
VIROBRICKS
Engineering
ROYALE
007

Introduction of AAVs to the Registry

Tools for Engineering DNA, RNA and Proteins

miRNA Binding Pattern Evolution

Software for Rational miRNA Binding Pattern Design

Virobricks: Engineering Royale

Threshold Device

Synthetic Viruses

VDJ

Measurement Standard – GFP Quantification

Virus Assembly

Virus based Selection

miR bdg. pattern Evolution

Transcription Factor based Sensing

miRNA Binding Site Pattern Selection

Promoter Optimization

Ago Protein Selection

Killer Application: Cancer Cell Killing

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Virus Assembly

Synthetic Standard Virus for iGEM Registry

Biobyte Subparts for rapid Modification and easy Use

Virus assembly

Virobyte library

Rapid assembly tool for Virus synthesis

Overview

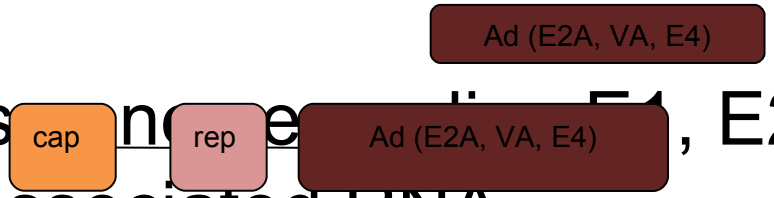
- Biobyte protocol
- AAV parts
- AAV production
- AAV for protein evolution

Presentation Thomas

AAV vectors

- Vector
  - Transgene flanked by ITR sites (for packaging)

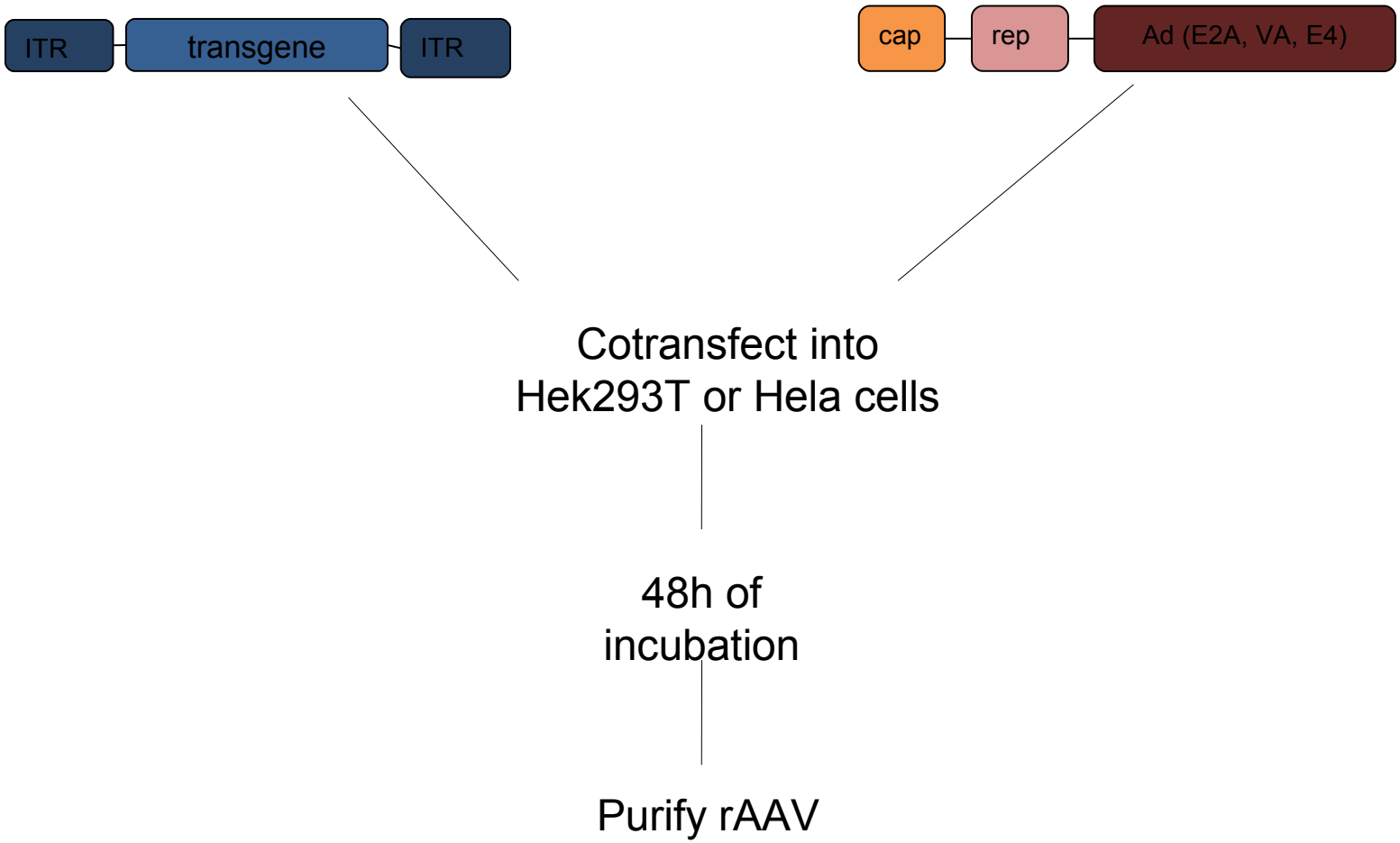
- Helper plasmid:
  - Rep: regulator of viral life cycle
  - Cap (VP1, VP2 and VP3): viral capsid proteins
  - Adenovirus genes encoding E1, E2A, E4 and virus associated RNA



AAV parts

- Rep and cap cassette of different serotypes
- Adenovirus helper cassette
- ITR element
- IRES
- T2A sites
- Transgene = GOI (i.e. Argonaute library elements)

AAV workflow



Materials

- CaCl, HBSS, DMEM
- Purification kit from Qiagen
- Cell line (HEK 293T, HeLa)

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RNA: miRNA Binding Motif Library Screening

DNA: Promoter Optimization

Protein: Ago Library Screening

Virus based Selection

RNA: miRNA Binding Motif Library Screening

Why miRNA?

