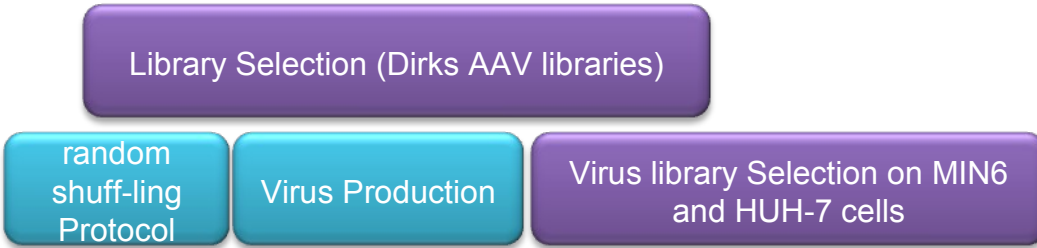
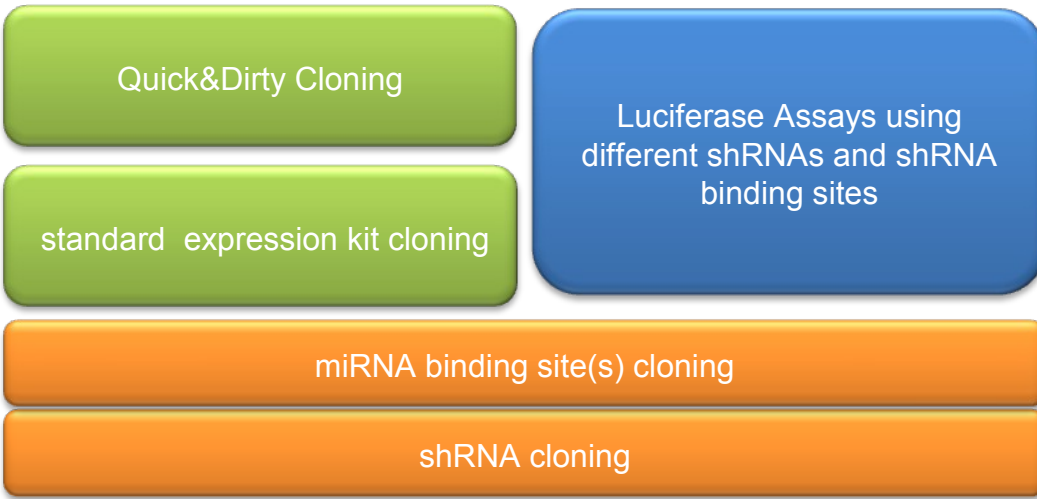




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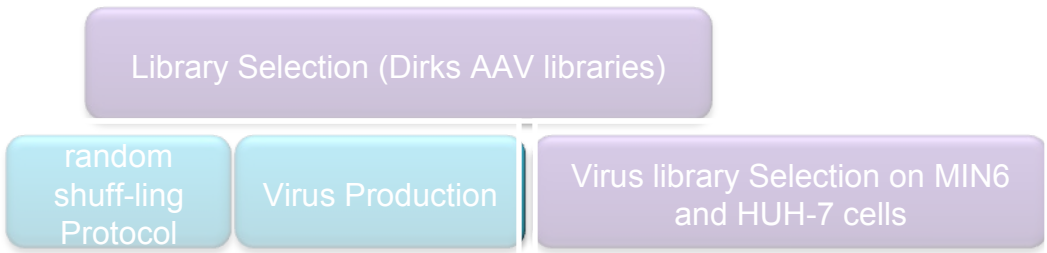


