Gene editing: From biblical times to the present

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Historical milestones towards gene editing I

- ~4000 years ago: Breeding of domestic animals
  Jacob (Genesis 30, 43)
- 1866 Inheritance of specific traits and their segregation in germ cells
  Gregor Mendel

- 1870-1902 Discovery of Chromosomes
  Walter Flemming, ***Eduard van Beneden***, ***Walter S. Sutton*** and others
- 1900: Rediscovery of Mendel's laws of inheritance
  Hugo de Vries and ***Karl Ehrich Correns***
- Gene mutations as drivers of evolution
  Hugo De Vries (plants); **T.H. Morgan, J. Muller** (flies)

Historical milestones towards gene editing II

- 1944 DNA as the carrier of genetic information
  Oswald T. Avery, Colin MacLeod & Maclyn McCarty

- 1953 The DNA double helix ➔ Duplication of DNA
  James D. Watson & Francis Crick

- 1961-1968: The genetic triplet code and its translation into the amino acid sequence of proteins (the "Central Dogma"). Control of gene expression.
- Since 1972: Recombinant DNA technology, mapping and sequencing of genes and genomes. Transgenesis.
- 1984-2003: The Human Genome Project

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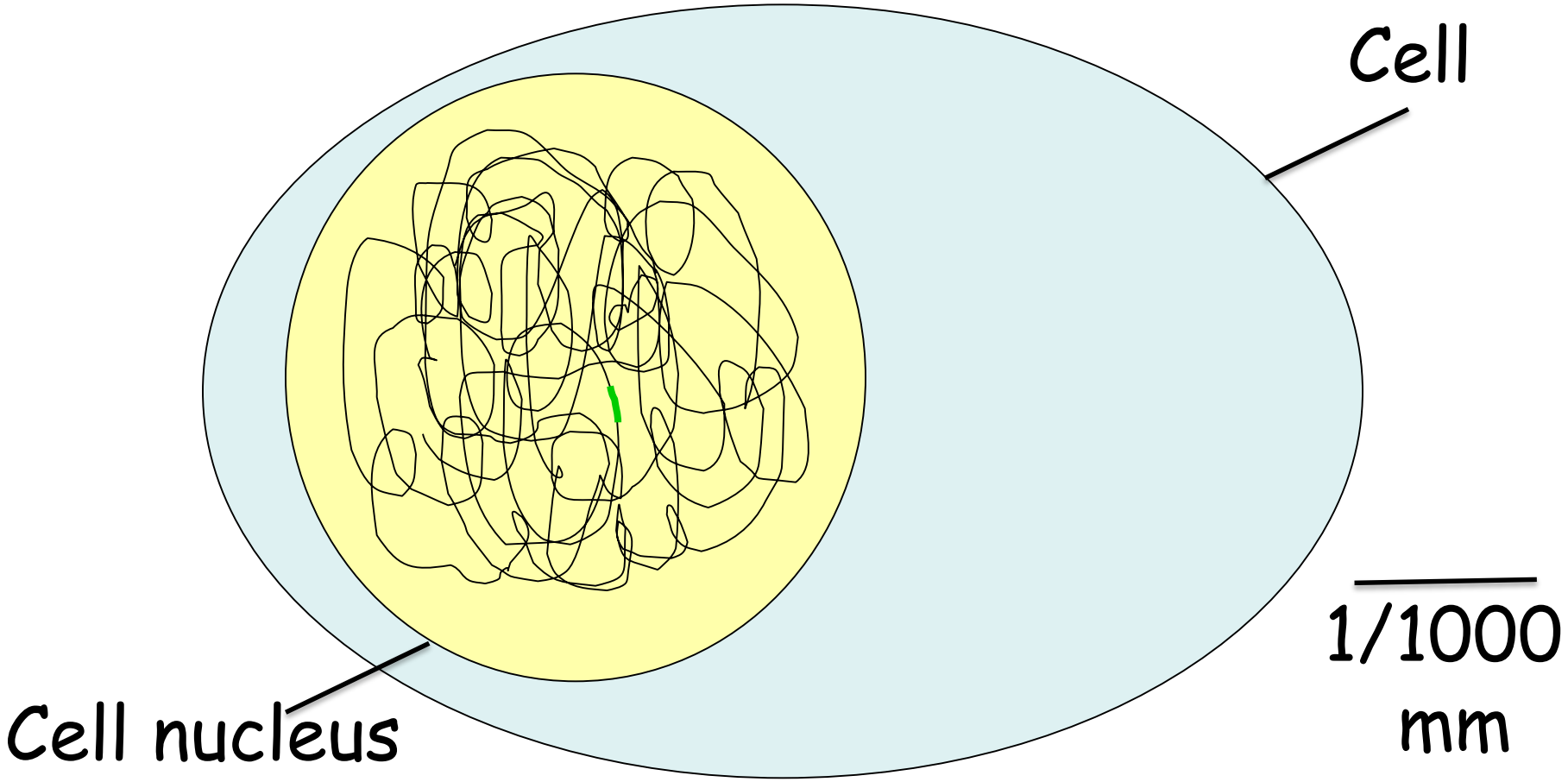
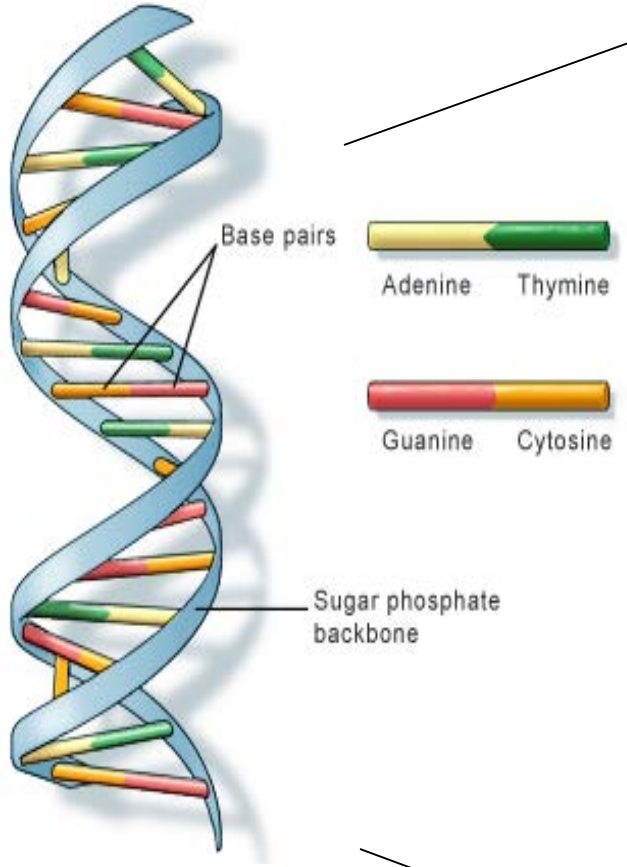
-> Gregor Mendel's "cell elements" now understood at the molecular level!

