What are the main components of a lysis buffer utilized to extract plasmids from bacteria? What is their function?Risposta obbligatoria. Testo su più righe.

SDS ,able to interact with the lipid part of the membrane, EDTA a chelant of covalent ions that essential cofactor for RNAasis and DNAasis,NAOH and KA to denaturate and rinaturate the DNA to separe plasmidic and genomic DNA, after is performed an ethanol precipitation and RNAasis is added to remove the RNA.

What are the "isoschizomers"?

Enzymes that are able to cut the same restriction site producing different ends, for example one produces 5' protruding sticky end an the other 3' protruding stiky end

You have to set up a ligase reaction. What are the reaction components that you need to insert into the test tube? What is the proper molar ratio between the insert and the plasmid vector you have to use?Risposta obbligatoria. Testo su riga singola.

(1 punto)

In the tube we should put the buffer (usually 10x) to recreate the condition in which the enzyme naturally work, the ligase the key enzyme of the reaction ATP which is essential for the enzyme to work. distilled water and last but not least insert and vector in a ratio of 3:1

How many colonies do you expect to obtain using 0.5 micrograms of plasmid DNA for a transformation of bacterial cells with the calcium chloride method? And how many with the same amount of DNA but using the electroporation method?Risposta obbligatoria. Testo su più righe.

(1 punto)

usually the efficiency of calcium chloride transformation is 10^6 for 1 microliter so i expect an efficiency of 0,5 x 10^6, for electroporation the yield is 10^9 so 0,5 x 10^9.

You have to clone a DNA fragment (200 bp) in a plasmid (2500 bp) using the XbaI restriction site. How many different types of recombinant DNA molecules do you expect to obtain? How can you discriminate between these using restriction enzymes? What are the expected dimensions of the restriction fragments in the different cases? Risposta obbligatoria. Testo su più righe.

(2 punti)

I expect two types of molecules since I'm cutting with a single restriction enzyme , one will have an orientation of the fragment that will put the EcoRI site closer to the right side of the vector (then closer to the other ecoRI restriction site on the plasmid itself) and another one with the insert oriented in the opposite direction so with the EcoRI site of the fragment closer to the PstI site of the vector, by using the position of the restriction site on the vector and the insert and the previous info I mentioned we can perform a digestion of the plasmid with EcoRI in order to separe the two type of orientation . In fact we expect to obtain two kind or reads from the electrophoresis: 1) a long linearized fragment that is around 450 bp, and a small fragment of few bw that would be the insert between eco RI (orientation of the fragment to the right) 2) a fragment of 200 bp more of less and one of 250 in case of orientation of the fragment with the Eco rI restriction site of the fragment itself closer to PstI of the vector.

Propose a cloning strategy to obtain the final construct, named VECTOR, using exclusively the restriction enzymes and the following plasmids. Risposta obbligatoria. Testo su più righe.

(5 punti)

The first thing i plan to do is to digest the gene vector with PstI and XbaI and the pBluescript with the same enzyme, to add more restriction site and i will obtain and unique kind of product(since I'm using two different enzymes) made in this way: XbaI, then the vector with SalI and HindIII site within and then PstI on the vector ,followed by SmaI, BamHI XhoI Eco RI SatI Not I. Then i would digest the obtained vector with XbaI and EcoRI and also with the same enzyme the vec. with the UTR and Promo inside. Performing the ligase i expect to obtain a uniquely oriented product (since i'm performing double digestion) with the gene placed in the middle of the vector itself in the small polylinker and the correct orientation of the gene seq compared to the utr and promo one.

What is the most used method to produce genomic DNA fragments to be cloned into phage vectors to create a genomic library? Risposta obbligatoria. Testo su più righe.

(1 punto)

Use restriction enzyme and partial digestion in fact in this way you can obtain not only various type of cut of the same DNA molecule, but the restriction enzyme produce sticky and it makes easier to clone he fragment

Respect to high-stringency molecular hybridizations, in low-stringency hybridizations the experimental conditions must allow the pairing of nucleic acid molecules only partially complementary to each other. What are the parameters that can be experimentally used to perform low-stringency hybridizations with heterologous probes? Risposta obbligatoria. Testo su più righe.

(1 punto)

We can keep a lower temperature and an higher salinity this will stabilize imperfect hybridization.

What is a Lamba ZAP vector and what is it usually used for?Risposta obbligatoria. Testo su più righe.

(1 punto)

Lambda Zap is a special kind of vector that have been produced introducing by a pBluescript plasmid cloned inside the two arms of the phagic vector, it also has inside a poli-linker. This allows us to infect like we would with a normal phage vector and screen the library like we would with a phage vect, but when needed we can take out from the phage the vector and make it become a proper plasmid using a helper phage encoding for a recombinase that is able to cut the cos sequences and make a circular plasmid, in this way I can work with a phage and switch when needed.

The following is the DNA sequence of the wild type allele of Gene Z that you want to amplify using the polymerase chain reaction (PCR). To amplify it through PCR what are the reaction components that you would absolutely need? Briefly state the function of each of these components.Risposta obbligatoria. Testo su più righe.

(1 punto)

Of course we would need the DNA template to be amplified, the two primer (forward and reverse) the will anneal to the denaturated DNA template fragment to allow the Taq polimerase to start the synthesis, Of course the enzyme so Taq polimerase , the dNTP used to built the complementary strand, distilled water and MgCl increasing and decreasing the MgCl concentration we can obtain an higher or lower stringency of the reaction (+MgCl> lower stringency) but we will raise the yeld. With big fragment rich in GC to be denaturated we may consider DMSO or BSA that will favor the denaturation

Select the set of primers from the options below, which you would use for a PCR reaction to amplify the gene Z. Risposta obbligatoria. Scelta singola.

(2 punti)

RIsposta 2, non riesco a copiare la sequenza

Calculate the annealing temperature of the two primers, A and B. The two primers amplify a 2Kb fragment. Project the PCR amplification cycle by defining the annealing temperature and the time of the extension step. Explain the reason for

your choices. A: 5'- GATGCTGCTAGTTGCTTAGG B: 5'- TCTTATGCTGTGCTGGATAG Amplification cycle 94 °C 5 min 94 °C 1 min] ___ °C 1 min] 30x 72 °C __min] 72 °C 10 min Risposta obbligatoria. Testo su più righe.

(3 punti)

My MT would be 58° because the primer mt is 60 and the other 58, so i chose the lower one aware that may be aspecific, for the extension step i wolud allow 2 min since is usually 1 min for 1kb

Imagine that the PCR of the previous exercise gives you as a result, in addition to the expected product of 2Kb, also 4 other non-specific products of various sizes, probably due to the incorrect pairing of one of the two primers. What amplification parameter, or which component in the reaction mixture can you modify to increase the specificity and thus obtain only the desired specific product?Risposta obbligatoria. Testo su più righe.

(2 punti)

i would lower the MgCl concentration i order to obtain a higher stringency, since i cannot raise the mt because otherwise i would not have the annealing of the 58° mt primer

Using the following table of the genetic code, draw the sequence of the degenerated oligonucleotide able to recognize the coding sequence for the amino acid box shown below. Would you use the inosine base? If yes, in what position? Please indicate it below. Nterm M - - I - - Y - - C - - R - - K - - W - - CtermRisposta obbligatoria. Testo su riga singola.

(4 punti)

my primer (the code IUPAC) i would create is : atgat(c/a)ta(t/c)tg(t/c)ag(a/g)aa(a/g)tgg , i would no use inosine since is used to substitute amino acid like Val which have a big variety of codons, in this case i have alteration of 2 nucleotide of the 3rd nucleotide of the codon itself

Draw and upload a picture of the constructs required to produce a GAL4-UAS binary expression system to obtain the expression of the GENE X only in adult Drosophila males.

(Domanda non anonima
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Risposta obbligatoria. .

(4 punti)