- Intracellular sensing
- Cell specificity
- Developmental stages
- **Very hot topic!**

→Specific tumor cell targeting

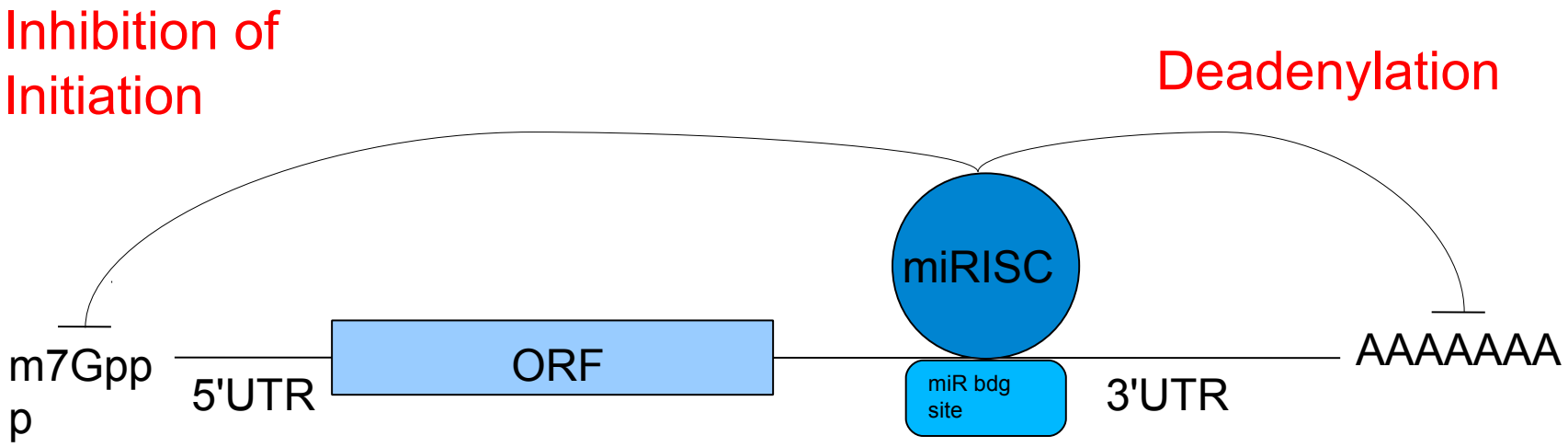
→**… many more**

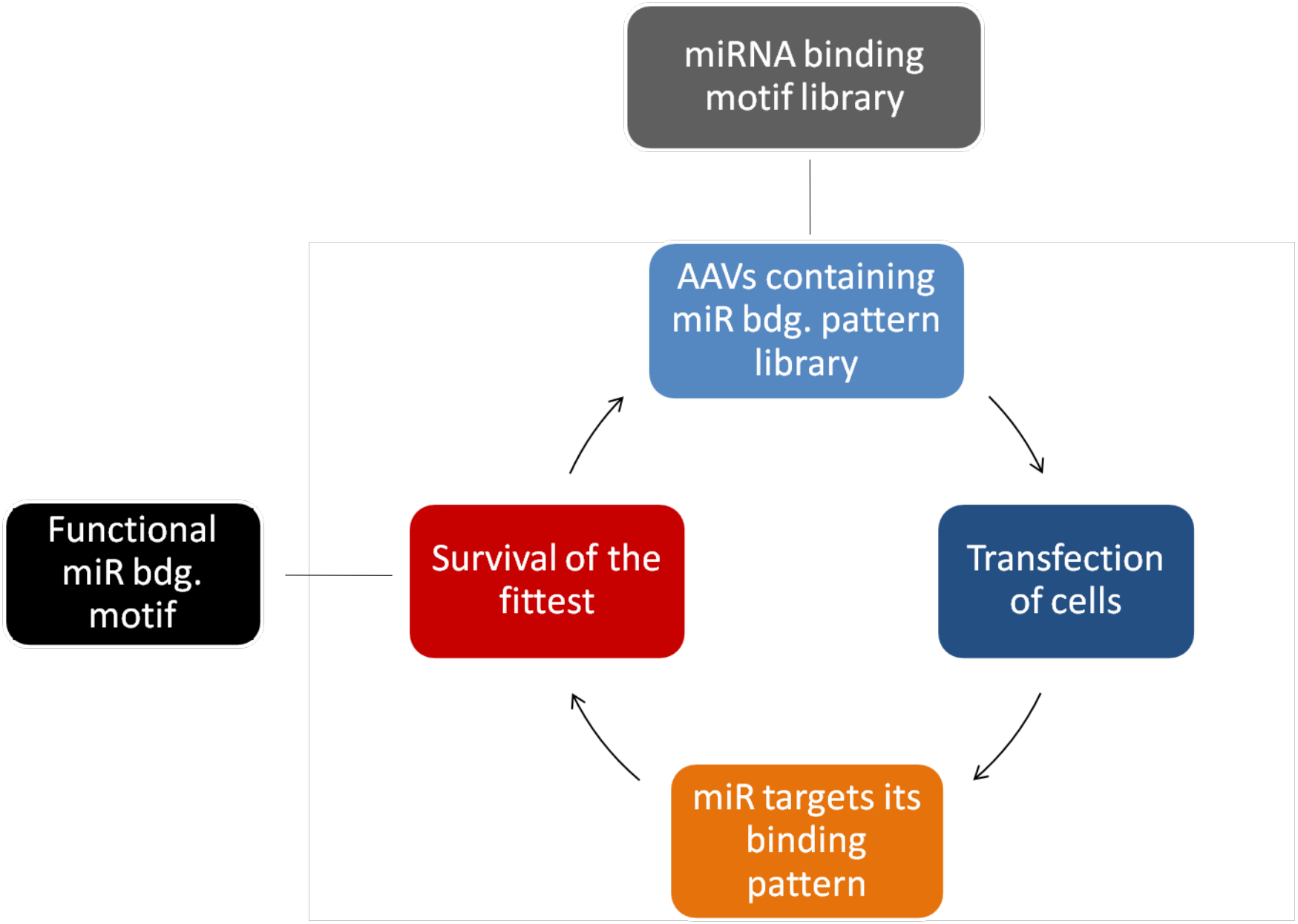
microRNA

- 22 nucleotides long

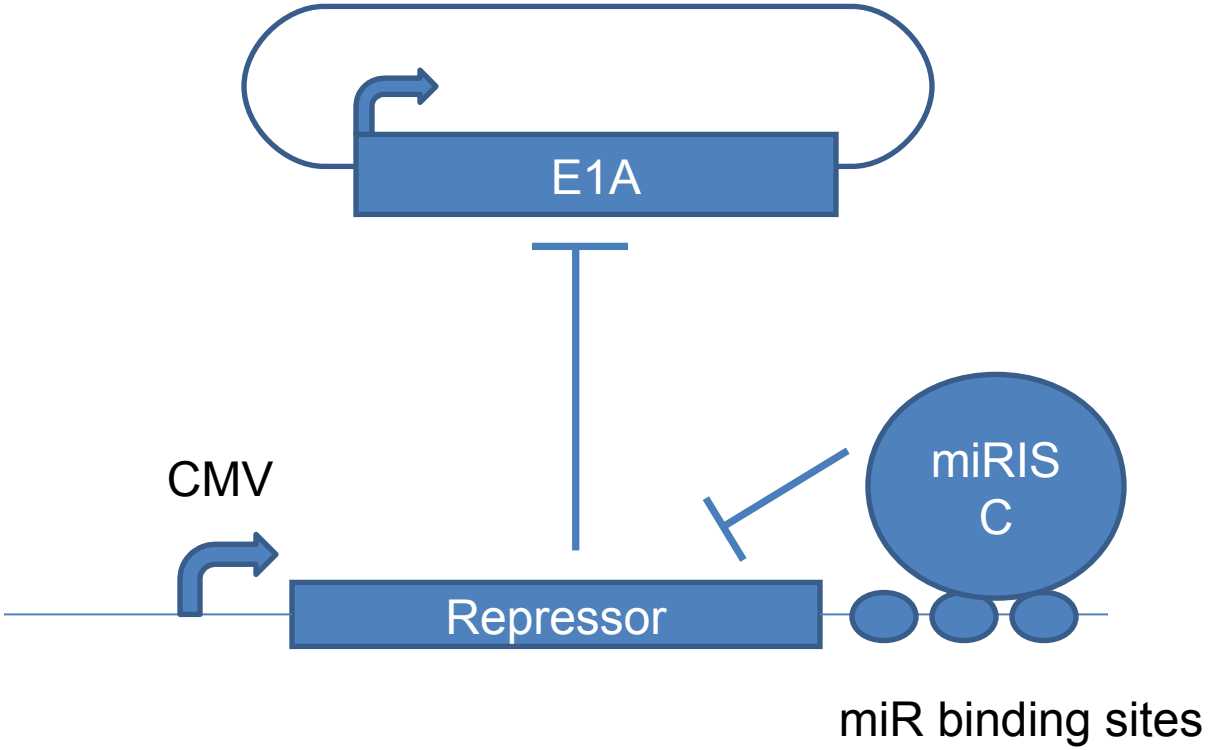
