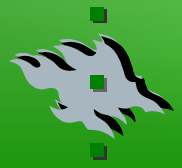
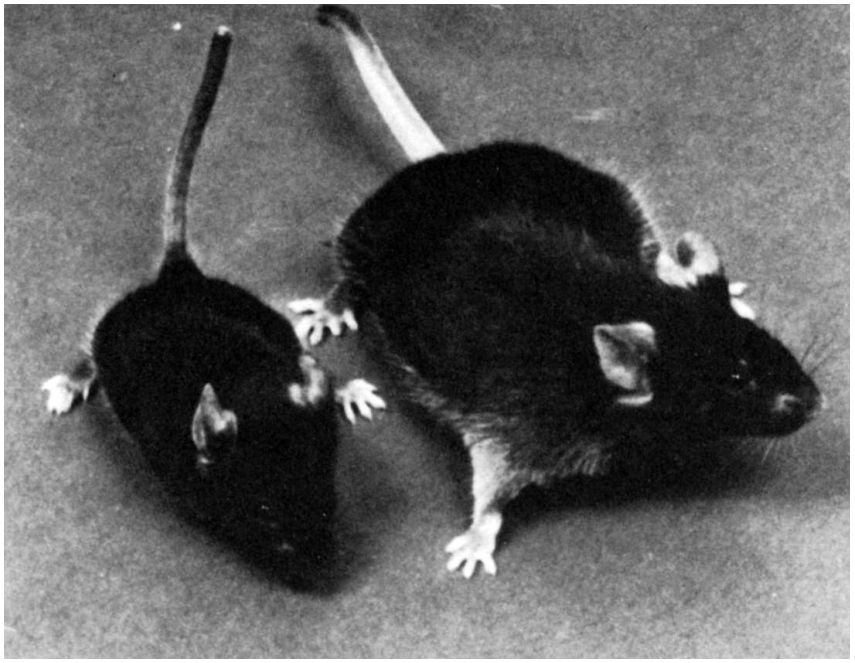


Theoretical cloning project

- Needed to get credits
- Make it up yourself, don't copy
- Possible to do in groups of 2-4 students
- If you need help or an idea, ask!
- If you have no idea what to clone, I can give you a cloning task!

Transgenic animals, knock-outs/ins, etc.



MT-hGH, 1982

K14-hVEGF-C, 1997

GloFish


Image by GloFish

- GloFish
- Only available in the USA and Taiwan
- Banned in the EU

Microinjection method

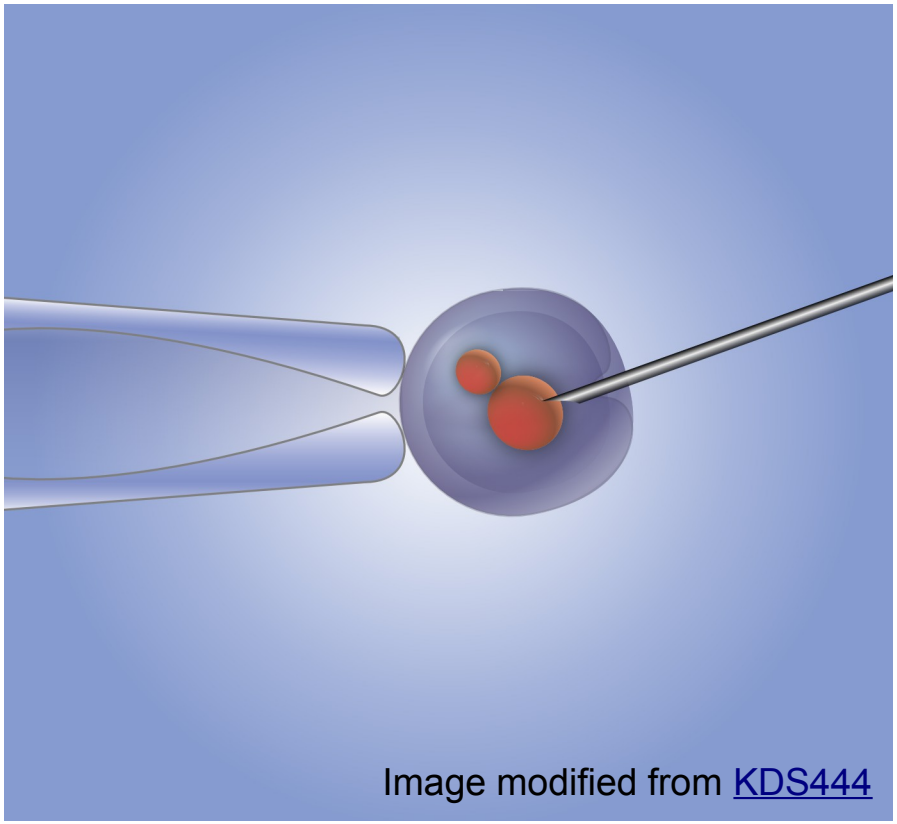


Image modified from KDS444

- In vitro fertilization
- Injection of purified, linear DNA into male pronucleus
- Implantation into pseudopregnant females
- F1 offspring is screened by PCR for DNA integration
- Successrate: 5-20% of F1 are positive
- Transfer of large DNA fragments possible
- Transgene integrates randomly as a tandem array

