Fluorescent Microscopy

This protocol was used to test for expression and uptake of fluorescent proteins.

Preparation of non-fluorescent cells

- 5 uL over-night cell culture was mixed with 25 uL purified protein in an eppendorf tube.
    - Protein purified from cells expressing the following vectors were used:
        i. Control (empty vector)
        ii. CPP
        iii. BFP
        iv. CPP-BFP
        v. YFP
        vi. CPP-YFP
- The sample was left at room temperature for 10 min.
- The sample was spun for 5 min at 13.000 rpm in a table centrifuge.
- The supernatant was removed and the pellet washed twice with 500 uL nuclease-free water.
- After removing water from the last wash the pellet was re-suspended in 10 uL nuclease-free water.

Imaging of cells

- For each sample 4 uL cell mixture was placed on a microscope slide and placed under the microscope.
- All images were taken with 100x resolution.
- The following setup was used for yellow fluorescence:
    - Excitation time: 250 ms
    - Excitation: 460 - 490 nm
    - Dicroitic Mirror: 505 nm
    - Barrier Filter: 510 - 555 nm
- The following setup was used for blue fluorescence:
    - Excitation time: 100 ms
    - Excitation: 330 - 385 nm

- Dicroitic Mirror: 400 nm
- Barrier Filter: >420 nm