Results

Pre day 6:

GFP plates, Master plates, restriction analysis,

Characterisation of mutants

Bradfort assay

UV/VIS absorption of E. coli mutants and wild types

The excitation (550 nm) and the emission (395nm and 470 nm) of the five chosen mutants and the wild type were measured. (Gerätetyp???)

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| **Fig.**  Combined excitation spectra (550nm) of all mutants and the wild type. Variant 2, variant 3, variant 4, variant 5, wild type. |
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| **Fig.**  Combined emission spectra (395 nm) of all mutants and the wild type. Variant 2, variant 3, variant 4, variant 5, wild type. |
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| **Fig.**  Combined emission spectra (470 nm) of all mutants and the wild type. Variant 2, variant 3, variant 4, variant 5, wild type. |
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| **Fig.**  Spectral properties of the chosen mutant: Variant 2. Excitation (550 nm), Emission (395 nm), Emission (470 nm) |

SDS PAGE

An SDS-PAGE was done with our lysed mutants and the wildtype to estimate the GFP production. The results of the Bradfort assay, were used to normalize the total protein amount of the samples. (Staining etc)

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| PAGE.jpg |
| **Fig.** SDS-PAGE gel of the proteins present in the chosen mutants and the wildtype. Induced (i) cultures were grown on a media containing IPTG, non-induced on a media without. The band at 25 kDa contains the produced GFP (26.9 kDa) |

Day 7

Bacterial Growth curves

The following growth curves were generated using the measured data. The optical density (600 nm) of a sample was used as an indicator for the number of bacteria in the culture

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| **Fig.**  This figure represents the growth curves of the GFP-wt in LB-Media (blue) and M9 media (red). The growth of the cultures was eastimated by measuring the optical density at a wavelength of 600nm in regular timesteps. |

To obtain the groth rates of the cultures a plot on a semi logatithmic scale was used:

Therefore the slope of the refression line is equal tot he growth rate of the culture

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| **Fig.**  This shows the natural logarithm of the optical densities of the cultures versus the growth time. A linear regression of the exponential growth phases was done to get the growth rates on different media. The culture on the LB media (blue) showed two growth phases with different rates. The initial velocity is thereby given by: OD = 0.021t – 3.273 (R2=0.99) At times over 200 min.: OD = 0.003t – 0.245 (R2=0.97).. The growth on the M9 media (red) can be described using: OD= 0.008t – 3.10 (R2=1.0) |

The following growth rates were found:

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| LB (initial) | 1.272 h-1 |
| LB(later) | 0.192h-1 |
| M9 | 0.498h-1 |

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| **Tab.**  The grow the rates of E. coli JM101, provided with the plasmid GFPuv\_6His, on LB and M9 media |

DNA sequencing

The plasmid DNA was extracted out of the four most promising mutants and sent in for sequencing. Afterwards the nucleotide sequence coding for the GFP was extracted, and translated into the amino acid sequence using ().The resulting sequence was aligned to the ancestral plasmid () (ClustalW) to make the mutations in each variant visible (Tab.) The DNA sequence of variant 5 could not be resolved, because the mutated sites were present in two different versions (stücheli erklären doppel mutant...)

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| Wild type | | 5’ 188 CT**TTCTCT**TA | 212 TT**TCC**CG 3’ | | |
| Variant 2 | | 5’ 188 CT**CTGGTC**TA | 212 TT**GCG**CG 3’ | | |
| Variant 3 | | 5’ 188 CT**TTAACA**TA | 212 TT**ACA**CG 3’ | | |
| Variant 4 | | 5’ 188 CT**TTTACA**TA | 212 TT**GGA**CG 3’ | | |
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| **Tab.**  DNA sequence of the mutated plasmids and their ancestor. The mutated sites are indicated in bold. | | | | |

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| Wild type | TT**FS**YGVQCF**S**RY |
| Variant 2 | TT**LV**YGVQCF**A**RY |
| Variant 3 | TT**LT**YGVQCF**T**RY |
| Variant 4 | TT**FT**YGVQCF**G**RY |
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| **Tab.**  Amino acid sequence of the mutated sites of the plasmids. Starting at Thr63 | | | |

Insert PyMOL and DNA seq.

Todays OD??

Product analysis

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| **Tab.**  Excitation (395 nm) spectra of our Product compared to the desired variant 2 and the wild type. Product, variant 2, wild type |

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| Purification factor | 3.3 ± 1 |
| GFP fraction | 19.5% ± 4% |
| GFP concentration / [mg/mL] | 6.78 ± 4.2 |