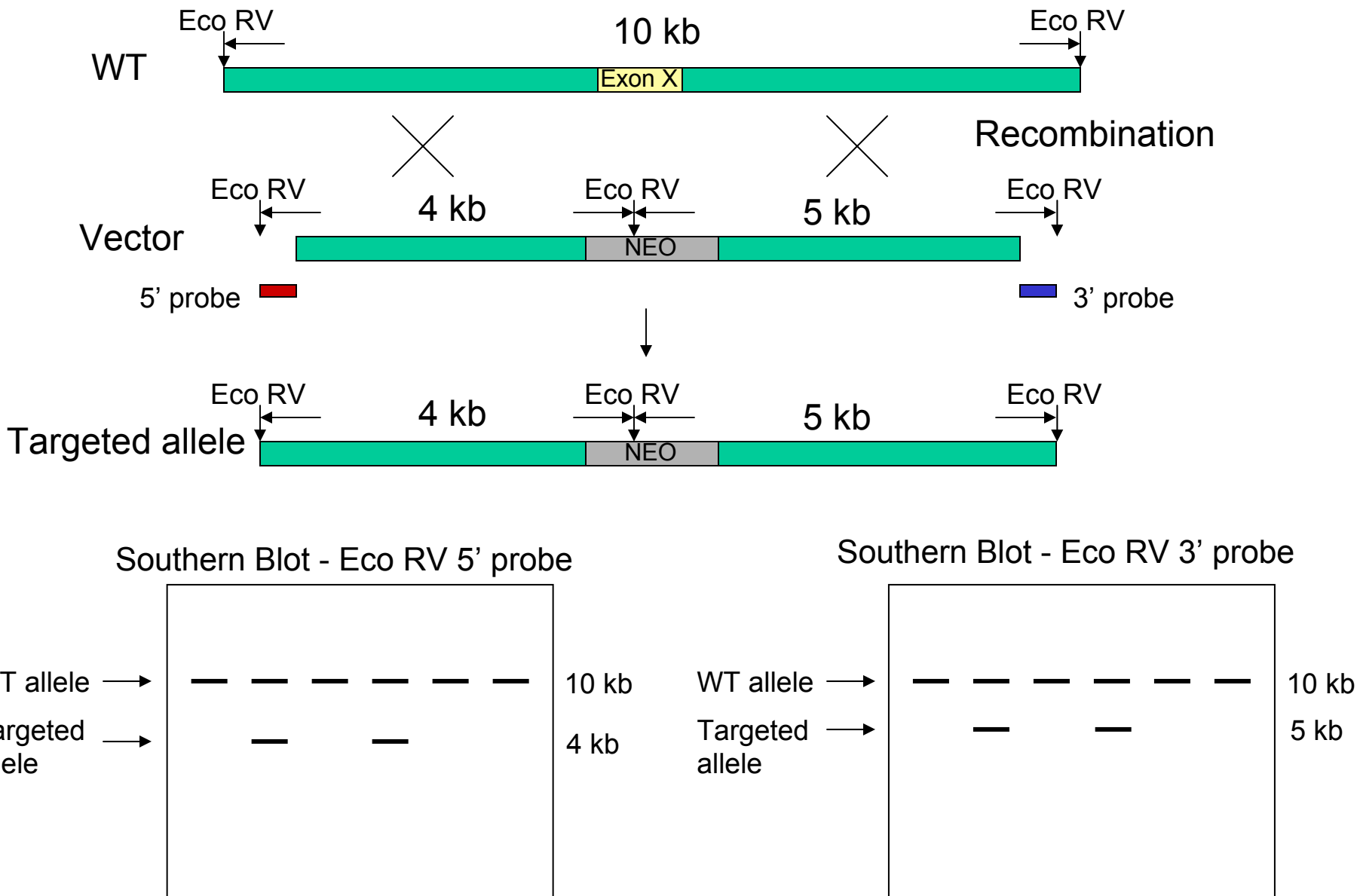


Gene targeting construct

Suggestions:

1. Use the same strain of DNA to generate the construct as that of the ES cells to ensure the highest frequency of recombination.
2. Each arm should be about 3 Kb. Shorter than 2 Kb might result in a reduced frequency of recombination.
3. It is important that the probe be specific for sequences outside of the targeting vector (non-overlapping with the recombination arms) so that it will detect homologous recombination at the correct endogenous site rather than random integration events.

Example



History: knockout (KO)

1981 Martin Evans and Matt Kaufman in Cambridge, U.K., isolate **mouse** embryonic stem cells, which can develop into the full range of tissues.

1987 Mario Capecchi's team at the University of Utah describes a method for making knockout mice, as does Oliver Smithies's group at the University of Wisconsin.