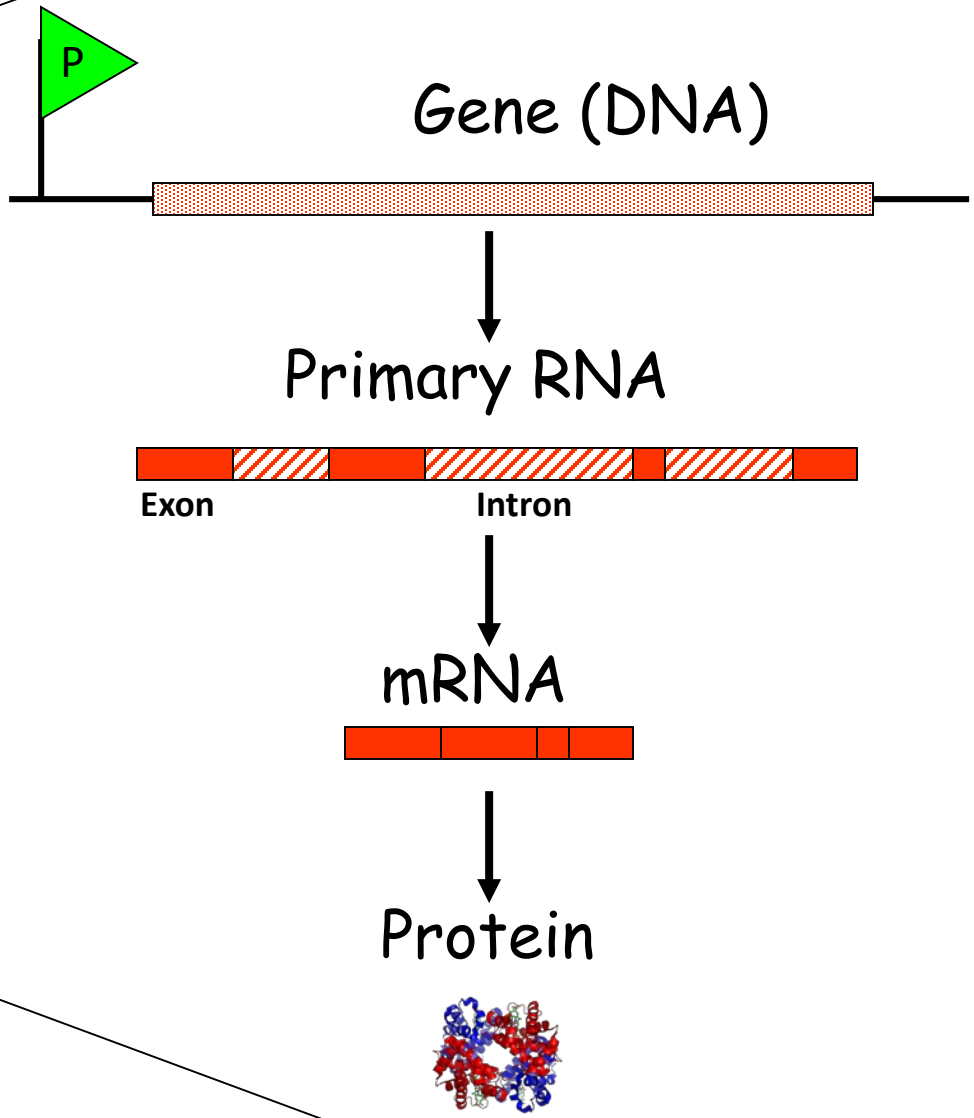
The human genome:
A 2-meter DNA filament organized into chromosomes, with ~25,000 genes



The Central Dogma



The DNA Double Helix



Genes and gene expression

- Each gene encodes a particular protein.
- Each cell in our organism contains the complete genome, but different cell types express different patterns of genes.

➔ The function of cells depends on an intact pattern of protein expression.

Mutations:

- Change the base sequence of genes
- Disturb protein function
- Occur spontaneously or
- Are caused by environmental cues

Most mutations are repaired by the cell!

