University of Melbourne iGEM 2014 Lab Procedure

Procedure	Name:	DNA Gel Purification		
	Description:	DNA Gel purification from agarose gel after electrophoresis. See also Sub-cloning protocol and training week video (from 49:00).		
	Trigger:	Having completed DNA gel electrophoresis, to purify a DNA sample from the gel		
Last updated	Name:	Robyn	Date:	15/8/14
	Version:	3		
You will need	Time:	1 hour		
	PPE:	UV protective gear Nitrile gloves		
	Equipment:	➢ UV light box ➢ Centrifuge ➢ 1000 µL pipette ➢ 100 µL pipette ➢ Scalpel ➢ Scale		
	Materials:	➢ DNA purification kit (Note: Will include this protocol) ➢ 2 x 1.5 or 2 µL microfuge tubes per DNA band ➢ Post-electrophoresis agarose gel containing DNA		

General Notes:

- *All purification steps should be carried out at room temperature.*
- *All centrifuges should be carried out in a table-top microcentrifuge at >12000 x g*
- *Initial microfuge tubes will be disposed of so label using any system you like that will help you not mix tubes up. Fresh tubes used from Step 17 will be stored for later use so use the correct labelling protocol.*

Step 1	Take one fresh empty microfuge tube for each DNA band being purified, weigh and record its mass.
Step 2	Place the agarose gel on the UV light box and locate the band for the desired DNA. Ensure you are wearing the UV protective gear (face guard) and nitrile gloves. *NOTE: Place the gel on the glass plate, not the plastic one because the plastic will reduce the intensity of the UV light making the bands more difficult to see.* *Note: If the bands cannot be seen try imaging the gel. See 'B2 DNA Gel Electrophoresis Protocol' for instructions on this.*
Step 3	Cut the band out of the gel using a clean scalpel very carefully. Cut as close to the DNA as possible to minimize the gel volume.
Step 4	Place the band into a microfuge tube. If there are multiple DNA bands to purify, place each type of band into a separate tube that is clearly labelled so they are not mixed up.
Step 5 ***Note: Linked video ends here.***	Reweigh each microfuge tube, now containing the gel slices, and calculate the mass of the gel pieces (final weight minus starting weight).
Step 6	Pipette 1 µL of Binding Buffer from the kit per 1 mg of gel (1:1 ratio). It is better to have slightly more binding buffer rather than less.
Step 7	Incubate the gel mixture at 50 - 60°C for 10 minutes or until the gel has completely dissolved in the microfuge tube, inverting gently every few minutes to aid the process. Ensure the gel has completely dissolved.
Step 8	Vortex the mixture briefly once dissolved and check the colour – yellow indicates it is at the correct pH for DNA binding. If it is orange or violet, add 10µL of 3M sodium acetate, pH 5.2 solution and mix. The colour of the mix will become yellow.
Step 9	Pipette the contents of the microfuge tube, up to ~800 µL, into a pink purification column, (from the purification kit). Use one column per microfuge tube, clearly labelling each tube so as not to mix them up. *NOTE: If there is more than 800 µL you will need run this step a second time with any remaining gel solution after step 11.*

Step 10	Spin the column(s) in a small desktop centrifuge for 1 minute at 13000 rpm, being careful to balance the centrifuge. The column will filter the DNA from the other contents of the solution, as only the DNA will bind to the column.
Step 11	Discard the flow-through, being careful to return the right flow through tube to the same purification column if working with multiples.
Step 12	Repeat steps 9-11 if there is any remaining gel solution in the original microfuge tube.
Step 13	Pipette 700 µL of Wash Buffer from the kit to the purification column *NOTE: Check that the "ethanol added" box on the buffer bottle lid has been checked before using. If this is the first time the kit has been used you will need to dilute the wash buffer with ethanol as per the kit instructions.*
Step 14	Centrifuge for 1 min at 13000 rpm.
Step 15	Discard the flow-through.
Step 16	Centrifuge the empty purification column to remove any residual wash buffer.
Step 17	Transfer each column into a fresh microfuge tube, clearly labelled with the lab book page number and details as per the labelling protocol, being sure to reference this in the lab book – this is the final tube that will be stored.
Step 18	Pipette 50 µL elution buffer (from the purification kit) to the centre of the column membrane.
Step 19	Centrifuge for 1 min at 13000 rpm.
Step 20	Discard the purification column and store the purified DNA (now in the microfuge tube) at -20°C in the box allocated for the stream, being sure to update the lab book with the details.
Version history	Original created by Sheryl
	Updated on 16.07.14 by Elizabeth Brookes
	Updated 15/8/14 by Robyn E.