Transfection Basics

Outline

1. Implications for the design of our biobricks

2. Transfection in General
    1. Popular Transfection Agents
    2. Lipid-mediated transfection
    3. Stable Transfection vs. Transient Transfection

3. Site specific recombination
    1. Overview
    2. Cre-LoxP-System

4. Questions

Implications for the design of our biobricks

- None!

- In the past, parts intended for use in eukaryotes have also been submitted as standard biobricks.
  - Slovenia used an altered Biofusion standard

Popular transfection reagents

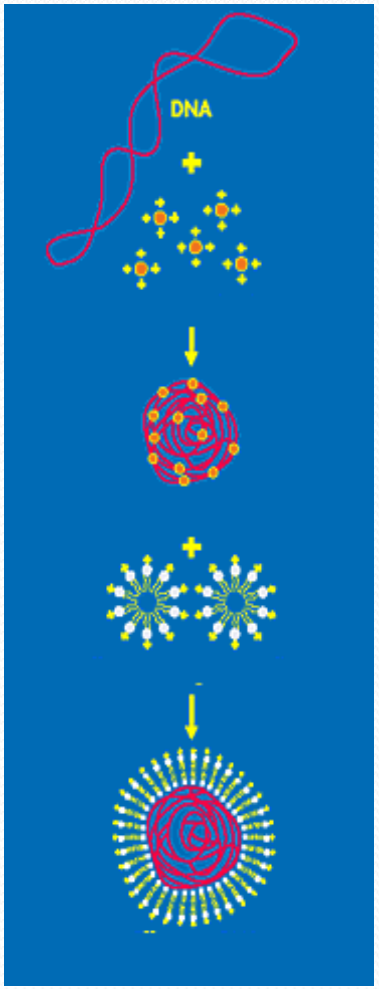
- Lipofectamine 

- Effectene 

- FuGENE 

Lipid-mediated transfection

- Cationic lipids:
  - positively charged head group
  - hydrocarbon chains
- Additional reagents:
  - Enhancer: promotion of DNA-condensation
- Interaction of the cationic lipids
  - with negatively charged DNA
  - Interaction with negatively charged cell membrane
- Uptake of the transfection complex by endocytosis



Transient vs. stable transfection

- Transient transfection
  - DNA is not integrated into a host chromosome
  - Sambrook and Russel: Method of choice for
    - Temporary, but high level of expression
    - Analysis of a large number of samples within a short period of time

Transient vs. Stable transfection

- Stable Transfection
  - The target gene is integrated into chromosomal DNA
  - This is usually done by site-specific recombination using recombinase-systems
- Sambrook and Russel:
  - Formation of stably transfected cells is much more difficult (at least 2 – 3 weeks)
  - Broader range of experimental applications

Some of many Questions

- Which cells will we be working with?
- Will we use stable and/or transient transfection?
- If we use the Cre-LoxP-system or something similar:
  - Are there already established cell lines we could use?
  - If we have to create our own cell lines: Can we do this beforehand?
- Do we have specials vectors which we could use?
  - Vectors with LoxP-sites, with established selection mechanisms, with strong constitutive promotors
- How time consuming is the work with mammalian cells going to be? Can this be done in three months?
  - For how long do we have access to the „clean-bench“?