Cell Harvest and Cell Disruption for Chlorophyll Determination

Screening of Transformants

- label 1.5 ml Eppendorf tube for each transformant
- transfer 1.5 ml of cultures to Eppi and centrifuge at 4000 G for 2 minutes at room temperature
    - cell pellet should be visible
- discard supernatant and centrifuge at 4000 G for 30 sec at RT
- residual medium can be removed with 200 µl pipette
- add DTT-Carbonate-Buffer (60 µl) and vortex, after that add SDS-Sucrose-Buffer (55 µl)
    - the above-mentioned measurements only work if cell count has been observed
    - otherwise add Buffer until a lighter green color can be observed
- vortex thoroughly (every sample at least 30 sec)
- cook for 2 min
- let cool down briefly and centrifuge for 2 min at maximum speed (RT)
- storage is possible for roughly an hour at RT

Chlorophyll determination

- label fresh 1,5 ml Eppis and add 190 µl water
- prepare on eppi as reference and add 200 µl water
- transfer 10 µl of supernatant to each eppi, vortex, then add 800 µl Aceton in each sample and the reference
- vortex
- centrifuge samples at maximum speed for 5 min
- determine the absorption to the samples at 645 nm and 663 nm
    - use glass cuvette
- put the results in the following formula

$$Chlorophyll\ [\mu g/\mu l] = \frac{[(A_{645}\ x\ 17.76) + (A_{663}\ x\ 7.34)]}{10}$$

- load the SDS-gel with 2 µg of Chlorophyll