into the fridge, not the freezer.**

EZ	L	M	N	+/+	-/-	-
20 $\mu$ L EZ Marker	25 $\mu$ L Linda's DNA	25 $\mu$ L Minh's DNA	25 $\mu$ L Nisha's DNA	25 $\mu$ L +/+ sample	25 $\mu$ L -/- sample	25 $\mu$ L negative control

3<sup>rd</sup> experiment

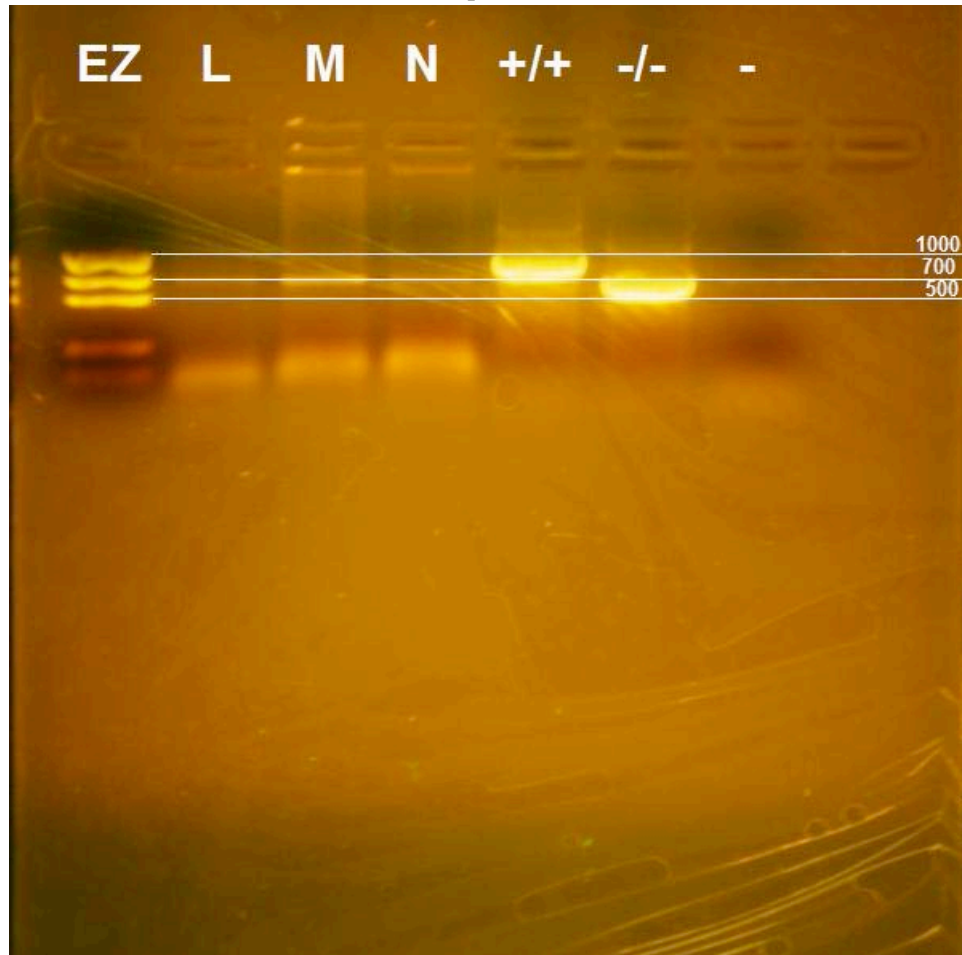


**Protocol used:** **New machine, old program** 40 cycles. 94°C (1mn) – 60°C (1mn) – 72°C (2min). With hot start.

**Result:** This experiment did not work either, as the negative control lane has band on it, the homozygous (-/-) lane has more than one band (the extra band is above the 1000bp range), and the DNA sample's lanes do not have clear band (with some junk in the well holes, which might be cause by Chelex).

