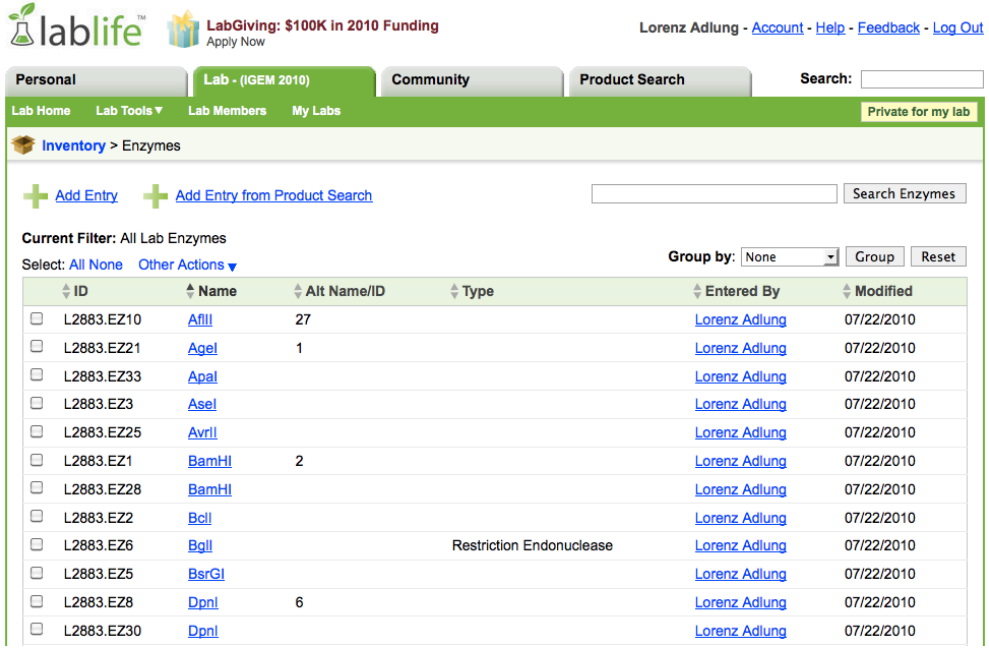
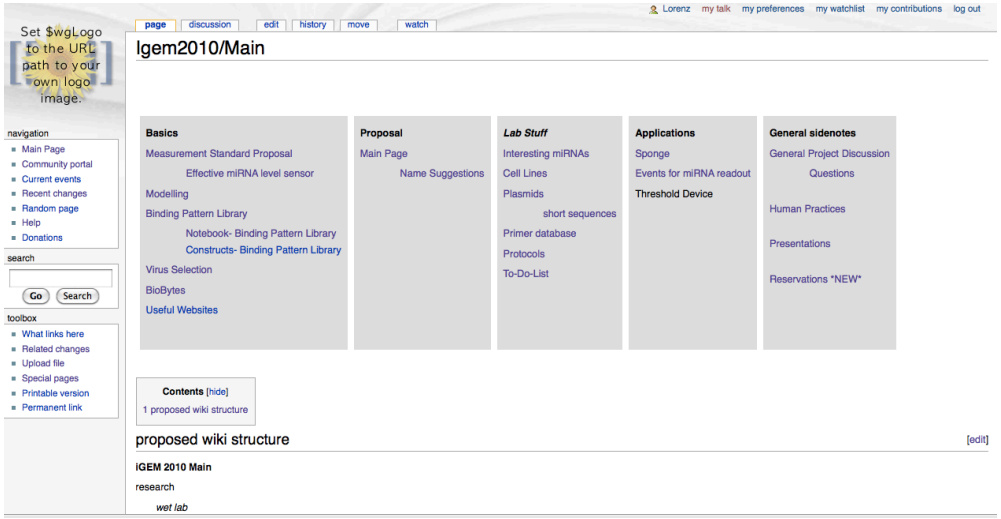
general setups

- trials to structure data integration and accessibility
  - benefit for all of us
- hierarchy for better overview
- redundant information: data twice listed in Lablife and in the Wiki



Lablife: enzyme inventory



internal iGEM wiki: structured overview

concept

- standard protocol
  - virus construction and selection
- viruses with best miRNA targets
  - positive & negative selection
- distinction between different cell lines
  - specific miRNA expression patterns