Embryonic stem cell method

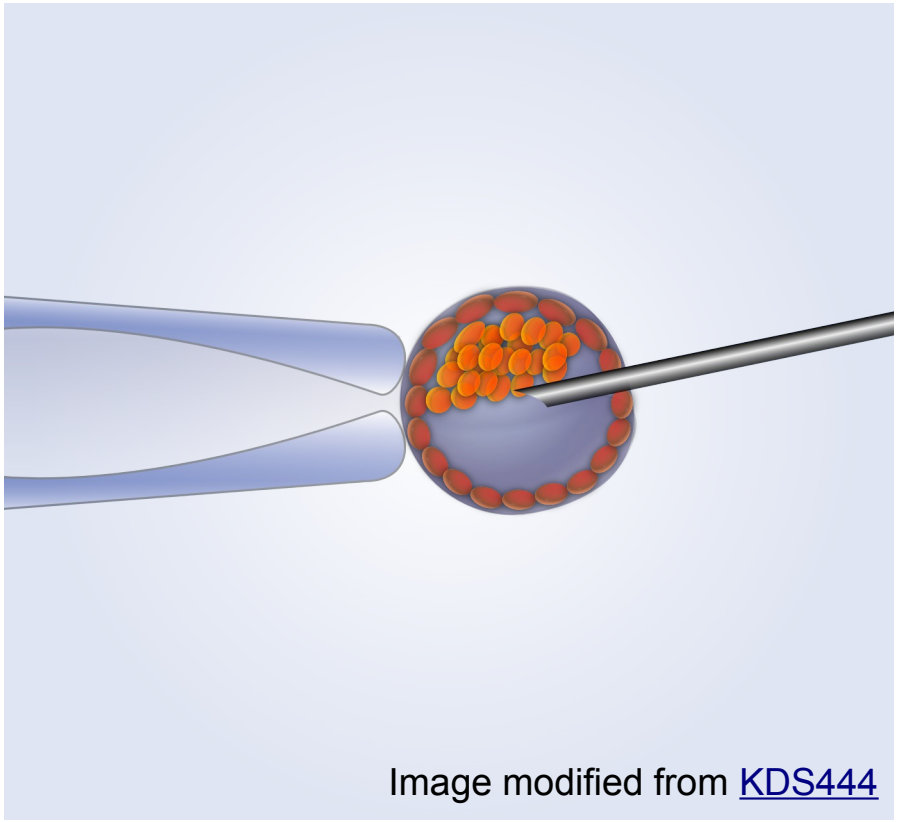
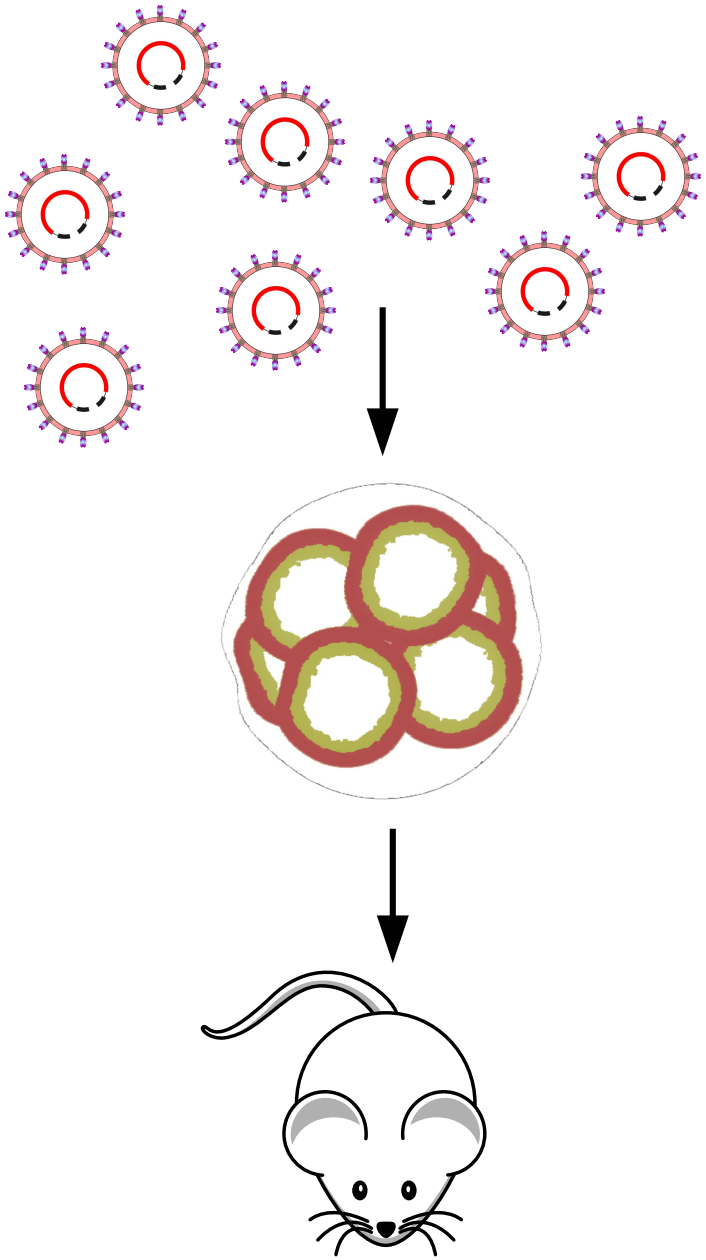


Image modified from KDS444

- Transgene is inserted into ES cells (linearized DNA, different methods: mostly electroporation)
- Stable ES cell line (= transgene has integrated into genomic DNA)
- Injection of transgenic ES cells into blastocyst
- Chimeric mice are borne, some of which have the transgene in their germ line (traditional transgene marker: fur color)

Retroviral method



- Infection of developing embryo at 8-cell-stage with recombiant retrovirus
- Implantation into pseudopregnant females
- “Retrovirus-infected” mouse
- Cargo-limit ~8kb

