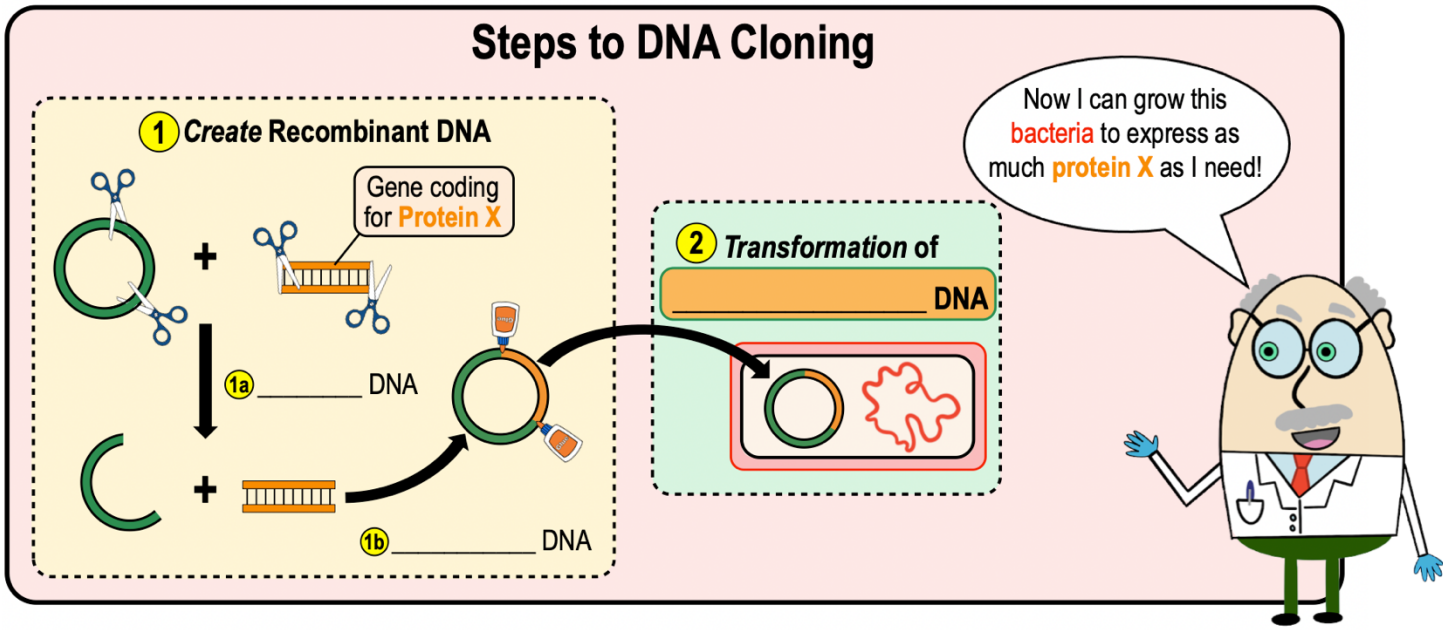
CONCEPT: STEPS TO DNA CLONING

●*Recall:* There are _____ general steps in *DNA Cloning*:

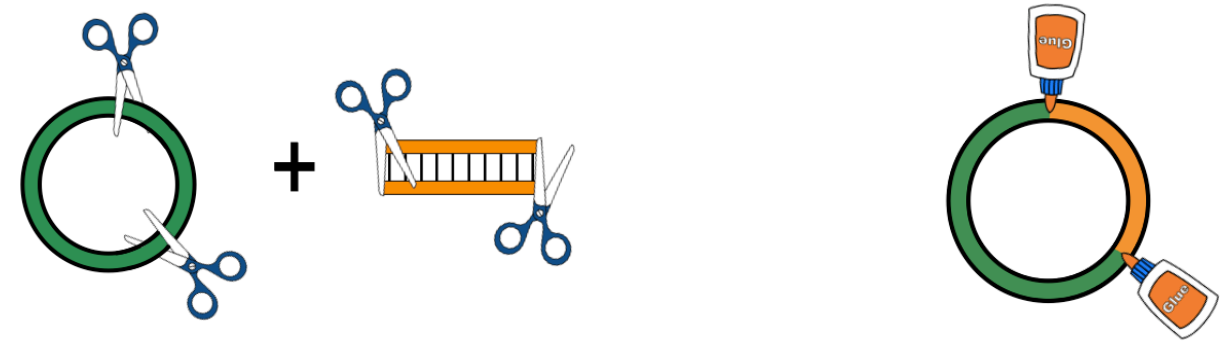
1 _____ the *recombinant DNA* 2 _____ the *recombinant DNA*



