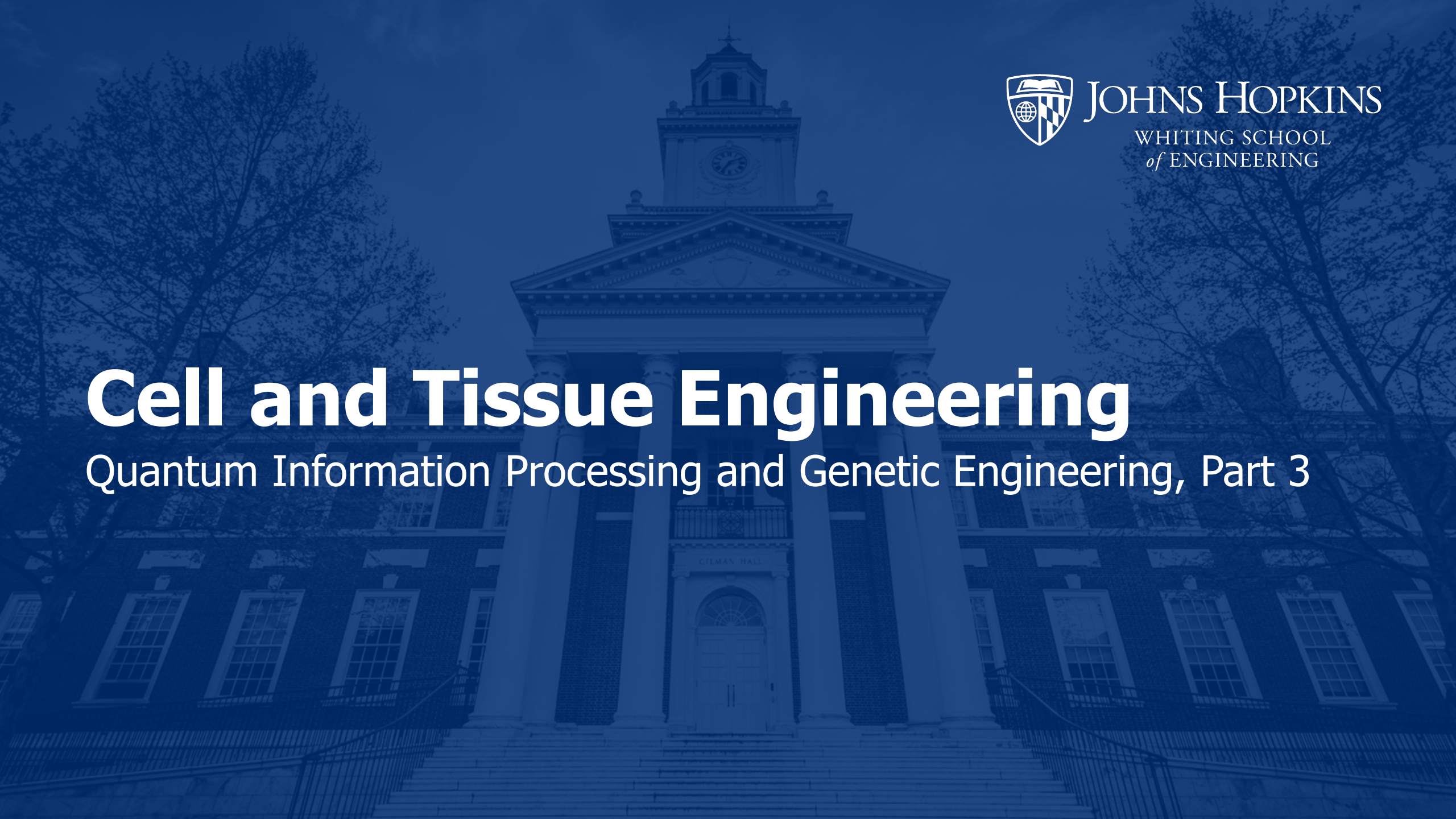


JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING

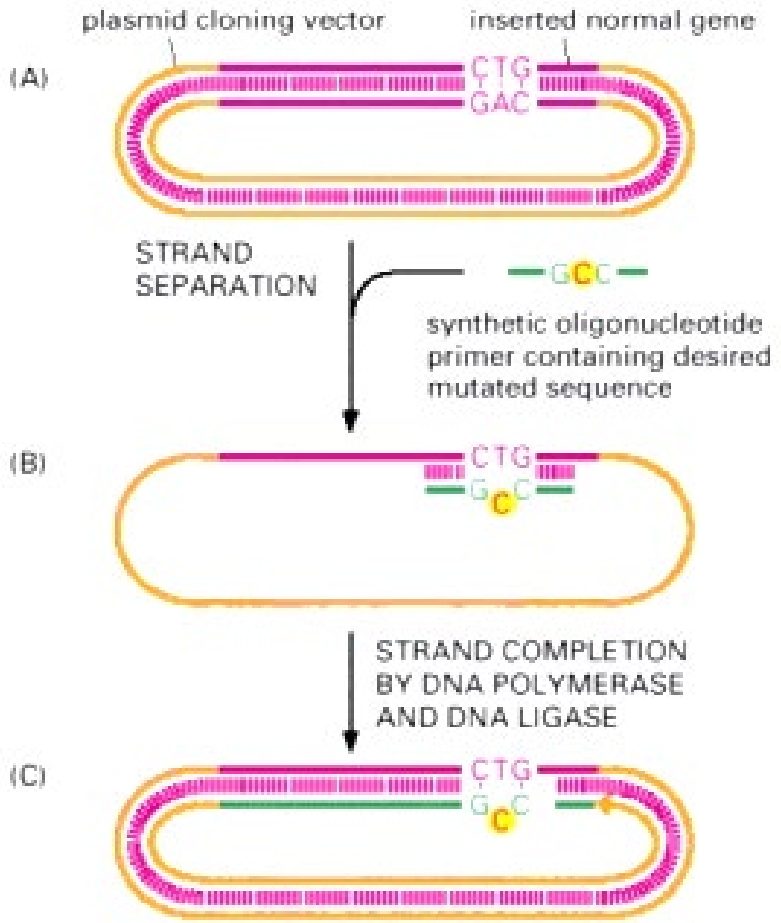
Cell and Tissue Engineering

Quantum Information Processing and Genetic Engineering, Part 3

5 Major Recombinant DNA Techniques

Restriction	cutting at a specific site using restriction enzymes
Hybridization	using a specific fragment of DNA or RNA to identify related sequences
Sequencing	determining the nucleotide sequence of a DNA fragment
DNA cloning	taking a single DNA molecule and making billions of identical copies
DNA engineering	altering DNA sequences to modify gene function