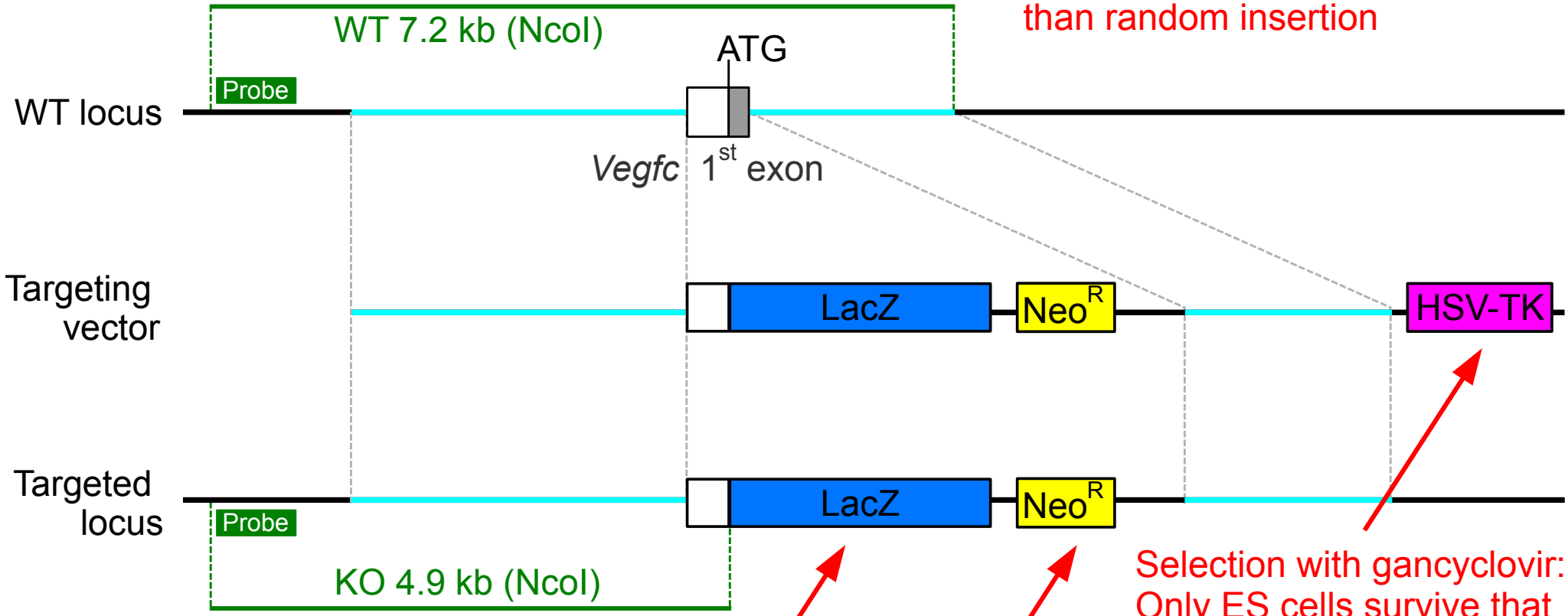
Transgenic animals

- PCR-screning vs. protein screening vs. RT-PCR screening (insertion is random and can happen into a transcriptionally inactive region), screening of mice (pronucleus injection) or screening/selection of ES cells (blastocyst injection); optional copy-number determination
- DNA insertion results in heterozygous transgenic animals: breeding can result in homzygous animals with increased expression levels
- Multiple insertions can happen into different locations, e.g. on different chromosomes (can segregate in subsequent generations)
- Pronuclear injection: Insertion can happen after nuclear fusion and mitosis resulting in mosaic animals which may or may not transmit the transgene to the next generation

Transgenic animals

- Phenotype can be due to insertional inactivation of a gene
- Expression levels: Analysis of several different founder mice to find the one with the right expression levels
- BAC (Bacterial Artificial Chromosome) transgenesis: Insertion of large fragments (>100kb) with entire genes and regulatory sequences
- To overome the random-insertion drawbacks of traditional transgenes: Targeted insertion

Gene targeting (“knock-outs and knock-ins”)



Homologous recombination in ES cells is ~1000x less frequent than random insertion

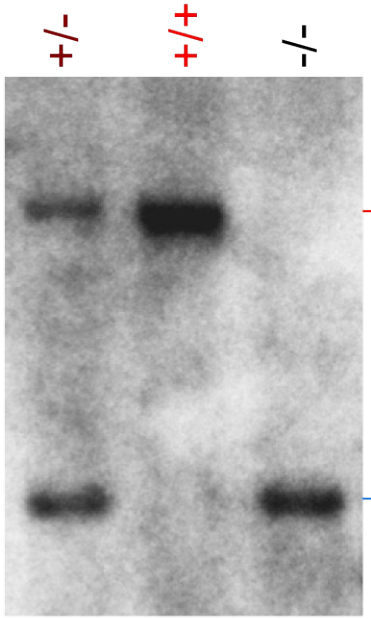
Technically a “knock-in”: A different functional gene replaces the knocked-out gene

Selection with G418: Only ES cells survive that integrated the targeting vector

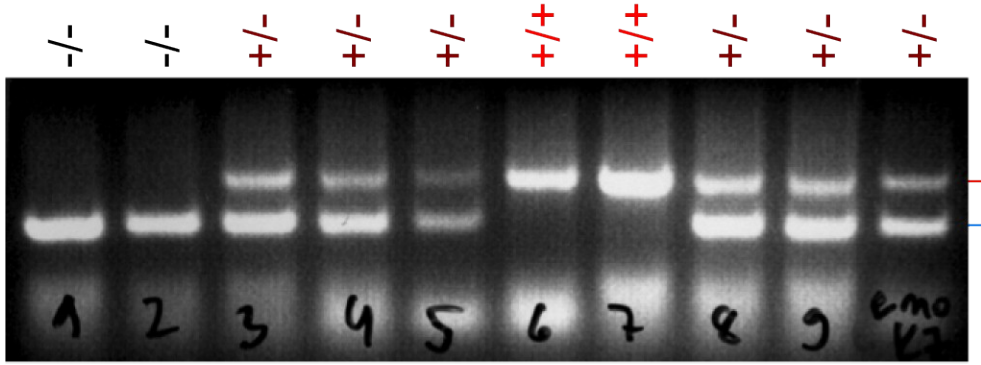
Selection with gancyclovir: Only ES cells survive that did not integrate HSV-TK by random integration

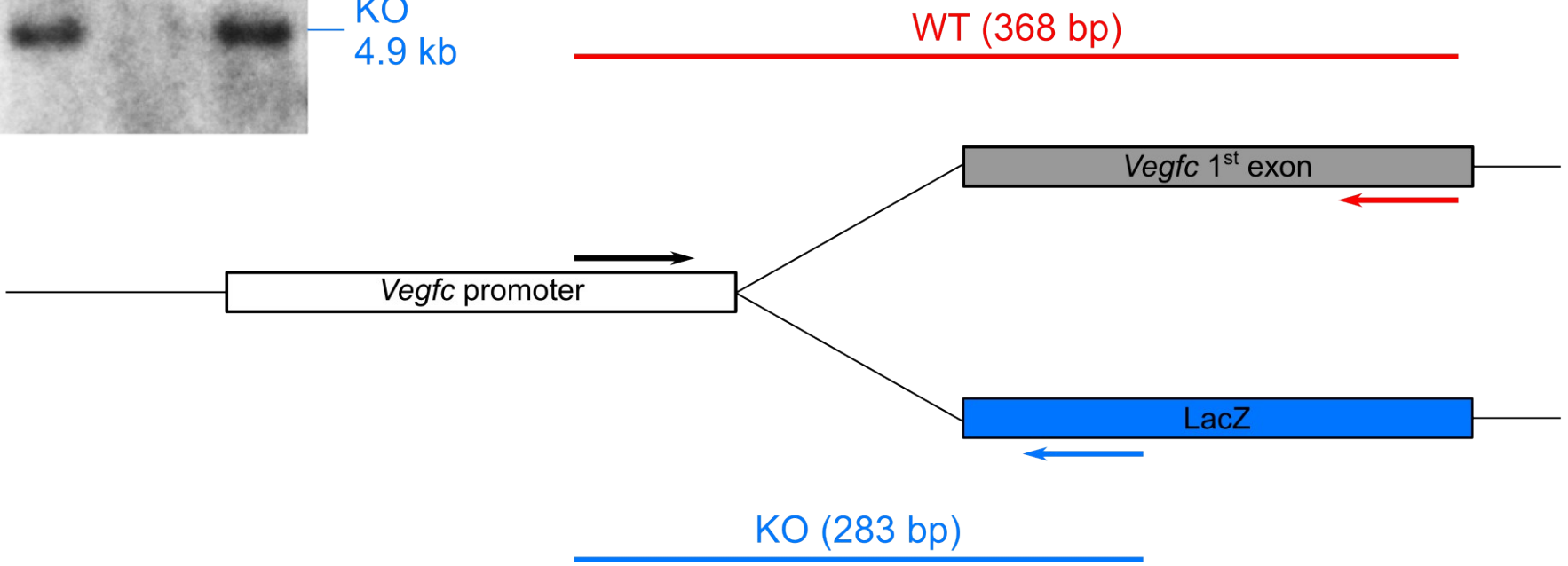
Screening (ES cells & mice)

