Ligation:

To ligate a level 1 construct, use BsaI and to ligate a level 2 construct, use BbsI. The Volume of the ligation is 20 µl and will be filled up with ddH_2O. To calculate the quantity of the constructs, use the concentration [ng/µl] and length of the construct in [kDa]. Follow the link: http://www.molbiol.ru/ger/scripts/01_07.html and calculate the amount of the construct in [ng] that is needed to get 40 fmol. Then, dived the result [ng] threw the concentration [ng/µl] to get the amount in [µl]. For high-fidelity enzymes, use cut-smart-buffer.

Ligation of a MoClo construct	
Ingredient	Amount [µl]
parts	40 fmol
Buffer	2
ATP	2
T4-ligase	0,5
Enzyme	0.5
ddH_2O	20-X

Place the ligation into the Thermocycler for

a) 1 min. -> 95°C
b) 30 sec. -> 95°C
c) 30 sec. -> 55°C
d) 30 sec. -> 72°C
e) 5 min. -> 72°C
f) Infinit -> 4°C

Cycle b),c),d) repeat for 30 times.

Transformation in *E.Coli*:

Use 100 µl of competent *E.Coli* Top 10 cells and handle carefully. Do not touch the Eppy at the botten and keep them on ice. Do not pipet them up and down. Put 10 µl of the ligation onto the competent cell and keep them for

a) 30 min. -> on ice
b) 90 sec. -> at 42°C
c) 5 min. -> on ice

Add 600 µl of LB-Media onto the cells and keep them for 45 min. at 37 °C.

Plating out:

Depending on the efficiency of the ligation use 200 µl to 400 µl of the LB-ligation mixture. Spread them carefully onto a LB-Agar plate (1.5%) under the clean-bench. Stroke out the mixture with a

bend tip of a pipette. Keep the Plate lid down at 37°C over night for 12-15 h. Do not keep the plate too long at 37°C to avoid satellite cultures.

Picking:

To pick colonies, prepare LB-media with the according antibiotics. For a level 1 construct, use ampicillin and for a level 2 construct, use kanamycin. For every colony you pick + one as a buffer, use 5 ml LB and 5 µl stock of the antibiotics. Add them sterile into a falcon and invert the falcon. Pour 5 ml of the LB-antibiotics mixture into a reaction tube (sterile) and pick a single colony with the tip of a yellow pipette.

Keep the reaction tubes shaking at 37°C over night (12-15 h).

Antibiotic stocks:

antibiotics	Stock concentration [mg/ml]	dissolved	Concentration of the media [µg/ml]
spectinomycin	100	ddH_2O	100
ampicillin	100	50% EtOH 50% ddH_2O	100
kanamycin	50	ddH_2O	50
hygromyin	50	SDS	50

Miniprep:

Perform the Miniprep according to the instruction.

Control digestion:

First, perform the control digestion with a computer program such as serial cloner. Chose up to two enzymes that create bands not smaller than 1000 bp. Chose the enzymes to get two bands that differ in size by 1000 bp. Use 500 ng of the construct and calculate the amount needed by dividing the 500 [ng] threw the concentration [ng/µl] of the construct.

Control digestion	
Ingredient	Amount [µl]
construct	500 ng
Buffer	2
Enzyme	0.5
ddH_2O	20-X

Keep the digestion for 1 h at 37°C.

Loading a gel:

Prepare a mastermix (MM) with ¾ Loading dye, ¼ Gel Red (1:1000 stock)

Mix 5 µl of the control digestion and 1.5 µl of the MM.

Use a 1% agarose-gel and place the gel in a gel chamber. Fill the chamber with 1XTAE buffer. Push out any air bubble of the chambers and run the gel with 100 mV and 150 mA for 45min.-55min.

Retransformation:

Perform a retransformation according to a transformation but only use 1 µl of your plasmid and only plate out 50 µl of the picked construct.

Transformation into *Chlamydomonas reinhardtii*:

Before the transformation:

Prepare some 2 ml eppis with glasbeat. Therfore, put a tip of glasbeats in the eppi and wrap the eppis airtight in tinfoil. Autoklave the eppis and dry them for 2 days at 50°C. Do not close the lids of the eppis until they are dry. Then close them and remove the tinfoil. One day before the transformation dilute the algae to a concentration of 2*10^6 cells/ml and use 100 ml for each transformation as well as the control. Two hours before the transformation, run a linearization of the construct. Chose an enzyme that cuts only once and that cuts in the backbone. Run a control gel of the linearization as described above. 30 min. before the transformation sterilize a 1000 µl an 20µl pipette by pipetting up and down 70% EtOH without a tip.

The actual transformation:

Work steril. Measure the concentration of the algae and use 1*10^8 cells per transformation. Centrifuge them down in a 50 ml falcon for 5 min. at 4000 g. Discard the supernatant and resuspend the pellet in 330 µl per 1*10^8 cells. Therefore, first use the remaining supernatant that was not successfully discarded. Full up the lid of the bottle of TAP-Media with the Media and put your pipette to 330 µl. Suck up the resuspended pellet and fill the rest of the tip with the TAP-Media from the lid, then put everything back in the falcon and resuspend again. For each transformation fill 330µl of the chlamys with 1*10^8 cells into a 2 ml glasbeat-eppi and add 10 µl of the linearized construct. For the control use TAP-Media. Do not touch the eppy on the insight of the lid. Vortex the cells for 15 sec. (UVM4) or 25 sec. (clip) by holding two eppies against each other at an angle. Plate out the cells carefully onto a TAP-spec-plate and try not to pick up the glasbeats. Stroke out the mixture with a bend tip of a pipette. Only tough every area one no not kill the algae. Let the plate dry and then close it with a strip of parafilm. Keep the plates dark over night and then place them lid down for 8-10 day under light.

Picking of chlamy-cultures:

When you see single colonies on your plate you can start picking them. Therefore, use a TAP-agar plate and label it with 1 to 30. Pick sterile under the clean bench. Be careful to only pick single colonies. Use sterile toothpicks or the yellow pipette tips to pick the colonies. Pick a colony in one

swipe and turn your picker to not destroy your cells. Place the colony on TAP-Plate. When finished, close the plate with parafilm and place it under light for 3-4 days. Transfer the colonies on a fresh plate every three weeks.