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Chapter 1 : Bioprocess Engineering Shuler Kargi Solutions Manual - Video Dailymotion

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We will practice transcription, translation, and post-transcriptional modification. Briefly, what is its function? Find the gene sequence from NCBI. Nucleotide sequence and of bases in the primary transcript. Erythropoietin is a protein secreted by the kidneys; it stimulates red blood cell production in bone marrow, treats anemia, and is an alternative to blood transfusion. It is one of the drugs that Lance Armstrong took. Pick from the pull-down menu "Nucleotide"; enter into the search field "human erythropoitin"; click "Search". A couple of DNA examples: Hereforth, we answer based on X Petpitde sqquence primary structure of protein CDS join These are marked red for EPO: N, N, and N and 1 O- glycosylation site S Reaction that converts one amino acid to another or to an organic acid. What is an auxotroph? How might a thymidine auxotrophic mutant of E. Thus, that nutrient must be provided for cell growth, unless the cell is provided with the missing gene via a plasmid that also contains the gene of interest say, insulin. Basically, whatever is missing in the chromosome, we provide it in a plasmid. Or, simply grow in nutrient containing thymidine to apply selection pressure. Given the gene of human erythropoietin from last homework, you are tasked to insert the gene into a plasmid named pUC19 or search for it at NCBI. Summary of sites in pUC19 To simplify purification, keep the signal peptide. Print out the nucleotide sequence from the intermediate steps. On the nucleotide sequence of the final recombinant plasmid, clearly mark: Ignoring the post-translational steps such as proper folding and glycosylation for now, will a bacterial host like E. Start with pUC19 and epo. Look for one restriction enzyme that cuts in: There is no one single restriction enzyme that can make these 3 cuts. Note that although both PspOMI and ApaI have the same recognition bases gggccc , the resulting sticky ends are incompatible. The sequence is chosen based on available restriction sites in pUC19 within beta-galactosidase CDS, usually within mcs Step 1c. The sequence is chosen based on available restriction sites in pUC19 within beta-galactosidase CDS, usually within mcs Step 1d. We find several combinations: There is no frame shift. Two different restriction sequences provie directionality, resulting in a higher probability of inserting EPO in the correct direction. The translation product has 41 extra peptides preceeding the EPO signal peptide.. EPO cc gggatgaggg cccccggtgt ggtcacccgg. We also add a stop codon "taa" to terminate the translation of beta-galactosidase and re-start the translation of EPO. Once the lacZ promoter is induced, there will be two translation products: Plate on a petri dish containing X-Gal 5-bromochloroindolyl galactopyranoside , which when digested by beta-galactosidase releases a blue product. After induction with and IPTG isopropyl thiogalactopyranoside , we select white colonies. The protein that is expressed has 41 extra peptides preceeding the EPO signal peptide. You decide to perform the PCR step to cut out the codons that correspond to these 41 extra peptides, so that the translation product is the human erythropoitin EPO , no more, no less. Provide the sequence of the forward and backward primers, say the standard 20 bases. Deleting bases means not copying these bases with PCR. The bases that correspond to the 41 extra peptide are stricken out. The flanking 20 bases are in red. If we intend to re-circularize the PCR product after we cut out the above bases , we might want to simultaneously introduce a common restriction site to both ends during PCR. Furthermore, we add a few extra bases "xxx" to help the restriction enzyme grab on to the end of to digest the linear PCR product to create sticky ends. After PCR, we digest with BamHI, then circularize and ligate; the new circular plasmid produces exactly the same primary translation product as human EPO, no more, no less. The PCR approach in Problem 3 of Homework 8, which results in the premature termination of the beta-galactosidase fragment and restarting EPO, works in a prokaryote host, but not in an eukaryote host. Thus, in practice, we perform this PCR-digestion-recircularization step after the initial cloning step if we are to place this plasmid in

an eukaryote host.

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