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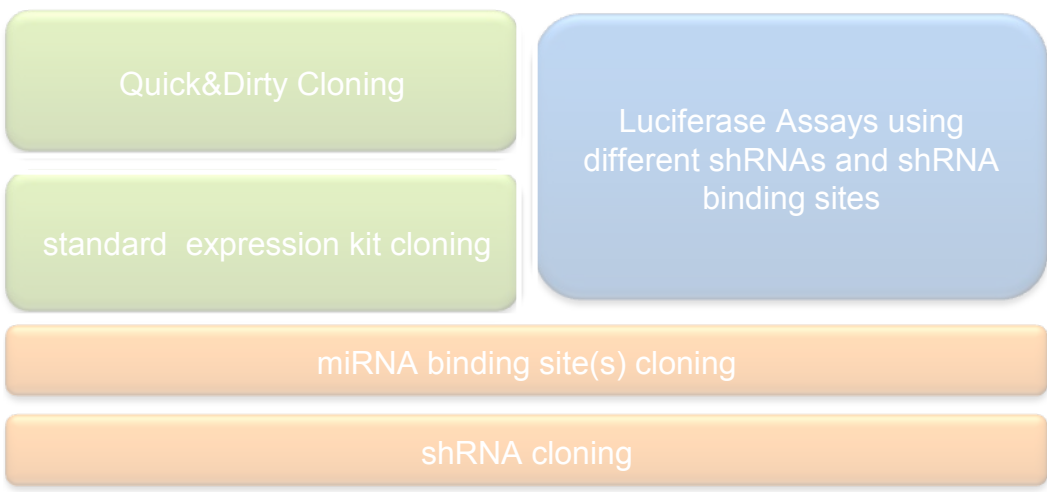




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Shuffling and Virus Production

Workflow

- 2 days shuffling
- 6-9 days virus production

Manpower

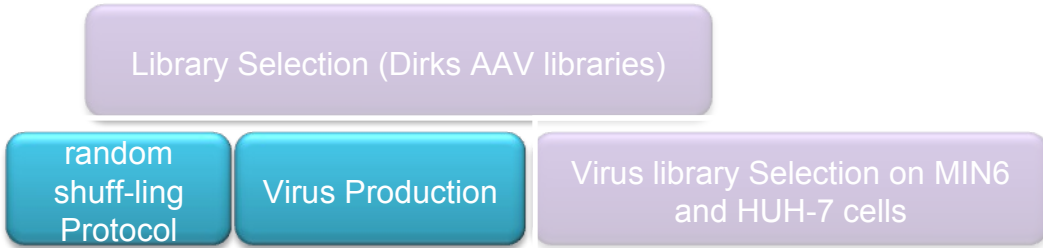
- 1 person, 14 days

Timeplan

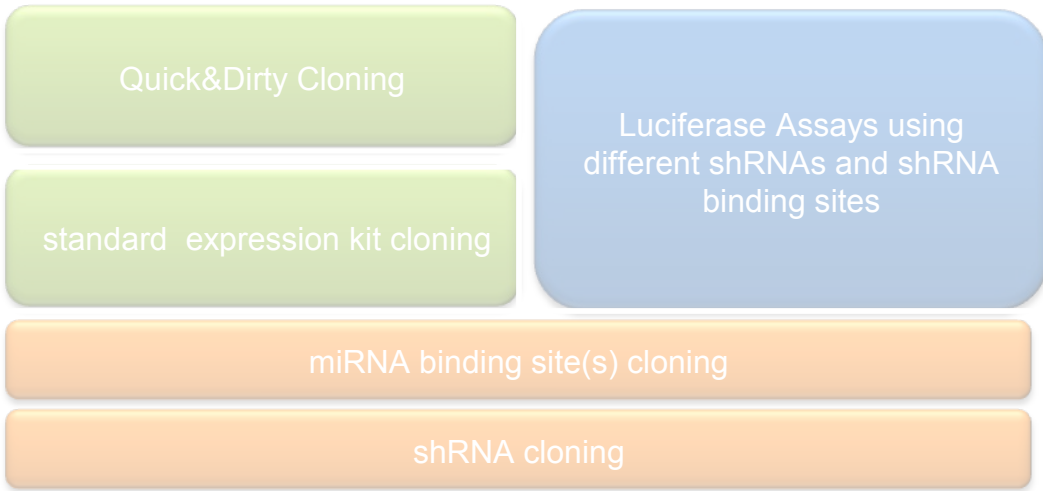
- Order primers today
- Start shuffling by Thursday/Friday



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Virus Selection

Workflow

- 2-3 weeks for selection of library

Manpower

- 1 person in the cell culture

Timeplan

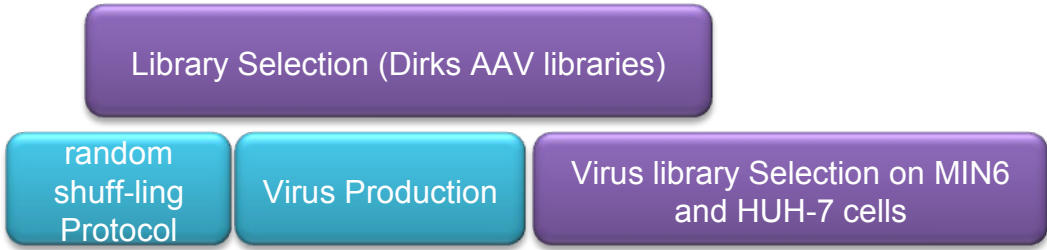
- Establishment of MIN6 cell line as soon as possible
- Start with Dirks library right away on HUH cells; on MIN6 cells in about 10 days