Southern Blot
NcoI 5' external probe



PCR





Targeting Rosa26

- On mouse chromosome 6
- Rosa26 promoter is (fairly) ubiquitously active in all tissues of the developing embryo and in most tissues of the adult mouse (but the locus contributes to uniformness of expression)
- Originally: β-Gal retroviral GeneTrap, which did not affect development or viability of the mice
- Now many other reporter genes with ubiquitous expression
- Many other transgenes were targeted (“knocked-in”) to the Rosa26 locus
- Transplant tracing, chimera analysis
- Conditional Rosa26 if you want reporter gene expression only in a (tissue/time)-specific fashion

Conditional transgenes and knock-outs

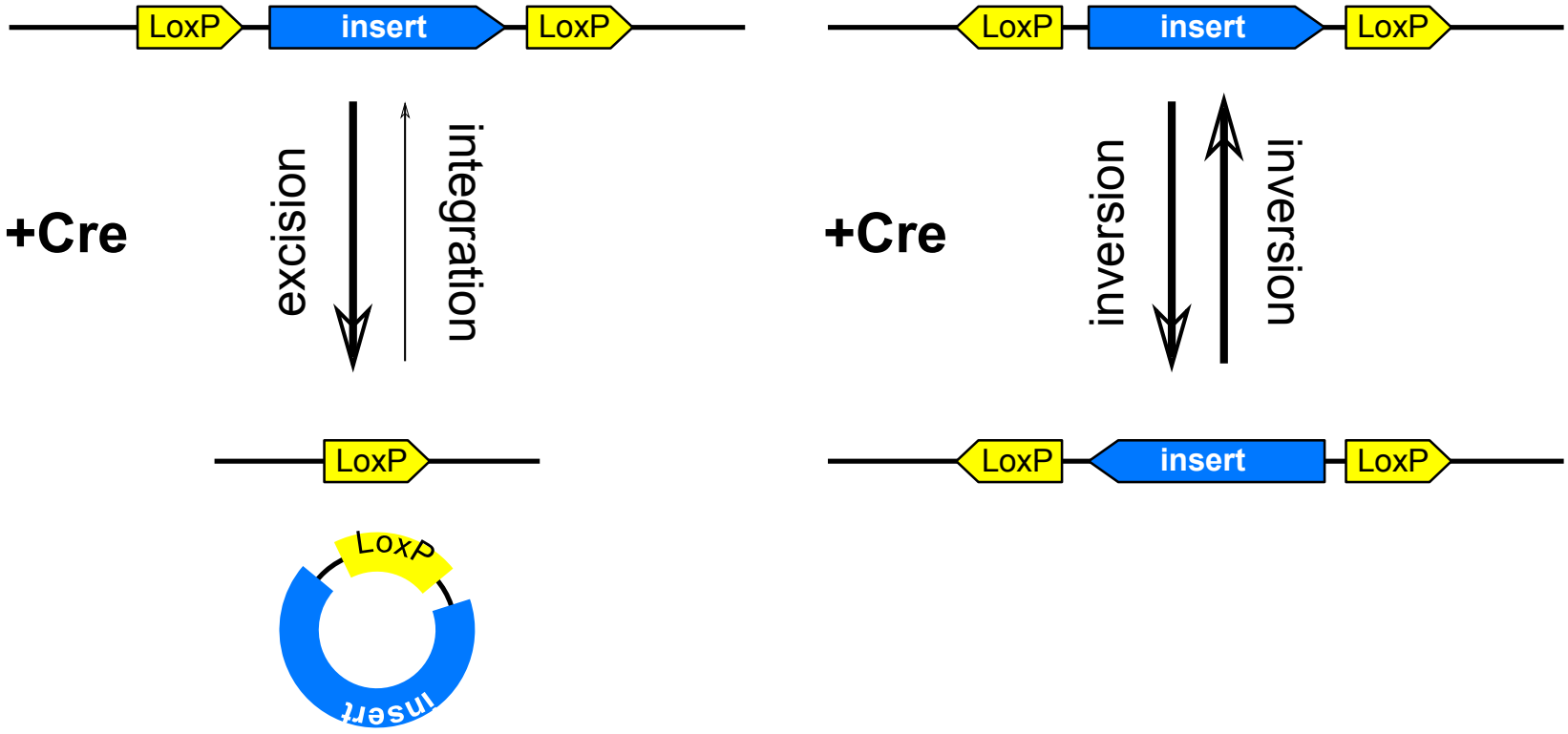
Cre-Lox recombination (from P1 bacteriophage)

LoxP site:

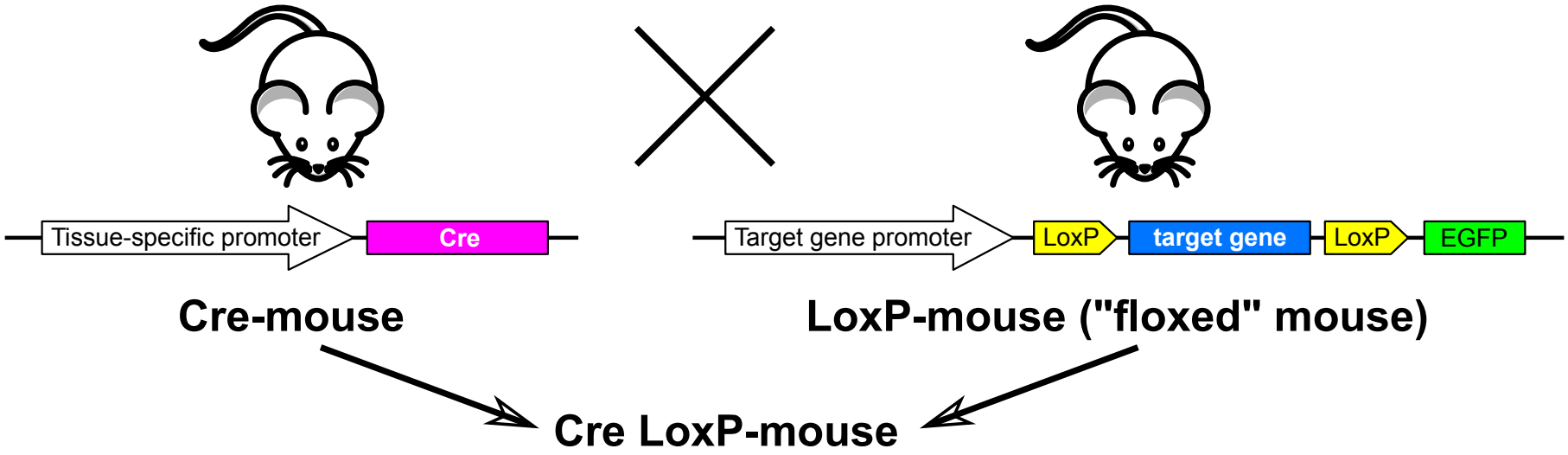
13 bp - 8 bp - 13 bp

ATAACTTCGTATA-NNNTANNN-TATACGAAGTTAT

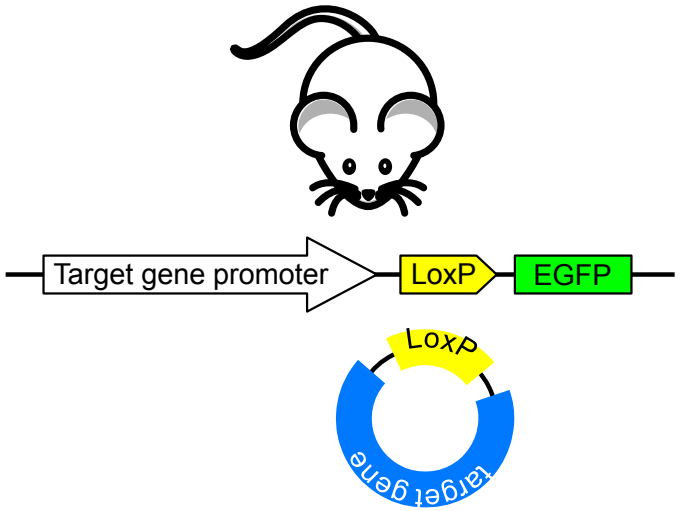
asymmetric 8 bp center



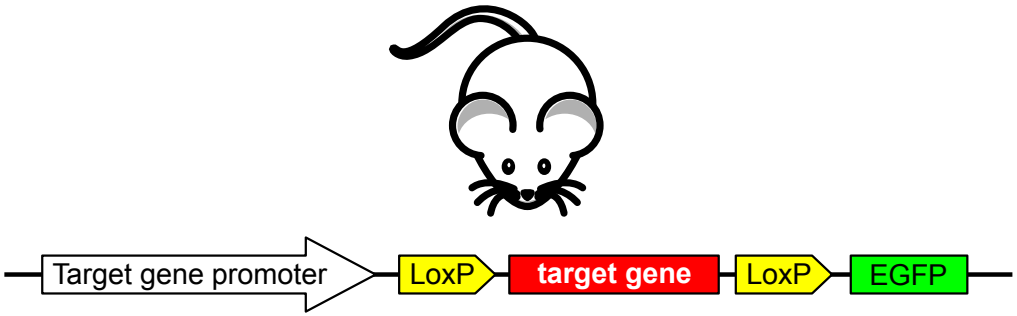
Conditional transgenes and knock-outs



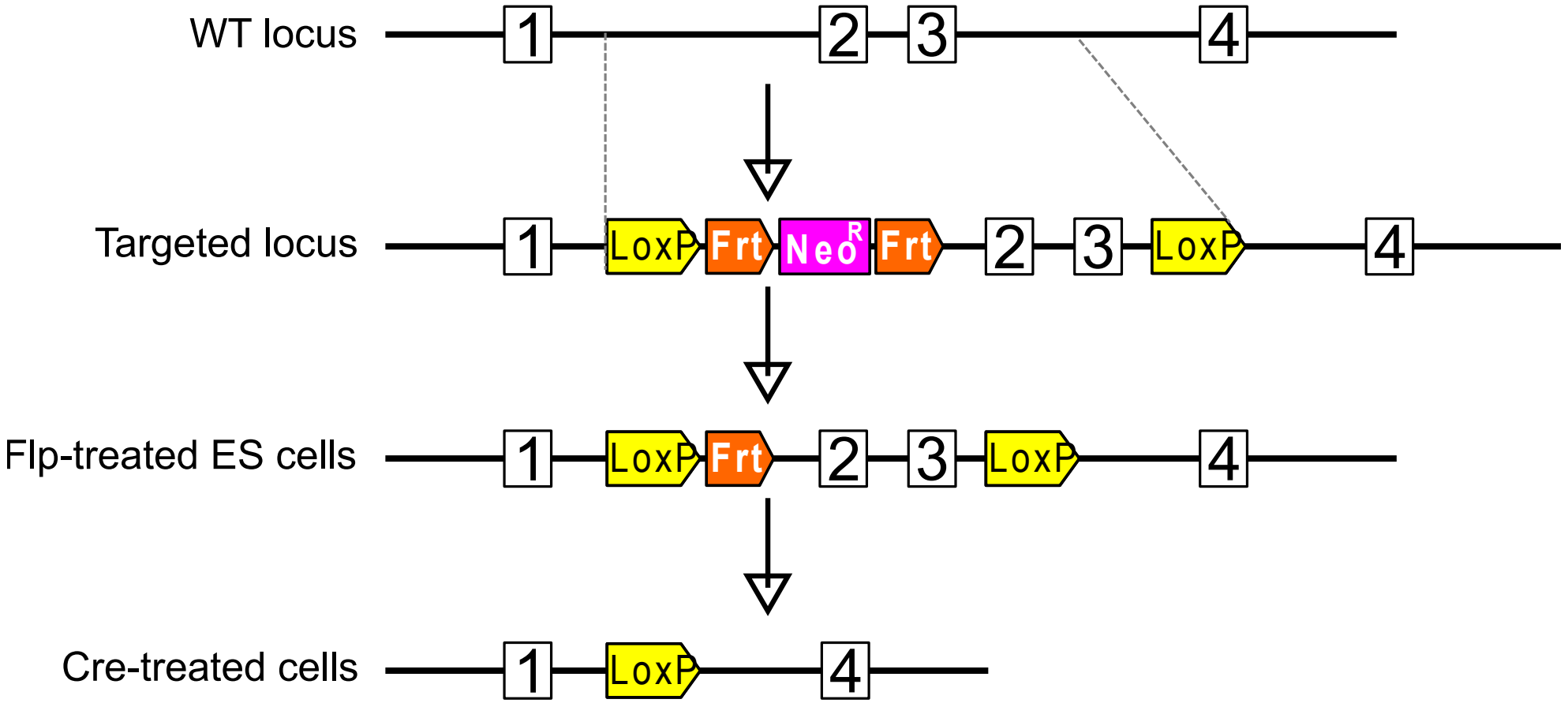
Tissue specific promoter active:
Target gene inactivation & EGFP activation