Step 1) Create Recombinant DNA

●Creating a *recombinant DNA* molecule is a 2-step process that requires:

1a _____ *enzymes* to cut DNA & 1b _____ *enzymes* to paste DNA



PRACTICE: Human DNA cut with restriction enzyme A can be joined to:

a) A bacterial plasmid cut with restriction enzyme A.

b) Human DNA cut with restriction enzyme B.

c) Human DNA that is uncut.

d) A bacterial plasmid that is uncut.

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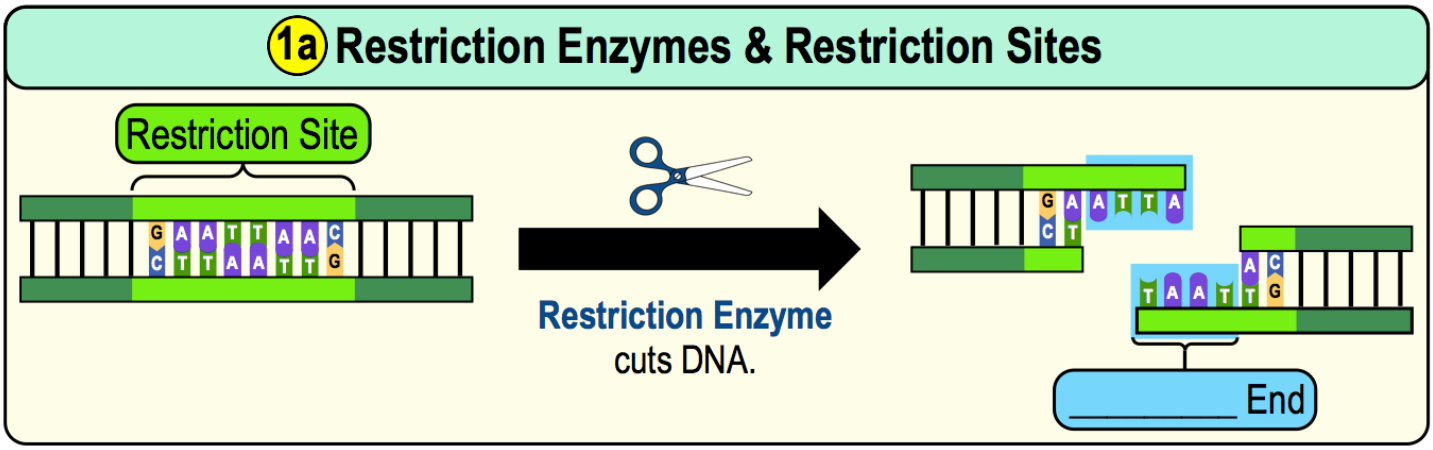
1a) Use Restriction Enzymes

- _____ **Enzymes:** cleave DNA at restriction sites producing *"sticky ends"*.
- **Restriction _____:** *specific* sequence of DNA where *restriction enzymes* bind & cut the DNA.
  - □ **Sticky ends**: a _____-stranded DNA "overhang" produced from a restriction digestion reaction.

EXAMPLE: Restriction enzymes cut DNA at the restriction site leaving a sticky end overhang.



PRACTICE: What is the enzymatic function of restriction enzymes?

a) To add new nucleotides to the growing strand of DNA.
b) To join nucleotides during replication.
c) To join sticky ends of DNA.
d) To cleave DNA at specific sequences.

