Purification of DNA with Agarose Gels

- determine the right agarose percentage for your separation problem

  (normally 1% for plasmid DNA and 2% for PCR products from 50 - 500 bases works well enough)

Agarose (w/v %)	DNA (kb)
0.3	5.0 - 60
0.6	1.0 - 20
0.8	0.5 - 12
1.0	0.3 - 8.0
1.2	0.2 - 6.0
1.5	0.1 - 3.5
2.0	0.05 - 2.0

- for the normal tray size (10cm x 6cm) you will need 100mL gel solution
- dissolve the required amount of agarose in 100mL 1xTAE buffer
- heat in the microwave till dissolved
- add 0.5 $^{\mu g}/_{mL}$ ethidiumbromide (= 5µL of 10 $^{\mu g}/_{\mu L}$ stock solution) and pour the gel

  (**USE GLOVES !!!**)
- make sure you did not puncture the gel when removing the comb

  (mend holes with melted agarose from the back)
- mix your samples with 6x agarose gel loading buffer and load on gel

  (use gel loading buffer without dye for small DNA fragments, because the dye will cover the band under UV light. Let loading buffer with dye run in a separate lane.)
- run the gel in 1xTAE buffer at 100V for 30min in the dark (cover with aluminium foil)
- cut out the desired band on the UV table and purify with gel extraction kit

  (the Eppendorf kit works very well even for small fragments ~70bp)

Recycling:

1) Gel: Cut out all other gel bands containing DNA / primers and dye and dispose of it as hazardous waste. Cut rest of the gel in small pieces and re-use it. For re-use do not melt agarose gel in microwave (as it still contains ethidiumbromide), but dissolve it on the heating plate under the hood. Add fresh ethidiumbromide.
2) TAE: Leave the running buffer (1xTAE) in the tray and use it multiple times (as long as you do not let your samples run off into the buffer). Filter used running buffer through active charcoal to remove ethidiumbromide. Dispose of the active charcoal (after multiple use) as hazardous waste.