Sanger Sequencing at EAWAG

Sanger sequencing is outsourced to Microsynth AG.
You can Sanger sequence either from PCR products or products cut out of a gel.

DNA clean up
- Clean up your DNA (PCR product or gel piece) by using the Promega Wizard kit (can be found in the MolGen lab in the cabinet on the back wall, to the left of the door of the gel room). The amount of purification columns you take is the amount of marks you have to make on the tick list! The protocol to follow can be found on the same shelf (use the side of the protocol that says purification by centrifugation, not by vacuum).

Tubes for samples
- On the shelf above Marco's desk there is a box with prepaid barcodes and the appropriate tubes to put your samples in (make sure to fill out the tick list in the box and write down how many you take of the following: barcodes, tubes and envelopes). Take an envelope to send the samples later.
- Stick the barcodes on the tubes and make sure you write down what barcode is being used for which sample. (There is a picture of how the barcodes must be stuck on to the tubes in the folder where the barcodes are found.)

Sample preparation
- It's easiest (and cheapest) to premix the primers with your sample. Take 12 µl of your purified DNA (after cleaning it with the Wizard kit) and add 3 µl of primer at 10 µM concentration (either forward or reverse, depending on which end you want to sequence; if you want to sequence from both ends you have to make two tubes per sample, one containing the forward and the other the reverse primer, each with 12 µl sample and 3 µl of the respective primer). The final primer concentration in the tube should be 2µM and the volume 15 µl, that is why you add 3 µl to 12 µl of product. If your primer is not at 10 µM concentration as we usually use them, you have to first produce the right dilution.

Sending the samples
- Fill out the excel sheet for sequencing (yellow columns have to be filled out, white ones are optional) with your sample and primer names and send it to Marco (marco.thali@eawag.ch). The sample sheet is available in the following folder on Q:
Q:\Abteilungsprojekte\eco\Lab Finances Orders Chemicals\microsynth\Economy Sequencing. Marco will print out the order form and give you two copies.
- Put the sample tubes in a plastic zip lock bag (can be found in the MolGen lab in the communal drawer) and then in the envelope and check barcode/economy (not premium!) on the envelope label as sending method.
- If you want to you can add a copy of the order form (printed by Marco) to the envelope with your samples to ensure that the correct samples are sent with the correct order number. The second printed order form is for you to keep.
- If you have packaged your samples before 2.15 pm, you can put the envelope in the "out" tray in the room across from the administrative office on the G-floor. Between 2.15 and 3.00 pm you can bring the envelope to the reception in the FC building. Anything later will be sent the next day (so it's probably better to keep your samples in the fridge at 4°C overnight and put the envelope in the "out" tray the next day).