PRACTICE: Each restriction enzyme has a specific sequence of nucleotides where it cuts the DNA. These sequences of DNA are unique to each restriction enzyme and are known as:

a) Restriction Sites.
b) Restriction Signals.
c) Restriction Sequences.
d) Restriction Sticky Ends.

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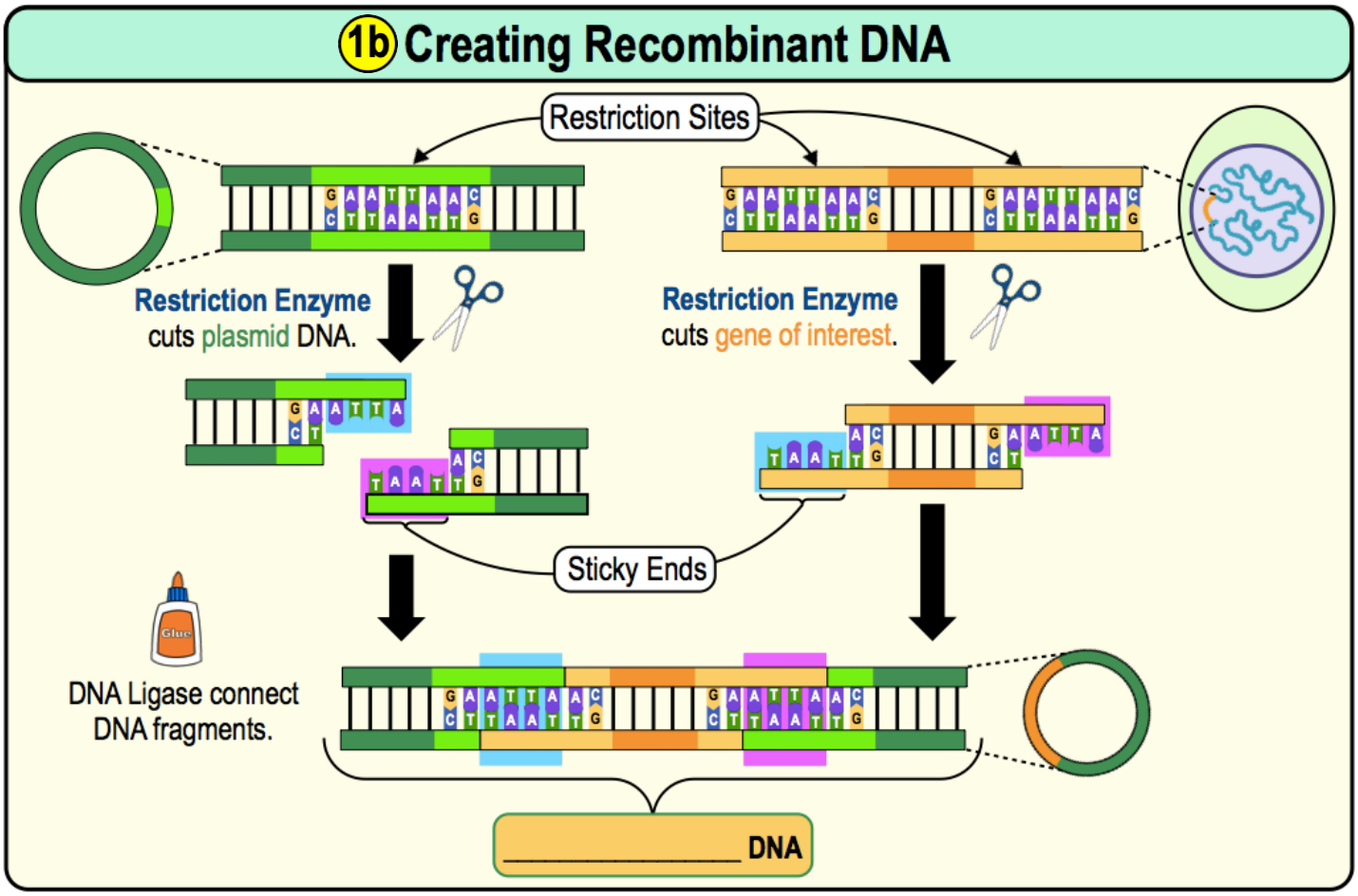
1b) Use Ligation Enzymes

●**DNA** _____**:** enzyme that *ligates* (or joins) the two *sticky ends* together creating the *recombinant DNA.*

□ Note: only DNA fragments cut by the _____ *restriction enzyme* can be ligated back together.