To Make a Knockout **Mouse**

Introduce a designer gene into **mouse** embryonic stem (ES) cells in culture.

↓
Screen ES cells and select those whose DNA includes the new gene.

↓
Implant selected ES cells into normal **mouse** embryos, making "chimeras" of mixed heritage.

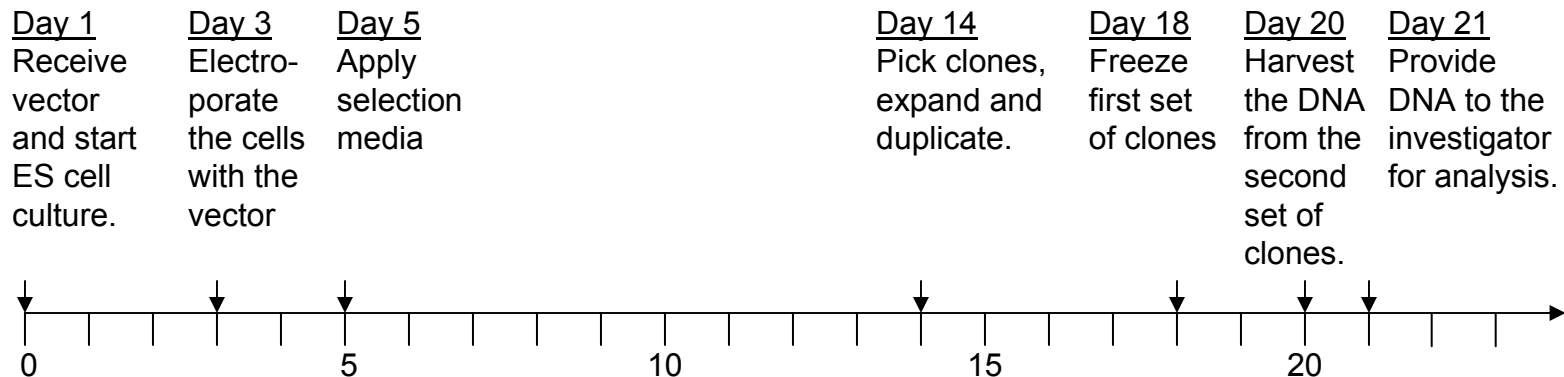
↓
Implant chimeric embryos in pseudopregnant females.

↓
Females give birth to chimeric offspring, which are bred to verify transmission of the new gene, producing a mutant **mouse** line.

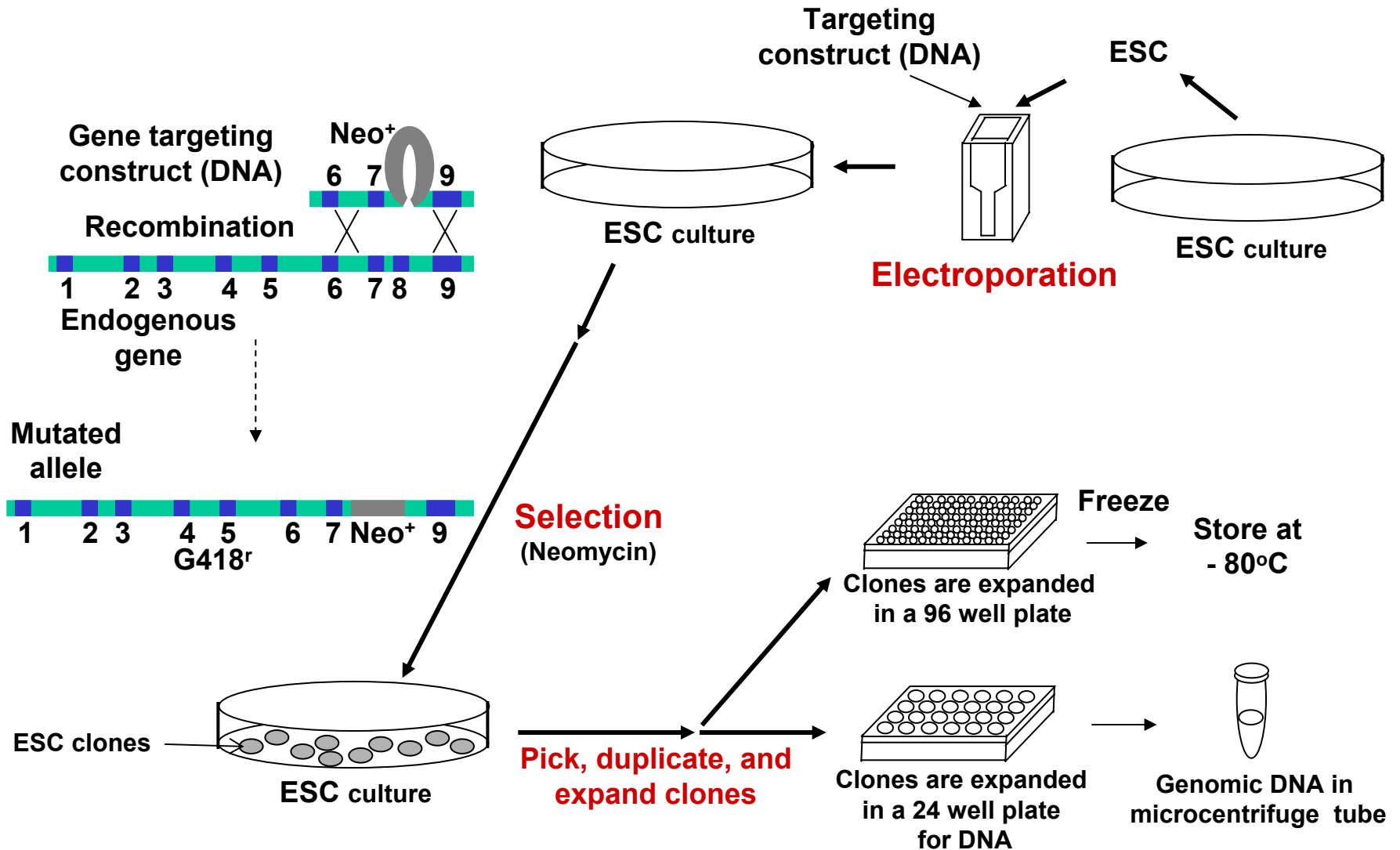
1991 Rudolf Jaenisch at MIT describe the first knockout **mouse** lacking the *b 2*-microglobulin-deficient mice, an instant sensation among researchers.

