

constructs 'n' measurements

implications
iubricstio2

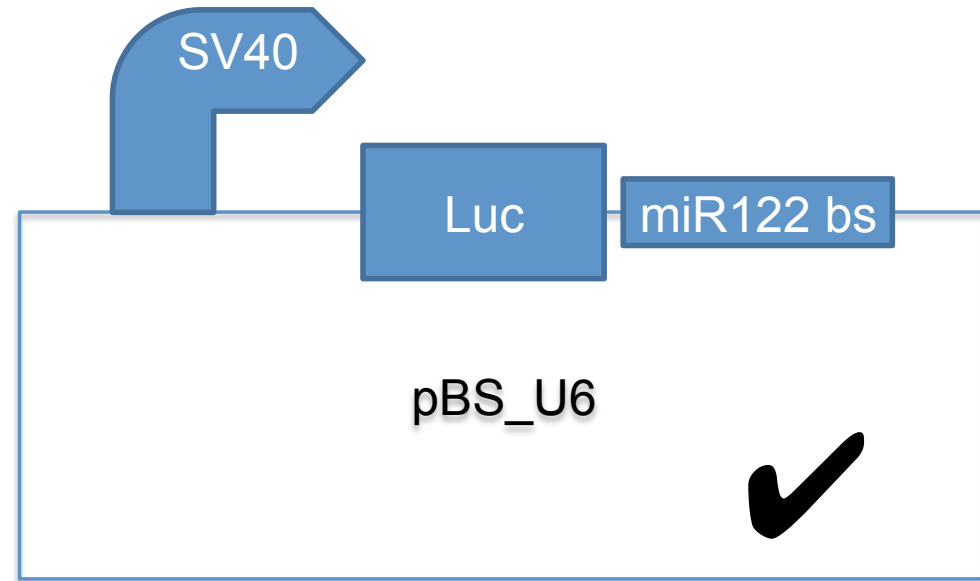
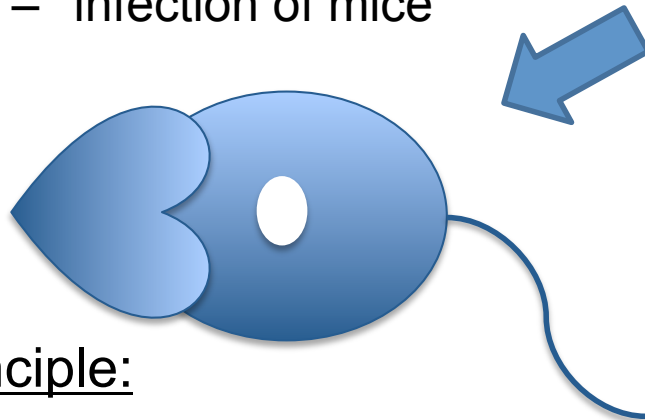


<http://2010.igem.org/Team:Heidelberg>

achievements (I)

- **off-target construct works!**

- viral context
 - virus production
- infection of mice

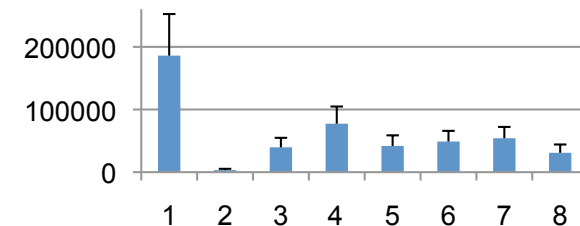


principle:

- knock-down of luciferase gene in the liver, exclusively
 - reason: expression profile of miR122
 - result: mouse is overall glowing but in the liver

