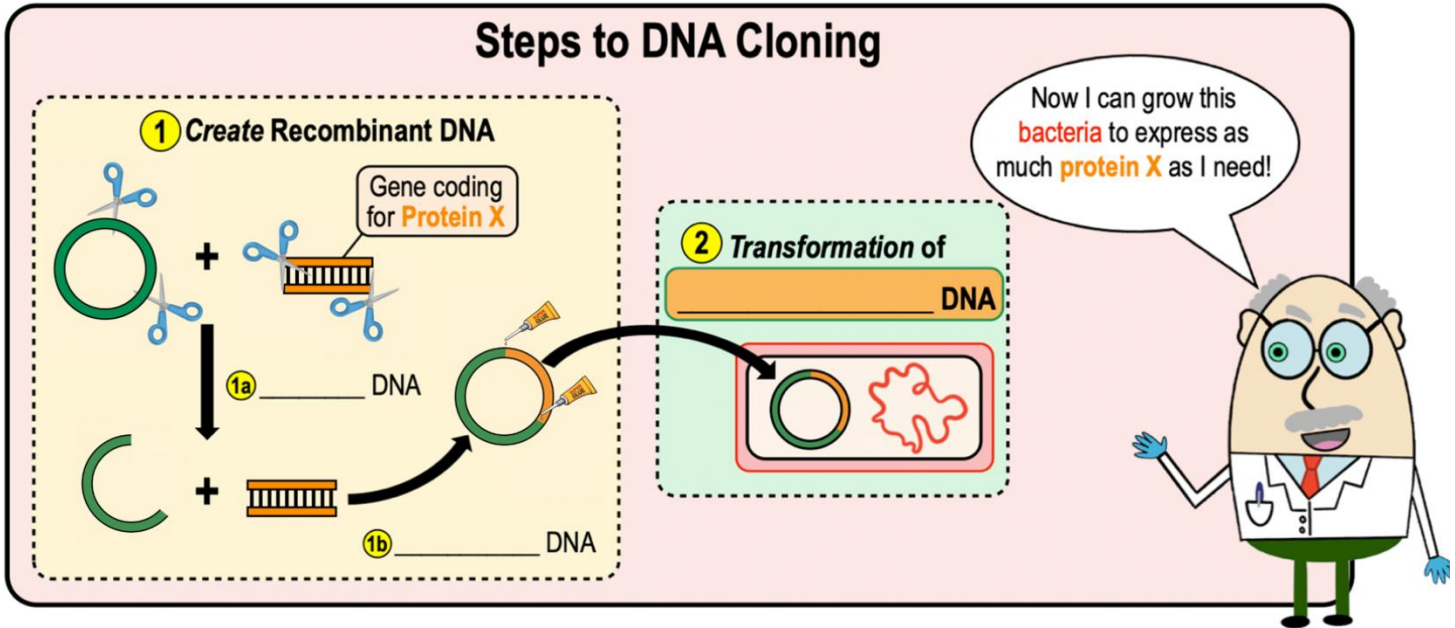


CONCEPT: STEPS TO DNA CLONING

• Recall: There are ____ general steps in DNA Cloning:

1 _____ the recombinant DNA

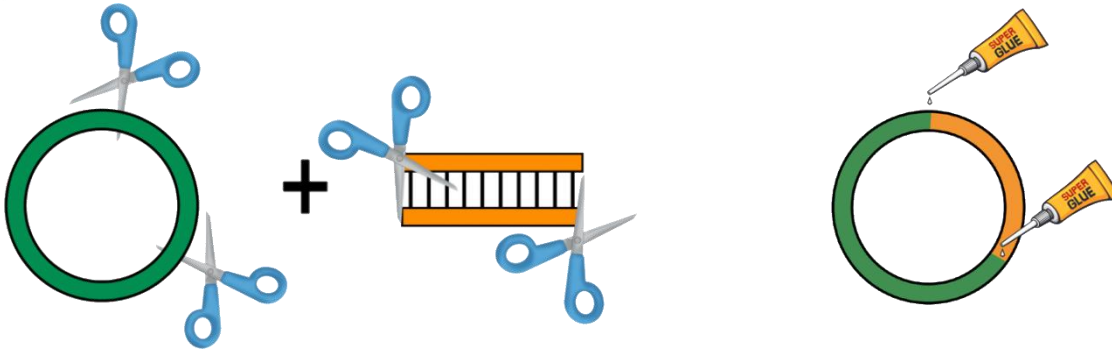
2 _____ the recombinant DNA



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:

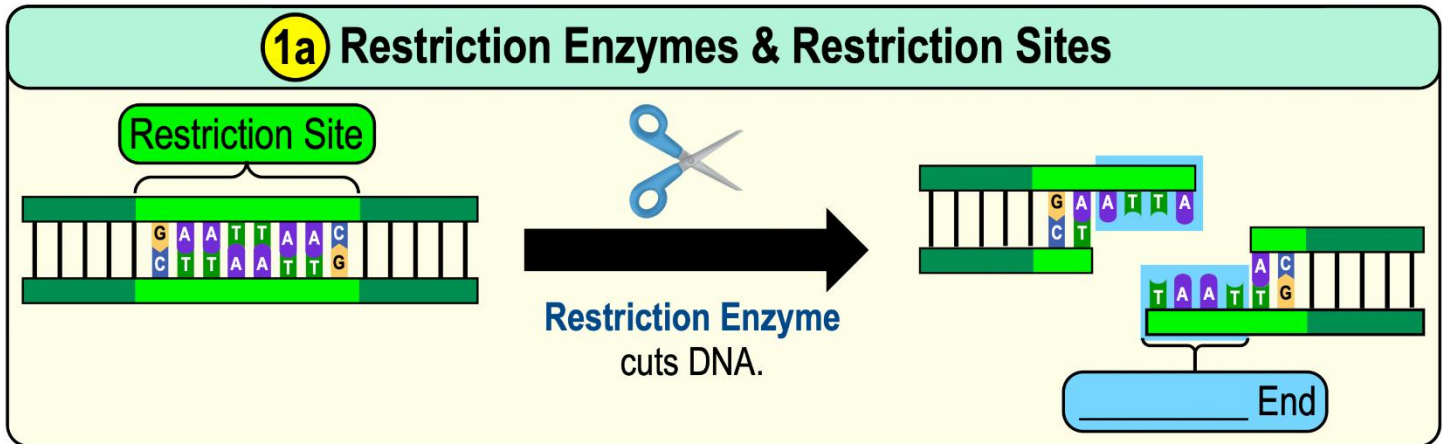
- A bacterial plasmid cut with restriction enzyme A.
- Human DNA cut with restriction enzyme B.
- Human DNA that is uncut.
- A bacterial plasmid that is uncut.

CONCEPT: STEPS TO DNA CLONING

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?

- To add new nucleotides to the growing strand of DNA.
- To join nucleotides during replication.
- To join sticky ends of DNA.
- 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:

- Restriction Sites.
- Restriction Signals.
- Restriction Sequences.
- Restriction Sticky Ends.

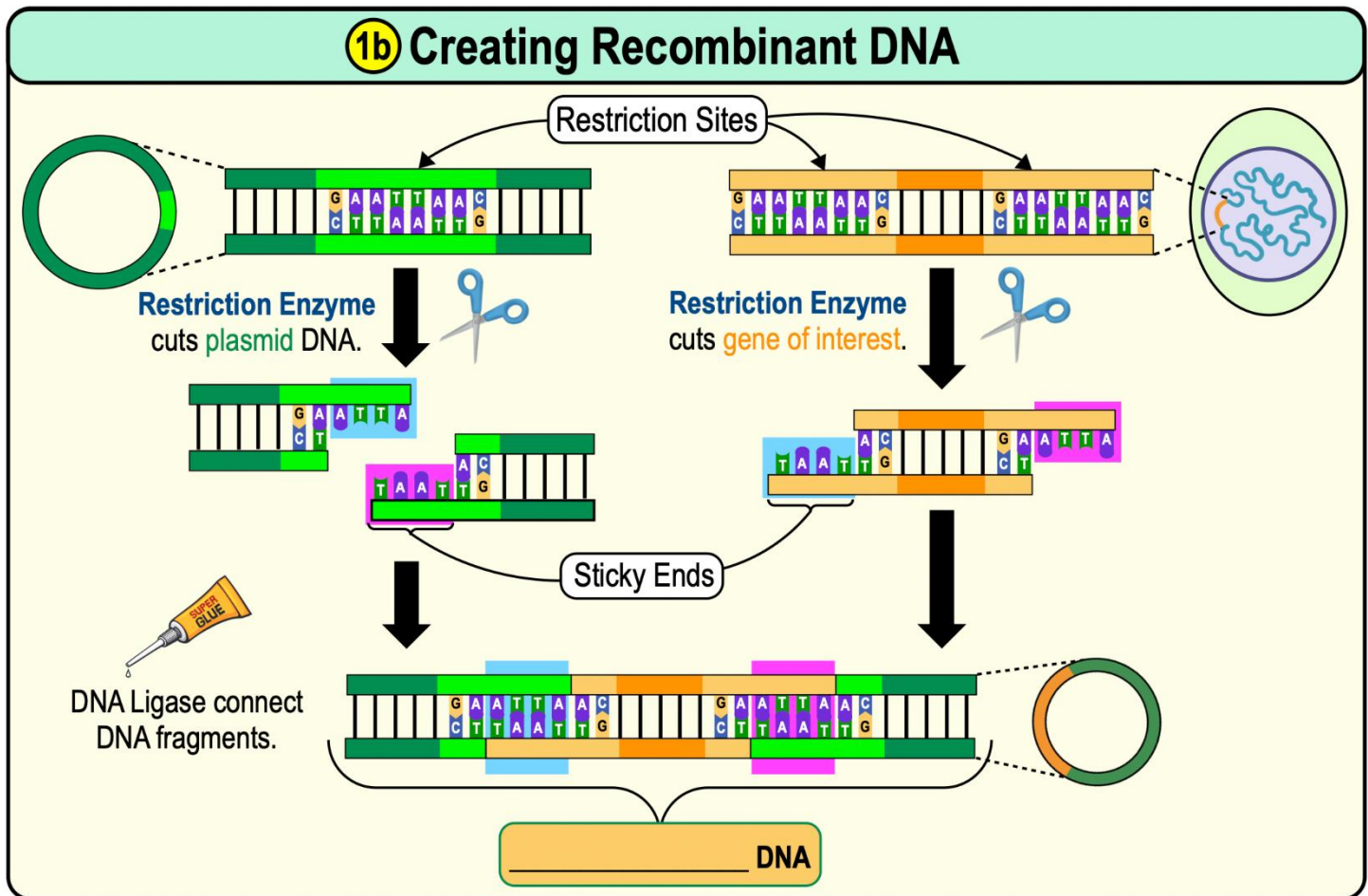
CONCEPT: STEPS TO DNA CLONING

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:

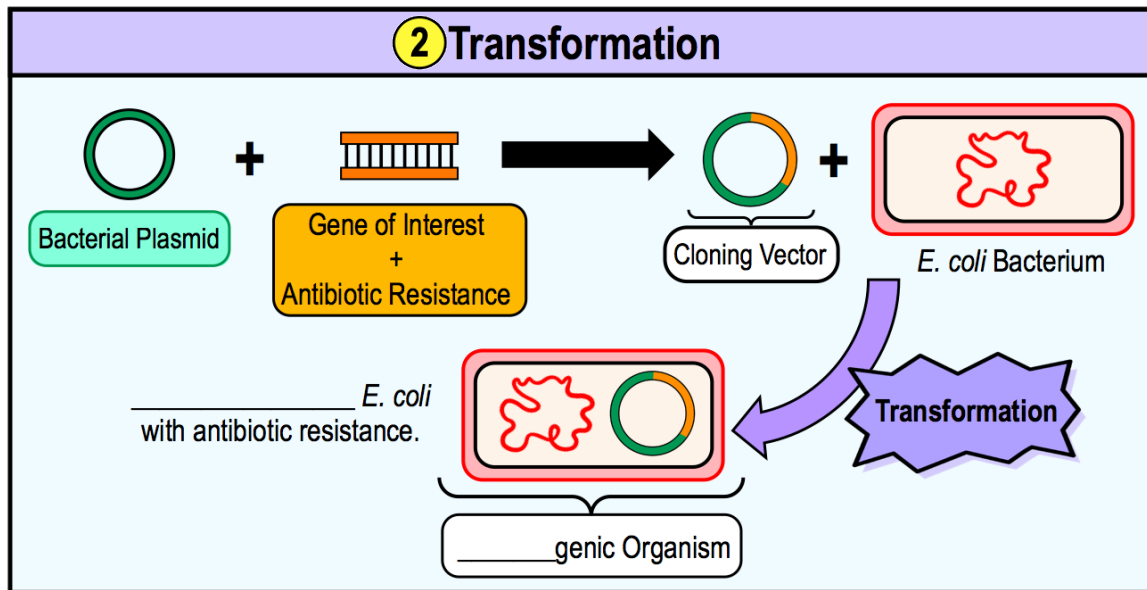
- a) Restriction Endonucleases.
- b) DNA ligase.
- c) DNA polymerase.
- d) Helicase.
- e) Primase.

CONCEPT: STEPS TO DNA CLONING

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:

- Translation.
- Transduction.
- Transcription.
- Transformation.

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

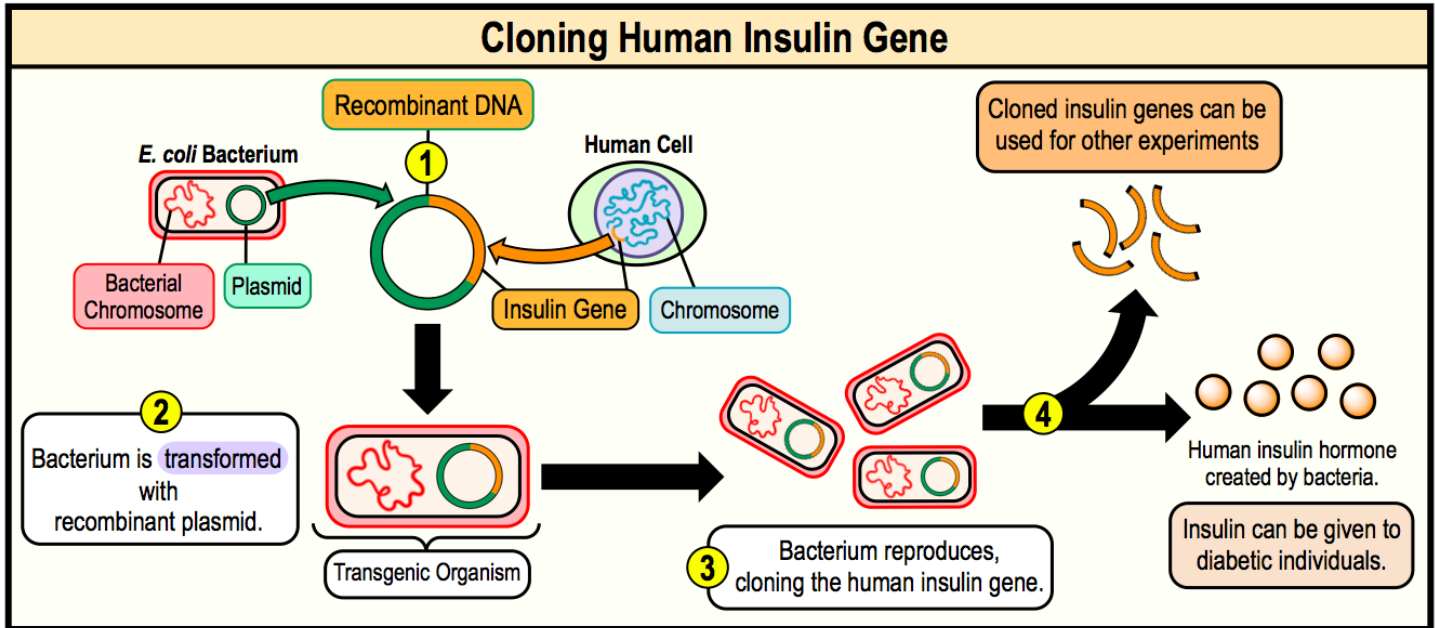
- Recombinant organism.
- Transgenic organism.
- Hybrid organism.
- Polyploid organism.

CONCEPT: STEPS TO DNA CLONING

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