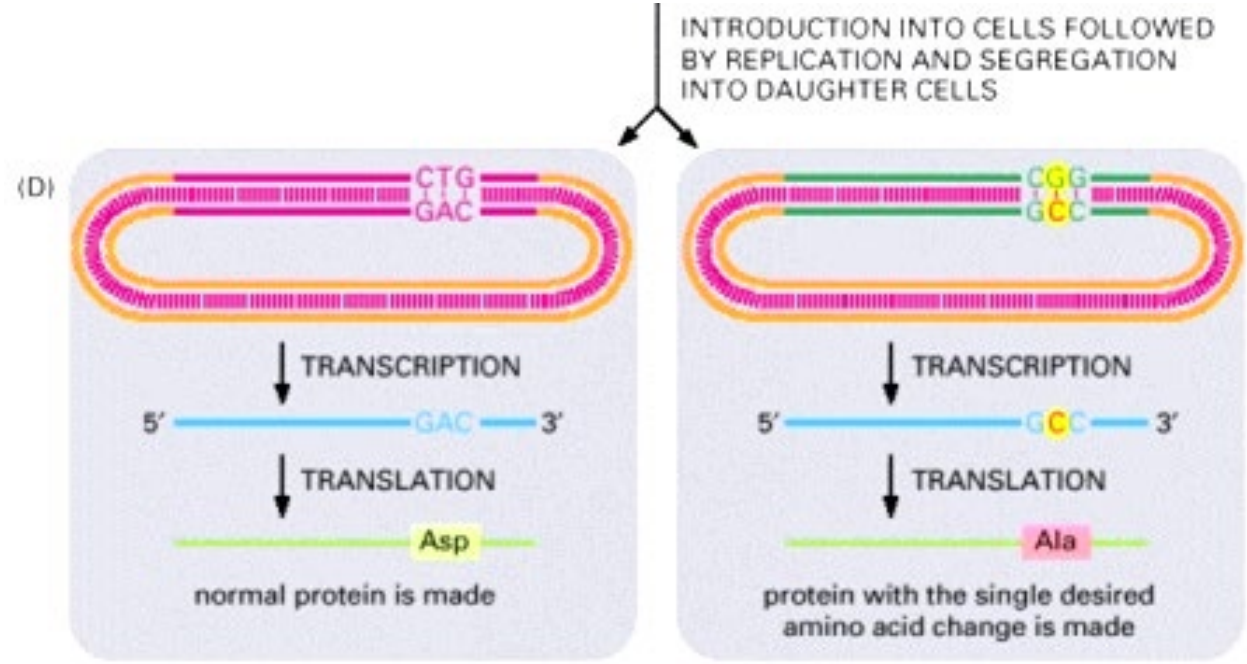
DNA Engineering - creating a mutation



Site-Directed Mutagenesis

Molecular Biology of the Cell, 4th edition. Alberts

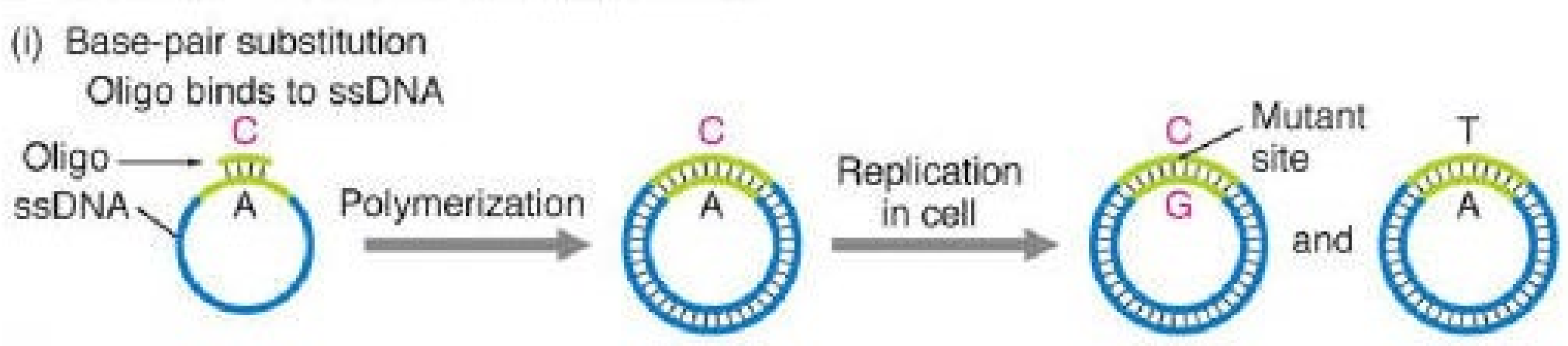
DNA Engineering - creating a mutation (cont.)



Site-Directed Mutagenesis

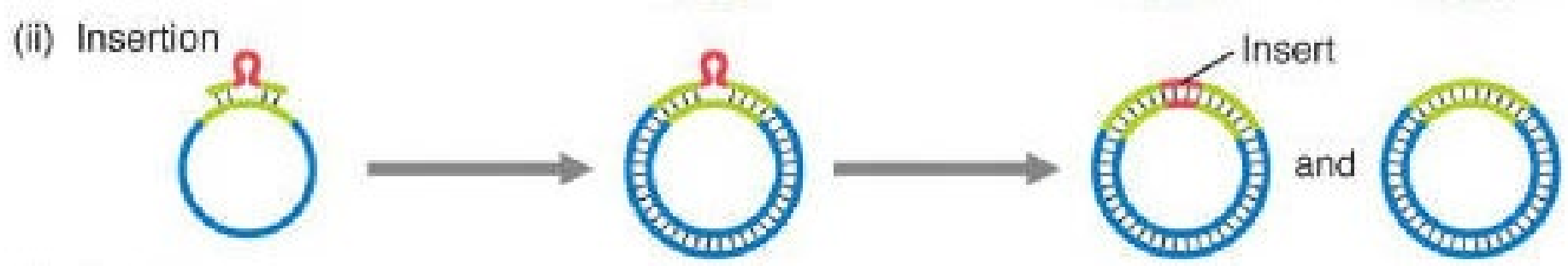
Molecular Biology of the Cell,
4th edition. Alberts

DNA Engineering - creating a mutation (cont.)



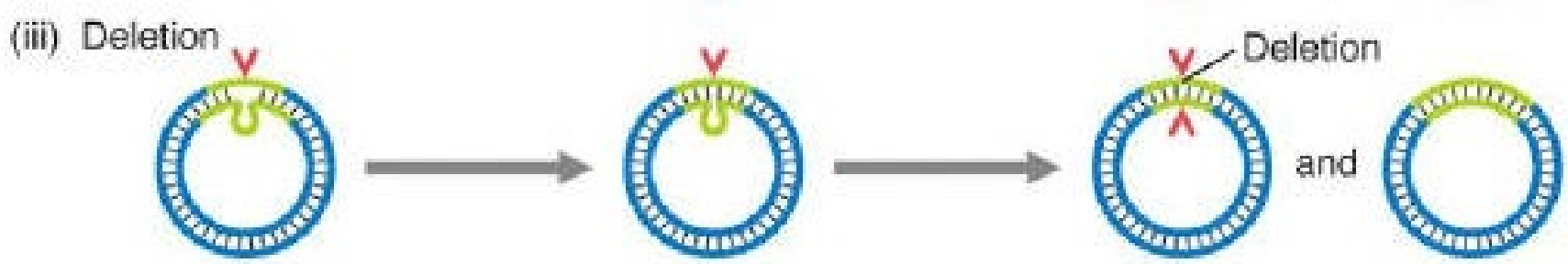
Site-Directed Mutagenesis

DNA Engineering - creating a mutation (cont.)



Site-Directed Mutagenesis

DNA Engineering - creating a mutation (cont.)



