iGEM Team Heidelberg

Meeting Protocol

2010/10/06

participants: Dima, Dirk, Dominik, Jude, Laura, Lea, Lorenz, Ola, Philipp, Rebecca, Rudi, Ting
recorder: Lorenz

mentioned aspects:

- what is our project all about?
  - virus selection purpose: perfect virus for infection of specific cell type in culture
    - comparison to wild type adeno-associated virus (AAV)
    - figures after FACS measurement of reporter expression
      - choosing viruses for distinct infection → "tuned target gene expression[1]"
      - selection pressure in primary cells is very high
        - Philipp contacts Marina to talk to the supplier of the cells
          - primary hepatocytes can be received only every Monday
      - two selection rounds have to be sufficient
        - harvest, PCR amplification, cloning
          - pick of clones randomly and transfection again
      - selection alternative: wash cells short time after infection
        - to keep only rapidly / effectively infecting viruses
- useful information about mouse injection and serotype tropism
  - see Zincarelli *et al.*, 2008
    - for tuning and ON-/OFF-system: usage of **AAV9** is recommended
      - broadest tropism, high and rapid infection
- cell culture measurements are not reliable for injection into mice
  - total numbers are different, but still: transduction in general will be seen *in vitro*
    - combination of both approaches (*in vitro* & *in vivo*) in parallel!
- **modeling**: program is required to color-code the shuffled cap genes!

final conclusions:

- ideal: combination synthetic viruses (shuffled capsids) with tuning construct shuttles
  - both systems should be tested independently at first
  - test *constructs* with wild type virus (serotype 9) and go with those into mice
    - see Dirks nine suggestions

reference:

- Zincarelli C, Soltys S, Rengo G , Rabinowitz JE (2008). Analysis of AAV serotypes 1-9 mediated gene expression and tropism in mice after systemic injection. *Mol Ther*. **16**, 1073-1080.

1 same purpose for miRNA construct: to specify gene therapy – even though selection is required. Originally, two levels to tune efficiencies: infection rate due to serotype, expression due to miRegulation.