Insufficient repair➔

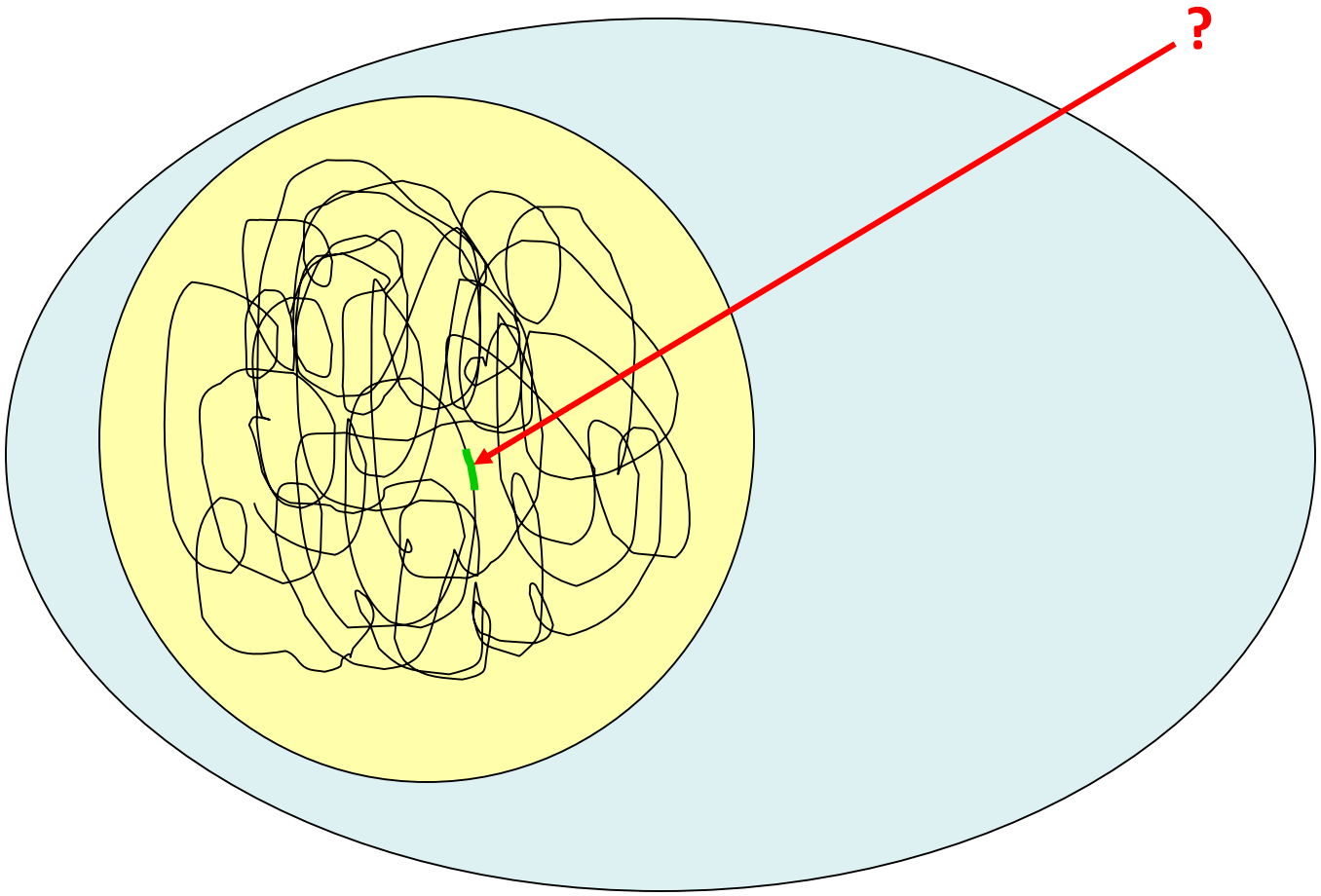
Cell damage, cancer, inherited diseases

Inherited diseases

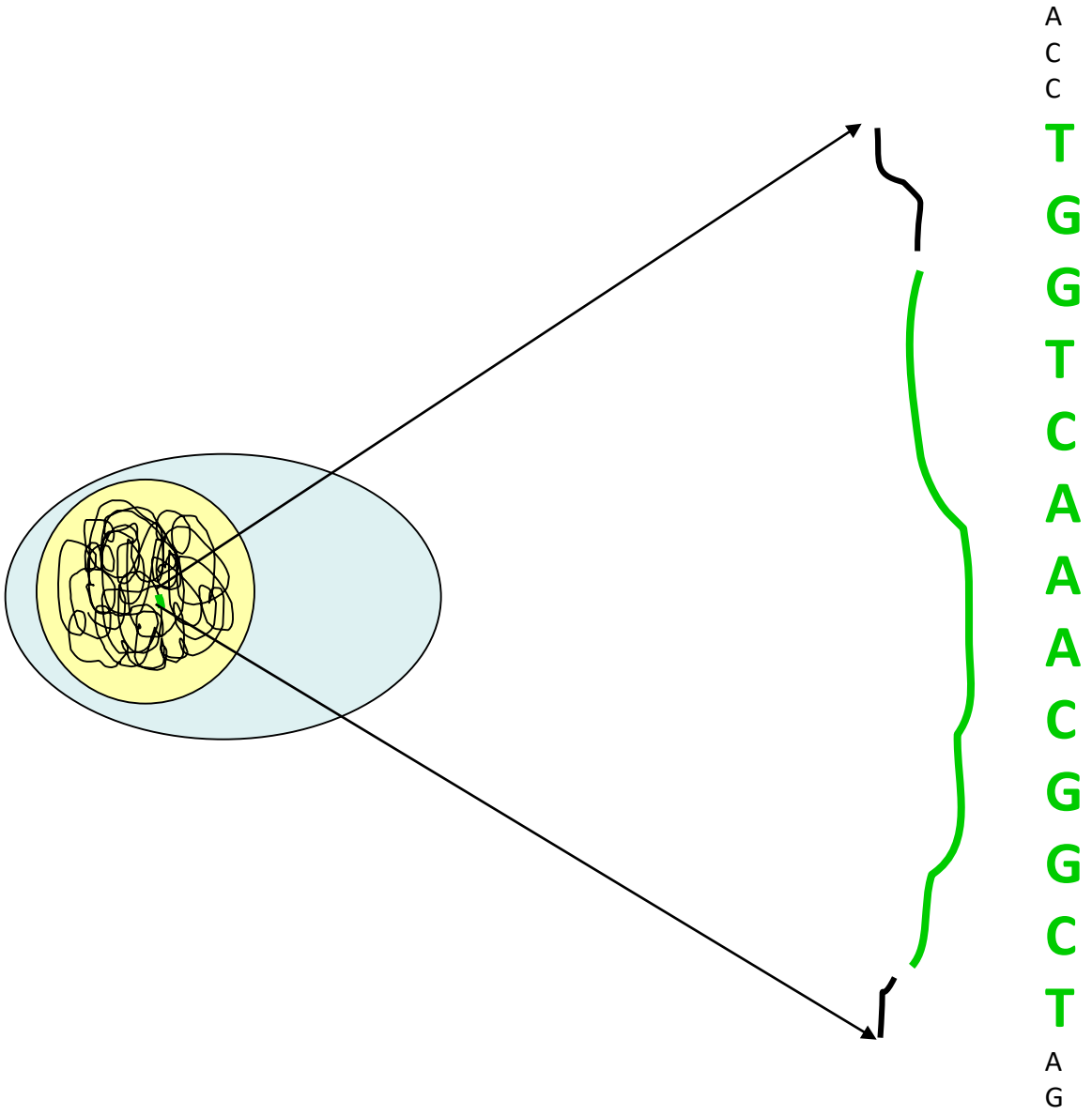
Caused by gene mutations,
transmitted through the germ line from generation to generation.
Inherited diseases can be mono- or polygenic.

How can we intentionally mutate or repair genes in the genome?

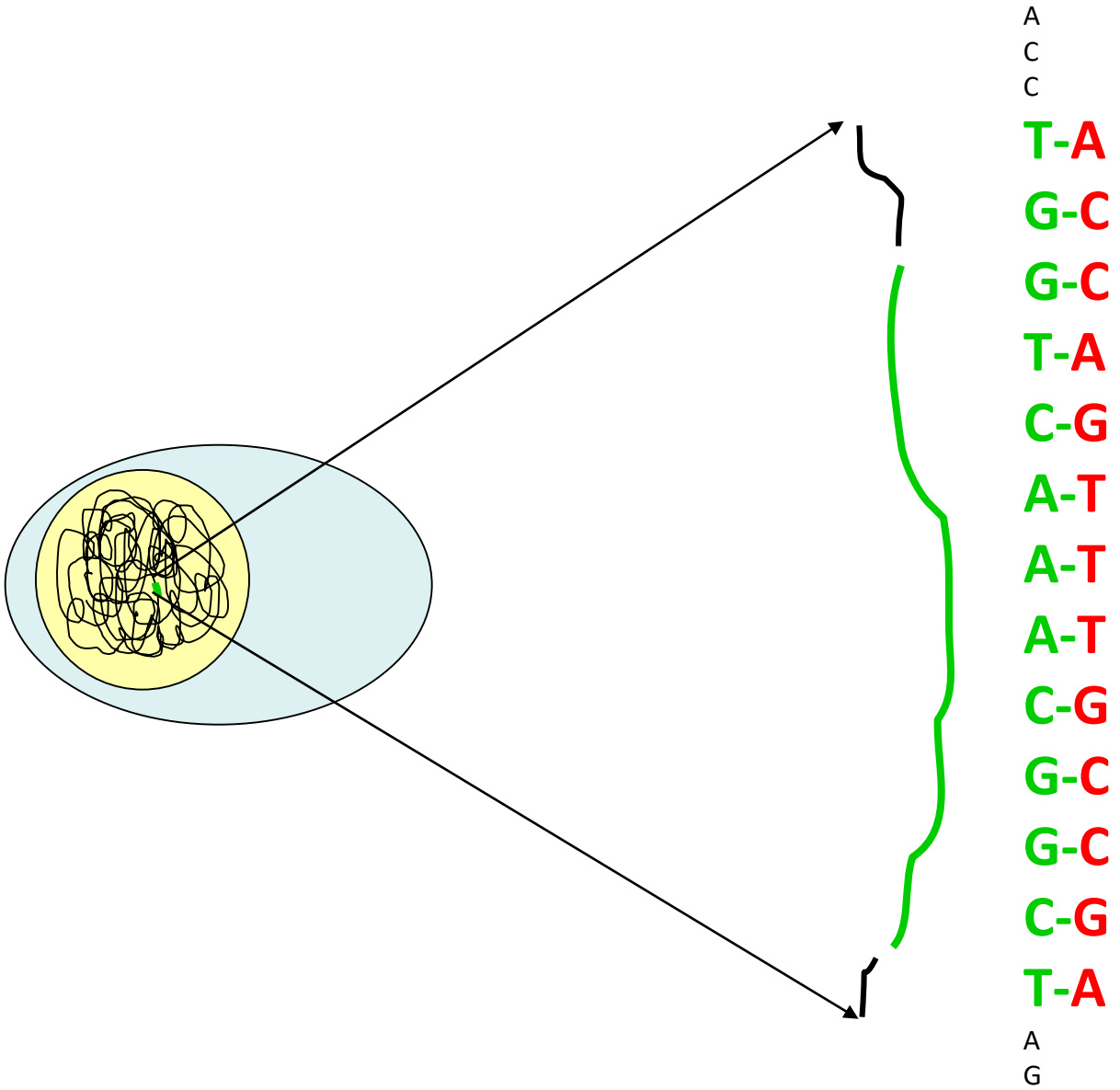
How to "find" a gene?