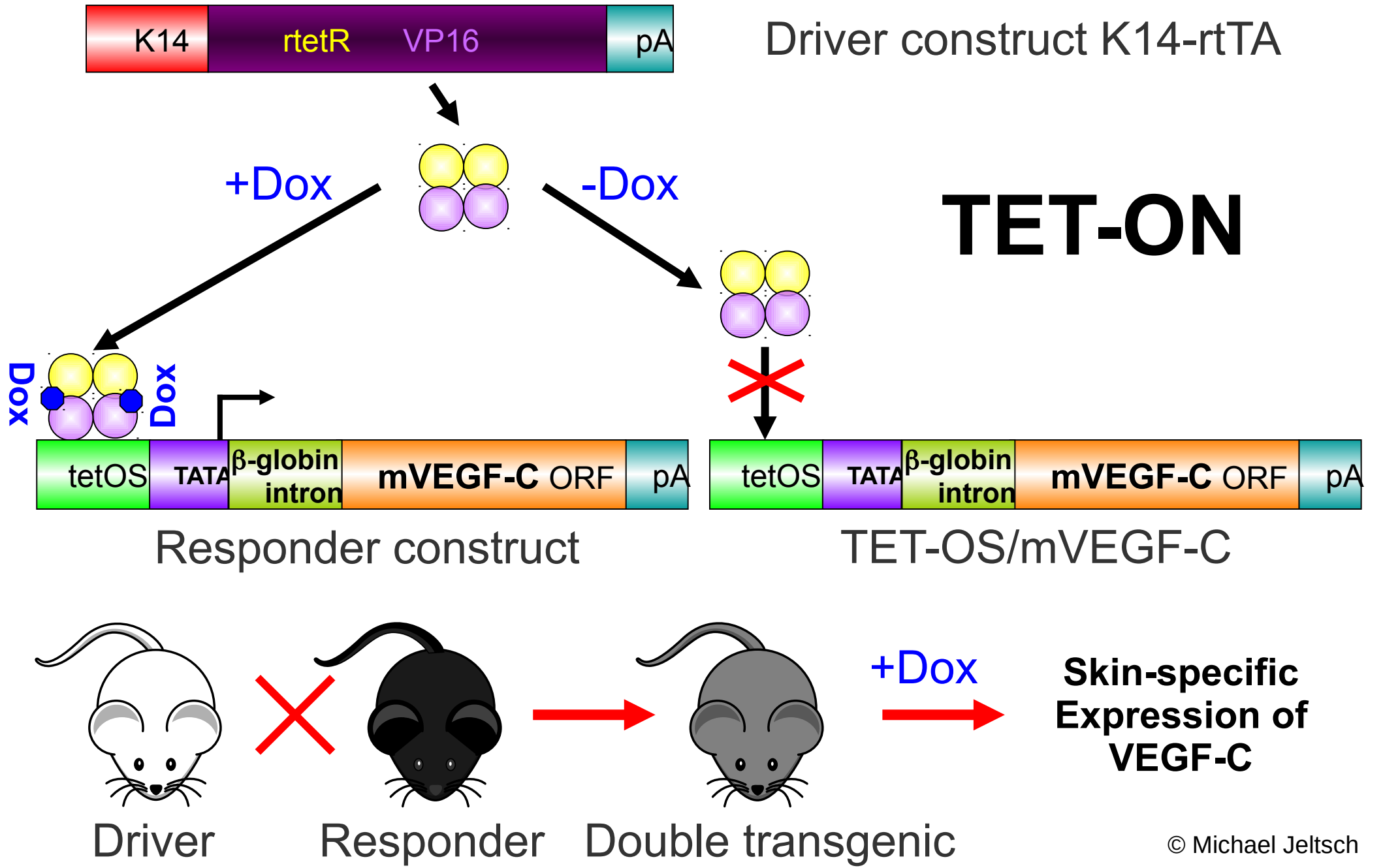
Tissue specific promoter inactive:
Target gene remains functional



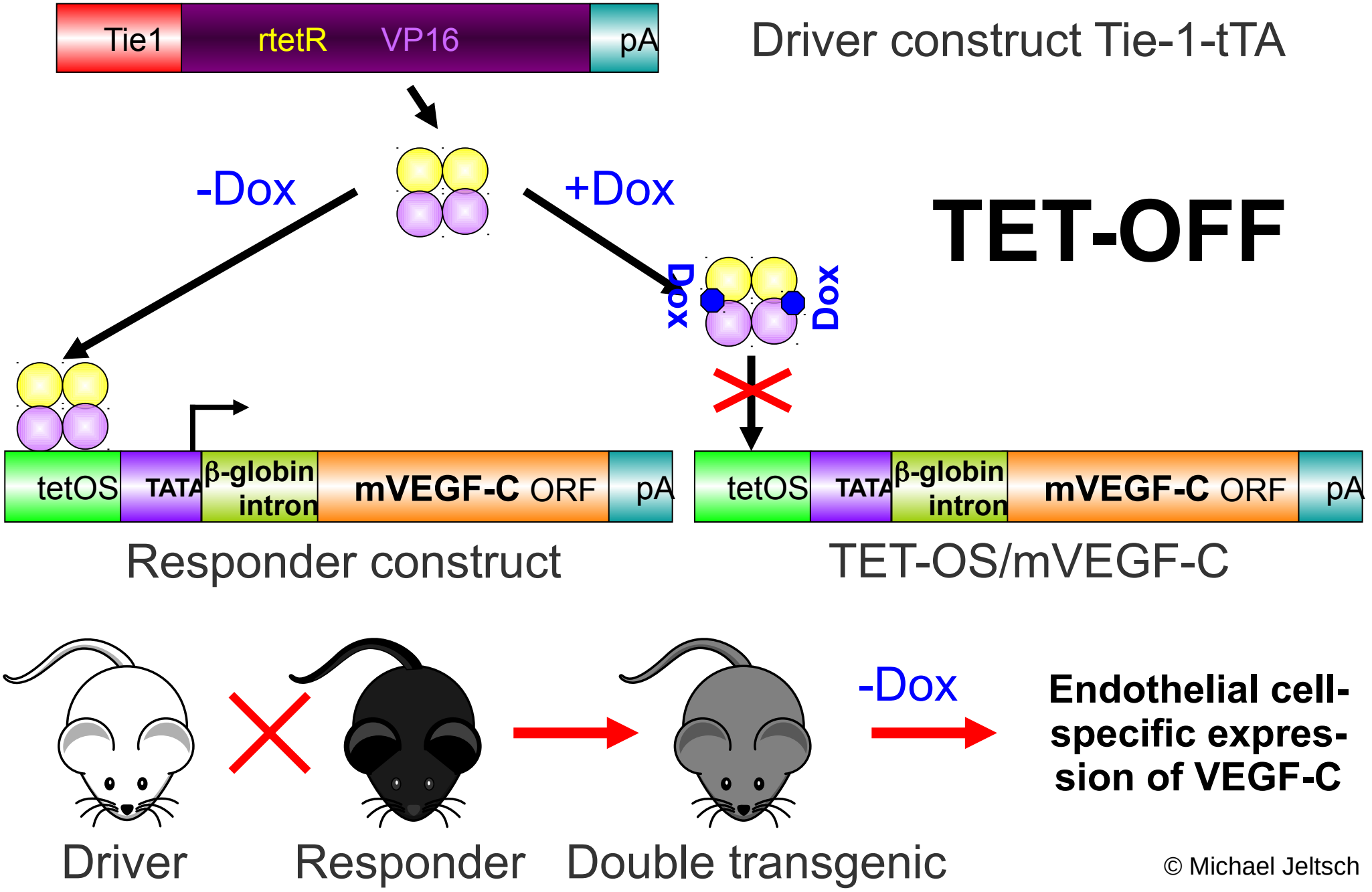
Conditional transgenes and knock-outs: FLP-FRT