regulator of gene expression

Seed region is important for binding of miRNA to target mRNA (~7 nt)

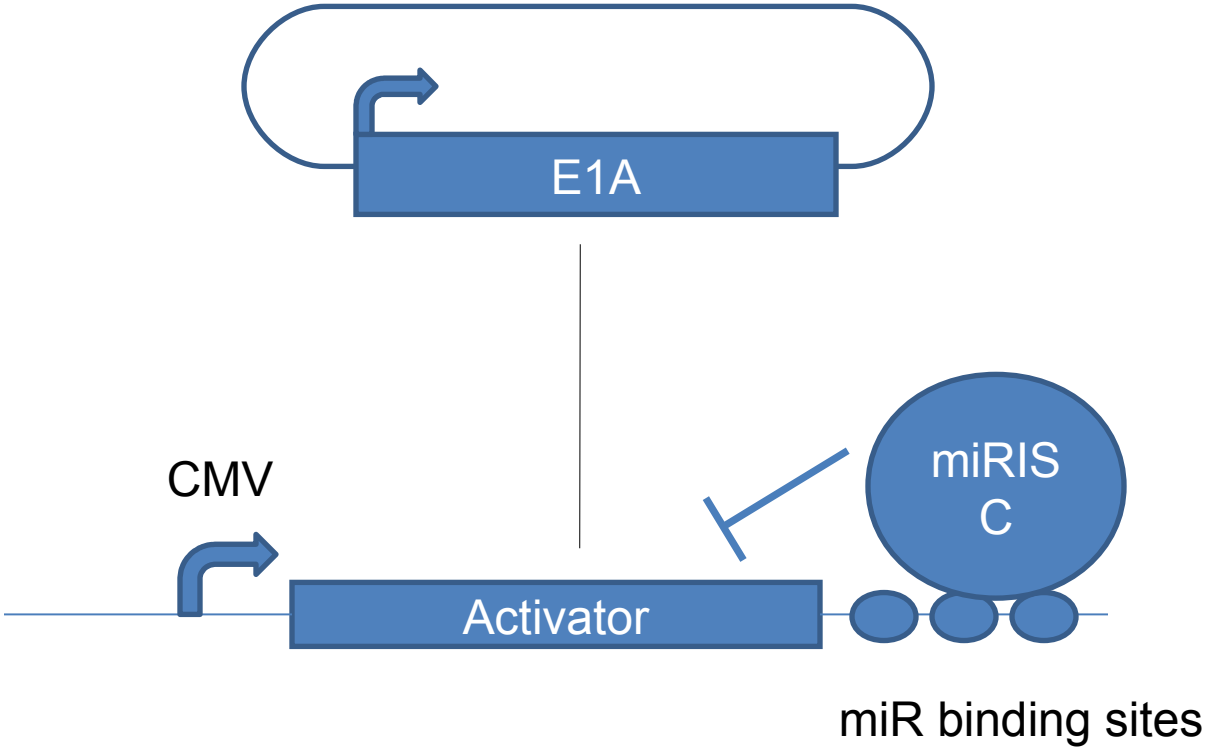



miRNA binding motif library
AAVs containing miR bdg. pattern library
Transfection of cells
miR targets its binding pattern
Survival of the fittest
Functional miR bdg. motif