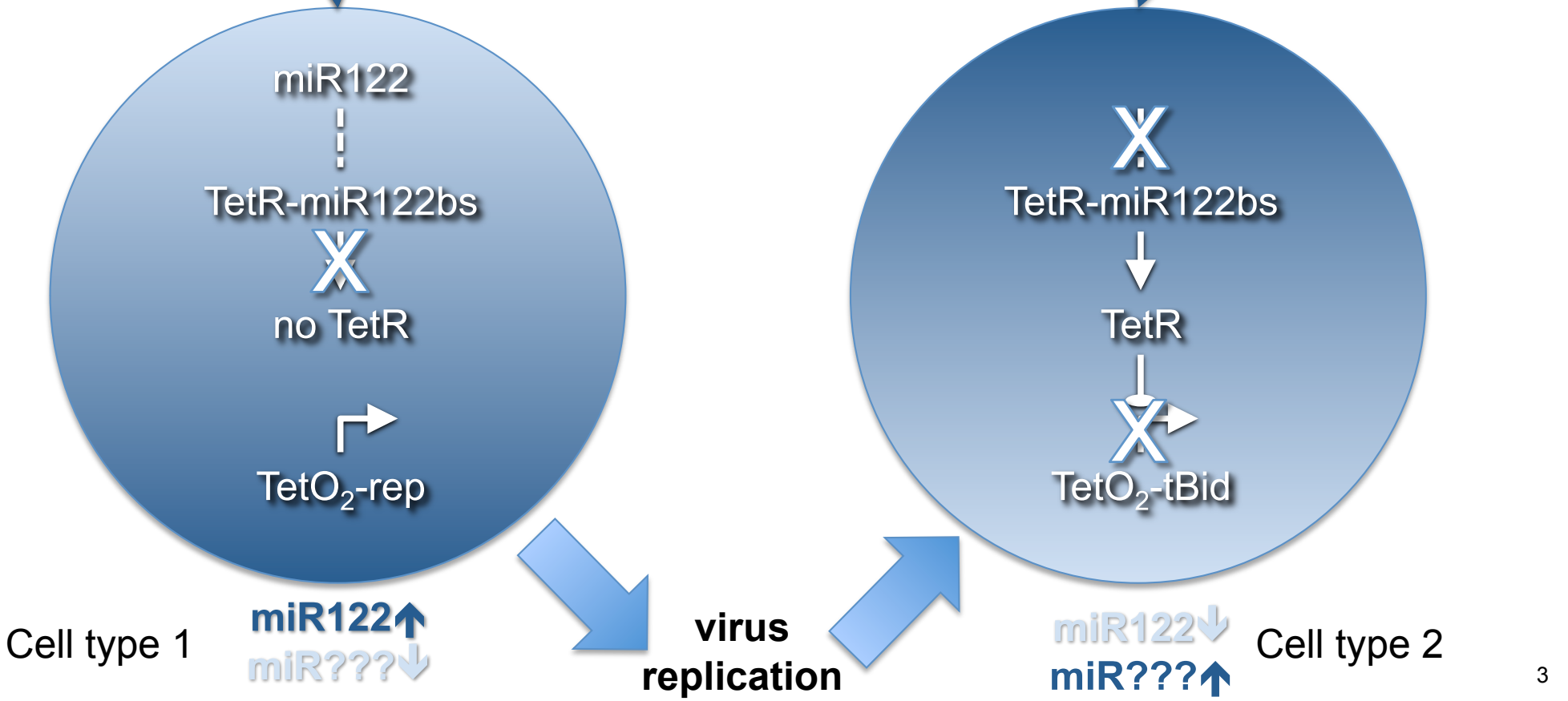
scene 1

cell survival

Virus 1: containing TetR-miR122bs (good affinity!)