Regulated expression: Tet-On



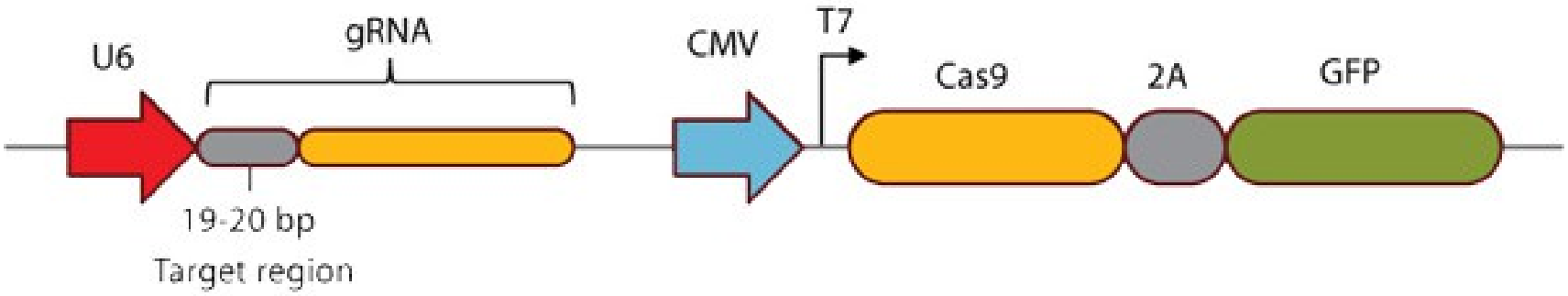
Regulated expression: Tet-Off



Gene Editing

- Technical literature older than 2 years is pretty useless because Crispr/Cas gene editing (e.g. in ES cells) is much simpler than all of the previously used methods.

CRISPR/Cas



CRISPR/Cas Single Plasmid Format

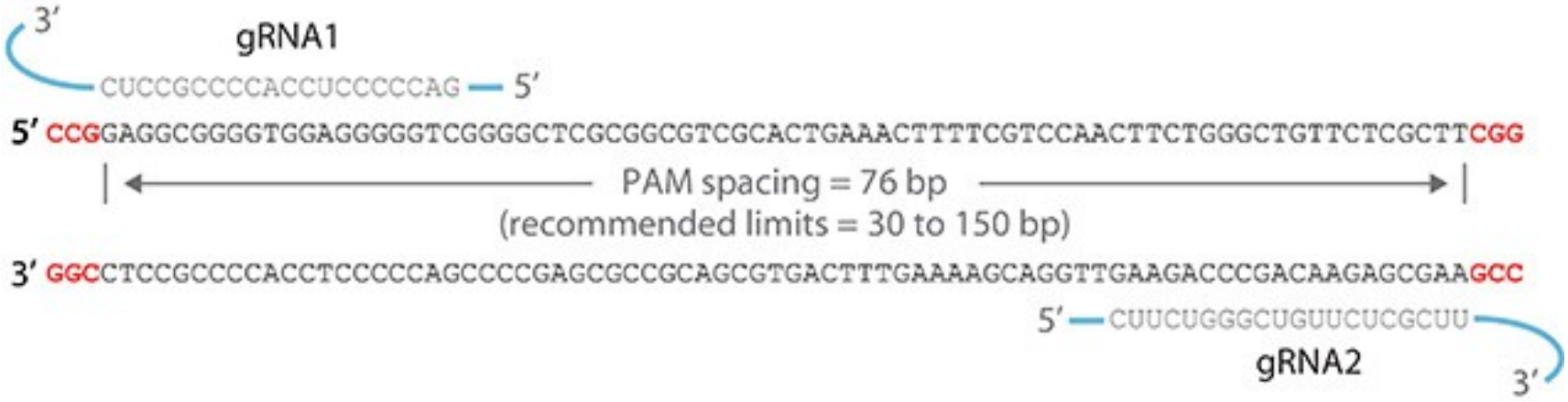


Figure by Sigma

The Cas9 nuclease is targeted to genomic DNA by an sgRNA containing a guide sequence