Gene targeting

- Prepare the gene targeting construct DNA for the ESCC. The investigator should provide about 100 μg of DNA (QIAGEN Maxi) of targeting construct (vector). This vector should be linearized and verified on a mini gel, precipitated in 70% ethanol, and sent to our facility.
- The ESCC will prepare ES and feeder cells, electroporate the ES cells with the investigator's vector, apply selection conditions to the cultures, and pick approximately 300 clones.
- The ESCC will then expand and duplicate clones; one set of clones will be frozen down, the other set will be used to isolate DNA samples for Southern blot or PCR screening by the investigator.
- The schedule is as follows:



Gene targeting in embryonic stem cells (ESC)



References

Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos.

Nature. 1981 Jul 9;292(5819):154-6.

Thomas KR, Capecchi MR. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells.

Cell. 1987 Nov 6;51(3):503-12.

Zijlstra M, Bix M, Simister NE, Loring JM, Raulet DH, Jaenisch R. Beta 2-microglobulin deficient mice lack CD4-8+ cytolytic T cells.

Nature. 1990 Apr 19;344(6268):742-6.

History: Cre-loxP system

1985 Brian Sauer's introduction of the Cre-loxP system for temporal control of transgenic gene expression draws little attention at San Francisco meeting (transgenic mice).

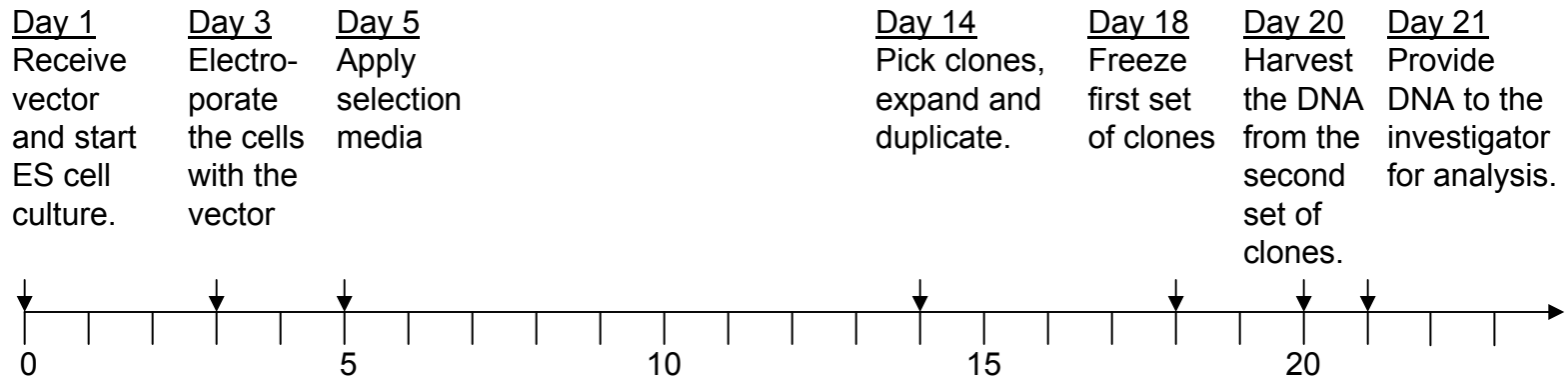
1981-1991 knockout mice are established.

