Screening of Secreting *Chlamydomonas reinhardtii*

Protocol iGEM 2019

Materials: 2x SDS-sample buffer, acetone, 95 % EtOH, MQ-Water, rubber bands, transparent nylon tights, 8 M urea

Freeze-drying / lyophilizer:

1. Transfer 2 ml of culture into a 2 ml reaction tube
2. centrifuge at 4000 G for 4 minutes. Transfer supernatant into a fresh tube.
3. centrifuge at 5000 G for 5 minutes. Transfer the supernatant into a 15ml centrifuge tube (Falcon)
4. cut transparent tights (nylon tights) it 4 x 4 cm sqares
5. to prevent loss of sample whilst lyophilization put on the squares and fixate them with a rubber band. close the centrifuge tube and put your samples in -80°C freezer for at least 30 min.
6. remove caps from the tubes and place tubes in the lyophilizer overnight

Protein precipitation (desalting):

1. Take the samples out of lyophilizer. Resuspend dried protein in 150 µl 1x SDS sample buffer
2. transfer sample to new 1.5 ml tube
3. add 600 µl freezing cold acetone
4. freeze for 20 min, -80°C
5. centrifuge for 30 min at 25000 g,4 °C
6. remove supernatant. Be careful, don't remove the pellet
7. let pellets dry under fume hood

Urea precipitation:

1. Resuspend pellet in 10 µL 8 M Urea
2. Sonicate in sonication bath for 10 – 15 min
3. Hey (2 min, 95 °C)
4. Repeat steps 2 and 3 until pellet is gone

Loading preparation:

1. resuspend in 45 µl 1x SDS sample buffer
2. boil for 1 min, 95°C
3. load samples (15 µl) and a negative and a positive control on SDS-gel and perform Western Blot

Protocol iGEM 2020

Materials: 2x SDS-sample buffer, acetone, MQ-Water, rubber bands, transparent nylon tights

Freeze-drying / lyophilizer:

1. Transfer 6 ml of culture into a 6 ml Falcon tube
2. centrifuge at 4000 G for 4 minutes. Transfer supernatant into a fresh tube.
3. centrifuge at 5000 G for 5 minutes. Transfer the supernatant into a 15ml centrifuge tube (Falcon)
4. cut transparent tights (nylon tights) ito 4 x 4 cm sqares
5. to prevent loss of sample whilst lyophiliation put on the squares and fixate them with a rubber band. close the centrifuge tube and pt your samples in -80°C freezer for at least 30 min.
6. remove caps from the tubes and place tubes in the lyophilizer overnight

Protein precipitation (desalting):

1. Take the samples out of lyophilizer. Resuspend dried protein in 150 µl 1x SDS sample buffer
2. transfer sample to new 1.5 ml tube
3. add 600 µl freezing cold acetone
4. freeze for 20 min, -80°C
5. centrifuge for 30 min at 25000 g,4 °C
6. remove supernatant. Be careful, don't remove the pellet
7. let pellets dry under fume hood

Loading preparation:

1. resuspend in 45 µl 1x SDS sample buffer
2. boil for 1 min, 95°C
3. load samples (15 µl) and a negative and a positive control on SDS-gel and perform Western Blot