EZ	L	M	N	+/+	-/-	-
20 $\mu$ L EZ Marker	25 $\mu$ L Linda's DNA	25 $\mu$ L Minh's DNA	25 $\mu$ L Nisha's DNA	25 $\mu$ L +/+ sample	25 $\mu$ L -/- sample	25 $\mu$ L negative control

4<sup>th</sup> experiment

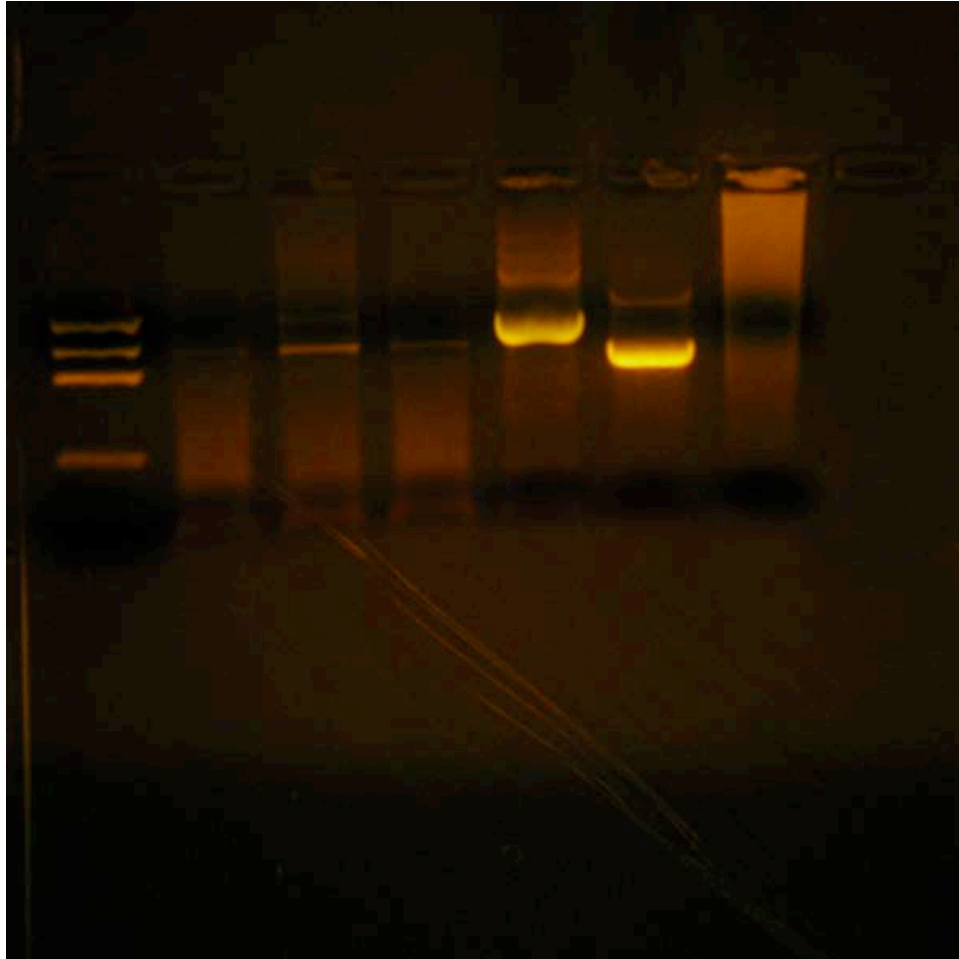


**Protocol used:** **New machine, old program**, 25 cycles. 94°C (1mn) – 60°C (1mn) – 72°C (2min). With hot start.

**Result:** This time, the negative control lane does not have any band. However, the lanes of Linda's and Nisha's DNA samples do not have any band either, only the lane of Minh's sample does have a faint band. Because for all the experiment from 1 to 4, we used the same cocktail mix, this might explain why the negative control lane and the DNA lanes keep having bands or do not have any band at the same time.

EZ	L	M	N	+/+	-/-	-
20 $\mu$ L EZ Marker	25 $\mu$ L Linda's DNA	25 $\mu$ L Minh's DNA	25 $\mu$ L Nisha's DNA	25 $\mu$ L +/+ sample	25 $\mu$ L -/- sample	25 $\mu$ L negative control

5<sup>th</sup> experiment



**Protocol used:** Old machine, 25 cycles. 94°C (1mn) – 60°C (1mn) – 72°C (2min). With hot start.

**Result:** The experiment failed as we can see several bands on +/+ lane and strange negative control lane

### Conclusion:

In 5 experiments/ runs that we did, only experiment 1 (which we used the old machine, 40 cycle and no hot start) gave the most decent result. The only problem is the band that the negative control lane has. This might be cause by the contaminated cocktail mix.