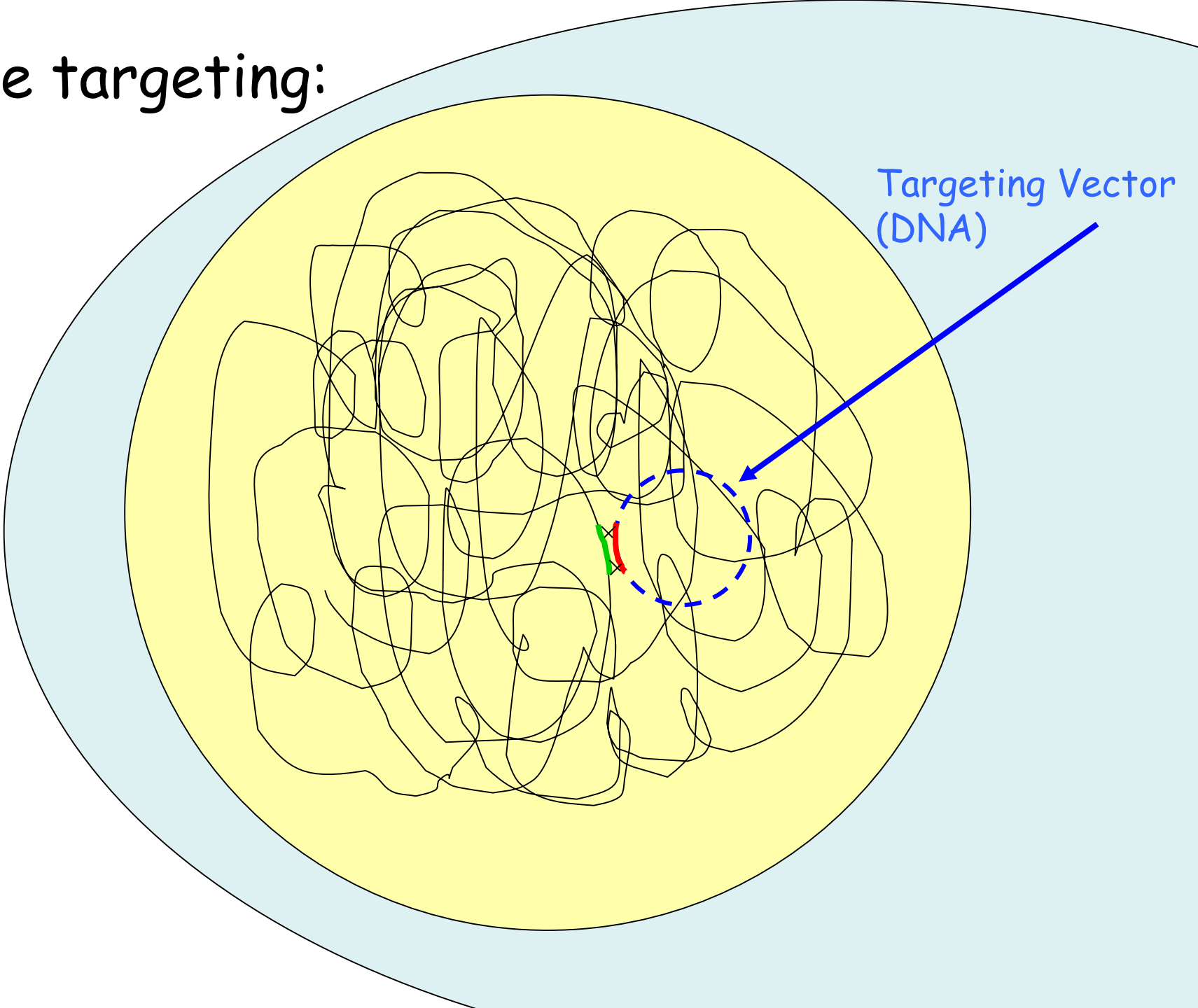
If one knows the base sequence…



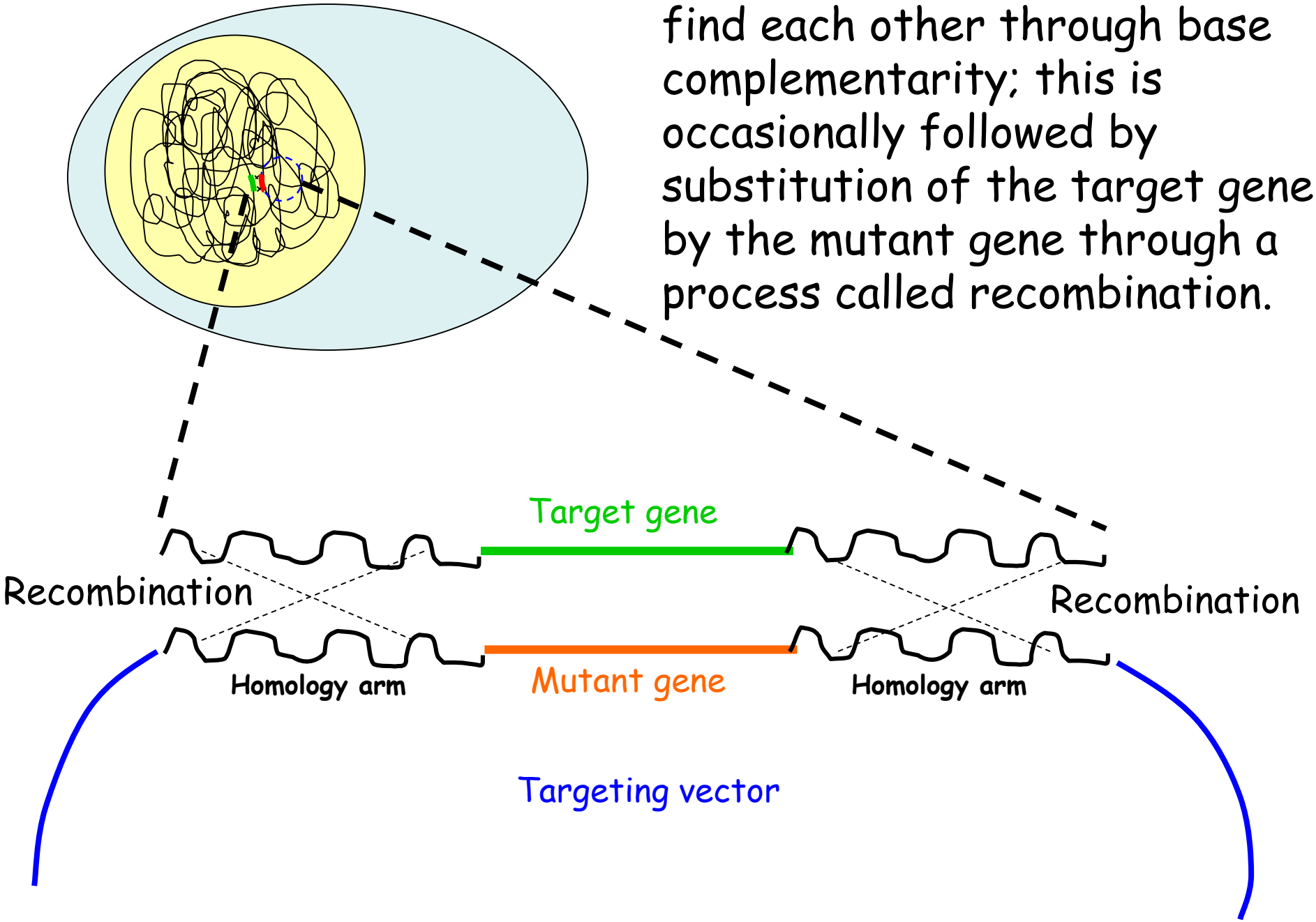
…through a DNA of complementary base sequence!



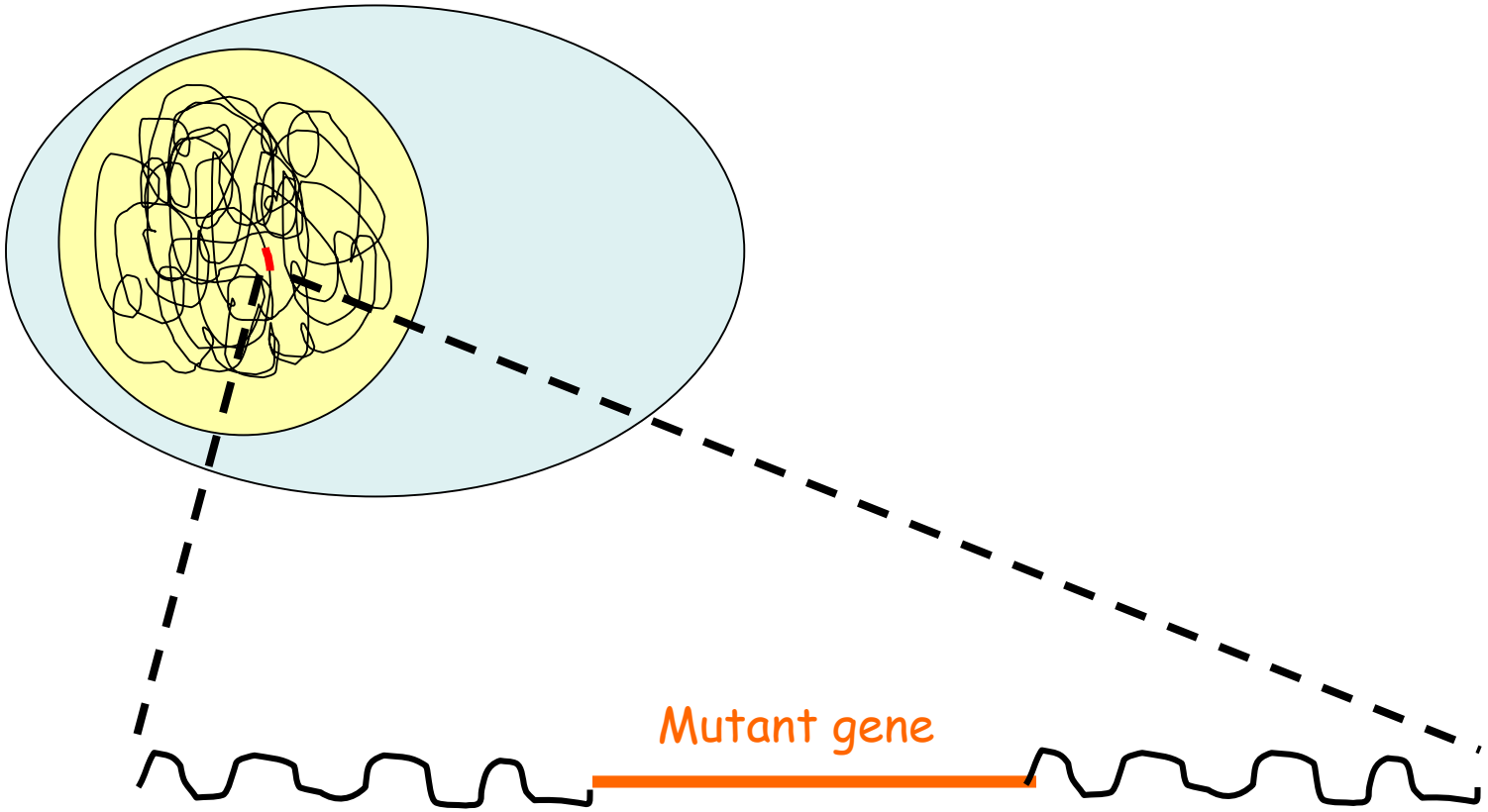
Gene targeting:



Target gene and mutant gene find each other through base complementarity; this is occasionally followed by substitution of the target gene by the mutant gene through a process called recombination.

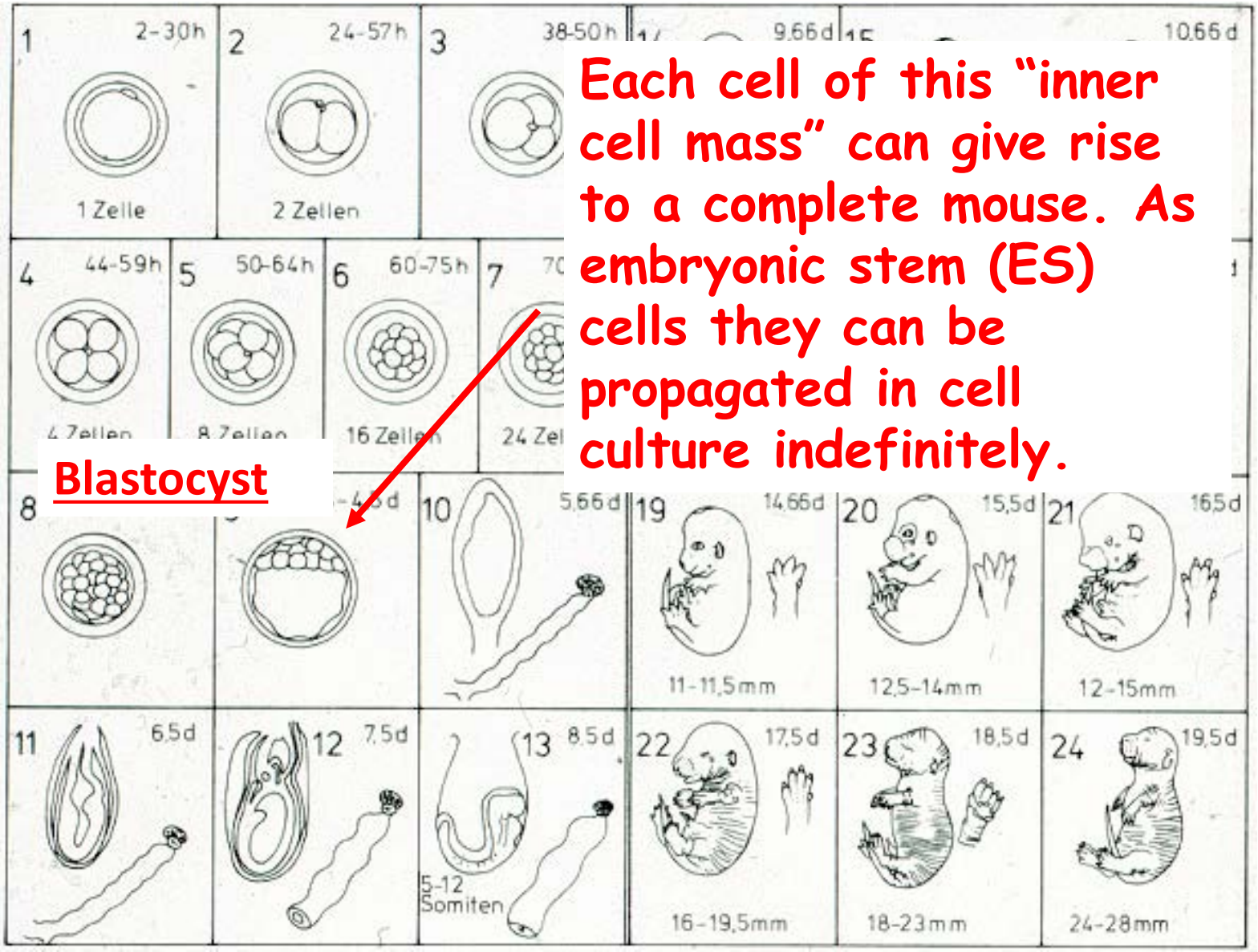


Gene substitution by a mutant gene



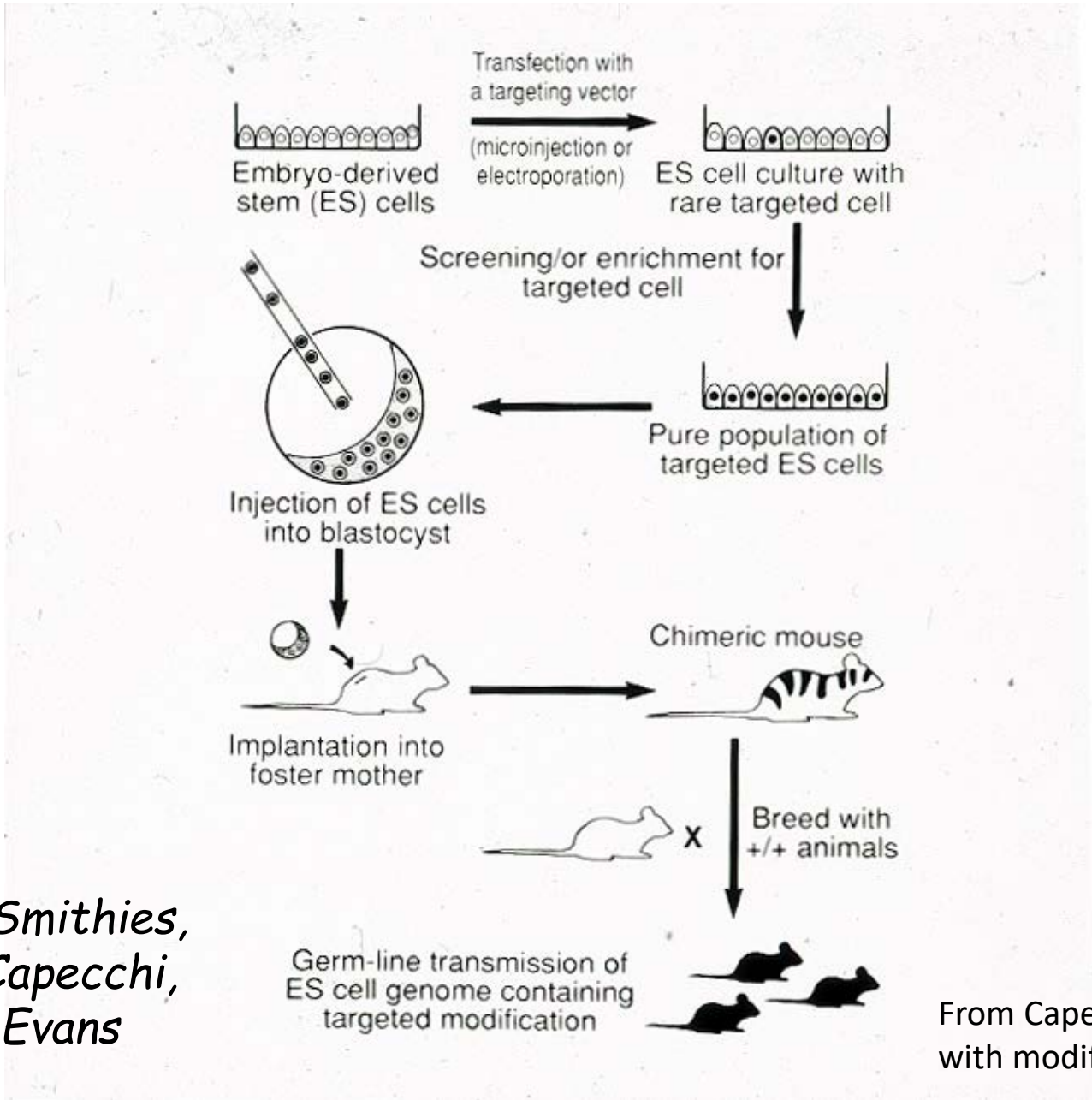
Classical gene targeting in mouse embryonic stem (ES) cells