Why MIN6 cells

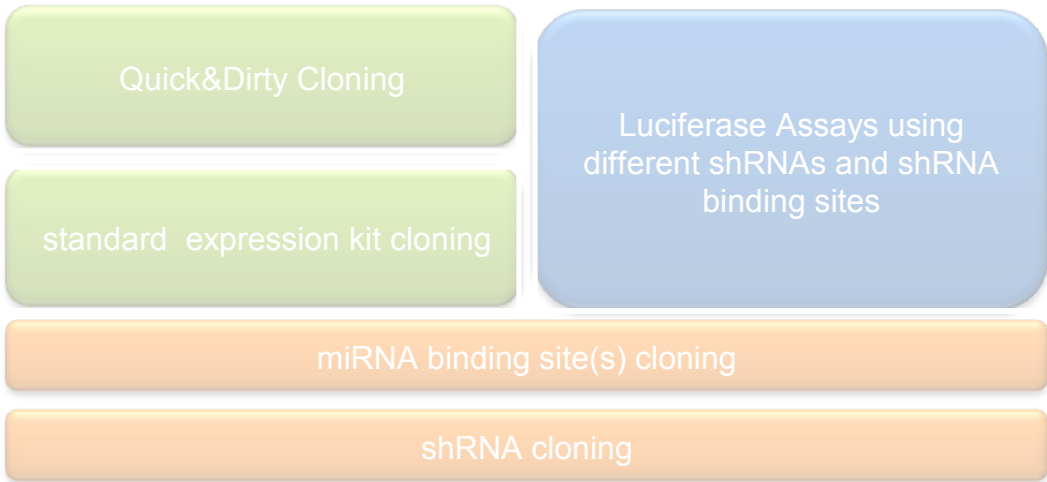
- Handling easy- good for virus selection
- clear miR candidates as targets (miR-375/6) → TetR-based on-system



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Quick&Dirty Cloning

Workflow

- Cloning of 3 fragments
- Ligation into final AAV-2 vector

Manpower

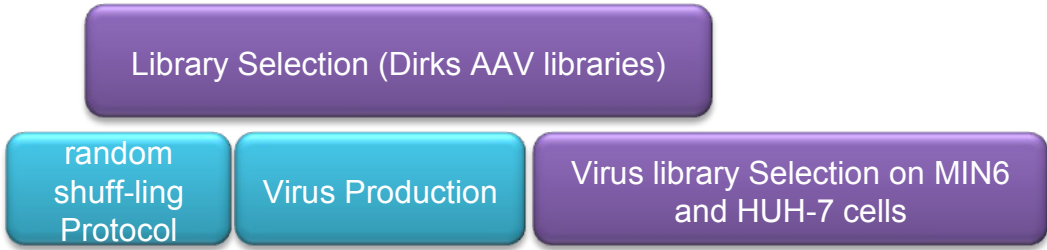
- 2 people, fulltime

Timeplan

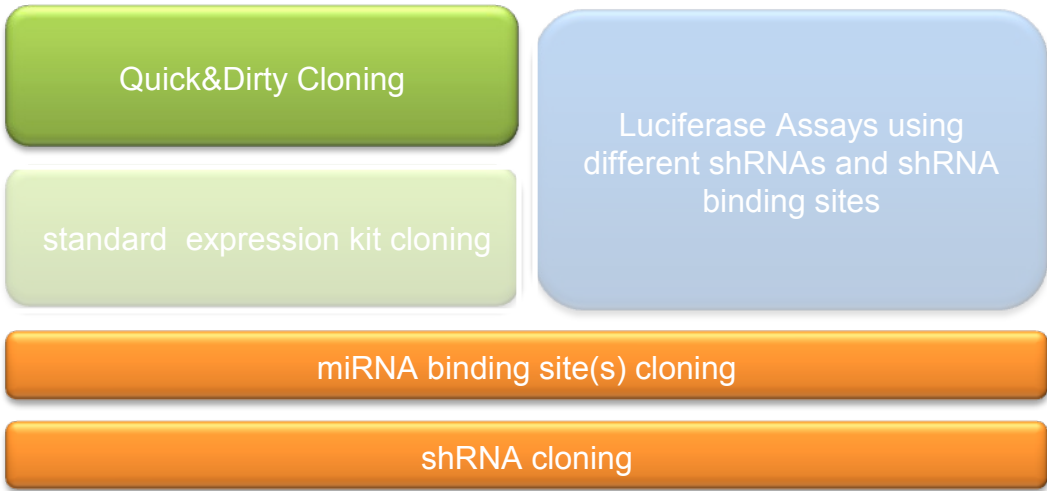
- Establishment of MIN6 cell line
- Start with Dirks library right away on HUH cells; on MIN6 cells in about 10 days