We also should have kept the DNA from experiment 1 and 2 in order to continue the experiment 3, 4 and 5. By that way, we could compare the results of different machines/ protocols.

Right now, we are re-doing the experiments with the new ingredients (that were not used before in order to eliminate contamination) and new DNA sample. We are looking forward to better results.

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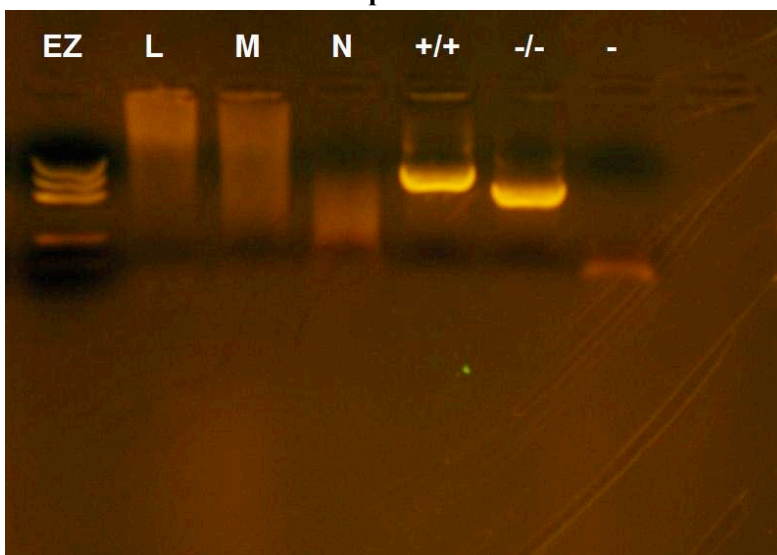
## [PROJECT: PV 92]

REPORT FOR THE NEXT 8 RUNS WHICH WERE DONE IN MAY 2009

**Note: In experiment 6 to 14, we used the same DNA samples. However, we filtered the DNA samples after doing experiment 8 and 9.**

EZ	L	M	N	+/+	-/-	-
20 $\mu$ L EZ Marker	25 $\mu$ L Linda's DNA	25 $\mu$ L Minh's DNA	25 $\mu$ L Nisha's DNA	25 $\mu$ L +/+ sample	25 $\mu$ L -/- sample	25 $\mu$ L negative control

**6<sup>th</sup> experiment**

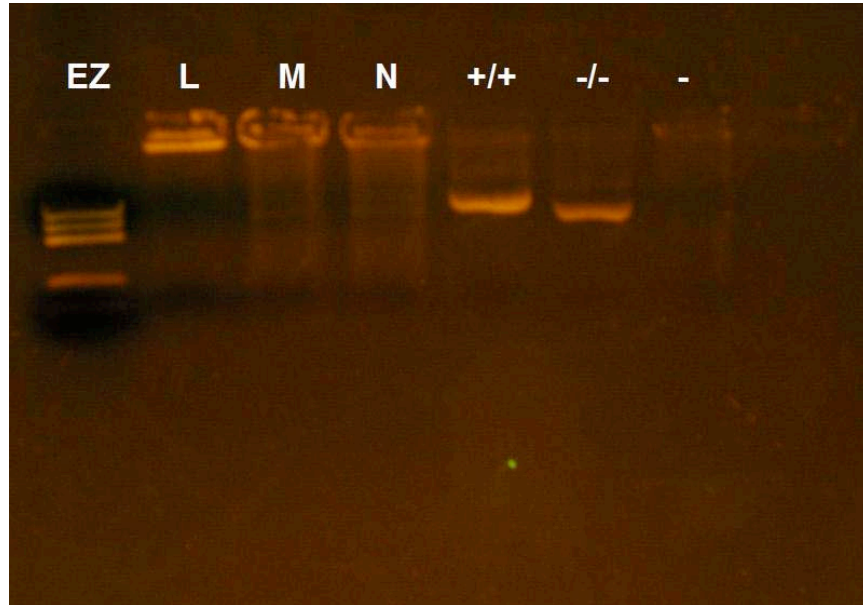


**Protocol used:** Old machine, 40 cycles. 94°C (1mn) – 60°C (1mn) – 72°C (2mn). Hot start.

**Result:** We could see 3 smear bands on 3 DNA samples' lanes. Those could be caused by the amount of cycles we ran the PCR. 40 cycles might be too much and during that time, primers might bind to some other sites and began replicating, resulting in the smear bands that we saw on the gel.