EXAMPLE: A restriction enzyme and DNA ligase are used to clone a recombinant DNA plasmid.



PRACTICE: The single-stranded ends of DNA molecules can be joined together by:

a) Restriction Endonucleases.

b) DNA ligase.

c) DNA polymerase.

d) Helicase.

e) Primase.

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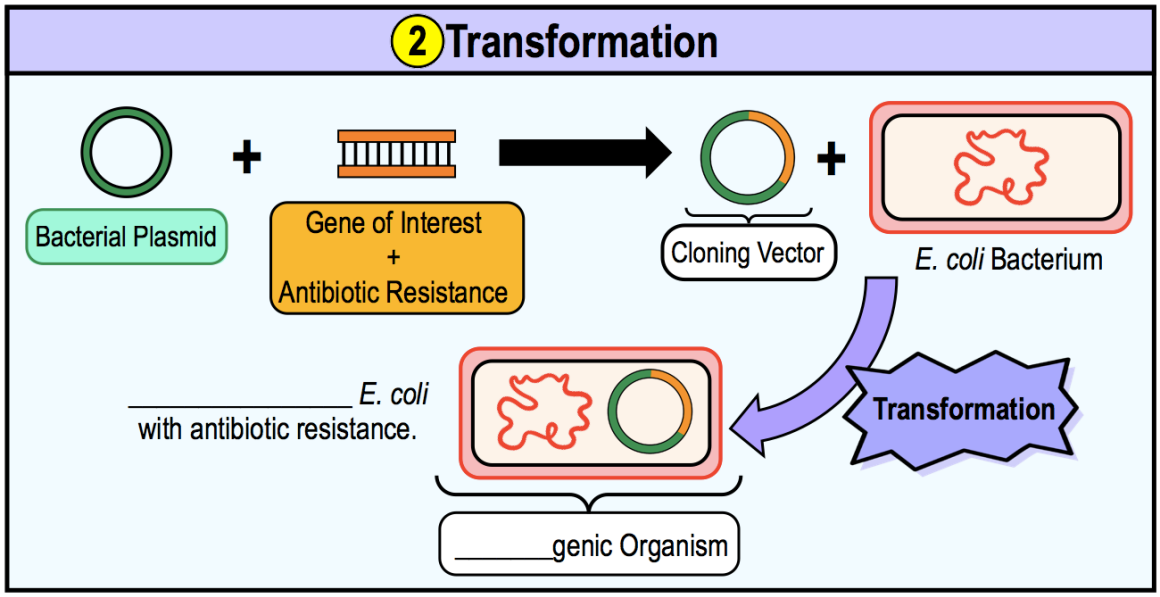
2) Transform Recombinant DNA into Bacteria

- The second and final step of DNA cloning is to _____ the *recombinant DNA.*
  - **Transformation:** process allowing cells to directly uptake foreign DNA (ex. *cloning vector*).
- _____ **Organism:** organism that receives & expresses recombinant DNA.
- *Phenotypic markers* (ex. _____ resistance) are used to confirm a ***positive*** *transformation.*

EXAMPLE: Creating a transgenic organism with antibiotic resistance by transformation of recombinant plasmid DNA.



- Transformed bacterial cells *replicate* & *express* the gene of interest, which the researcher can then purify & study.

PRACTICE: The process of using DNA from one organism to alter the characteristics of another is called:

a) Translation.
b) Transduction.
c) Transcription.
d) Transformation.

PRACTICE: An organism which has foreign genes incorporated into its genomes is known as a:

a) Recombinant organism.
b) Transgenic organism.
c) Hybrid organism.
d) Polyploid organism.