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Standard Kit Cloning

Workflow

- Cloning of all parts
- 3A strategy for assembling parts in 3 rounds
- Standardization (BBb)

Manpower

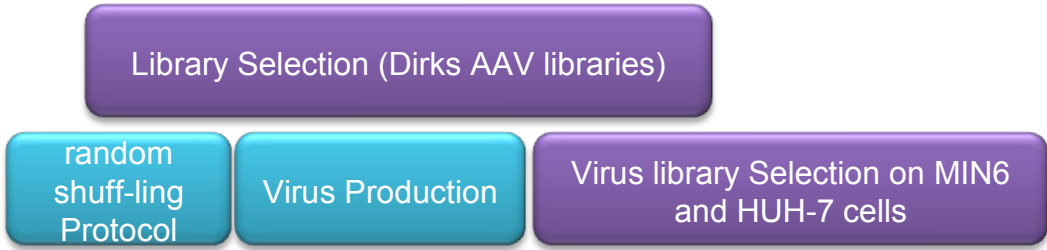
- 2 people, fulltime

Timeplan

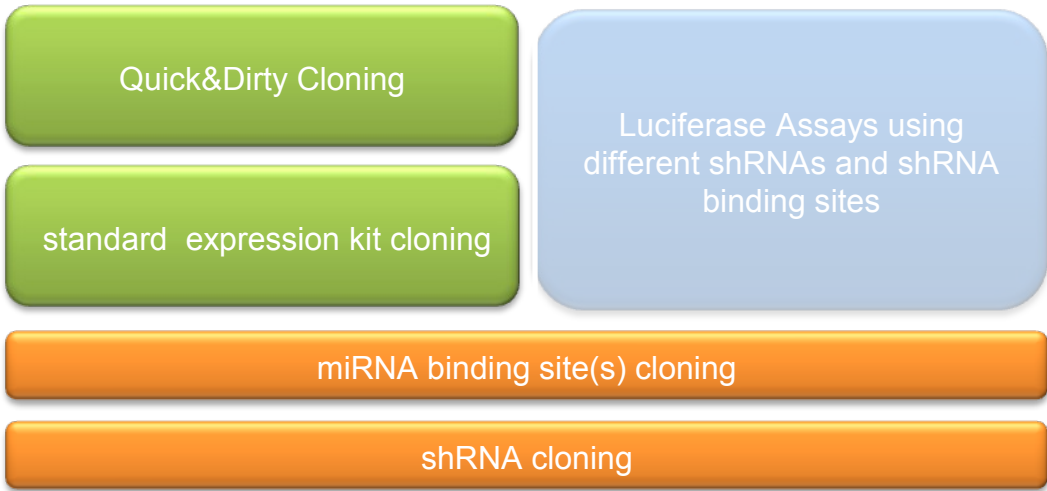
- Standart Part Cloning and Sequencing till Friday/Saturday
- Complete Assembly till mid september



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Luciferase based Measurements

Workflow

- Start with transfecting Quick&Dirty construct (3 days procedure)
- Test standardized construct mid September
- Virus production and selection for interesting candidates

Manpower

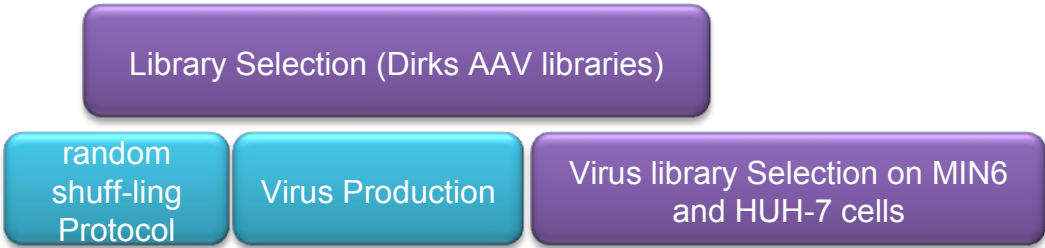
- 2 people, cell culture and assay setup

