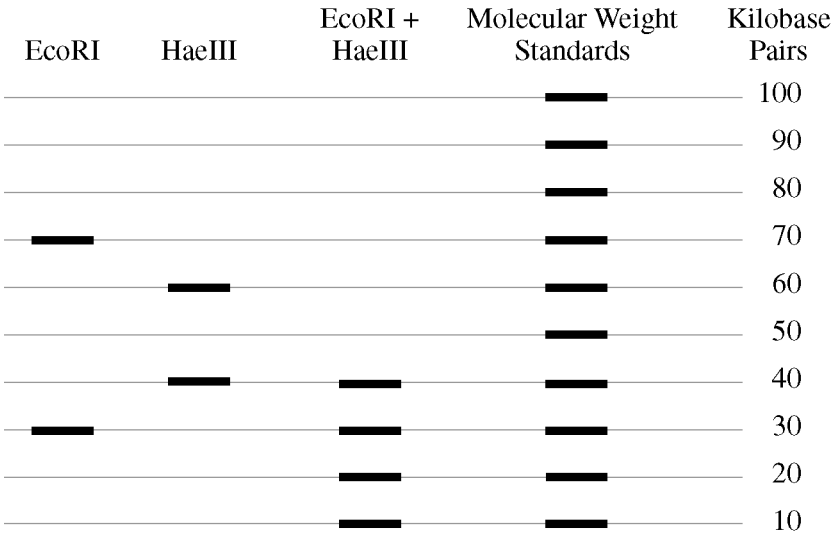
4. A bacterial plasmid is 100 kb in length. The plasmid DNA was digested to completion with two restriction enzymes in three separate treatments: EcoRI, HaeIII, and EcoRI + HaeIII (double digest). The fragments were then separated with electrophoresis, as shown.

RESULTS OF GEL ELECTROPHORESIS

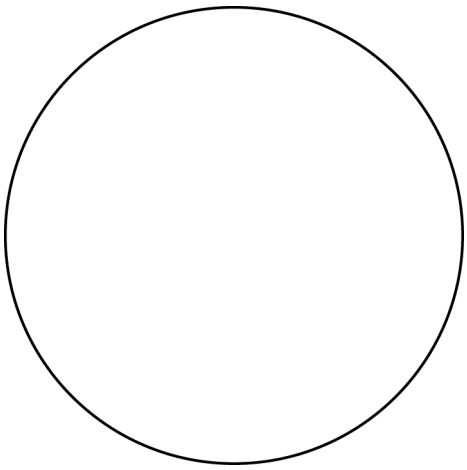


(a) Using the circle provided, **construct** a labeled diagram of the restriction map of the plasmid. **Explain** how you developed your map.

(b) **Describe** how:

- recombinant DNA technology could be used to insert a gene of interest into a bacterium
- recombinant bacteria could be identified
- expression of the gene of interest could be ensured

(c) **Discuss** how a specific genetically modified organism might provide a benefit for humans and at the same time pose a threat to a population or ecosystem.



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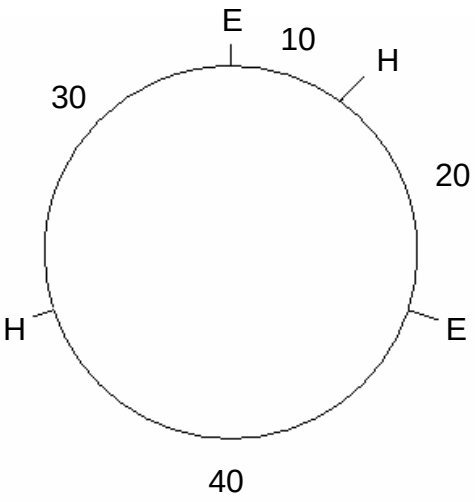
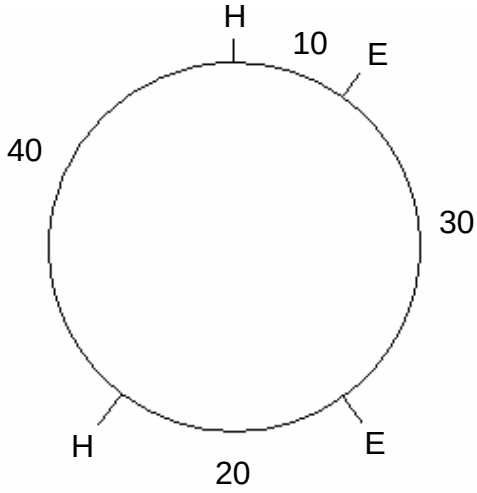
Question 4 (continued)

(a) Using the circle provided, **construct** a labeled diagram of the restriction map of the plasmid. **Explain** how you developed your map.

Construct a labeled map and **explain (3 points maximum)**





E = EcoRI Restriction Point
H = HaeIII Restriction Point

- Restriction sites correctly placed and kilobase sizes shown **(2 points)**

- Explanation **(1 point)**
  (NO POINTS for explanation with incorrect or missing map OR for interpreting gel only)
  - trial and error discussion
  - restriction site within larger fragment

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Question 4 (continued)

(b) **Describe** how:

- Recombinant DNA technology could be used to insert a gene of interest into a bacterium
- Recombinant bacteria could be identified
- Expression of the gene of interest could be ensured

Describe how to: (6 points maximum)

(1) Insert gene of interest (4 points maximum)

- Cut gene of interest from source and/or cut plasmid with restriction enzyme
- Use SAME restriction enzyme on both
- Anneal/ligate/mix/combine gene of interest with vector (plasmid/virus/phage)
- "Sticky ends"/bp matches/complementarity
- Treatment for competent cells ($CaCl_2$/heat shock); incubate together
- Chemical modification can prevent restriction enzyme activity (e.g., methylation)
- Gene = cDNA (without introns) to fit into plasmid

(2) Identify recombinant bacteria (1 point)

- Phenotypic selection (antibiotic resistance/blue-white colony selection/"glo" gene, product produced [e.g., insulin])
- Radioactively/fluorescently labeled probe (tag/dye) / mRNA
- Electrophoresis of cut recombinant vs. original (gene/plasmid) **OR** with sequence comparison of recombinant vs. original (gene/plasmid) **(Not bacterial genome)**

(3) Ensure expression of gene of interest (1 point)

- Promoter [for prokaryote]
- cDNA/removal of introns for prokaryotic expression
- Operon (e.g., nutrient/arabinose induced)

(c) **Discuss** how a specific genetically modified organism might provide a benefit for humans and at the same time pose a threat to a population or ecosystem. **(3 points maximum)**

Discuss GM, benefit to humans, and threat to population/ecosystem

- Nonhuman organism with specific, heritable GM trait
- Plausible benefit to humans related to the GM trait
- Plausible or unknown threat to population/ecosystem related to GM trait/modified organism