

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™ LabGiving: \$100K in 2010 Funding Apply Now Lorenz Adlung - Account - Help - Feedback - Log Out

Personal Lab - (iGEM 2010) Community Product Search Search: [input]

Lab Home Lab Tools Lab Members My Labs Private for my lab

Inventory > Enzymes

+ Add Entry + Add Entry from Product Search [input] Search Enzymes

Current Filter: All Lab Enzymes Select: All None Other Actions Group by: None Group Reset

ID	Name	Alt Name/ID	Type	Entered By	Modified
<input type="checkbox"/> L2883.EZ10	AflII	27		Lorenz Adlung	07/22/2010
<input type="checkbox"/> L2883.EZ21	AgeI	1		Lorenz Adlung	07/22/2010
<input type="checkbox"/> L2883.EZ33	ApaI			Lorenz Adlung	07/22/2010
<input type="checkbox"/> L2883.EZ3	AseI			Lorenz Adlung	07/22/2010
<input type="checkbox"/> L2883.EZ25	AvrII			Lorenz Adlung	07/22/2010
<input type="checkbox"/> L2883.EZ1	BamHI	2		Lorenz Adlung	07/22/2010
<input type="checkbox"/> L2883.EZ28	BamHI			Lorenz Adlung	07/22/2010
<input type="checkbox"/> L2883.EZ2	BclI			Lorenz Adlung	07/22/2010
<input type="checkbox"/> L2883.EZ6	BglI		Restriction Endonuclease	Lorenz Adlung	07/22/2010
<input type="checkbox"/> L2883.EZ5	BsrGI			Lorenz Adlung	07/22/2010
<input type="checkbox"/> L2883.EZ8	DpnI	6		Lorenz Adlung	07/22/2010
<input type="checkbox"/> L2883.EZ30	DpnI			Lorenz Adlung	07/22/2010

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igem2010/Main

navigation

- Main Page
- Community portal
- Current events
- Recent changes
- Random page
- Help
- Donations

search [input] Go Search

toolbox

- What links here
- Related changes
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- Printable version
- Permanent link

Contents [hide] 1 proposed wiki structure

proposed wiki structure [edit]

iGEM 2010 Main
research
wet lab

internal iGEM wiki: structured overview

Lablife: enzyme inventory

concept

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

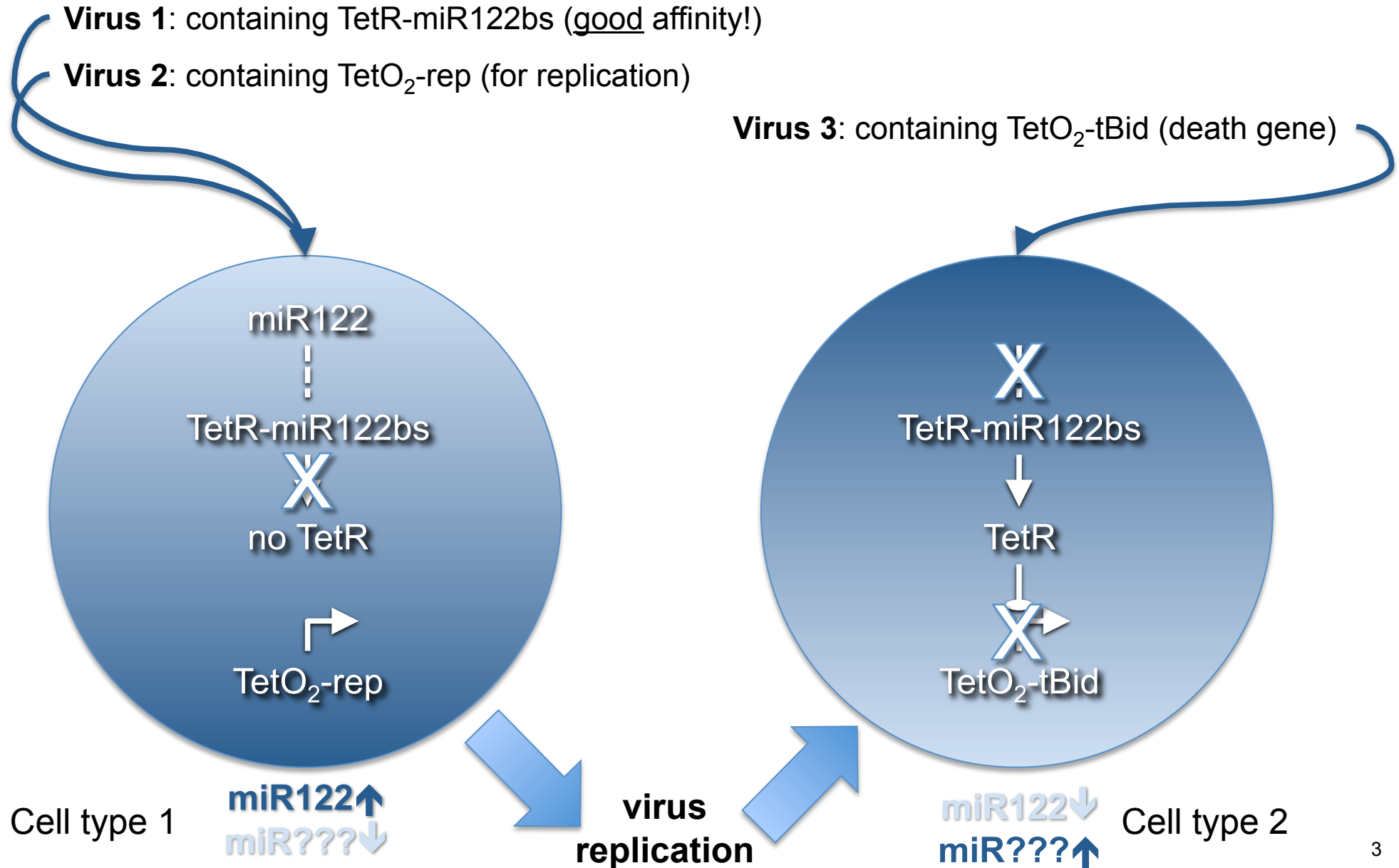
scene 1

cell
survival

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

Virus 2: containing TetO₂-rep (for replication)

Virus 3: containing TetO₂-tBid (death gene)

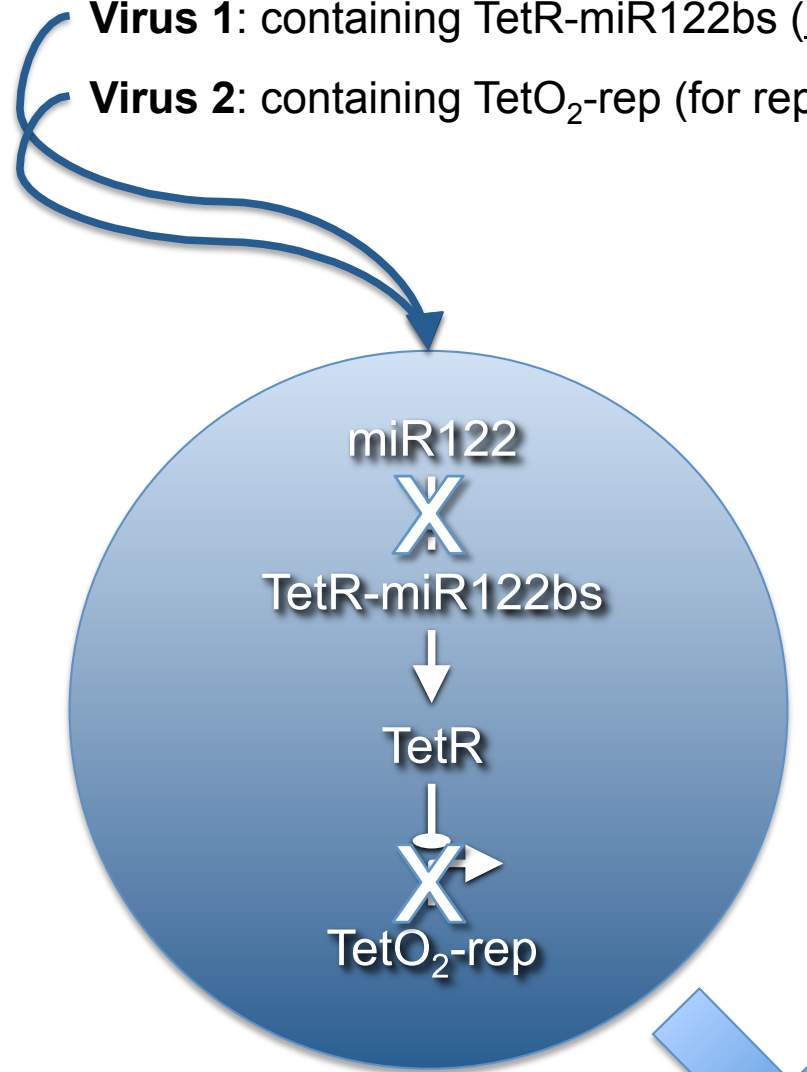


scene 2

no
replication

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

Virus 2: containing TetO₂-rep (for 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

Cell type 1

miR122↑
miR???

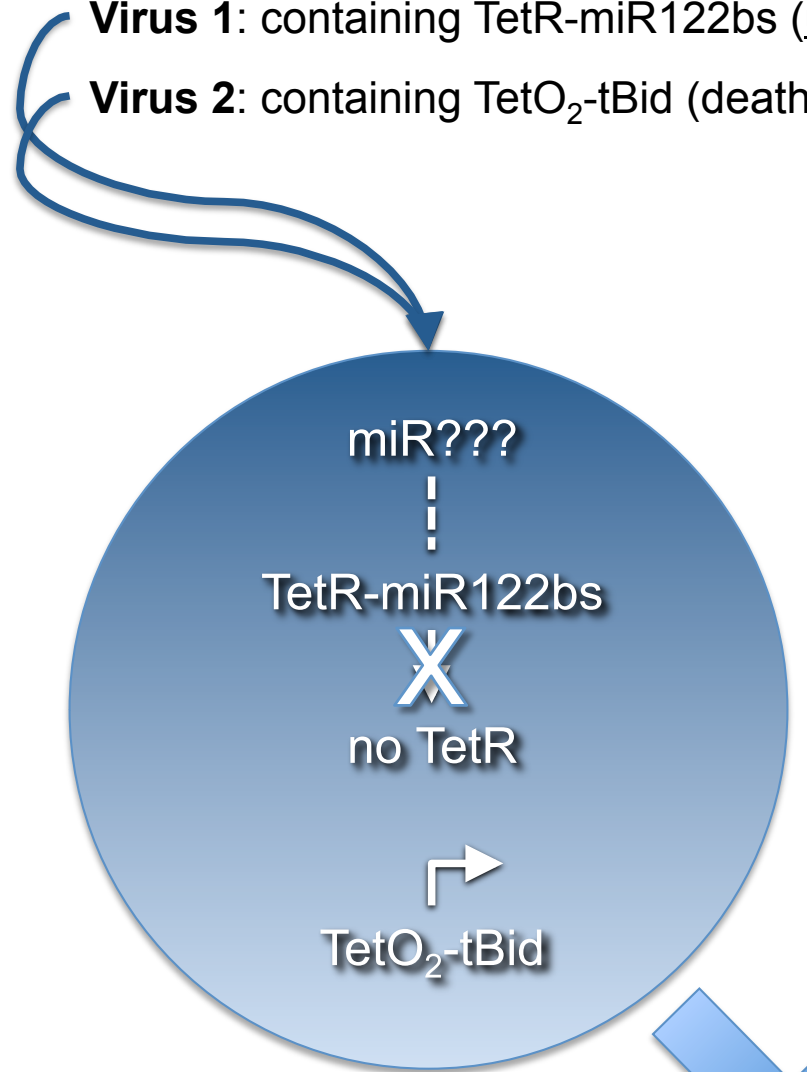
no virus
replication

scene 3

cell
death

Virus 1: containing TetR-miR122bs (unspecific!)

Virus 2: containing TetO₂-tBid (death gene)



- 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

Cell type 2
miR122↓
miR???↑
no virus replication

selection systems

Tet system ("T-REx", invitrogen)			E system (Weber & Fusenegger)		
TetR			E repressor		
flanked by miRNA target sites			flanked by miRNA target sites		
TetO₂			EO		
death gene	rescue gene	GFP	death gene	rescue gene	GFP
CASP8	rep		CASP8	rep	
tBid			tBid		
shRNA (virus replication)	cap		shRNA (virus replication)	cap	

- **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 outside 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