Timeplan

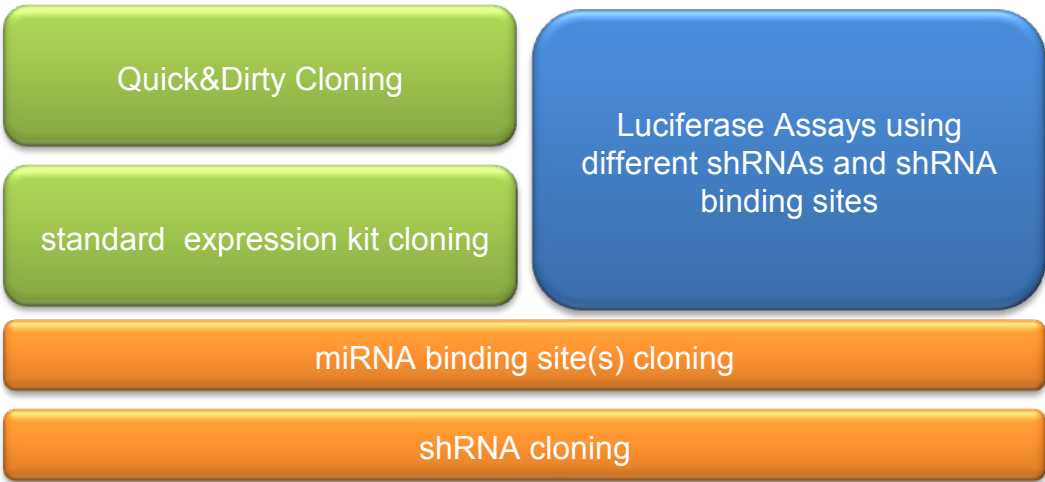
- start first measurements in about 12 days
- Do as many different high-quality measurements (8-12 replicates) as possible (transfection & infection based)

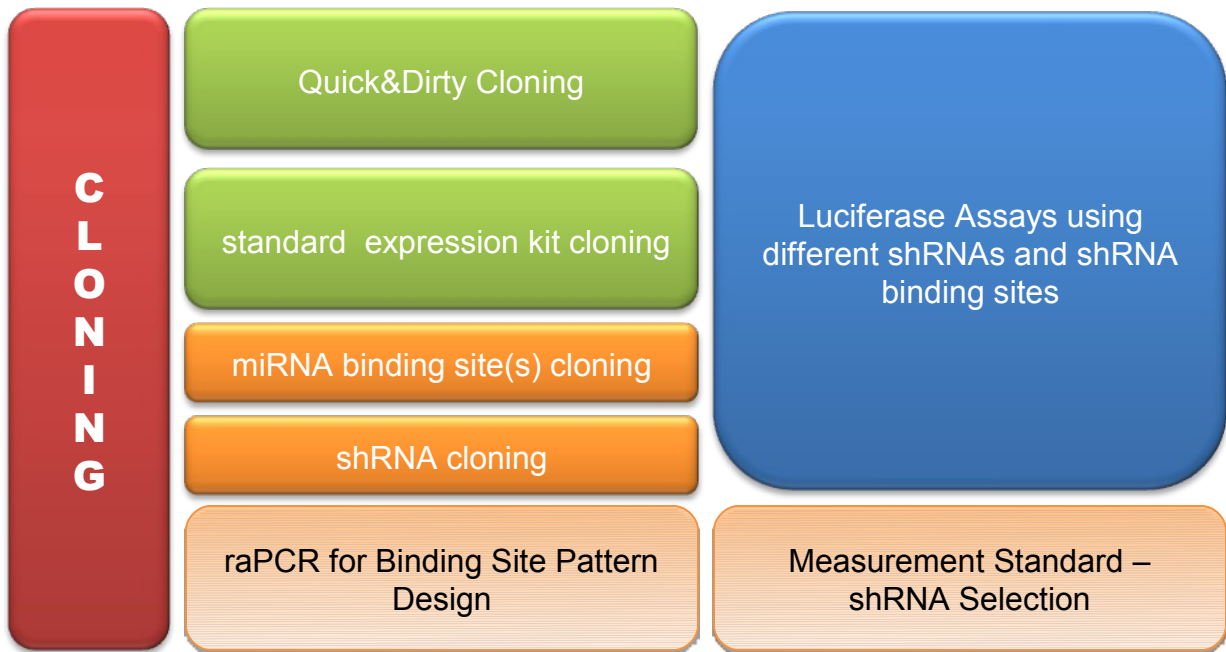
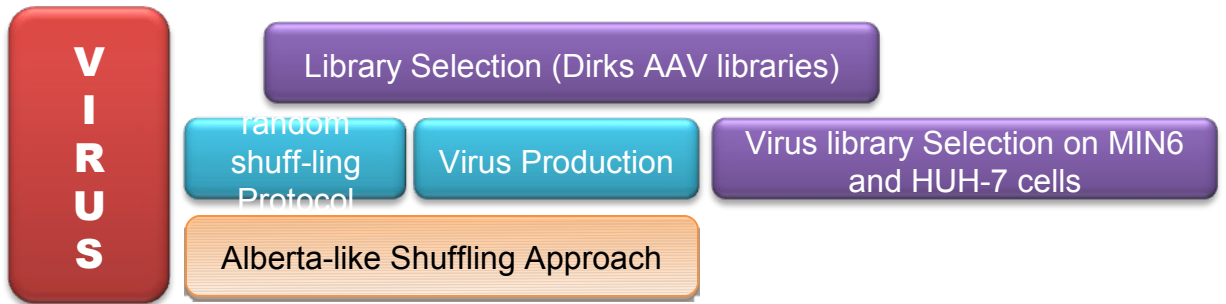