Mouse embryonic development and embryonic stem (ES) cells



Each cell of this "inner cell mass" can give rise to a complete mouse. As embryonic stem (ES) cells they can be propagated in cell culture indefinitely.

Classical gene targeting in the mouse

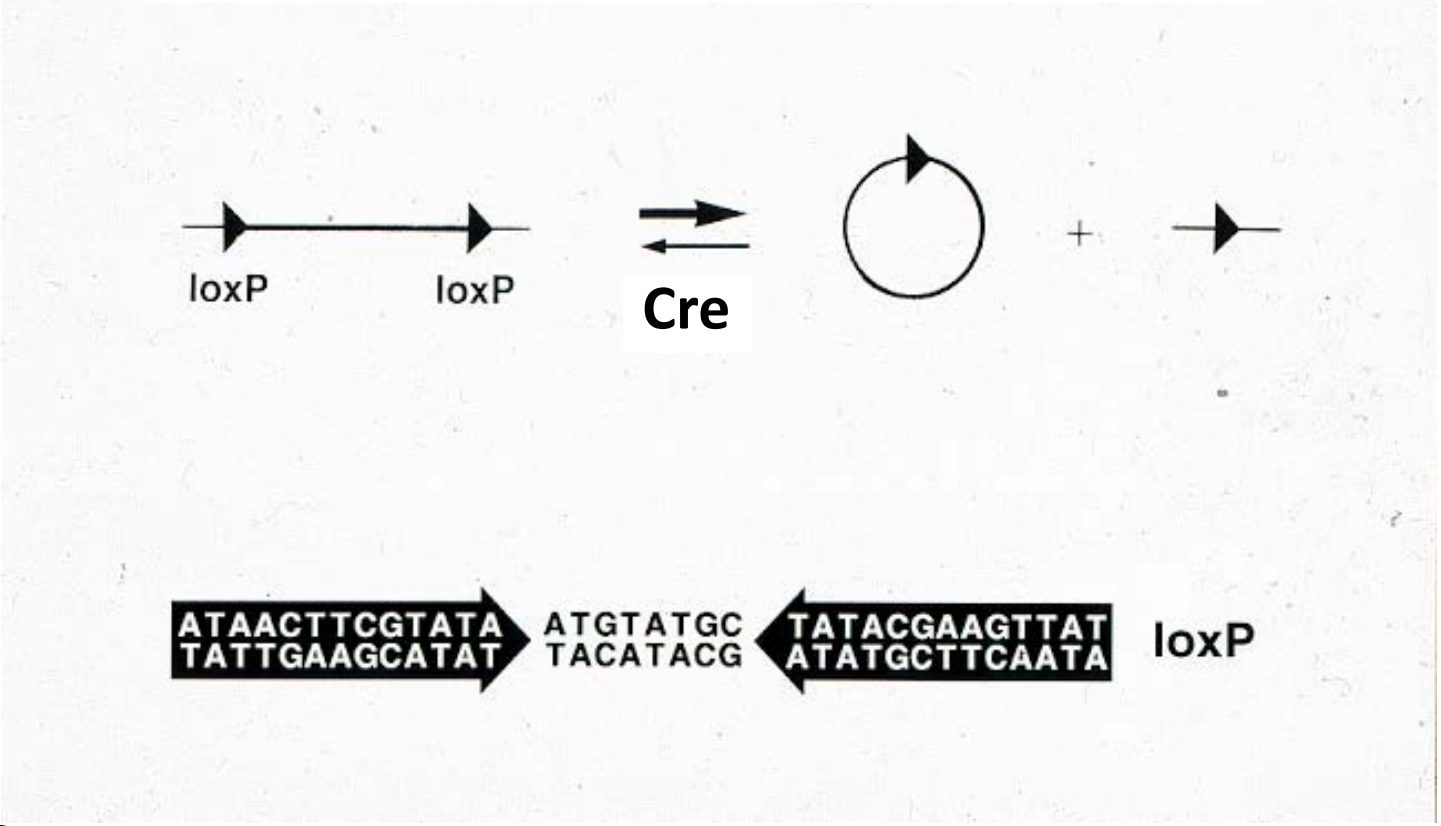


Oliver Smithies,
Mario Capecchi,
Martin Evans

From Capecchi M.1994, with modifications

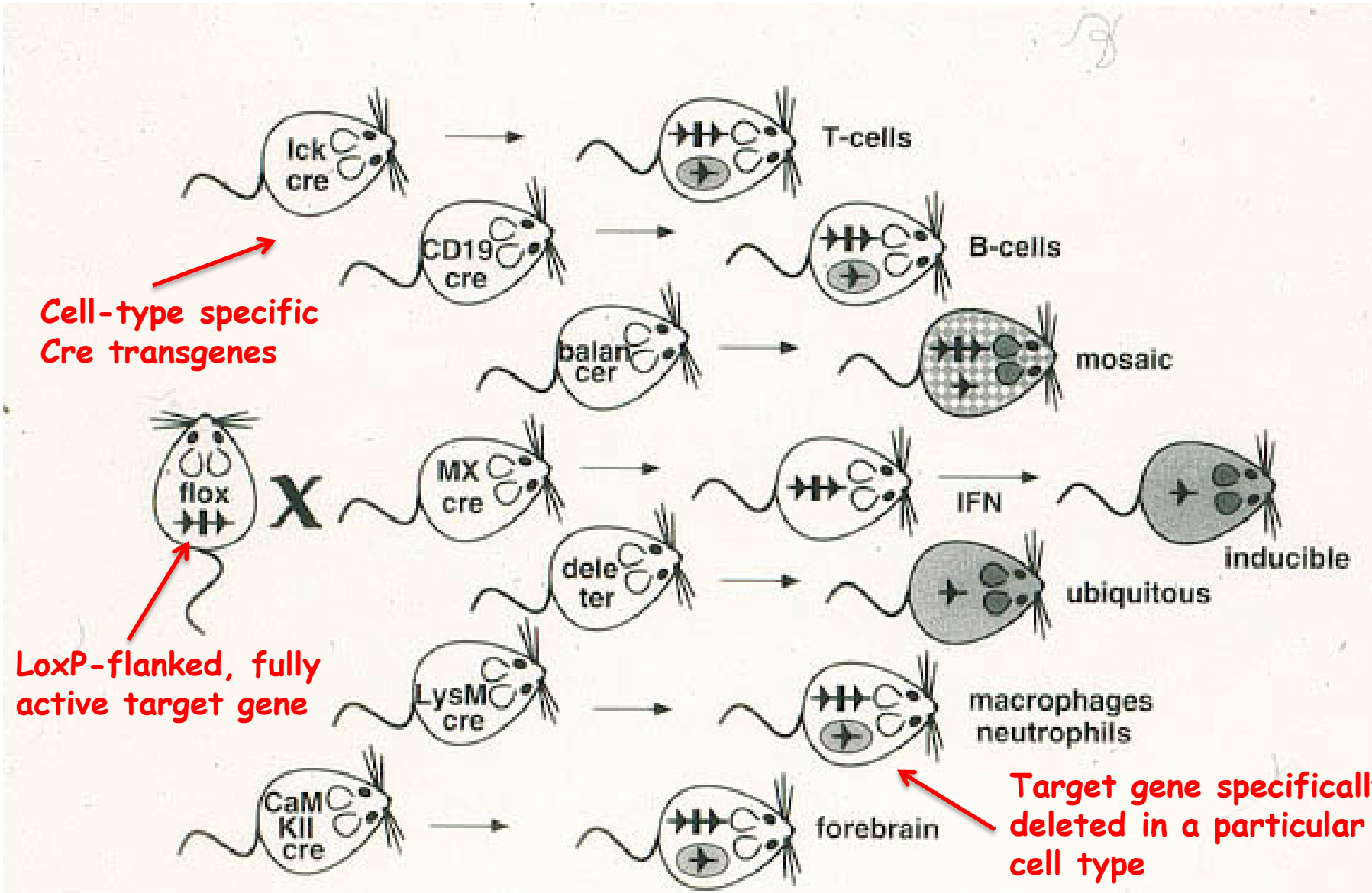
Recombinase-assisted targeted mutagenesis
Conditional gene targeting