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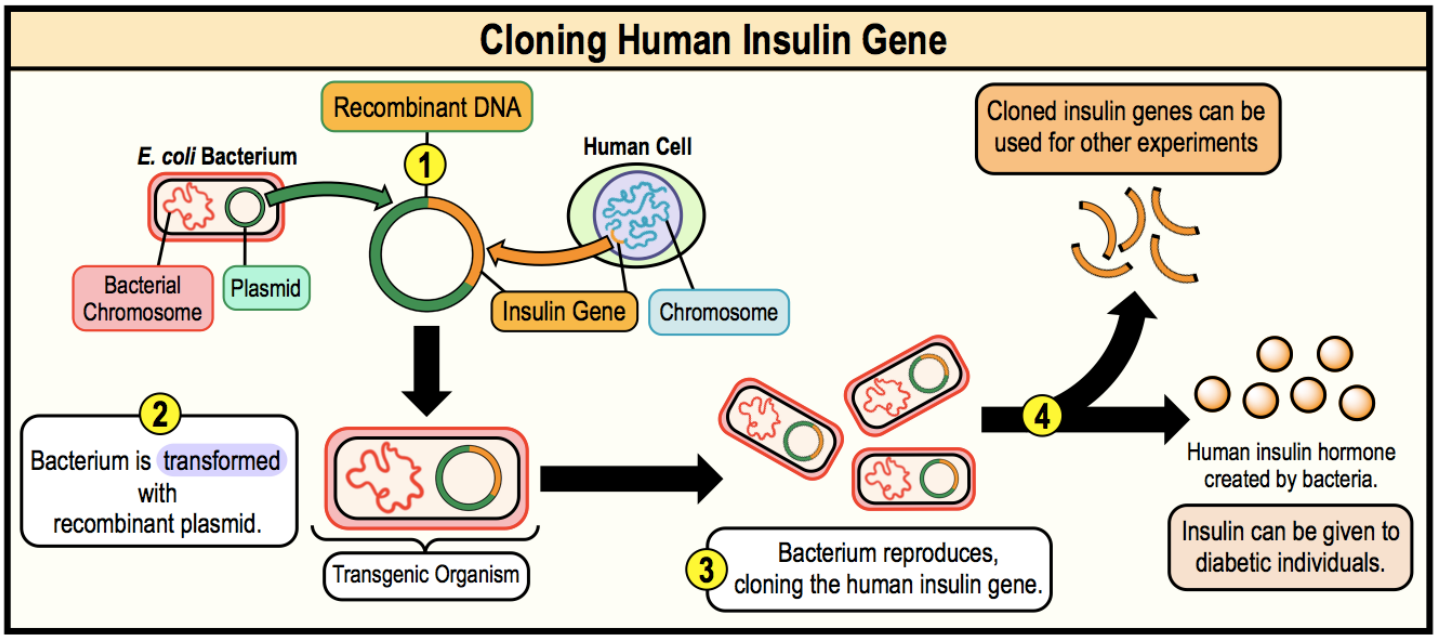
Review & Application of DNA Cloning in Medicine

●Now that we have discussed the techniques used in DNA cloning, let's see how they are used together.

●*Diabetics* do not produce enough _____ **protein** to metabolize blood glucose & they require daily injections.

□ Researchers have found a way to use _____ *organisms* to mass produce insulin for patients.

EXAMPLE: Human Insulin Protein is expressed & purified in large amounts using transgenic *E. coli*.



PRACTICE: What is the most logical sequence of steps for splicing foreign DNA into a plasmid and inserting the plasmid into a bacterium?

I. Transform bacteria with a recombinant DNA molecule.

II. Cut the plasmid DNA using restriction enzymes (endonucleases).

III. Extract plasmid DNA from bacterial cells.

IV. Hydrogen-bond the plasmid DNA to non-plasmid DNA fragments.

V. Use ligase to seal plasmid DNA to non-plasmid DNA.

a) II, III, V, IV, I
b) III, II, IV, V, I
c) III, IV, V, I, II
d) IV, V, I, II, III