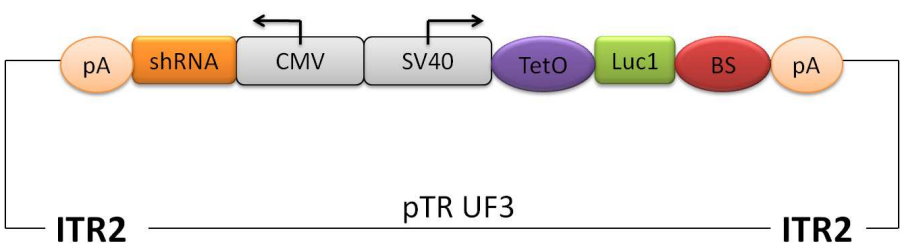
quick ‘n’ dirty cloning

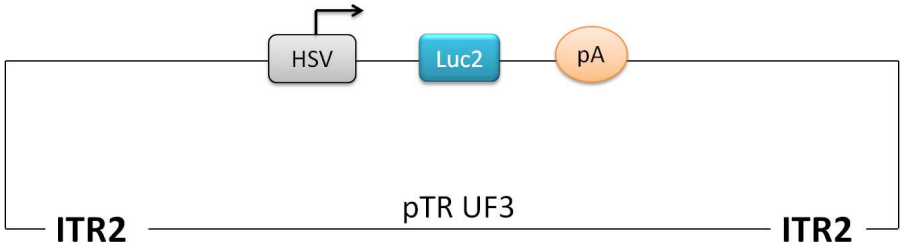
short notifications

overall aim

- **to have at least some constructs ready as soon as possible**
  - *in vivo* testing by transfection (one large construct) and infection
- no standardization but still modularity
  - exchangeable "parts": **shRNA**, **TetO**, **miR Binding Site**
- cloning scheme on the Wiki
  - **operator**: first trial with $TetO_2$, afterwards with 4x $TetO_2$
  - TetR fused to miR binding sites is expressed separately



"sensing plasmid"

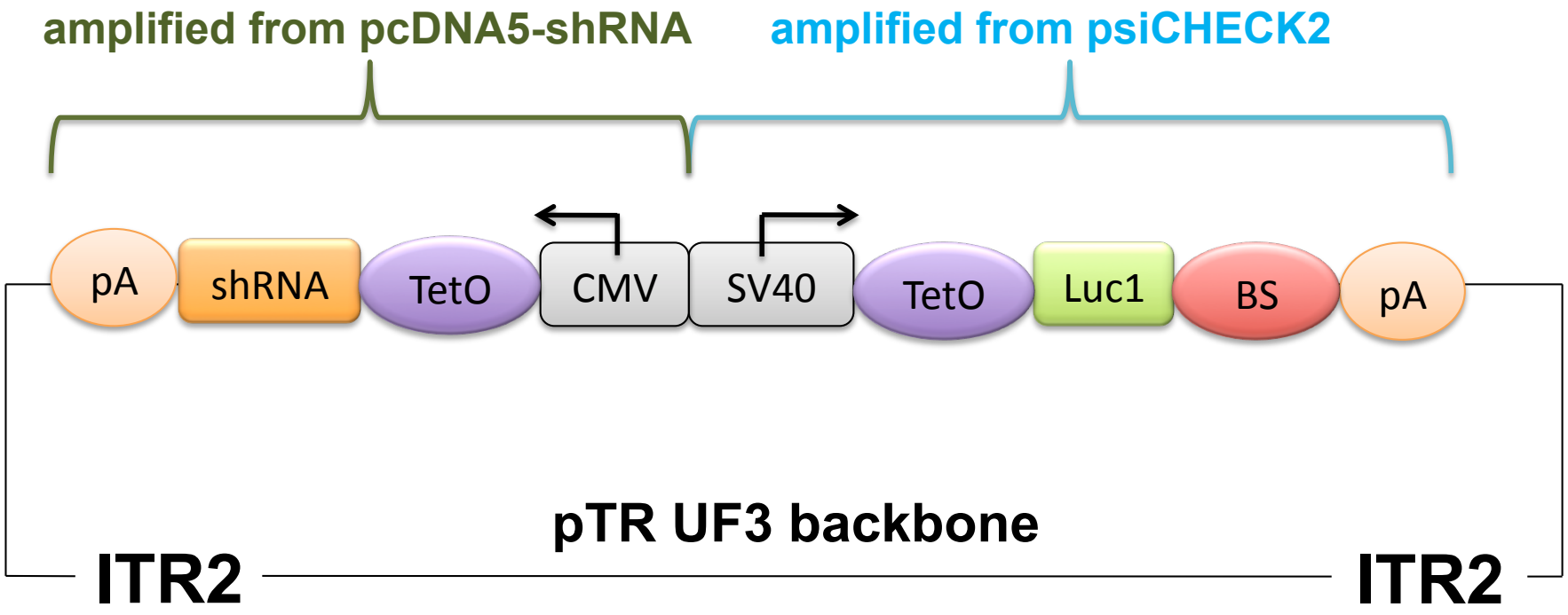


"normalization plasmid"

http://igem.bioquant.uni-heidelberg.de/mediawiki/index.php/Igem2010/Main/Protocols/Cloning/Virus_strategy

details

- PCR amplification of two inserts (plasmids ready, yet)
- directed ligation of two inserts into pTR UF3 (backbone ready, too)
  - sub-cloning of **TetO** and **miR Binding Site** into **psiCHECK2**



"sensing plasmid"

time frame

	Sat	Sun	Mon	Tue	Wed	Thu	Fri
			PCR and Purification				
	backbone and preparation			Ligations and Transformations			
August	21	22	23	24	25	26	27

	Ligations and Transformations			
	Miniprep and Sequencing			
August	28	29	30	31

taken into account:
team meeting, Weimar retreat, experimental failures, sickness, unpredicted weddings
AND your support!