1 20 native PAGE-Gel in 15 ml

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Material:

10 ml	30% acrylamide
300 μl	50% TAE
150 μl	10% APS
10 μl	Temed
1 μl	Sybr Gold
25 ml	1x TAE

⇒ Refill solution of acrylamide, TAE, APS, Temed with water so that the **endvolume is 15 ml**: 4,5 ml water are required.

1 Prepare your workplace:

- 5ml/10ml pipette, pipette boy, glass pipette
- Gel cassette, gel caster, comb and holder
- Rotiphorese Gel 30 (37, 5:1) (right fridge
- Temed (left floor unit on the left side of the fridge)
- APS (right fridge
- 50 % TAE
- 1 Falcon
- Parafilm

⇒ Take all of it under the hood **Rotiphorese Gel 30 and Temed are toxic ingredient**

2 Begin to carefully pipet Rotiphorese Gel 30 into the falcon.

3 Add water (One can also start with water, it does not matter)

4 Add the 50 % TAE buffer

Now it's important to work fast. Once one add Temed and APS the solidification starts from the point of injection. In order to allow the solidification center to continuously spread, one has to immediately vortex the sample after injecting Temed and APS.

5 Add Temed ⇒ Vortex for at least 30 sec

6 Add APS ⇒ Vortex for at least 30 sec

7 Then quickly fill the solution into the Gel cassette. Insert the comb. There should be enough sample volume so that there is a surplus. With this surplus one can test if the gel is already solidified. Concerning this concentrations of APS and Temed, the gel should be solid after 10 min. Once the gel is solid, it is not toxic anymore.

8 Clean the hood during the solidification process and prepare your DNA samples.

9 If the Gel is solid put the gel cassette into the gel caster and remove the comb.

10 **Firstly fill the gel camber with the buffer one has used for the gel preparation**. In this case: 50% TAE. After the gel chamber has been filled, one can stock up the box to the marker depending how many gels one wants to run. Be careful: The gel chamber should be completely filled up with buffer!!

11 Put on the lid and connect the gel box with the power source.

12 Let the gel run for 1,5-2h with 150 V (depending how short/long your DNA samples are. Be carefull: If the samples are quite short than the running time is not so long. There is the risk that the samples leak. Running time 05.06.15: 1 h 41 min)

13 After the Gel has run, it has to be stained in order to make the separated sample visible.

14 Therefore take the gel out of the cassette.

15 Put 1x TAE and sybr gold into a bowl and put it on the shaker for 15 min (gently shaking). Attention: Sybe Gold is toxic. Don't touch the gel with the hands, use the spatula in order to put the gel into the solution. Sybr Gold is 10 000 concentrated. Mixture with 1x TAE should lead to 1x Sybr Gold.

16 Destaining the gel by putting the Gel in new TAE buffer and shake it again for 15 min.

17 Afterwards, the gel can be observed in the UV box.

18 Testing the UV box with the following settings:

Transillumination controls	Overhead Light
White light on	White on

With this settings one can see if Gel is in the right position etc.

19 Exposure with the following settings:

Transillumination controls	Overhead Light	Emission Filter
UV safety	Off	Sybr Gold