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Manpower

Subgroup	Manpower (- mid September)
Quick&Dirty Cloning	2-3 People
Standard KIT	2 People
DNA Shuffling & Virus Production	1 Person
Virus Selection	1 Person (cell culture)
Wiki	1 Person

Subgroup	Manpower (- mid October)
Luciferase Measurements	2 People
Cell Culture	2 People
Virus Selection	2 People
Cloning	3 People
Wiki	!!!

Discussion

Measurment Standard	diraPCR	Alberta-Like Shuffling
<ul style="list-style-type: none">• Cloning of Measurement Standard simple• time-laps experiments possible• easy screen of shRNA binding site• cloning for stable cell lines running anyways (bit of extra work)	<ul style="list-style-type: none">• raPCR protocol set up; allows for binding site pattern construction• for strong effects we may need more than one binding site• for MIN6 cells, miR375/6 binding site patterns very promising	<ul style="list-style-type: none">• our own protocol• seems streight forward• allows for a more rational influence on shuffling (p/c)

Each of the options is a one person task, but would need further screening.

Kit for modular expression triggering

- shRNAs
- Promoters
- Terminators
- Genes of interest (Luciferases or any other gene from the Registry)
- miR binding sites/binding site patterns

- Repressor construct

Quick Assembly via iGEM 3A Strategy



Cut with
E & S



Cut with
X & P



Cut with
E & P



Mix &
Ligate

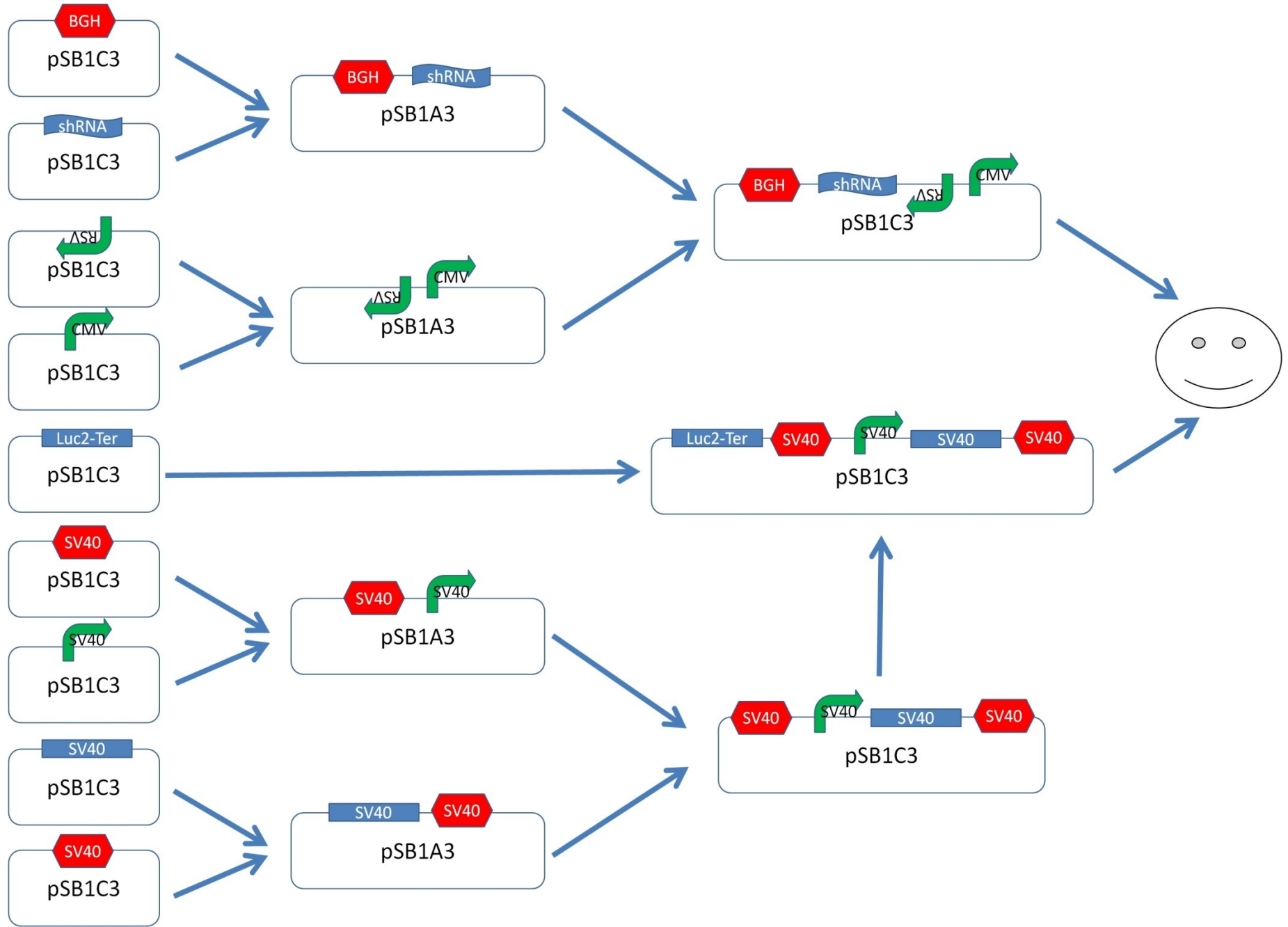


08/27

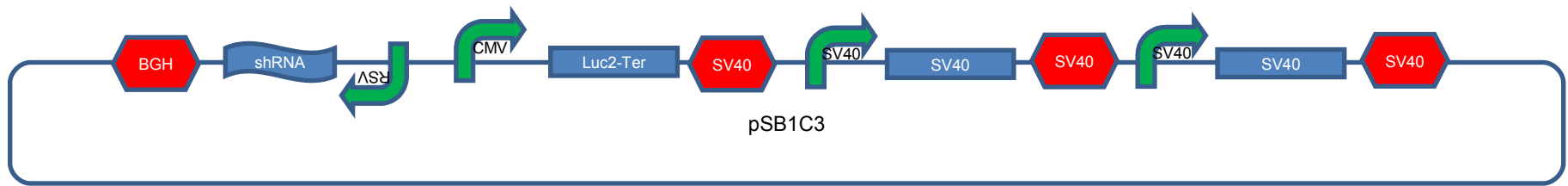
08/31

09/04

09/08



09/15



Parts

KIT

- Construction parts in BBb (promoters, terminators, luciferases, shRNAs, binding sites, CMV_Tet0, TetR)
- Standardized dual luciferase construct

AAV Parts

- Standardized AAV vector with ITRs and BBb sites??
- What else? (standardized Capsid?)

Library&Measurement parts

- shRNAs
- Binding sites
- pSMB_miMeasure?
- Binding site patterns

Protocols/Methods

- diraPCR protocol/diraPCR designer tool
- Alberta-Like Virus Shuffling Protocol
- pSMB_miMeasure protocol