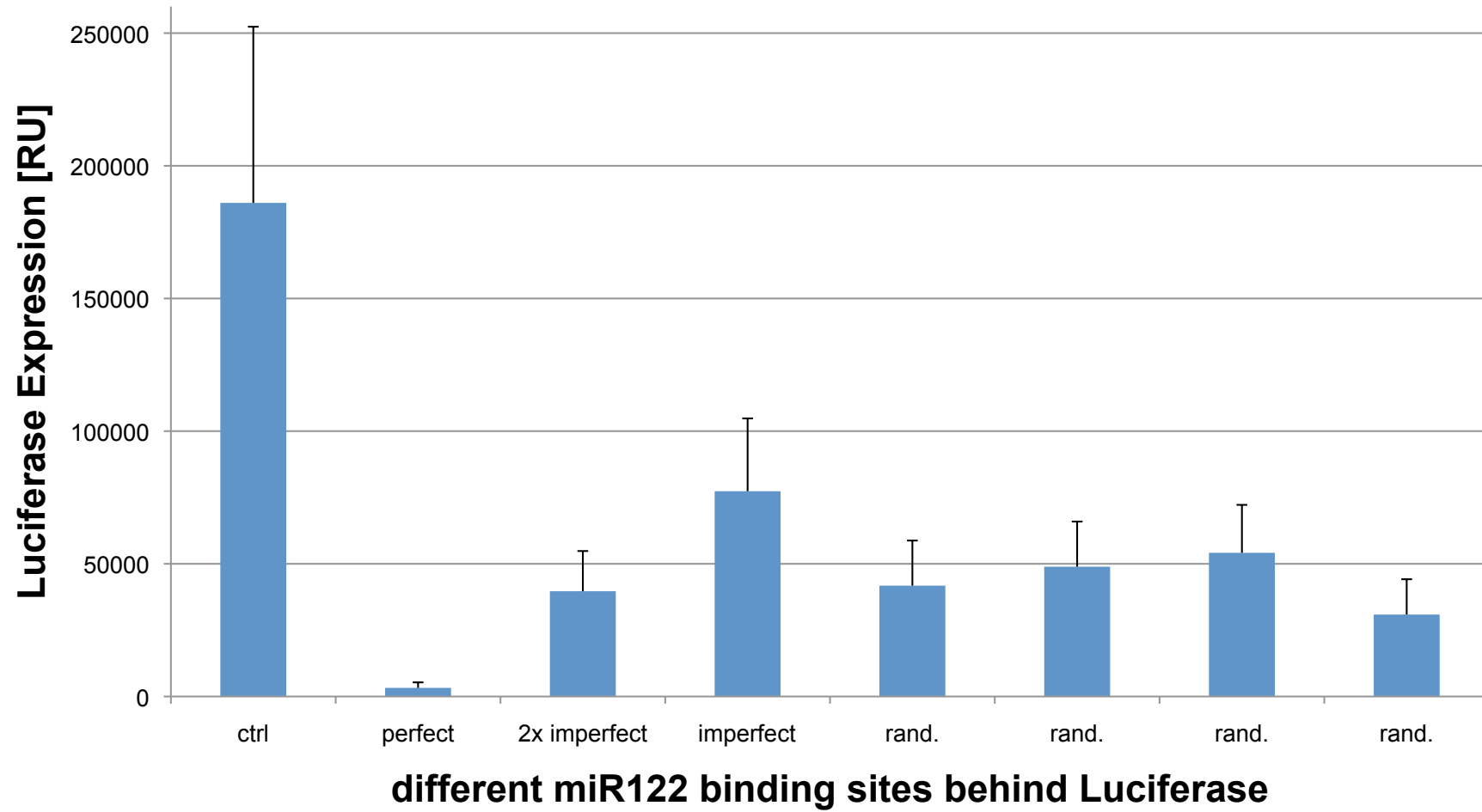
in vitro measurements:

- luciferase assay reveals suppression
 - in case: miR122 is expressed



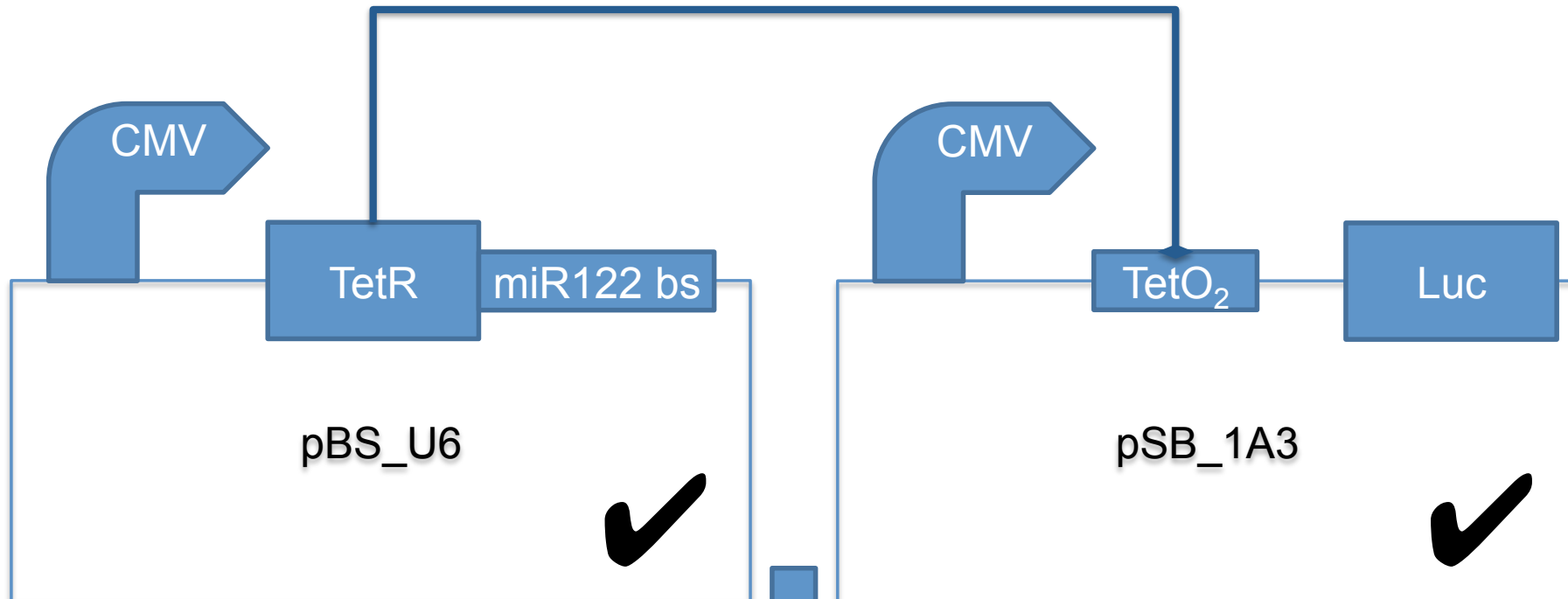
off-target *in vitro*

Luciferase Assay



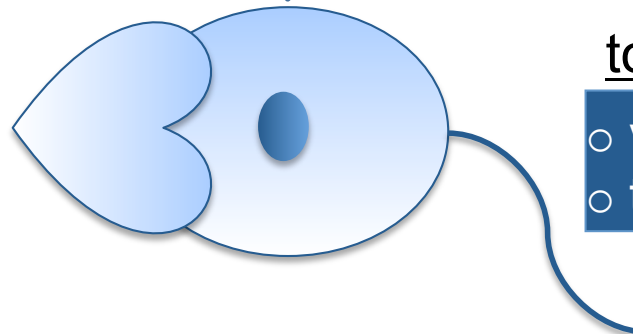
achievements (II)

- on-target works!



positive:

- ✓ twice perfect miR122 bs
- ✓ TetR: thanks to Eike
- ✓ precious data *in vivo*

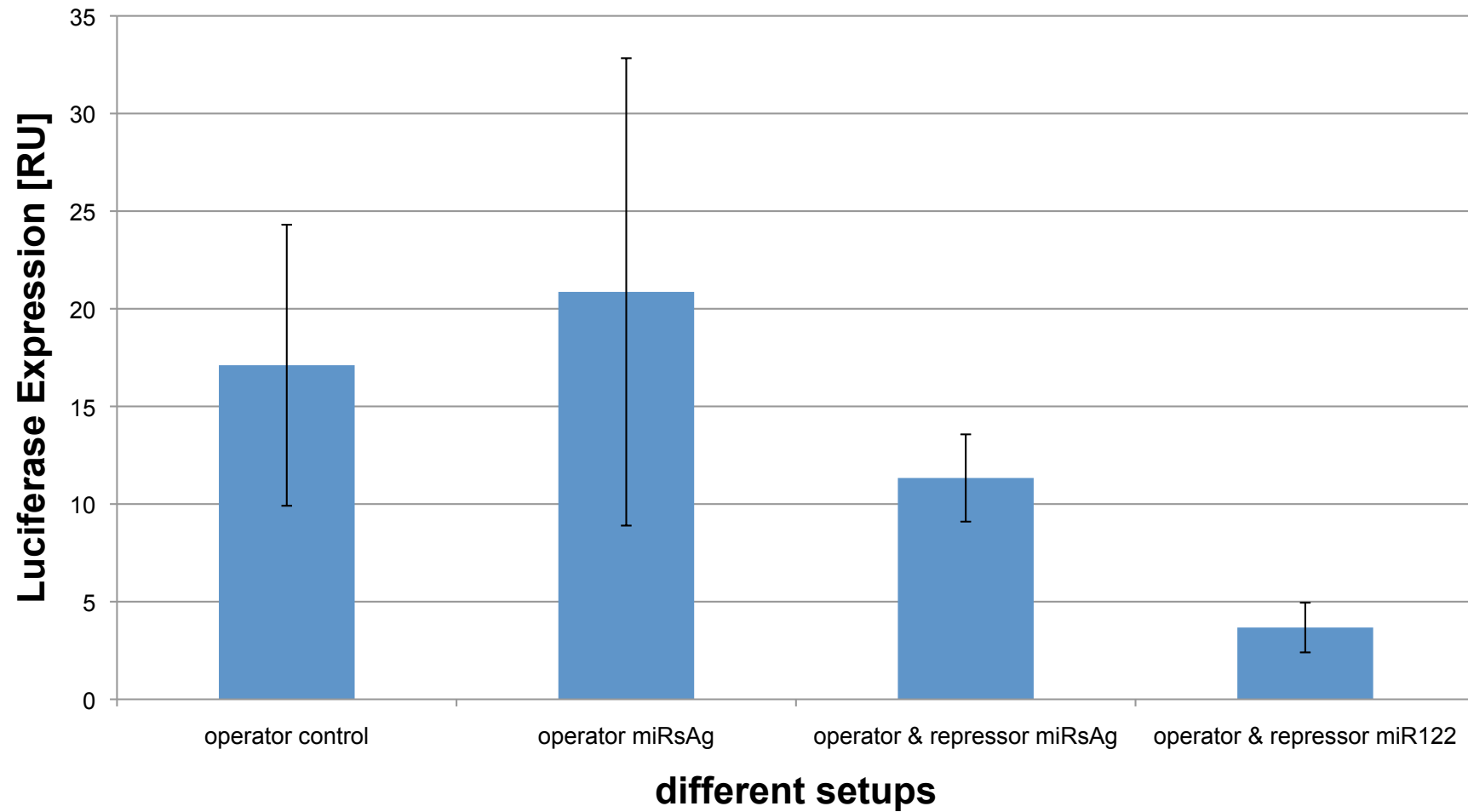


to do / to establish:

- viral backbone cloning
- transfection ratios

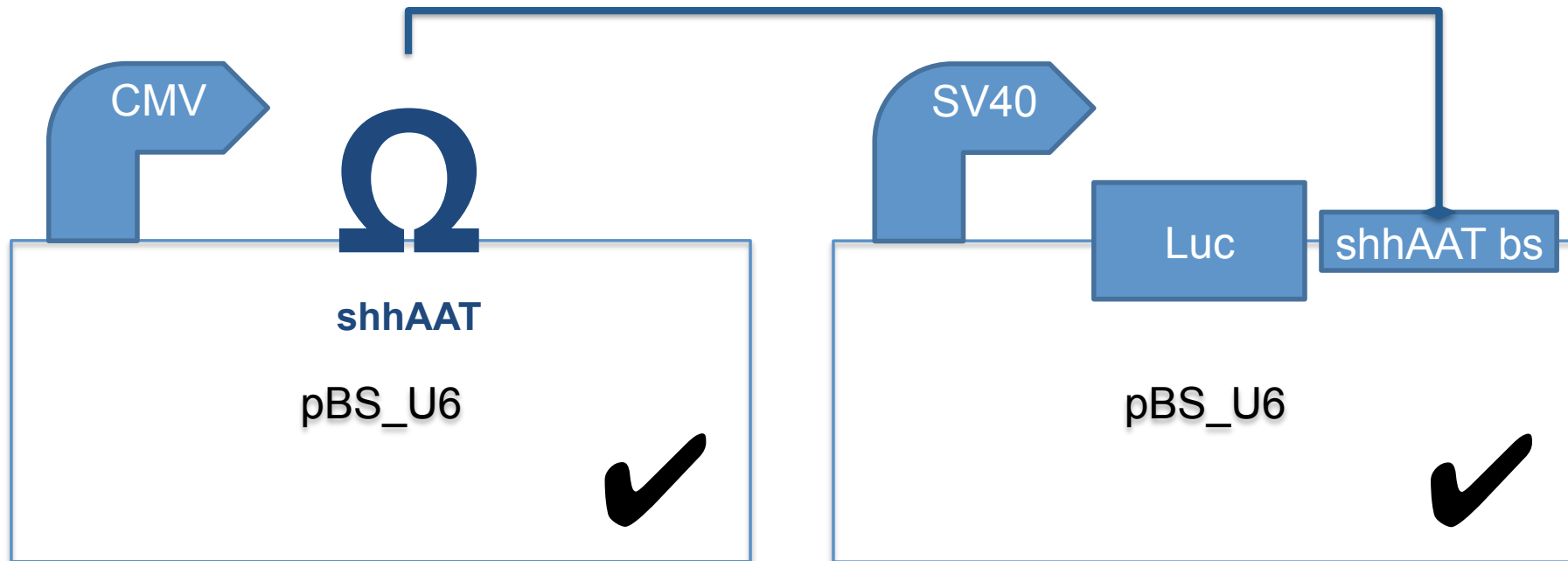
on-target *in vitro*

Luciferase Assay



achievements (III)

- new insights into a proper tuning system




problems:

- GUI based on single plasmid tuning
- system becomes more complex but with more degrees of freedom

achievements (IV)

reference binding site	measured binding site	purpose
-	-	test
-	miR perfect binding site	high range knock-down
miR perfect binding site	miR perfect binding site	low ratio
miR perfect binding site	miR random binding site	characterization
miR perfect binding site	-	check



additions:

- transfection of a miR expressing construct
 - to see knock-down with time
 - note: use fluorescence proteins with high temporal stability
- multiple binding site approach
 - to include miraPCR products
 - such binding site patterns would lead to higher complexity

critical points and outlook

- preparation of *in vivo* experiments needs a lot of work
 - **manpower** required!!!
 - maximum of ten constructs for mouse experiments
 - two off-target constructs
 - four on-target constructs
 - four tuning constructs
- **agreement**: virobyte & miraPCR subgroups will shrink to a minimum
 - few effort on minor aspects
- **encouragement** for the final steps: we still can reach our goal
- **crucial**: documentation
 - responsibilities and tasks will be delegated now!
 - advisors offered their help
- **motivation**: parts will be submitted by the **whole team!**

**Together we stand!
United we fall!**