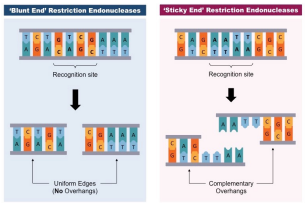
Restriction enzymes – Reference Summary

Genetic engineering = recombinant DNA technology (RDNA) = DNA construction made in the lab
Molecular cloning = making many copies (clones) of the same DNA

Basic step of building recombinant DNA

1) Identify your **gene of interest (GOI)** that you want to reproduce on the corresponding chromosome
2) Cut your GOI DNA from a larger piece and paste it into a vector
Vector = carrier = DNA that replicates very avidly
Grows lots of your clone DNA

Restriction endonucleases (REs) (enzymes)
REs are enzymes that precisely cut DNA at specific locations ("molecular scissors")
An RE cut may leave two types of DNA ends:



Blunt ends
Sticky ends

A blunt end RE cut leaves DNA pieces in which both strands terminate in a base pair.

For example:

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5'-GATCTGACTGATGCGTATGCTAGT-3'
3'-CTAGACTGACTACGCATACGATCA-5'
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A sticky end RE cut leaves DNA pieces in which both strands with overhangs. An overhang is a stretch of unpaired nucleotides in the end of a DNA molecule. These unpaired nucleotides can be in either strand, creating either 3' or 5' overhangs. These overhangs are in most cases palindromic.

For example:

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5'-ATCTGACT      + GATGCGTATGCT-3'
3'-TAGACTGACTACG        CATACGA-5'
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There are > 3,000 REs, named after the bacterium they were discovered in
You can find an RE that cuts the DNA exactly where you want to

Enzyme	Source organism	Restriction recognition site	Structure of the cleaved products
EcoRI	*Escherichia coli*	5′—G—A—A—T—T—C—3′ 3′—C—T—T—A—A—G—5′	5′—G A—A—T—T—C—3′ 3′—C—T—T—A—A G—5′ 5′ overhang
PstI	*Providencia stuartii*	5′—C—T—G—C—A—G—3′ 3′—G—A—C—G—T—C—5′	5′—C—T—G—C—A G—3′ 3′—G A—C—G—T—C—5′ 3′ overhang
SmaI	*Serratia marcescens*	5′—C—C—C—G—G—G—3′ 3′—G—G—G—C—C—C—5′	5′—C—C—C G—G—G—3′ 3′—G—G—G C—C—C—5′ Blunt ends