ENZYMATIC ACTIVITY

The incubation medium utilized to test an enzymatic activity contains (total volume = 100 µl) : 50 mM Tris, 2 mM DTT, 0.4 mM $MgCl_2$, 0.5 µg of oligonucleotide deoxypolyadenosine, 0.02 mM dGTP and a labeled nucleotide 0.5 µCi of [^{3}H]dGTP in addition to the enzyme whose activity is being tested.

1) Calculate concentration of each of the original solutions if you have to add 10 µl from each one into the incubation medium.

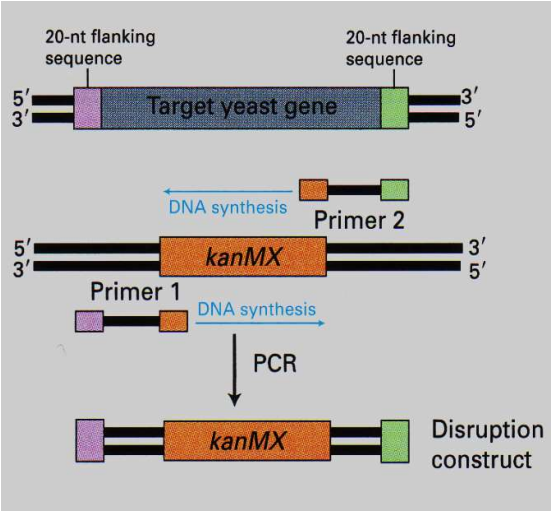
2) After 30 minutes of incubation at 37°C, the reaction is stopped by addition of EDTA. Solution is extracted with phenol/chloroform and the aqueous phase is precipitated with alcohol and salt. The precipitated material, which is radioactive, is dissolved in a buffer and radioactivity is measured. What is the product of the enzymatic reaction and the nature of the enzyme ?

Exercise: Give clearly a procedure to convert a 3'-overhang end of a DNA molecule into a blunt end.

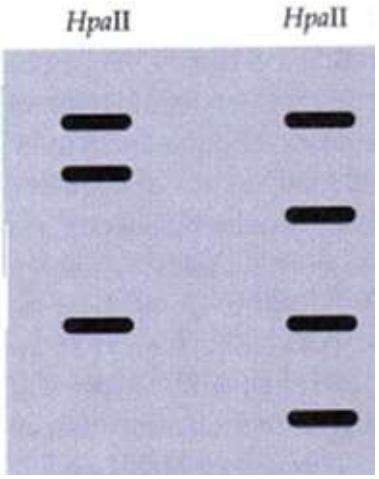
Exercise: Explain how to delete the underlined nucleotide (ATCGATCCTATTAAC); this sequence belongs to a cloned DNA inserted in a plasmid.

Exercise: give an experimental plan to determine whether a certain specific DNA sequence is methylated or not.

Exercise: comment the gene knock-out construct in the figure:



exercise: Digestion of DNA from the pancreas (right lane) and the intestine (left lane) with HpaII restriction enzyme followed by Southern blotting, using insulin gene probe, shows the result in the figure. Conclude and justify. (3 points).



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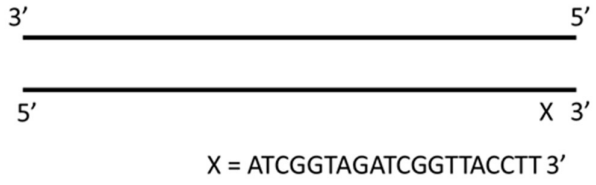
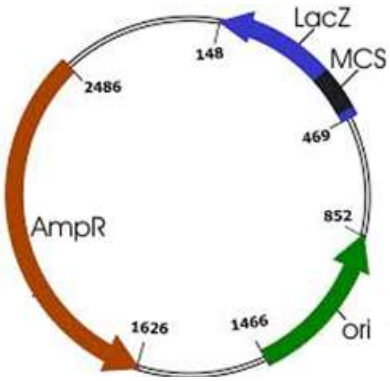
Exercise: Your aim is to amplify the following DNA fragment by PCR (the fragment is in a mixture of DNA types). You know the sequence of one extremity "X" that is indicated. You have no possibility to know the remaining sequence neither from database nor from partial peptide sequence.



Explain your method and the suitable molecular biology tools (enzyme(s), their substrate(s), the primers and their precise sequences) to reach your goal. (2.5 pts)

exercise: A genomic library is prepared using *Staphylococcus* genomic DNA. The used vector is shown in the figure. The vector is used to transform *E.coli* host cell.



Give the general name the used vector (1 point).

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Define clearly the *ori* sequence. (1 point)

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How can you select the *E. coli* cells that have received the vector? (1.5 points)

How to differentiate *E. coli* colonies containing the vector without insert from those containing a vector + insert? (1.5 points)

Exercise: You have to label a cloned DNA sample (ds) in your laboratory. The only available nucleotide is alpha ^{32}P-dATP. No other dNTP, no hexa-primer, no DNase-I are available. However, all DNA polymerase types are available in your laboratory. Choose one of them to perform labeling and explain.

exercise: Use the suitable molecular biology tools and techniques (<u>except</u> restriction enzymes) to insert AB segment of molecule (I) into (II). The final product (III) is <u>intact</u> circular dsDNA (answer by step). 5 pts.

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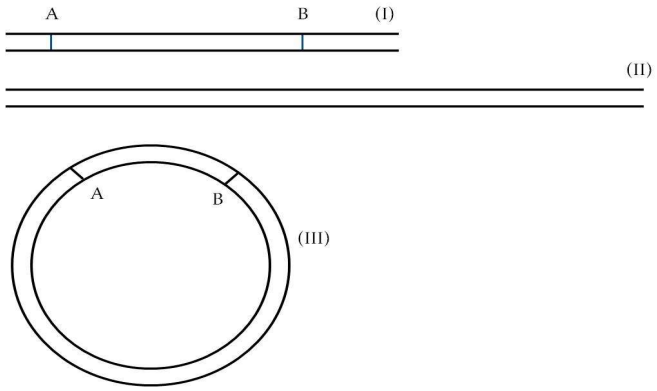


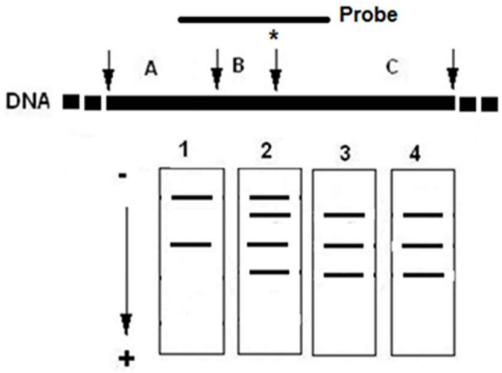
Ex: -The figure (next) shows the map of actin gene exon 1. The symbol "*" indicates a restriction enzyme site that results from a point mutation. All arrows refer to the same restriction enzyme site. The indicated probe is used to determine the genotype of 4 patients by RFLP-Southern blotting. The conclusion is:

a. patients 1, 3 and 4 carry only the normal allele.

b. patients 3 and 4 are homozygous mutant.

c. patient 1 is homozygous mutant.

d. patient 1 is heterozygous. e. the result is not conclusive.



ex: Exonuclease III is added to this DNA sample. Justify what may happen.

Is another enzyme able to remove the 3' overhangs? 5' overhangs?

A ligase is added to the reagents in the figure. What happens? Justify 1.5 pts.