Cre is a bacteriophage-derived enzyme which binds paired DNA target sequences called loxP and excises the DNA between them – also in mammalian cells



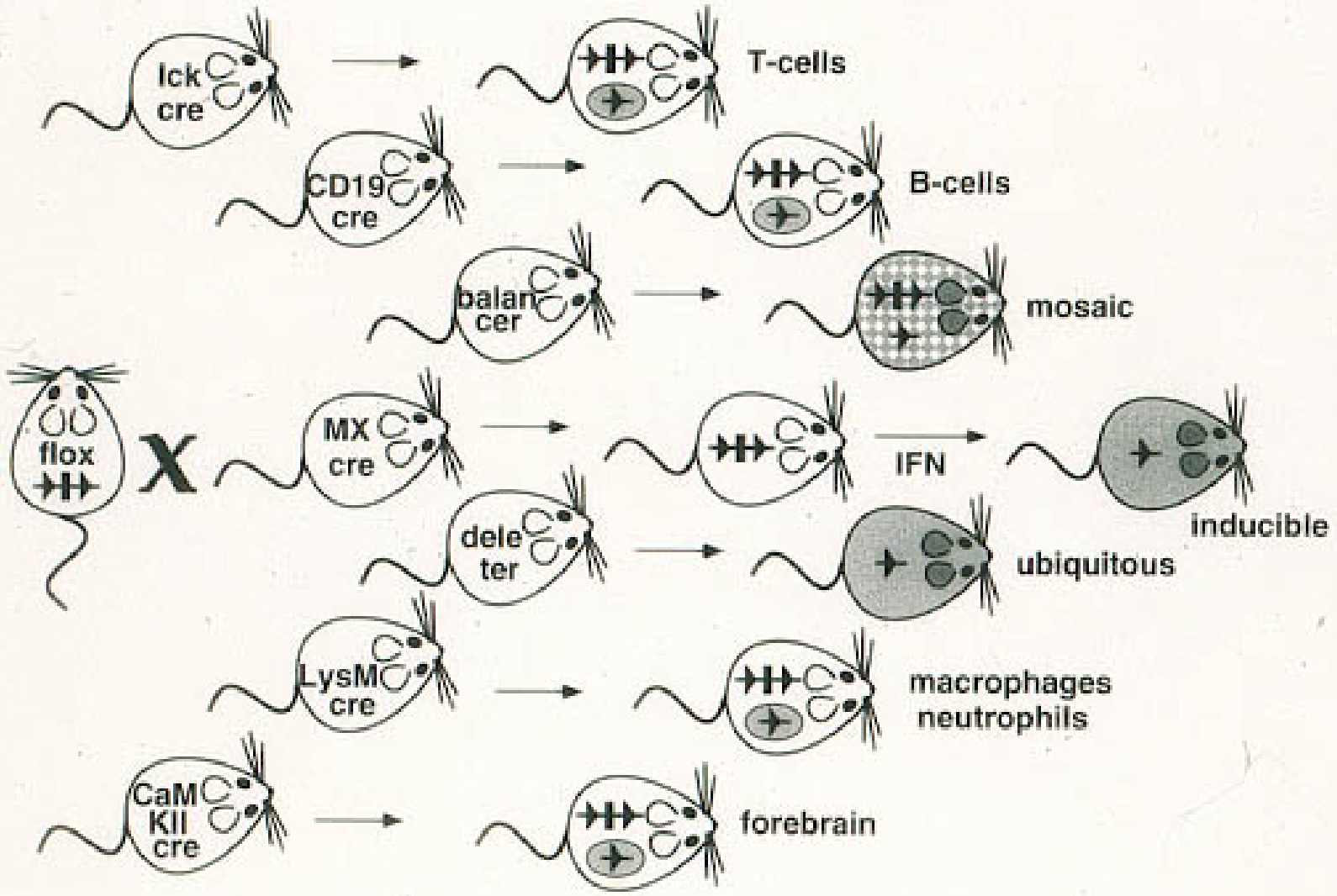
Brian Sauer,
Heiner Westphal,
Jamey Marth

Conditional gene targeting: The Cre Zoo 1996



From Rajewsky, K. et al. 1996, with modifications

Conditional gene targeting: The Cre Zoo 1996



Allows gene editing in somatic cells in vivo

Classical gene targeting is a very inefficient process because of the low frequency of spontaneous recombination.

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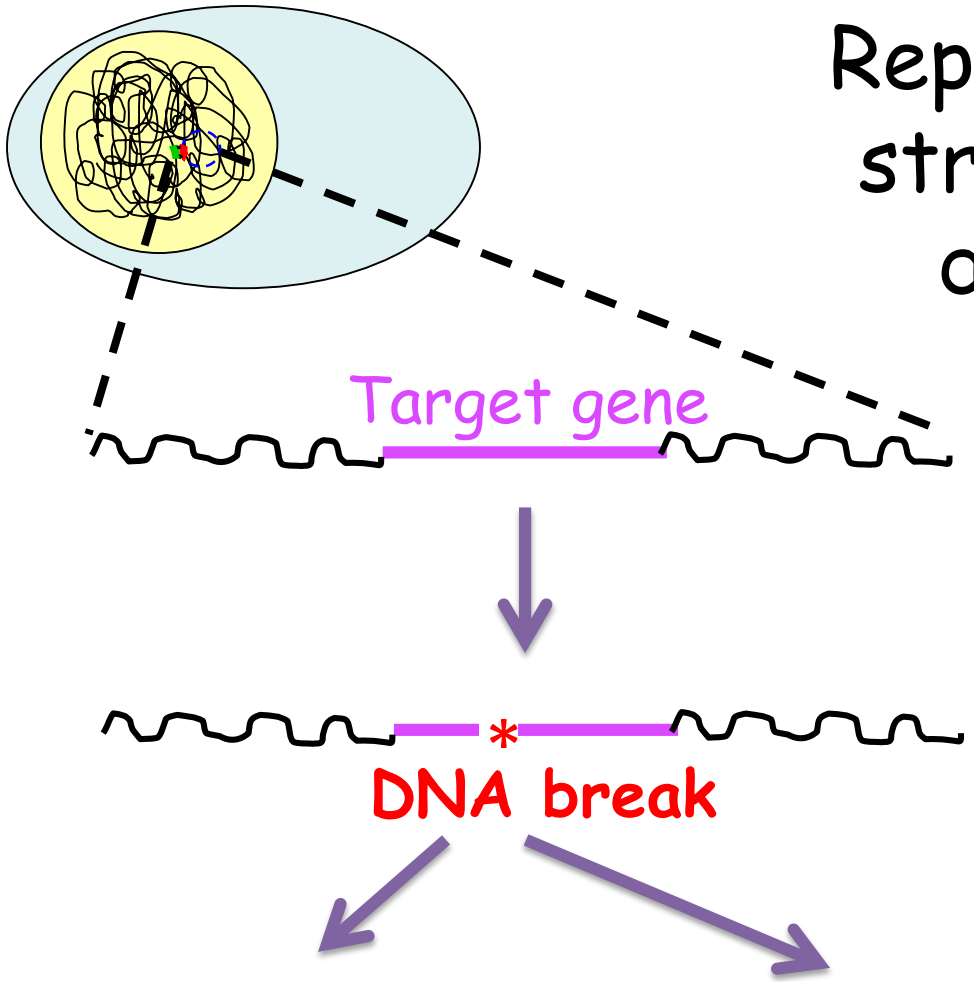
But the rate of recombination can be dramatically increased by the introduction of a DNA break in the target gene!