Virus Rescue – positive Selection



Virus Rescue – negative Selection



miRNA binding pattern Selection

- miRNA binding pattern library
  → raPCR
  → VDJ
- Rational design of library

- Characterization by standardized GFP quantification methods (explained later)

Random Assembly PCR

2. Oligonucleotide (“Oligos”) Design

Random Assembly PCR

Gradually from low to high Strength

5. Cloning in Cyanobacteria
6. Expressing in Mammalian cells

raPCR

- ordering of miR binding sites and spacer sequences (bioinformatics)
- PCR under different conditions

  → constant seed regions on one side

  → concentration of different oligos (binding sites, spacers)

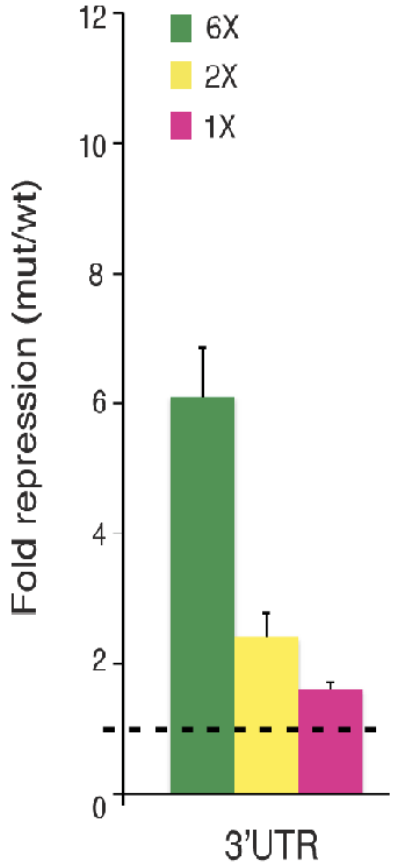
  → length of spacer oligos

→ miR binding site patterns resulting in different translational inhibition levels

Rational design of miRNA binding site pattern

- Cloning of different amounts of binding sites

→ translational inhibition



Virus based Selection

DNA: Promoter Library Screening

Why NFkB Promoter Optimization

- fine-tuning of promoter activation
- Important for threshold device

→ needed for Killer-Application

NFkB Promoter Optimization

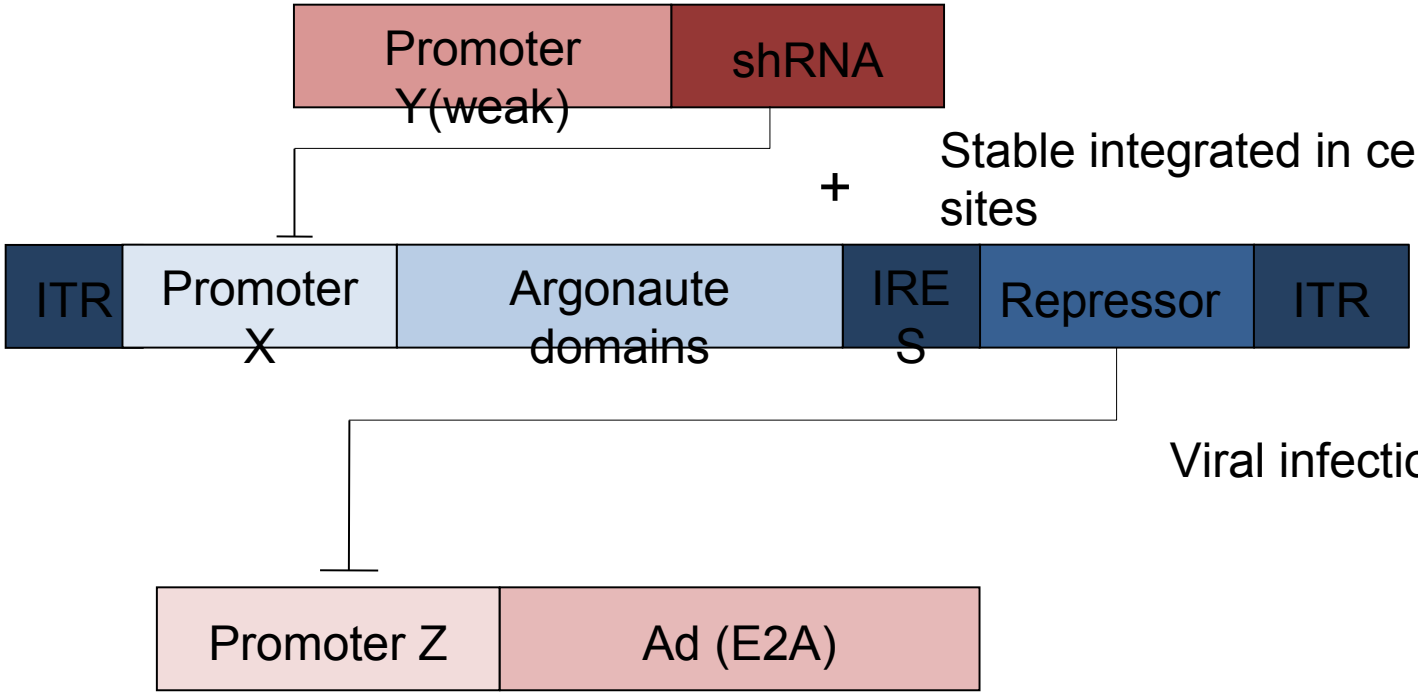
- Cloning of NFkB Promoter Library in AAVs
- Viral Selection cycles (positive and negative)

→ Optimized Promoter that can be used to fine-tune threshold device

Virus based Selection

Protein: Ago Library Screening

AAV for protein evolution



→ Functional Argonaute leads to viral replication

Virobricks: Engineering Royale

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Why Threshold Device

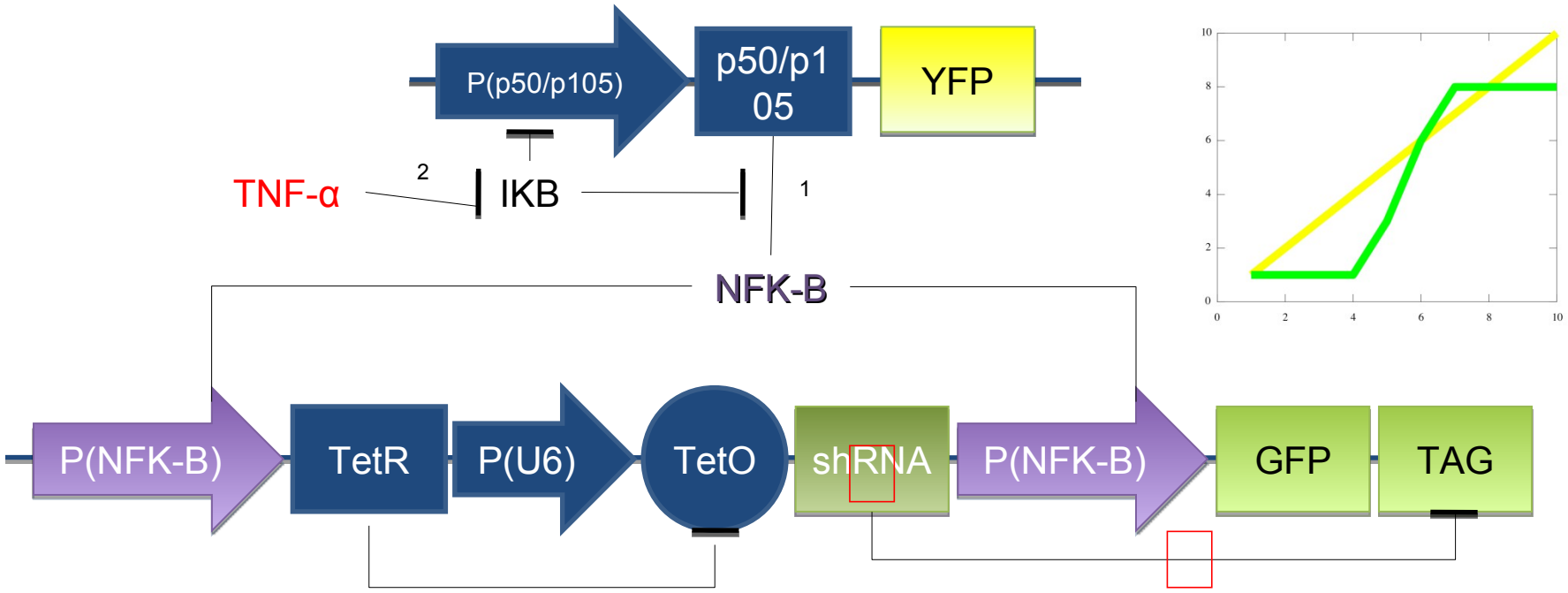
- Generates digital output from non-digatal input

→Important for many applications, including our killer-application

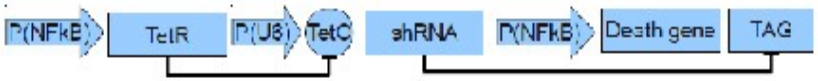
- Most parts in the registry
- Optimization easy by viral selection

threshold device principle

- defined induction level (YFP reporter) of NFK-B via input signal (i. e. TNF-α)
- measurement of output signal as promoter response (i.e. GFP)




Heidelberg 2009: NF-kB responsive promoter



Insertion of a strong synthetic NF-kB promoter

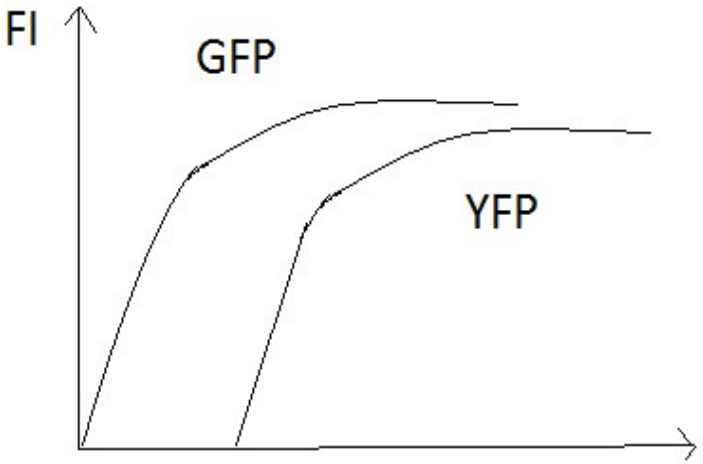
NF-kB Induction

Transfection of our threshold device

Input

Cell

Output



Measurement standard to measure two expression levels in vivo through GFP/YFP quantification in time lapse

Standardized GFP Quantification

- Measurement standard in time-lapse (TECAN)
- Good characterization for *in vivo* transcription factor activity, mRNA stability and miRNA inhibition level
- Different cell lines
- Good input for model

Modeling

Automated miRNA binding pattern evolution

Threshold Device Modeling

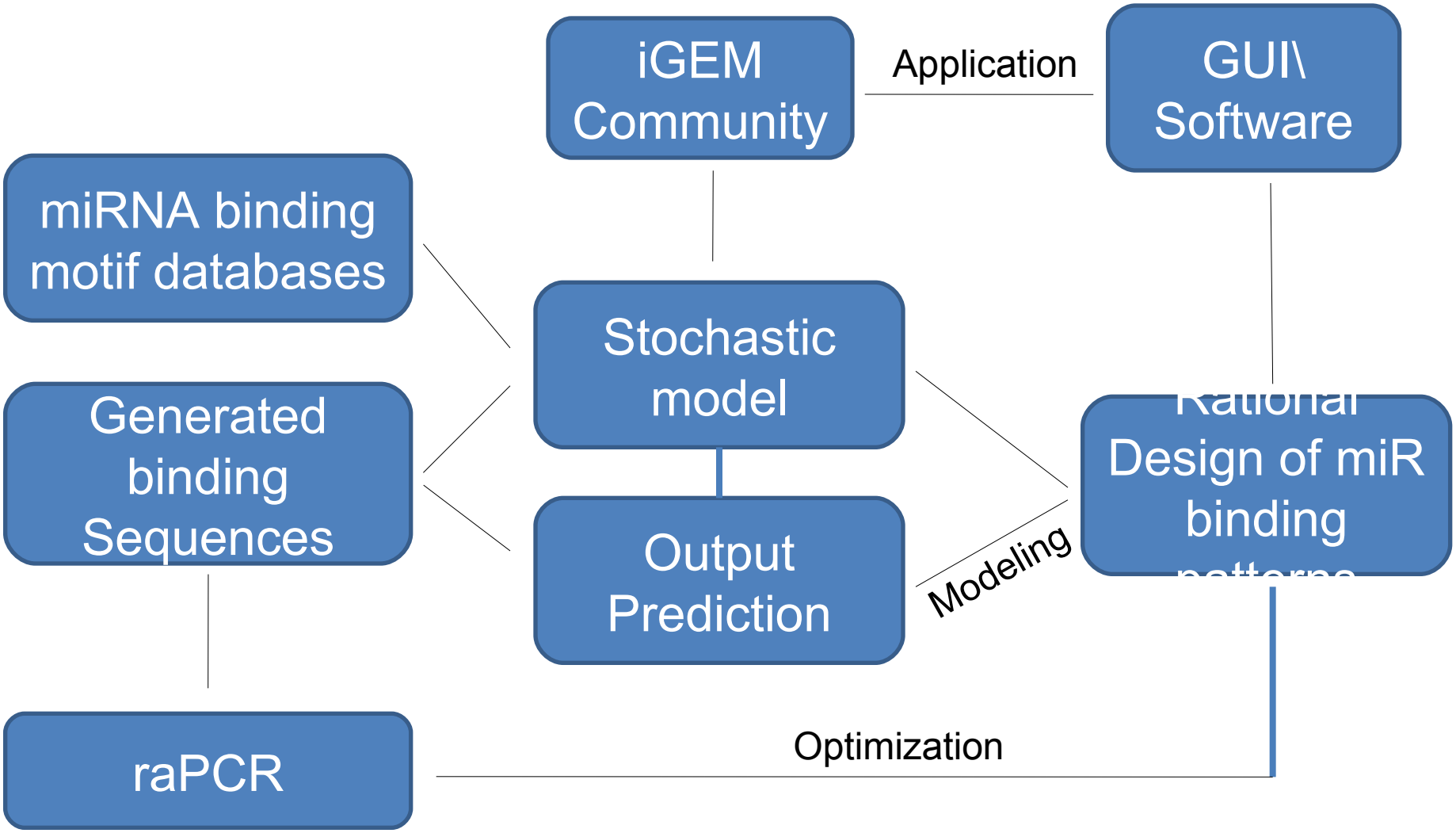
Predicting miRNA binding patterns

- Number of binding sites
- Spacial distribution of (different) binding sites
- Spacer Sequences between binding sites
- miRNA expression in different cell types

→Data available or easy to get

→Functional miRNA binding patterns for intracellular sensing

miRNA binding pattern model



Threshold Device Modeling

- Modeling the activation curve of threshold device under different conditions (different promoters, transcription factor concentrations…)
- Prediction of output produced by promoters of different strength

→enabling easy re-use by iGEM community

Killer Application

Intracellular miRNA sensing

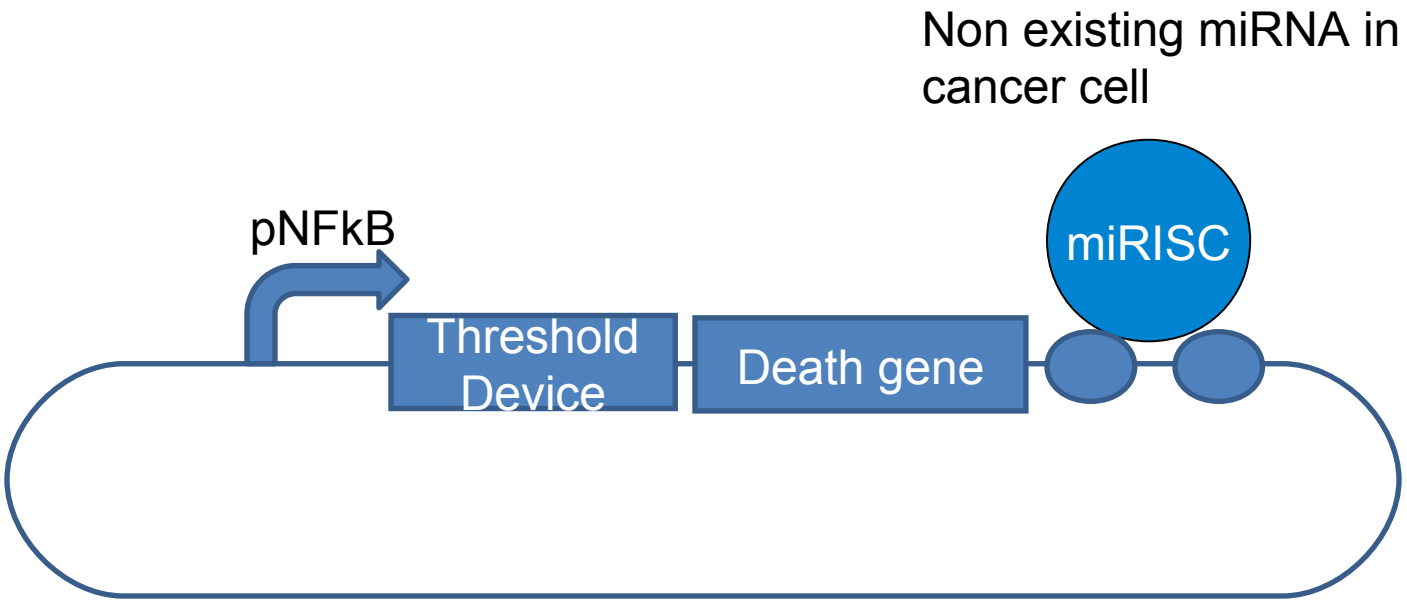
Selective Killing of Cancer Cells

Specific Cancer Cell Killing

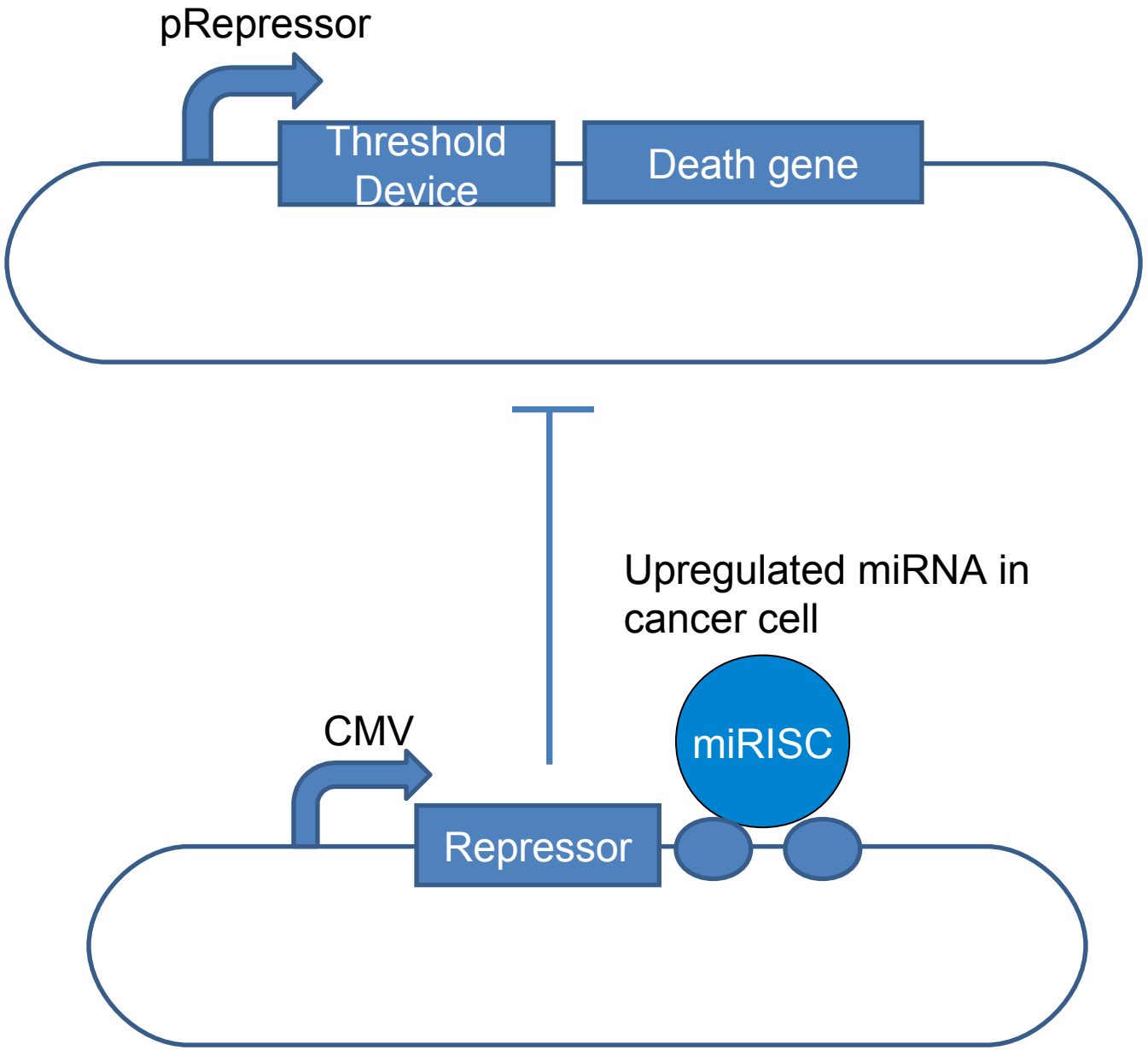
- miRNA expression pattern differ highly in different cell types and developmental stages
- Intracellular sensing of miRNAs can be used for selective cancer cell killing

- Two different Viruses for selective cell targeting possible

Negative Selection



Positive Selection



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V(D)J based miRNA binding pattern engineering