Virus 2: containing $TetO_2$-rep (for replication)

Virus 3: containing $TetO_2$-tBid (death gene)

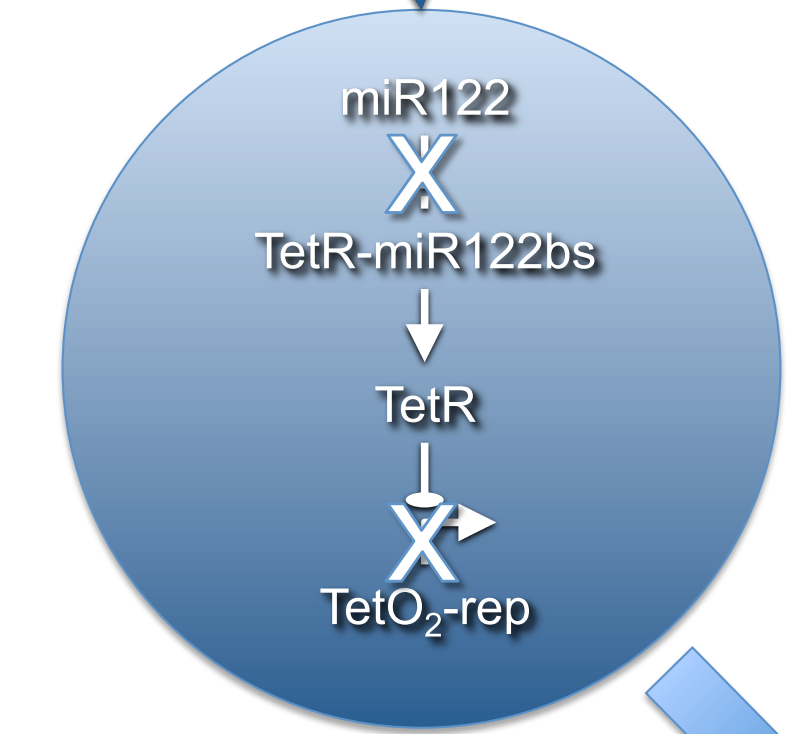


scene 2

no replication

Virus 1: containing TetR-miR122bs (bad affinity!)

Virus 2: containing $TetO_2$-rep (for replication)



Cell type 1

miR122↑
miR???↓

no virus replication

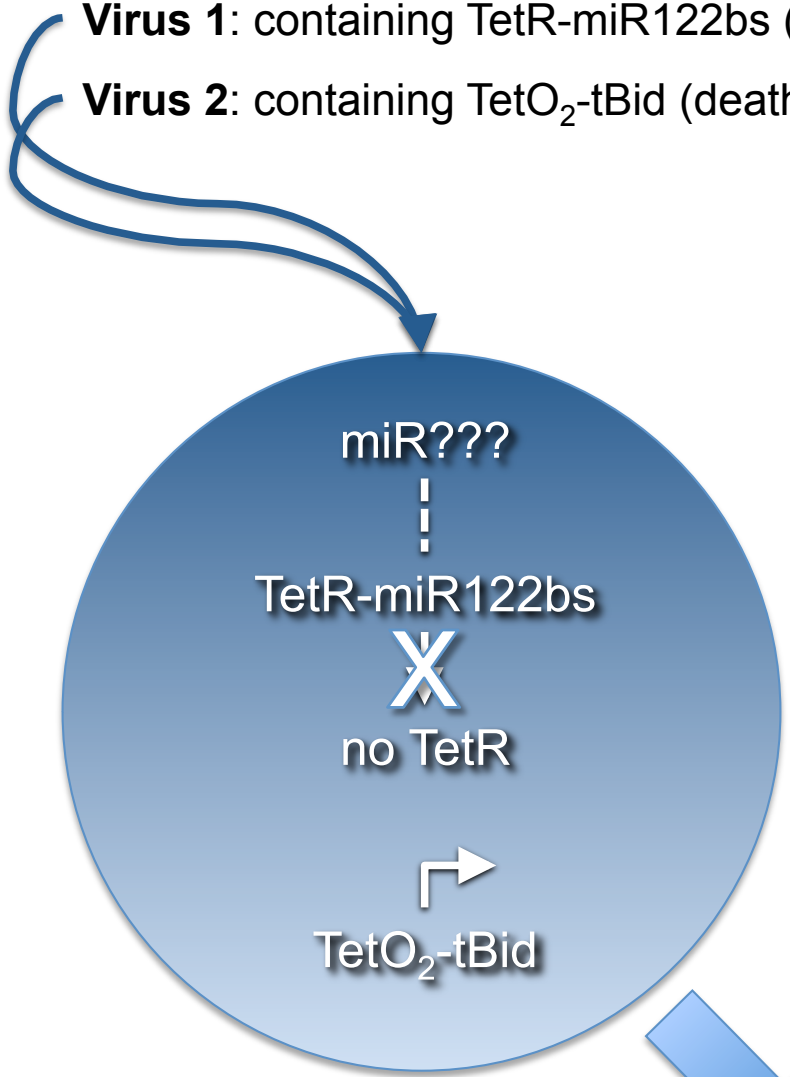
- no interaction
  - between miR122 and its target
- TetR is ordinarily produced
- inhibition at Tet operator
  - no transcription of rep gene
  - no replication of the virus