Site-Directed Mutagenesis

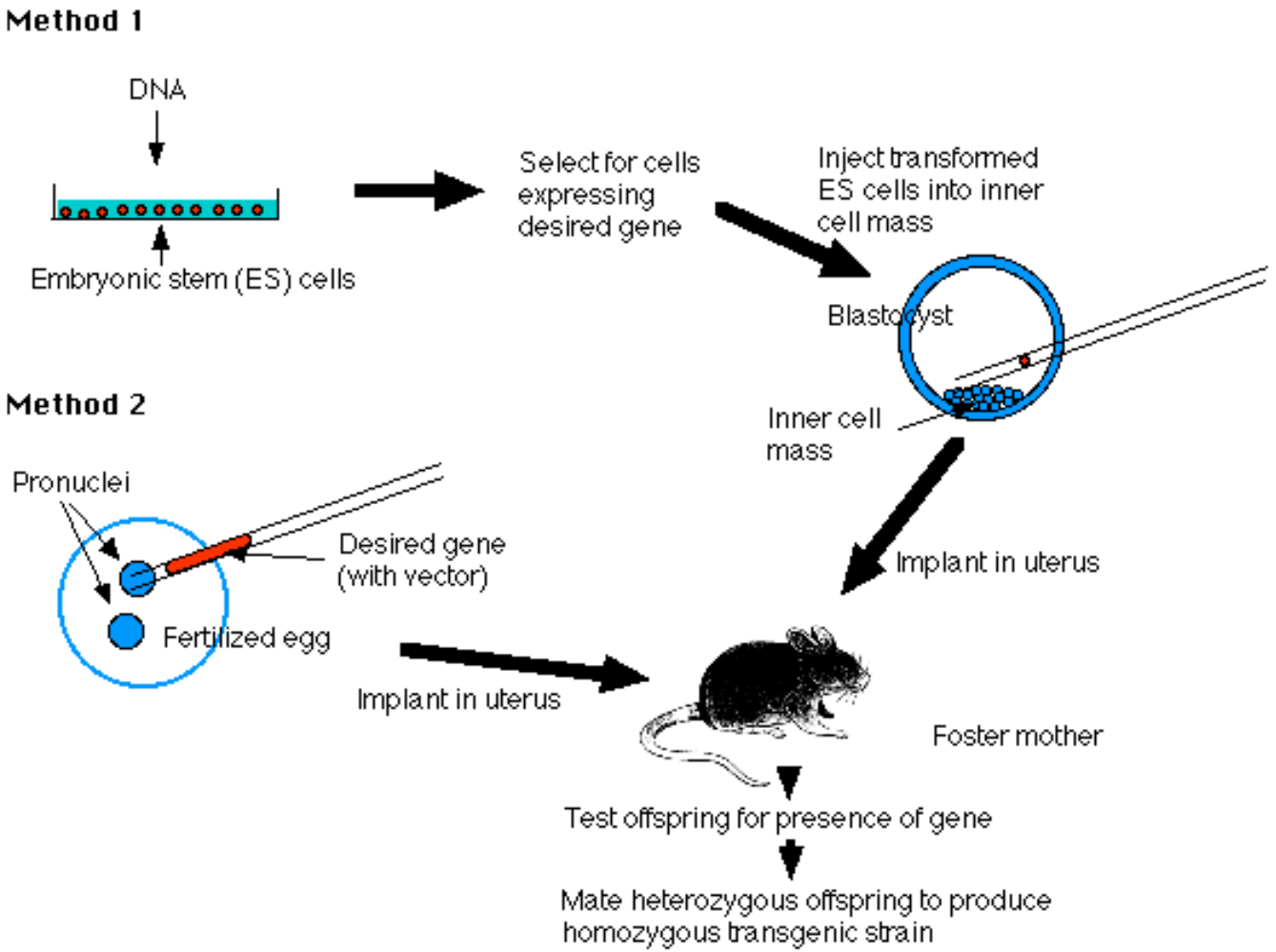
Making a transgenic animal

- Method 1 - Embryonic stem cell method
- Method 2 – Pronucleus method

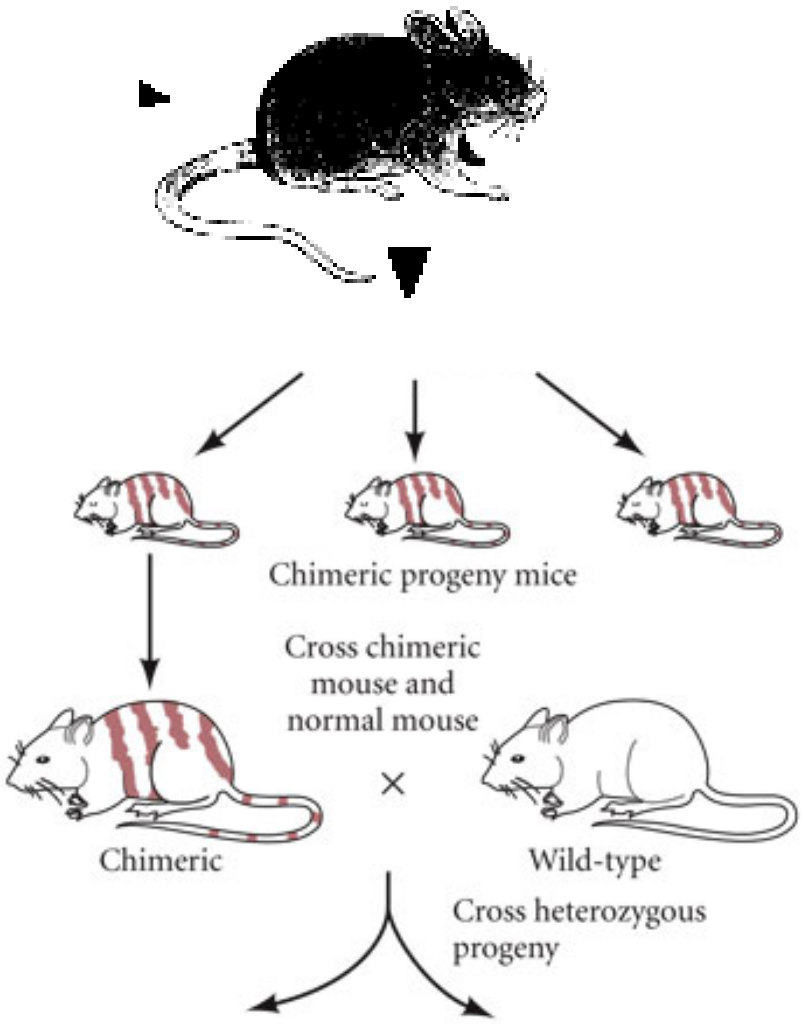
Making a transgenic animal

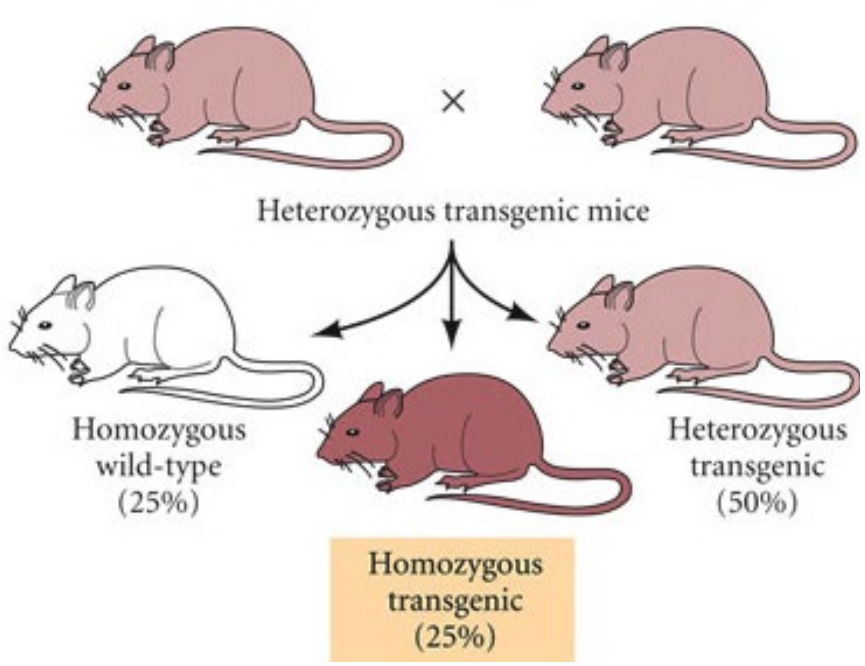