→ Initiation of cellular DNA repair

Rouet, Smih & Jasin 1994
Puchta, Dujon & Hohn 1993

Repair of a DNA double strand break with two different possible outcomes



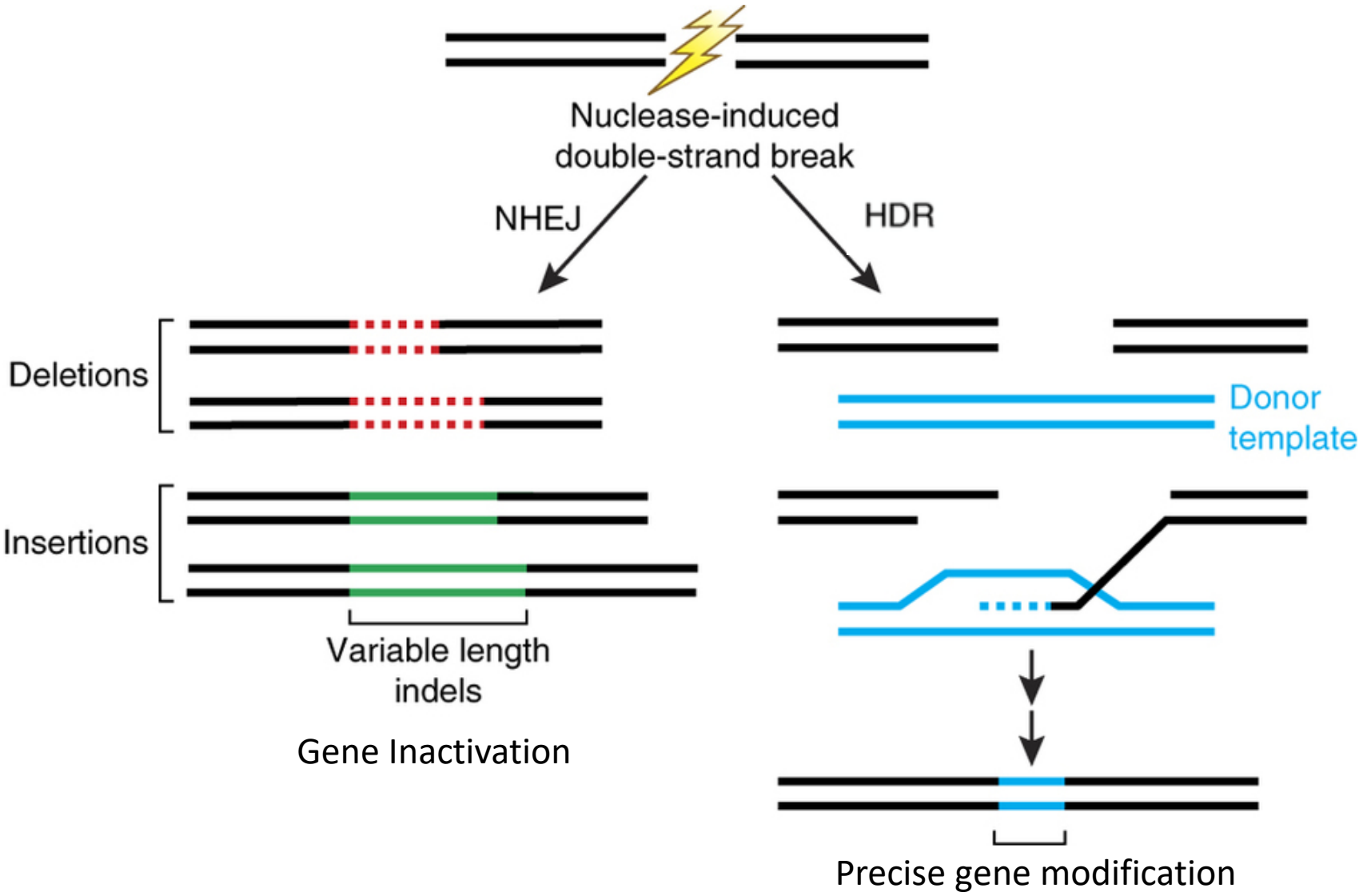
Non-homologous end joining:

➔ **Gene inactivation**

Homology with an added donor template:

➔ **Gene correction**

Two pathways of repair of a DNA double strand break



Since then: Search for and engineering of sequence-specific DNA nucleases

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Meganucleases, Zinc finger nucleases, TALE nuclease fusions,

And finally:

The CRISPR/Cas9 system:

Cas9 is a bacterial DNA nuclease associated with a guide RNA that docks the nuclease to a target gene through base complementarity.

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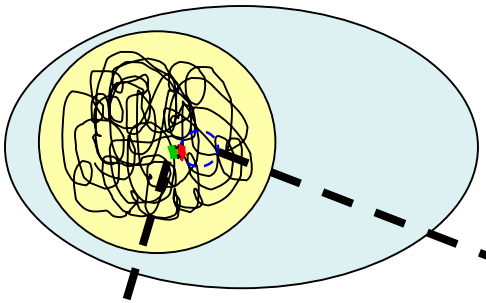
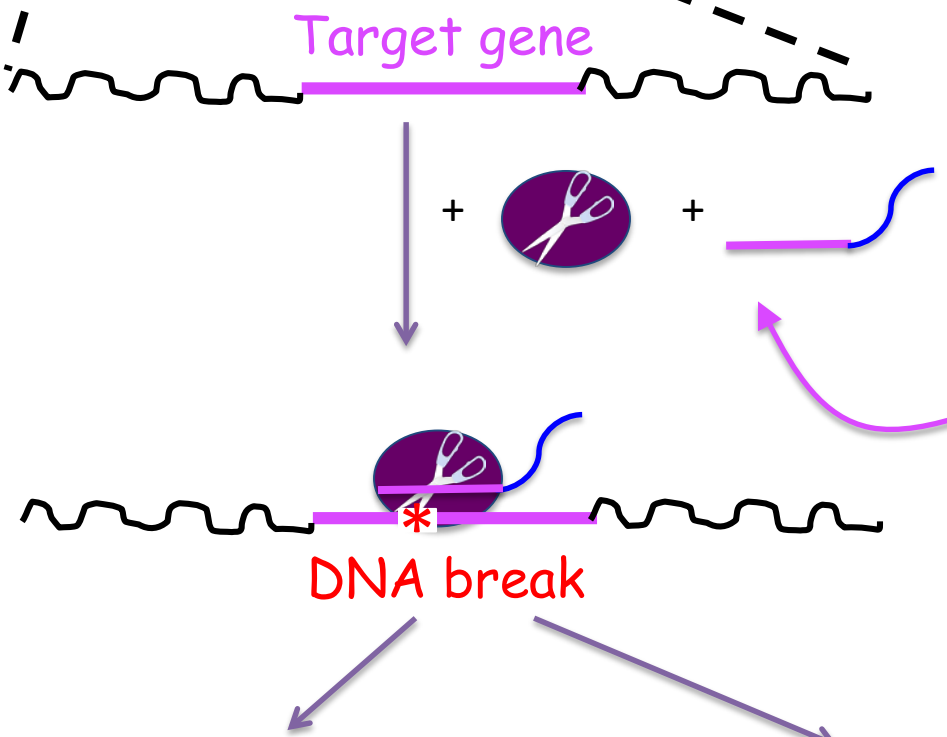
Cas9 is a bacterial DNA nuclease associated with a guide RNA that docks the nuclease to a target gene through base complementarity.
The base sequence of the guide RNA can be freely chosen, therefore the nuclease can be targeted to any target gene in the genome.

CRISPR/Cas9 finds and cuts (almost) any target gene in mammalian cells!





Guide-RNA allows the introduction of specific DNA breaks

Repair through non-homologous end joining:
Gene inactivation

Repair through homology with a donor template:
Gene repair

Where to go with this amazingly efficient technology in the human?

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We have become masters in the art of manipulating genes, but our understanding of their function and interaction is far more limited.

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