scene 3

cell death

Virus 1: containing TetR-miR122bs (unspecific!)

Virus 2: containing $TetO_2$-tBid (death gene)



Cell type 2

miR122↓
miR???↑

no virus replication

- unspecific interaction
  - between miR??? and miR122 target
- TetR expression is knocked down
- no sufficient inhibition at Tet operator
  - transcription of tBid gene
  - induction of apoptosis

selection systems

<table>
<tr><th colspan="3">Tet system (“T-REx”, invitrogen)</th><th colspan="3">E system (Weber & Fusenegger)</th></tr>
<tr><td colspan="3">TetR</td><td colspan="3">E repressor</td></tr>
<tr><td colspan="3">flanked by miRNA target sites</td><td colspan="3">flanked by miRNA target sites</td></tr>
<tr><td colspan="3">TetO₂</td><td colspan="3">EO</td></tr>
<tr><td>death gene</td><td>rescue gene</td><td>GFP</td><td>death gene</td><td>rescue gene</td><td>GFP</td></tr>
<tr><td>CASP8</td><td rowspan="2">rep</td><td></td><td>CASP8</td><td rowspan="2">rep</td><td></td></tr>
<tr><td>tBid</td><td></td><td>tBid</td><td></td></tr>
<tr><td>shRNA
~~(virus replication)~~</td><td rowspan="2">cap</td><td></td><td>shRNA
~~(virus replication)~~</td><td rowspan="2">cap</td><td></td></tr>
</table>

- **GFP** as a reporter allows **FACS** in case virus selection fails
- different **death genes tested** for induction of apoptosis in different cell lines
  - resistance of HEK cells against CD95 induced apoptosis

  ➔ usage of **shRNA against** proteins required for **viral replication**

  ➔ induced **silencing** will avoid propagation of virus genes

hurdles

- the theme is **far more complicated** than presented here
- work has to be improved in terms of **coordination**

- many virus **constructs**: elaborate infection process
  - not straight forward
- separate **testing** steps (including control experiments)
- **a lot** of cloning required
- fine-tuning of the **whole system**

However, there are future plans!

plans

Testing of Death & Rescue Genes	Cloning & Testing of Virus Constructs	Constructing a Standard Vector
Transfection → Cells dead? Virus replication?	First testing without miR recognition sites → Do the repressor constructs work?	RFC12 standard restriction sites between repressor and miR recognition sites → Generation of a MCS
Time lapse of transfected cells (from GFP expression to apoptosis) → time & amount needed	Second testing with one complementary recognition site for miR122	Site directed mutagenesis of the vector to eliminate standard restriction sites outsite the MCS
Insertion into Operator constructs	Virus selection	Testing of standard construct (virus selection)

TO DO

- follow a defined strategy and time plan
  - need for more protocols and standards
- establish a wiki structure to ease work
  - contemporary update of LabLife inventory, too
- finish constructs for the viruses
  - deadline: Monday, 16th August 2010
- support of / by all team members!
  - stay in touch even if not in the lab

We are grateful for great guidance through the advisors!

what to do next?

1) give feedback
2) contribute somehow
3) read the proposal (hopefully again)
4) look through the wiki
5) decide what you want to work on
6) getting the (paper) work started
7) come to the lab
8) ask for task
9) start on your own
10) get into discussion
11) present your results
12) import your data into Lablife or the wiki
13) become an appreciated iGEM seminar speaker