Embryotic stem method

Pronucleus method

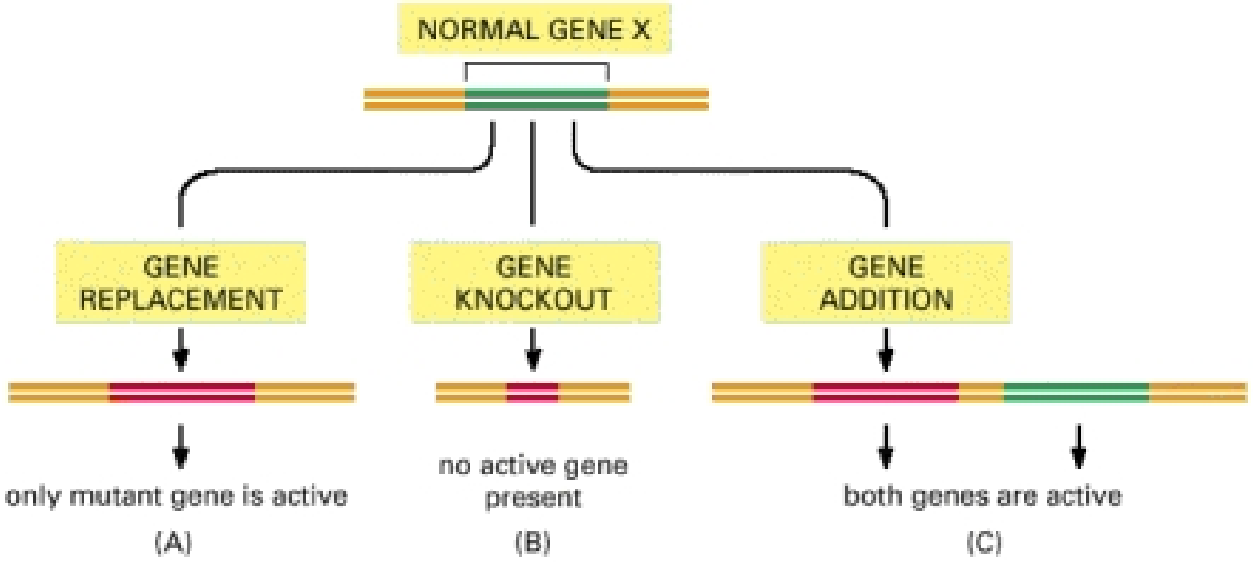


Making a transgenic animal





Types of Mutations



Random vs. Targeted Mutations

In order to knock-out or knock-in a gene you must be able to **target** the location of that gene in the genome.