EZ	L	M	N	+/+	-/-	-
20 $\mu$ L EZ Marker	25 $\mu$ L Linda's DNA	25 $\mu$ L Minh's DNA	25 $\mu$ L Nisha's DNA	25 $\mu$ L +/+ sample	25 $\mu$ L -/- sample	25 $\mu$ L negative control

7<sup>th</sup> experiment

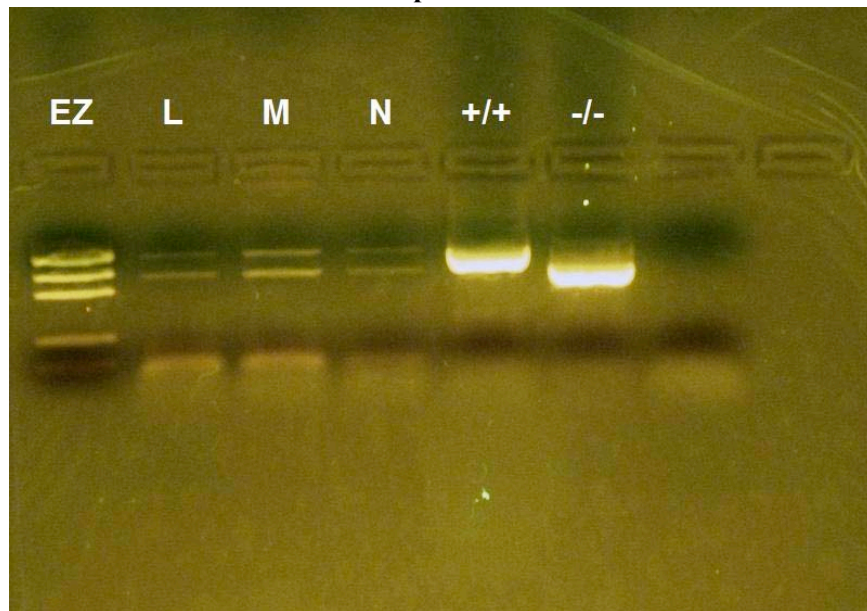


**Protocol used:** New machine, old program 40 cycles. 90°C (1mn) – 60°C (1mn) – 72°C (2mn). Hot start.

**Result:** We can also see smear bands on this gel. However on Minh's sample and Nisha's sample lanes, there are faint bands at PV92 strand band's positions (one found in 1000-7000kb range, the other in 700-500kb range). These bands agree with the genotype heterozygous that we found in the 1<sup>st</sup> experiment (1<sup>st</sup> lab report).

EZ	L	M	N	+/+	-/-	-
20 $\mu$ L EZ Marker	25 $\mu$ L Linda's DNA	25 $\mu$ L Minh's DNA	25 $\mu$ L Nisha's DNA	25 $\mu$ L +/+ sample	25 $\mu$ L -/- sample	25 $\mu$ L negative control

### 8<sup>th</sup> experiment



**Protocol used:** Old machine, old program 25 cycles. 94°C (1mn) – 60°C (1mn) – 72°C (2min). Hot start.

**Result:** This was the best that we have ever done: the negative control worked (resulting in no band); every band of DNA sample lanes is clear and show the genotype of each of us is heterozygous (which agrees with the results we got from the previous experiments)