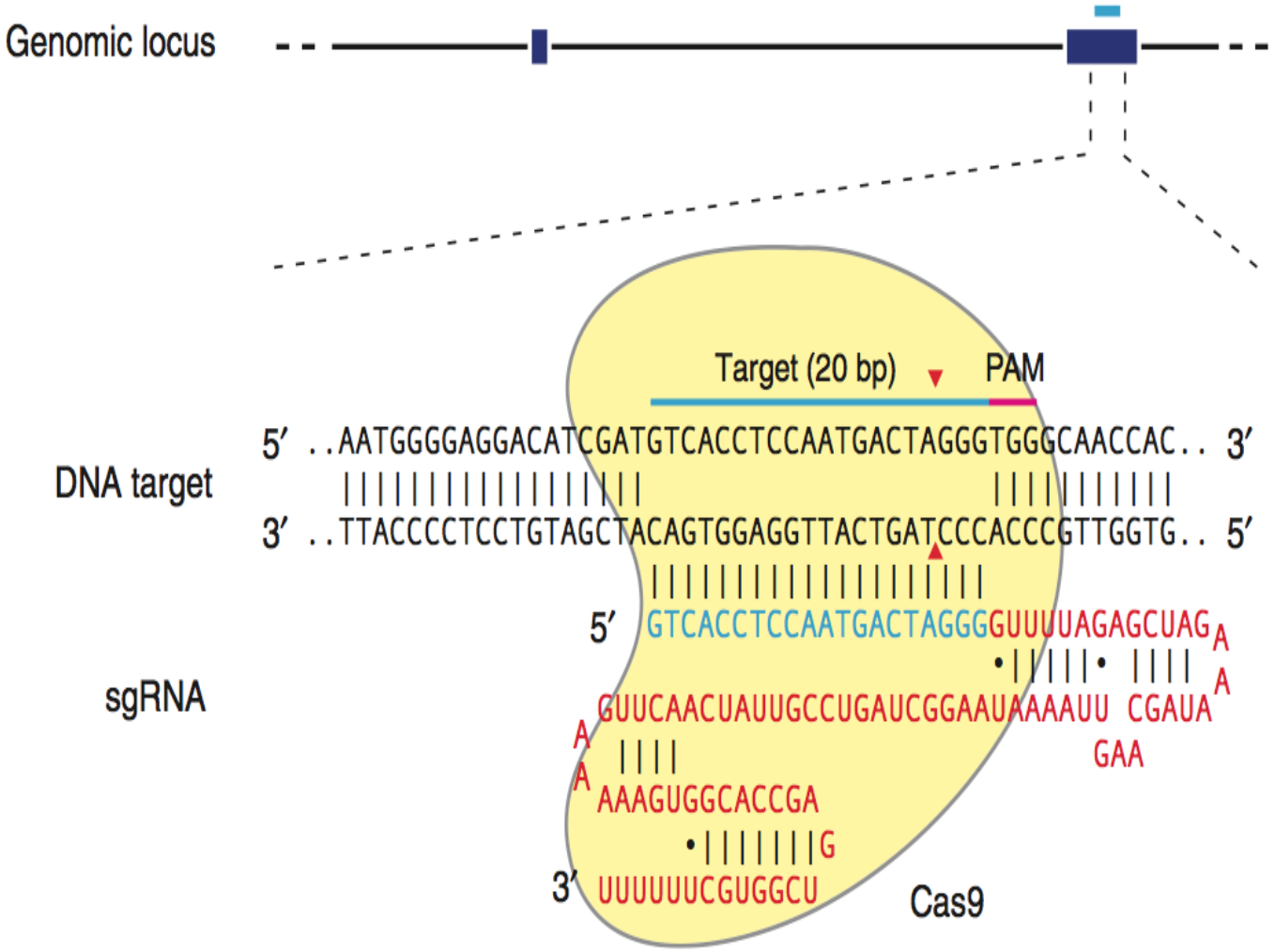


Image by: Ran et al. Nat Protocols (2013)

Cas9-mediated cleavage promotes gene knockout or precise gene editing

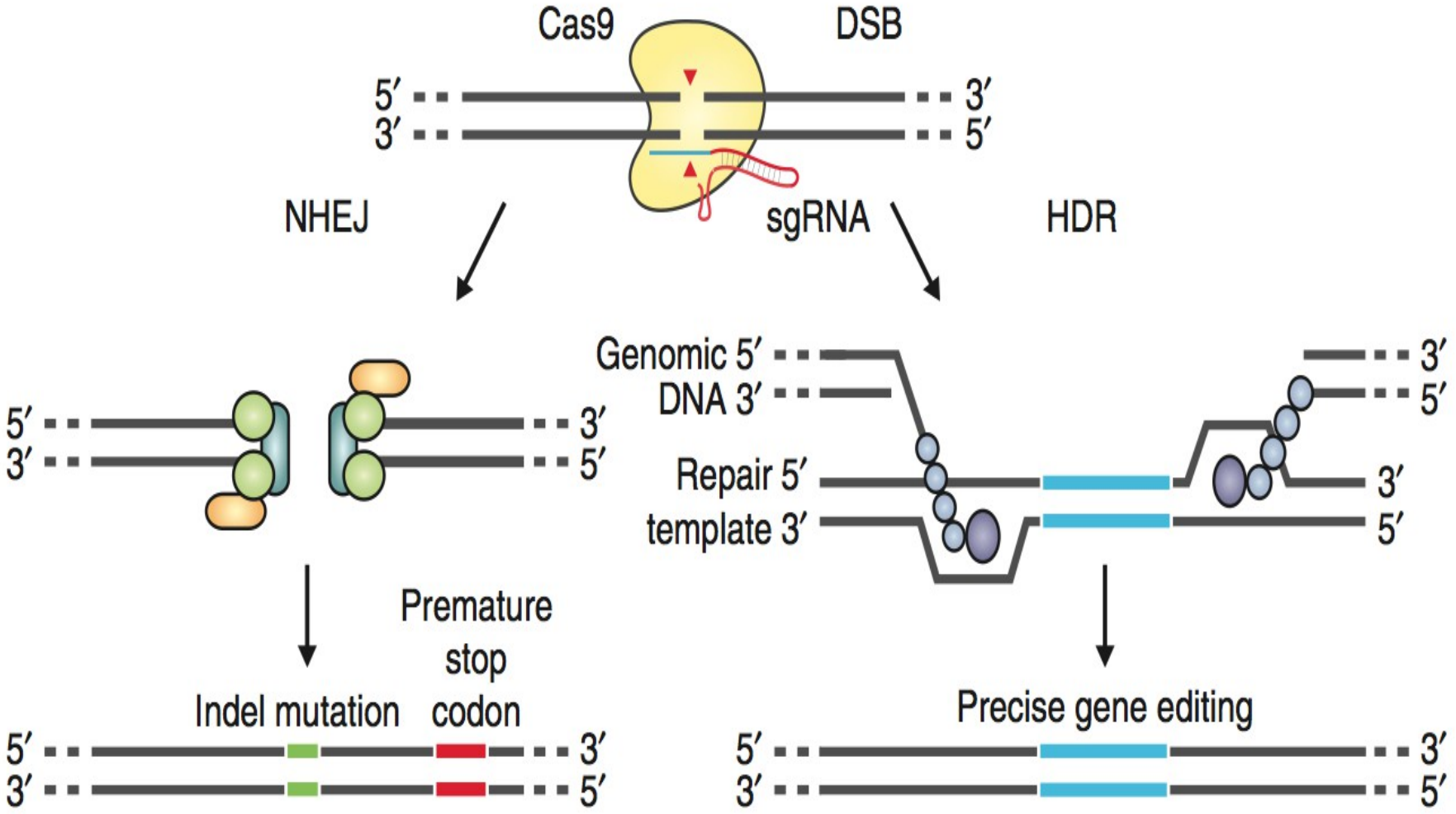
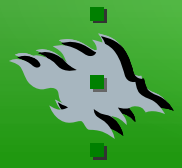


Image by: Ran et al. Nat Protocols (2013)



Next week: final seminar

- Is there a topic you want to be covered?
- No DNA ladder cloning suggestions?
- Topic: Viruses