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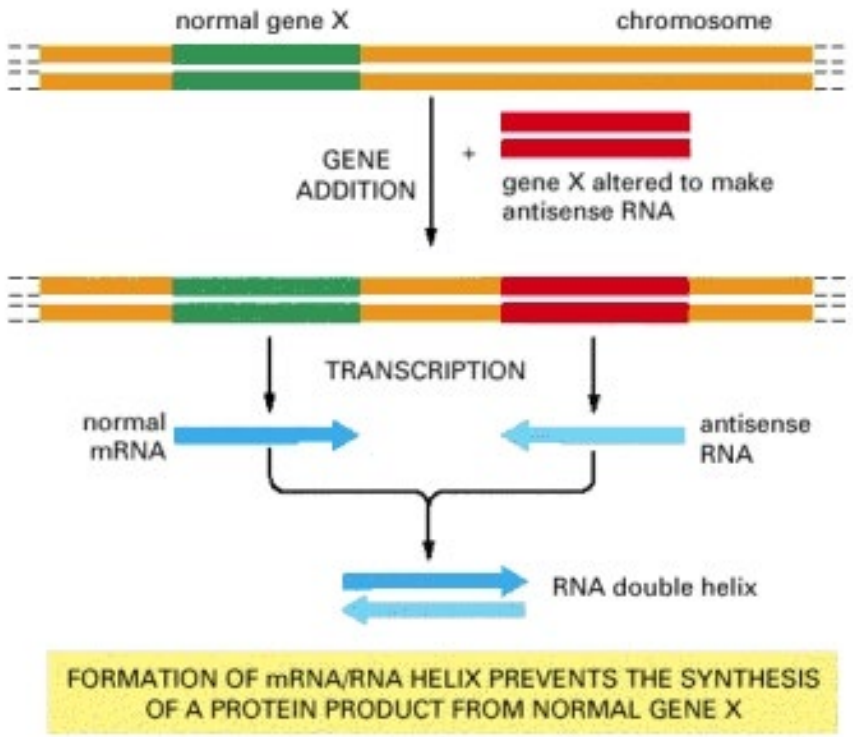
Types of Mutations (cont.)



In diploid organisms (humans, mice) it is much more difficult to control the integration site – and there are 2!

Easier to get something in than take something out.