### 9<sup>th</sup> experiment

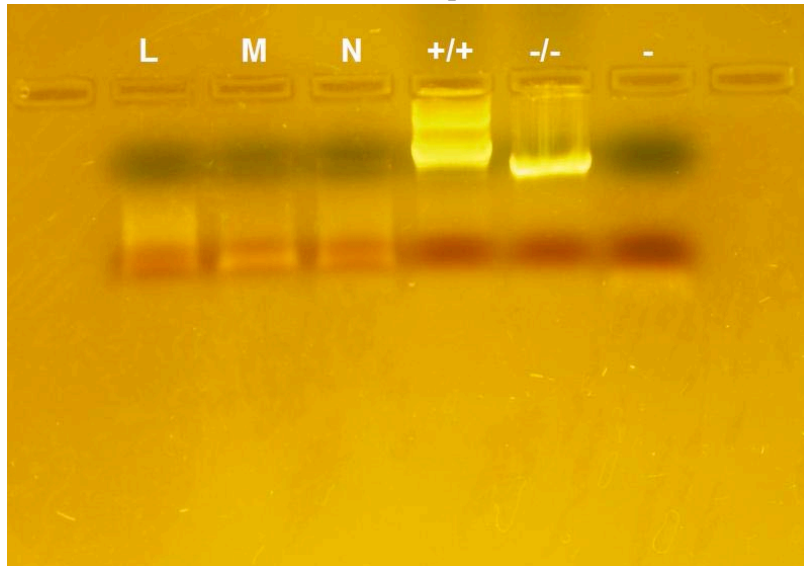
We also did the same thing as the previous experiment. We got the same result, but we did not save the picture by mistake. The results of experiment 8 and 9 made us strongly believed that the old machine 25 cycles protocol with hot start gave decent results, but the results were inconsistent in some cases (as we still see faint and unclear bands). The results of experiment 8 and 9 also eliminated the hypothesis “There are difference in using the old machine and the new one”. The difference is not the machine itself, but it is the protocol that we used, and the protocol of the old machine (with long extension) is better and gives better results.

In addition, because we did not filtered the DNA sample before doing these experiments but still got good results in experiment 8 and 9, we can conclude that the smear bands seen in the previous experiments were not caused by extra Chelex in the DNA sample.

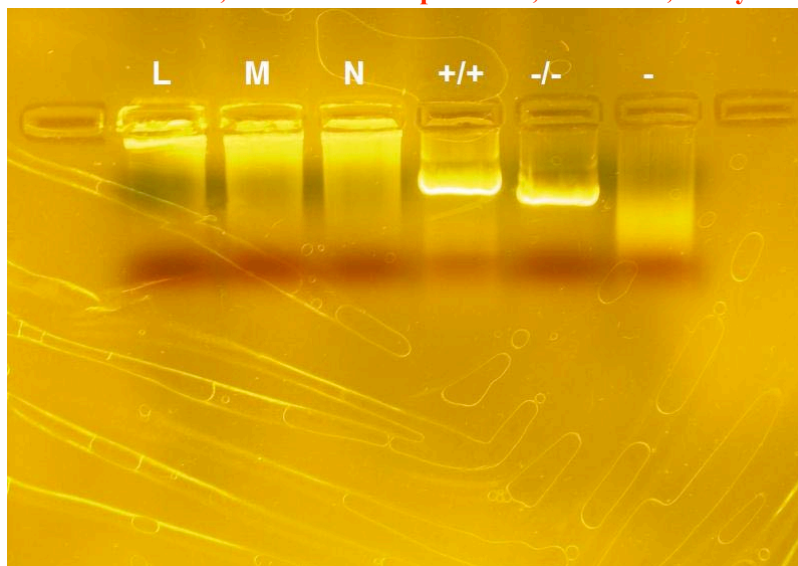
**Note: the DNA samples that we used in the following experiments were filtered. However, the old machine was taken, so we had to use the black machine that we had never used.**

L	M	N	+/+	-/-	-
25 $\mu$ L Linda's DNA	25 $\mu$ L Minh's DNA	25 $\mu$ L Nisha's DNA	25 $\mu$ L +/+ sample	25 $\mu$ L -/- sample	25 $\mu$ L negative control

