PCR.

1. Transfer DNA from plates to stock wells.
2. Transfer 10µto PCR plates.
3. Perform a 1 in 20 dilution thus 190 µl MilliQ Water.
4. Prepare Master Solution:

		Master Mix x1	Master Mix x105
MilliQ Water		5.4 µl	567 µl
10x NH_4		1.0 µl	106 µl
50 mM $MgCl_2$		0.3 µl	31.5 µl
10 mM dNTP		0.2 µl	21 µl
Forward	0.5 µl	1 µl	105 µl
Reverse	0.5 µl		
Taq		0.1 µl	10.5 µl
DNA		2.0 µl	210 µl

5. Remove Taq last (Keep in fridge till use).
6. Run PCR according to the Marker.
7. Add dye to PCR – approx. 1 – 2 µl
8. Store in Fridge till use.

Gel.

1. Clean Tray.
2. Pick a clean glass plate –the less chips the better.
3. Add Bind Saline – to cover plate and leave for 10 min.
4.

Acryl Biss	15 ml
Urea	15 ml
10 x TBE	6 ml
APS	600 µl
Temed	60 µl To be added just before pouring the Gel.

   Make up Cylinder to 60 ml.
5. Note which side was the top of the plate.
6. Rinse plate with MilliQ Water.
7. Dry Plate.
8. Clean Back plate and spacers with Ethanol and tissue.
9. Put Rainox on back plate and spacers.
10. Place spacers on back plate and place front plate face down on the back plate.
11. Get claps that match.
12. Clamp and place in pouring try.
13. Insert the comb and make sure that half the gap is visible.
14. Prep Syringe and then add Temed to Gel mix.
15. NO air bubbles in syringe and inject firmly between plates.
16. Make sure that half the comb is in the gap.
17. Let Gel sit for 40 min.

Running the Gel.

1. Run Gel at 60W for 15 min.
2. Add ladders:

20 bp	1.5 µl Ladder	1.5 µl Dye
100 bp	1.5 µl Ladder	1.5 µl Dye

3. Load Gel and Run.
4. Treat Gel with Acetic Acid for 10 min and rinse 3 times with MilliQ Water.
5. Place in Silver stain for 20 – 40 min.
6. Place in Developing solution made up during the running of the gel.

Gel Scan.

1. 15 ml Gel mix – 6.4 ml Poly Acrylamide and 3ml 10x TBE made up to 50 ml with MilliQ.
2. Vacuum pump.
3. Get plates ready – wash with Windex and 70% Ethanol.
4. Place in caster and push in.
5. Rinse spacers with MilliQ.
6. Bind Saline wipe to 1cm from wells (Bind Saline = 2ml Bind + 25 ml Ethanol).
7. Add 100 µl APS to Gel mix.
8. Add 10 µl Temed to Gel mix.
9. Pour on Plate and close.
10. Clamp the bottom of plates.
11. Insert Comb.
12. Clamp to of plates and let sit for 30 min.

Setup of Gel Scan.

1. Insert bottom tray and connect electrode.
2. Add buffer (0.6x TBE) close to the red line (NOT above).
3. Add 1 drop Dye (Athidium Bromide).
4. Insert plate.
5. Attach top Buffer tank, connect and add buffer.
6. Turn Gel Scan on.
7. Press “Next” to Temp/Volts.
8. Start – Dec – Pre run 900 V for 30 min.
9. Load Gel.
10. Pulse for 15 sec and Check.
11. Setup Computer.
12. Run the gel at 1200 V.