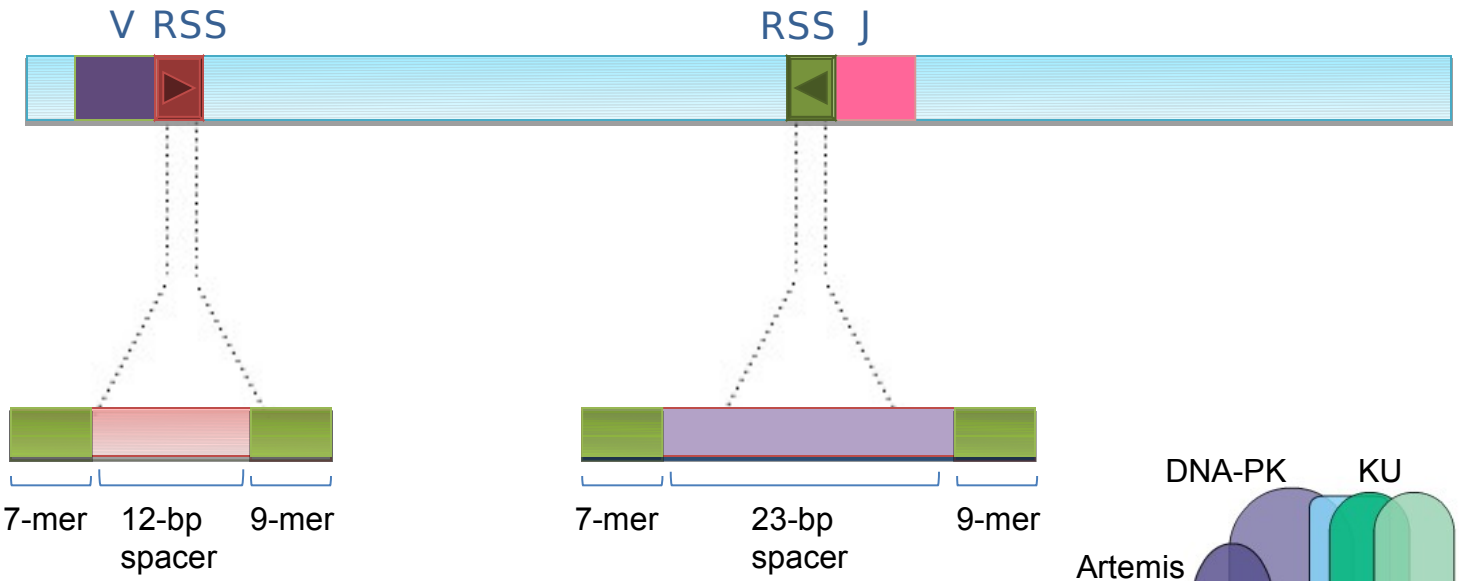
Automated miRNA binding pattern evolution

miRNA binding pattern engineering

- Recombine different miRNA binding motives
- Selection using Flow Cytometry

V(D)J Recombination

RAG1 + RAG2



Problems

- V(D)J system is not completely understood
- Histone acetylation required for starting of recombination process
- Sequence length and spacial distribution might be important for correct recombination
- Limited applications

→ risky!

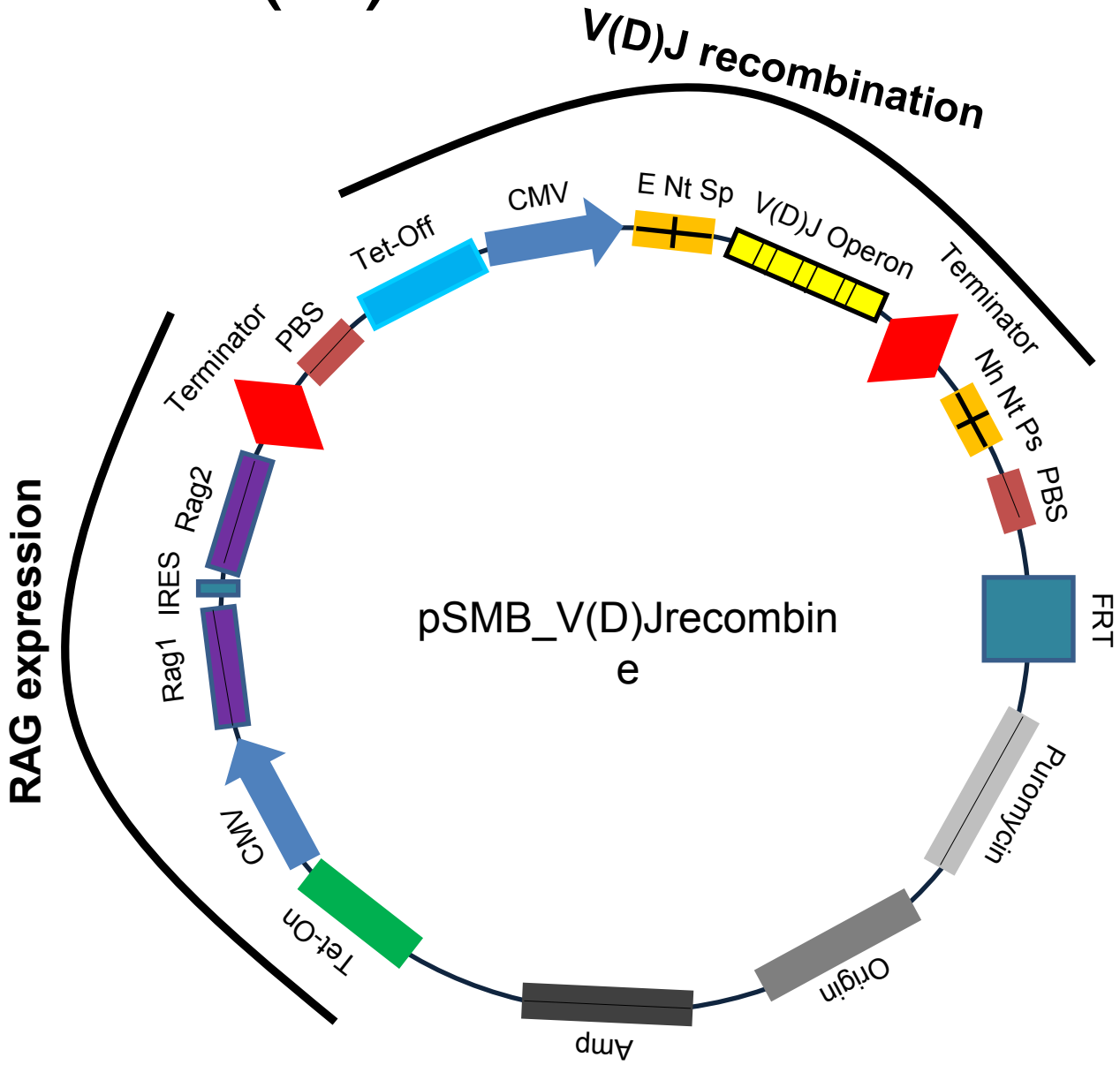
But…

- Construct short, can be ordered
- Rag cloning easy
- Stable cell line needed anyway
- Very interesting if it worked!
- Not much manpower needed
- Stop, if it does not work after a reasonable amount of work and time

→ Cloning Rag1 and Rag2 with Weimar, integrate Life-Science Lab (Human Practices)

VIROBRICKS – the Parts

V(D)J Construct



Project Workflow