**10<sup>th</sup> and 11<sup>th</sup> experiment**



**Black machine, the same old protocol, hot start, 25 cycles**

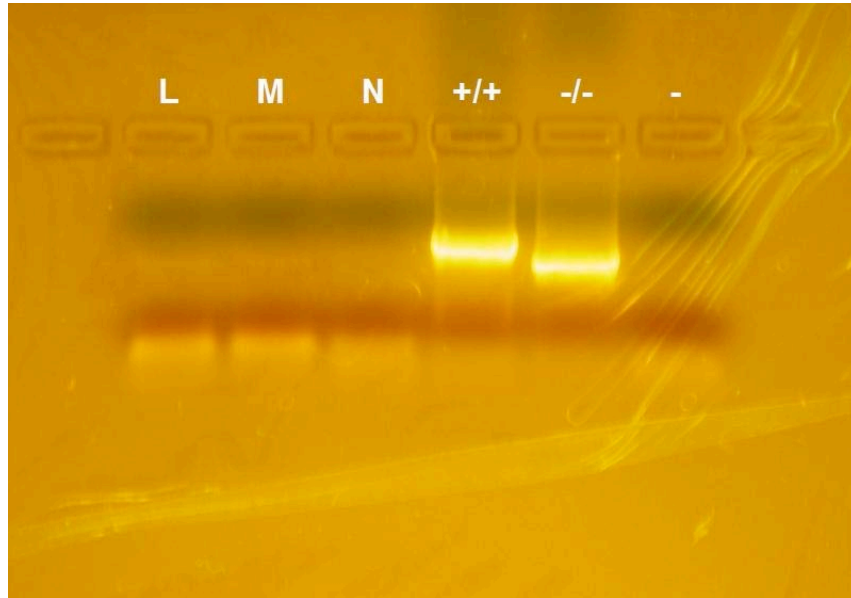


**Black machine, the same old protocol, hot start, 40 cycles**

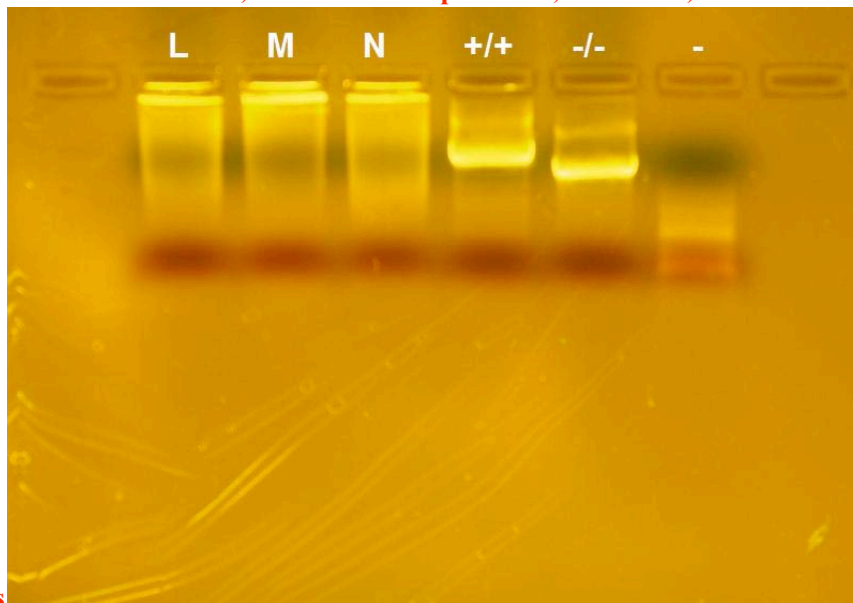
**Result:** There is a clear difference between 25 cycles and 40 cycles. 40 cycles give smear bands, but 25 cycles give faint band.

L	M	N	+/+	-/-	-
25 $\mu$ L Linda's DNA	25 $\mu$ L Minh's DNA	25 $\mu$ L Nisha's DNA	25 $\mu$ L +/+ sample	25 $\mu$ L -/- sample	25 $\mu$ L negative control

12<sup>th</sup> and 13<sup>th</sup> experiment



New machine, the same old protocol, hot start, 25



cycles

New machine, the same old protocol, hot start, 40 cycles

**Result:** The same results as black machine's.

## **Conclusion:**

1. 25 cycles give better results than 40 cycles (Which give smear band every time), but the 25 cycles' bands are still faint. We should try 30 cycles or so.
2. There is no difference in using the new machine and the old machine. However, the old machine's protocol **(25 cycles, 94°C (1mn) – 60°C (1mn) – 72°C (2min), Hot start)** works, while the new machine's protocol doesn't work well.
3. We shouldn't make large amount of cocktail at once. For 6 PCR tubes, we only need 200 µL master mix and 4 µL primer. For 2 set of 6 PCR tubes (12 tubes), 300 µL master mix and 6 µL primer are enough. Don't make higher amount than this (the original ratio is 400 µL master mix /8 primer and it is hard to calculate, mix and pipet the amount of 140 µL cocktail for 6 tubes)
4. Hot start is necessary.
5. Everything should be kept on ice before running PCR machine.
6. The DNA samples should be filtered before pipetting into PCR tubes to eliminate Chelex.