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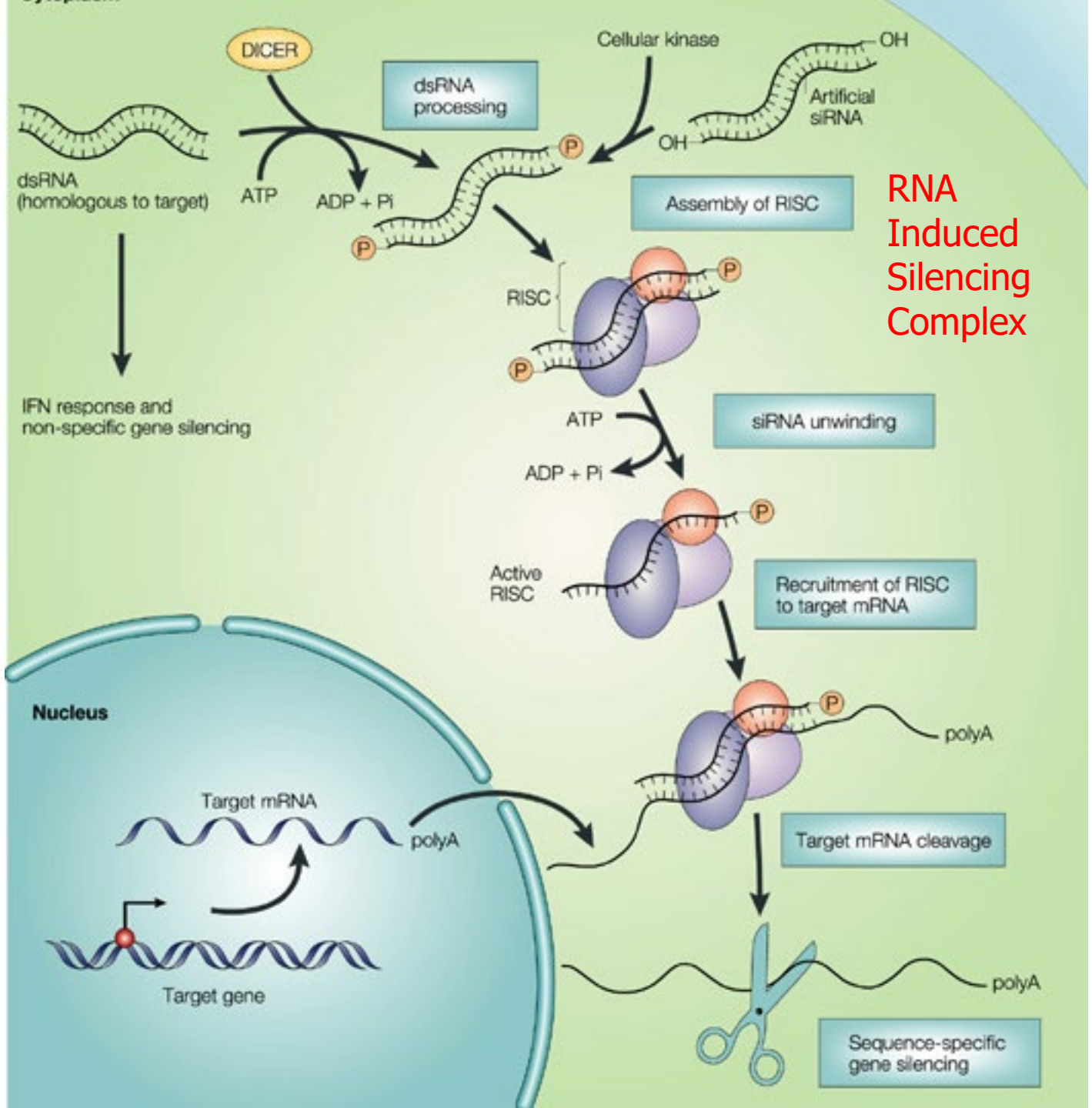

RNA Induced Silencing Complex

Mutations via RNA interference

Stevenson, 2003

Central dogma of Molecular Biology