1995 K Rajewsky published the [Science](#) article “Inducible gene targeting in mice”(Cre-loxP, conditional knockout).

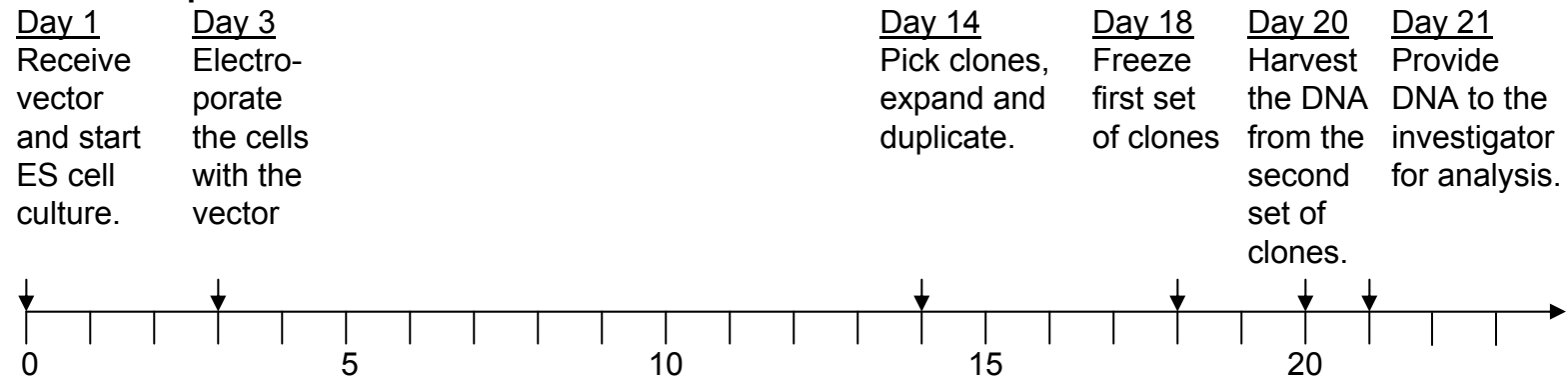
Gene targeting using Cre-loxP system

- The first step is the same as regular gene targeting (see Gene targeting).
- The second step is to electroporate the targeted ES cells from the investigator with the Cre vector and pick approximately 100 clones.
- The ESCC will then expand and duplicate clones; one set of clones will be frozen down, the other set will be used to isolate DNA samples for Southern blot or PCR screening by the investigator.
- The schedule is as follows:

First step



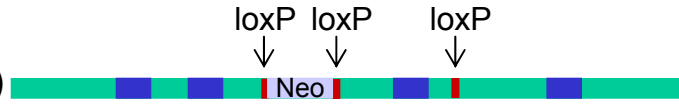
Second step



Gene targeting in embryonic stem cells (ESC) using Cre-loxP system

**First step
(Gene targeting)**

**Gene targeting
construct (DNA)**



Recombination

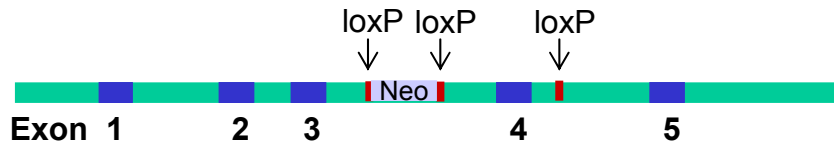


**Endogenous
gene**

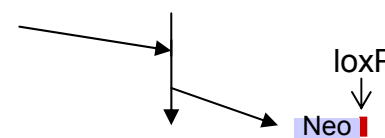


**Second step
(Cre expression
and Neo excision)**

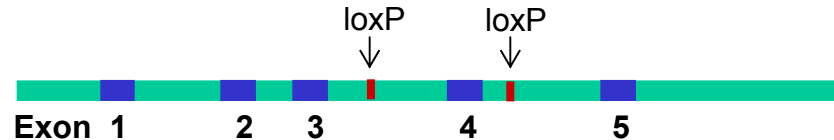
**Modified
allele**



Cre



**loxP
allele**



References

Sauer B. Functional expression of the cre-lox site-specific recombination system in the yeast *Saccharomyces cerevisiae*. Mol Cell Biol. 1987 Jun;7(6):2087-96.

Kuhn R, Schwenk F, Aguet M, Rajewsky K. Inducible gene targeting in mice. Science. 1995 Sep 8;269(5229):1427-9