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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 transform bacteria so that it now expresses a new gene and produces a new protein. 1. The white strip represents the plasmid puc18 2.

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AAAGCTTTGC..... GGTCGAAAGC.....

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[Book] Biology Lab Cloning Paper Plasmid

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Paper Plasmid activity - Liberty Union High School District

In its simplest form, PCR based cloning is about making a copy of a piece of DNA and at the same time adding restriction sites to the ends of that piece of DNA so that it can be easily cloned into a plasmid of interest.

Addgene: Plasmid Cloning by PCR (with Protocols)

1) To recover the DNA, use clean gloves and cut the marked circle area that contains the dried plasmid DNA. 2) Using clean forceps, insert the filter paper into a 1.5 ml micro centrifuge tube. Add 100 μ l of TE buffer (Or molecular biology water), tap on the tube to mix, and incubate at room temperature for 10 minutes.

Shipping and Receiving Plasmids on Filter Paper

Plasmid Cloning by Restriction Enzyme Digest (aka Subcloning) Summary. The following technique can be used to easily move any piece of DNA from one vector to another as long as it is...

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Background. Subcloning by restriction digest is a commonly used lab technique. For the purposes of this tutorial ...

Addgene: Plasmid Cloning by Restriction Enzyme Digest ...

A second popular approach uses PCR to amplify the region of interest from the plasmid. The resulting PCR product is then cloned into the desired vector. TA cloning or blunt-end cloning methods can be used as described in the PCR cloning section, but neither approach maintains directionality of the insert. To achieve directional cloning, restriction sites that are present in the destination vector for subcloning can be incorporated into PCR amplicons by using PCR primers designed with the ...

Common Cloning Applications and Strategies | Thermo Fisher ...

In general, cloning vectors are plasmids that are used primarily to propagate DNA. They replicate in *E. coli* to high copy numbers and contain a multiple cloning site (also called a polylinker) with restriction sites used for inserting a DNA fragment.

Cloning Vector - an overview | ScienceDirect Topics

The plasmid is often transformed into a bacterium like *E. coli*. Ideally when the bacterium divides the plasmid should also be replicated. In the best case scenario, each bacterial cell should have several copies of the plasmid. After a good number of bacterial colonies have grown, they can be minipreped to harvest the plasmid DNA.

Subcloning - Wikipedia

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

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linking the pooled fragments to autonomously replicating circular bacterial genetic elements known as plasmids, and introducing the resulting recombinant DNA molecules into bacteria . Boyer and I ...

DNA cloning: A personal view after 40 years | PNAS

Scientists working in Boyer's lab recognized the need for a general cloning plasmid, a compact plasmid with unique restriction sites for cloning in foreign DNA and the expression of antibiotic resistance genes for selection of transformed bacteria. In 1977, they described the first vector designed for cloning purposes, pBR322 (20).

Foundations of Molecular Cloning - Past, Present and ...

In a typical cloning experiment, a target gene is inserted into a circular piece of DNA called a plasmid. The plasmid is introduced into bacteria via a process called transformation, and bacteria carrying the plasmid are selected using antibiotics.

Overview: DNA cloning (article) | Khan Academy

PCR cloning differs from traditional cloning in that the DNA fragment of interest, and even the vector, can be amplified by the Polymerase Chain Reaction (PCR) and ligated together, without the use of restriction enzymes. PCR cloning is a rapid method for cloning genes, and is often used for projects that require higher throughput than traditional cloning methods can accommodate.

PCR Cloning Method | NEB

Cloning is generally first performed using *Escherichia coli*, and cloning vectors in *E. coli* include plasmids, bacteriophages (such as phage λ), cosmids, and bacterial artificial chromosomes (BACs). Some DNA, however, cannot be stably maintained in *E. coli*, for example very large DNA fragments, and other organisms such as yeast may be used.

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Cloning vector - Wikipedia

When using a cloning vector, it is critical that the cloning vector and the desired gene both have the same restriction enzyme site. This allows for the creation of the same "sticky" DNA ends as shown in the video to allow for the gene to stick to the plasmid. It's also important that you know where this restriction enzyme is cleaving.

DNA cloning and recombinant DNA (video) | Khan Academy

In some transformation experiments, a color-processing gene such as LacZ gene is utilized for confirmation of the molecular cloning (inserting a DNA fragment of interest into a plasmid vector). Plasmids with an uninterrupted LacZ gene turn their bacteria blue.

Recombinant Plasmid - an overview | ScienceDirect Topics

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