Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Period: \_\_\_\_\_\_\_

Making a Restriction Map of Plasmid DNA

**Introduction**: This activity demonstrates a method by which a map of a plasmid can be made. A plasmid (pTOM1, from *Exampelicus tomasinii)* is treated with two restriction enzymes that have different recognition sequences. Each restriction enzyme is used individually (a single digest) and then the two are used at the same time (a double digest). This way, the distance between restriction sites can be determined by analyzing the sizes of the plasmid fragments produced.

**Materials and Methods**: First, the plasmid is replicated by cloning or PCR. Next, three samples of the plasmid are digested: one with just EcoRI, one with just HindIII, and a third with both EcoRI and HindIII. Finally, the fragments produced by the three digestions samples are separated by gel electrophoresis to determine the size of each fragment. By analyzing the size data, it is then possible to construct a plasmid map that illustrates the relative positions of restriction sites on the plasmid.

**Data**: The following diagram is a simulation of what the electrophoresis gel produced by the experiment might look like:

Electrophoresis Gel of Restriction Fragments from pTOM1:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Standard fragment sizes | Size Standard | EcoRI | HindIII | EcoRI + HindIII |
|  |  |  |  |  |
| 8 kbp |  |  |  |  |
| 6 kbp |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
| 3.5 kbp |  |  |  |  |
| 3 kbp |  |  |  |  |
|  |  |  |  |  |
| 2 kbp |  |  |  |  |
|  |  |  |  |  |
| 1 kbp |  |  |  |  |
|  |  |  |  |  |
| 0.5 kbp |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
| 0.1 kbp |  |  |  |  |

**Results**: Use the size standard to determine the fragment sizes (kbp = kilobase pair) produced by each restriction enzyme digest shown in the gel electrophoresis data

|  |  |
| --- | --- |
| **Digest** | **Fragment sizes (kbp)** |
| EcoRI |  |
| HindIII |  |
| EcoRI & HindIII |  |

**Conclusion**: Use the fragment size data to produce a plasmid map. Indicate the distances between restriction sites (in kbp) on the map. Use a single line (|) to show EcoRI sites and a double line (||) to show HindIII sites.

Restriction map of pTOM1: