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PLASMID In this exercise you will use paper to simulate the cloning of a gene from one organism into a bacterial plasmid using a restriction enzyme digest. The plasmid (puc18 plasmid) can then be used to transform bacteria so that it

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LAB: CLONING

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this exercise you will use paper to simulate the cloning of a gene from one organism into a bacterial plasmid using a restriction enzyme digest. The plasmid (puc18 plasmid) can then be used to transform bacteria so

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that it now expresses
a new gene and
produces a new
protein. 1. [Book]
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PROCEDURE Isolate
(cut out) the pBR322
DNA and circularize it
into a small plasmid

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by using tape to connect the free ends. Be sure... Isolate (cut out) the Vaccinia DNA fragment. Examine the DNA sequence for restriction enzymes that can be used to cut... Identify the restriction endonuclease used to ...

CRACKING THE

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CODE/CLONING PAPER PLASMID

LAB: CLONING
PAPER PLASMID In this exercise you will use paper to simulate the cloning of a gene from one organism into a bacterial plasmid using a restriction enzyme digest. The plasmid (puc18 plasmid) can then be used to

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Plasmid transform bacteria so that it now expresses a new gene and produces a new protein. 1.

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Plasmid plasmid) can then be used to transform bacteria so that it now expresses

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a new gene and produces a new protein. 1. The white strip represents the plasmid puc18 2. Take the white strip and tape the ends together to make a loop to simulate the circular DNA of a plasmid. 3. The red strip

Answers Lab

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What is a plasmid? A circular-shaped bacteria that is capable of taking in new DNA and making it circular. Why did we cut both segments of DNA with the same restriction enzyme? Because both segments of DNA

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Plasmid
have the same
recognition site so
they are cut by the
same restriction
enzyme.

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exercise you will use

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paper to simulate the cloning of a gene from one organism into a bacterial plasmid using a restriction enzyme digest. The plasmid (puc18 plasmid) can then be used to transform bacteria so that it now expresses a new gene and produces a new protein.

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gene cloning; In a PNAS paper entitled “Construction of Biologically Functional Bacterial Plasmids In Vitro,” my colleagues

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A. C. Y. Chang, H. W. Boyer, R. B. Helling, and I reported in November 1973 that individual genes can be cloned and isolated by enzymatically fragmenting DNA molecules, linking the pooled fragments to autonomously replicating circular bacterial genetic

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Plasmids known as plasmids, and introducing the resulting recombinant DNA molecules into bacteria . Boyer and I ...

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One method is to conduct 2 ligations for each plasmid you are

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trying to create, with varying ratios of recipient plasmid to insert. It is also important to set up negative controls in parallel. For instance, a ligation of the recipient plasmid DNA without any insert will tell you how much background you have of uncut or self-ligating recipient

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Addgene: Plasmid Cloning by PCR (with Protocols)

The DNA cloning in the desired host can still be achieved via the employment of shuttle vectors containing the plasmid origins of replication for both the *E. coli* and the target

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Plasmid. Thus, the initial cloning of the desired DNA fragment within a shuttle vector in *E. coli* is followed by the introduction of the selected recombinant plasmid into the target species.

**Recombinant
Plasmid - an
overview |**

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Topics

We recommend using a 1:1, 1:3 or 3:1 molar ratio of vector:insert DNA when cloning a fragment into a plasmid vector. The following example of a ligation reaction consists of a 3.0kb vector and a 0.5kb insert DNA uses the 1:3 vector:insert ratio.

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Typical ligation reactions use 100–200ng of vector DNA.

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A multiple cloning site (MCS), containing sequences recognized by common restriction enzymes, and designed to allow simple insertion of a desired gene

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Plasmid sequence. An origin of replication (ORI) , allowing the plasmid to be simply and rapidly duplicated by the host organisms replication machinery.

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