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Relative mitogenic activities of wild-type and retinoblastoma binding-defective SV40 T antigen in serum-deprived and senescent human diploid fibroblasts. Dissociation of retinoblastoma gene protein hyperphosphorylation and commitment to enter S Phase. Human fibroblast commitment to a senescence-like state in response to histone deacetylase inhibitors is cell cycle-dependent MCB, Ogryzko V, Schiltz R. The transcriptional coactivators p and CBP are histone acetyltransferases Cell , Avantaggiati M L, Ogryzko V. Ogryzko V, Wong, P. WAF1 retards S phase progression primarily by inhibition of cyclin-dependent kinases. Ogryzko V, Howard B. A histone deacetylase inhibitor potentiates retinoid receptor action in embrional carcinoma cells PNAS, Mol Cell Biol, 18 9: J Biol Chem Sep 18; Xenopus NF-Y pre-sets chromatin to potentiate p and acetylation-responsive transcription from the Xenopus hsp70 promoter in vivo. Molecular Cell, , 4 5: Mol Cell Biol,19 An IFN regulatory factor-2 DNA-binding domain dominant negative mutant exhibits altered cell growth and gene expression. J Biol Chem Mol Endocrinol 14 7: Mammalian histone acetyltransferase complexes. Medicina B Aires , 60 Suppl 2: The p complex is an essential E1A transformation target. Ssdp proteins interact with the LIM-domain-binding protein Ldb1 to regulate development. The retinoblastoma protein binds the promoter of the survival gene bcl-2 and regulates its transcription in epithelial cells through transcription factor AP Ogryzko Use of protein biotinylation in vivo for chromatin immunoprecipitation Anal. Coactivators p and PCAF physically and functionally interact with the foamy viral trans-activator. Codon optimization of the BirA enzyme leads to higher expression and an improved efficiency of biotinylation of target proteins in mammalian cells. Biotechnology, Mar 30; 3: Oligoribonuclease is a common downstream target of lithium-induced pAp accumulation in Escherichia coli and human cells. Analysis of the human histone H2AZ deposition in vivo argues against its direct role in epigenetic templating mechanisms. Mol Cell Biol, Jul;26 Deciphering of Formamidopyrimidine-DNA glycosylase interaction with its substrate by chemical cross-linking and mass spectrometry J. Regulation of an inducible promoter by an HP1beta-HP1gamma switch. Analysis of interaction partners of H4 histone by a new proteomics approach. Proteomics, Nov;9 Exploring dimethylsulfate for in vivo proteome footprinting. S pdf 3. Immuno-affinity purification of mammalian protein complexes Methods in Enzymology, ; Use of in vivo biotinylation for chromatin immunoprecipitation.

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Chapter 2 : Molecular and Cellular Biology - Wikipedia

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Chapter 3 : Tulane University -Cell & Molecular Biology

Molecular and Cellular Biology Â® (MCB) is devoted to the advancement and dissemination of fundamental knowledge concerning the molecular biology of all eukaryotic cells. For Authors ASM Author Center.

Relationship to other biological sciences[edit] Schematic relationship between biochemistry , genetics and molecular biology Researchers in molecular biology use specific techniques native to molecular biology but increasingly combine these with techniques and ideas from genetics and biochemistry. There is not a defined line between these disciplines. The figure to the right is a schematic that depicts one possible view of the relationships between the fields: Biochemists focus heavily on the role, function, and structure of biomolecules. The study of the chemistry behind biological processes and the synthesis of biologically active molecules are examples of biochemistry. This can often be inferred by the absence of a normal component e. The study of " mutants " â€" organisms which lack one or more functional components with respect to the so-called " wild type " or normal phenotype. Genetic interactions epistasis can often confound simple interpretations of such " knockout " studies. The central dogma of molecular biology where genetic material is transcribed into RNA and then translated into protein , despite being oversimplified, still provides a good starting point for understanding the field. The picture has been revised in light of emerging novel roles for RNA. In the early s, the study of gene structure and function, molecular genetics , has been among the most prominent sub-fields of molecular biology. Increasingly many other areas of biology focus on molecules, either directly studying interactions in their own right such as in cell biology and developmental biology , or indirectly, where molecular techniques are used to infer historical attributes of populations or species , as in fields in evolutionary biology such as population genetics and phylogenetics. There is also a long tradition of studying biomolecules "from the ground up" in biophysics. For more extensive list on nucleic acid methods, see nucleic acid methods. Molecular cloning Transduction image One of the most basic techniques of molecular biology to study protein function is molecular cloning. A vector has 3 distinctive features: Located upstream of the multiple cloning site are the promoter regions and the transcription start site which regulate the expression of cloned gene. This plasmid can be inserted into either bacterial or animal cells. Introducing DNA into bacterial cells can be done by transformation via uptake of naked DNA, conjugation via cell-cell contact or by transduction via viral vector. Introducing DNA into eukaryotic cells, such as animal cells, by physical or chemical means is called transfection. Several different transfection techniques are available, such as calcium phosphate transfection, electroporation , microinjection and liposome transfection. The plasmid may be integrated into the genome , resulting in a stable transfection, or may remain independent of the genome, called transient transfection. A variety of systems, such as inducible promoters and specific cell-signaling factors, are available to help express the protein of interest at high levels. Large quantities of a protein can then be extracted from the bacterial or eukaryotic cell. The protein can be tested for enzymatic activity under a variety of situations, the protein may be crystallized so its tertiary structure can be studied, or, in the pharmaceutical industry, the activity of new drugs against the protein can be studied. The reaction is extremely powerful and under perfect conditions could amplify one DNA molecule to become 1. The PCR technique can be used to introduce restriction enzyme sites to ends of DNA molecules, or to mutate particular bases of DNA, the latter is a method referred to as site-directed mutagenesis. Proteins can be separated on the basis of size by using an SDS-PAGE gel, or on the basis of size and their electric charge by using what is known as a 2D gel electrophoresis. DNA samples before or after restriction enzyme restriction endonuclease digestion are separated by gel electrophoresis and then transferred to a membrane by blotting via capillary action. The membrane is then exposed to a labeled DNA probe that has a complement base sequence to the sequence on the DNA of interest. These blots are still used for some applications, however, such as measuring transgene copy number in transgenic mice or in the engineering of gene knockout embryonic stem cell lines. Northern blot Northern blot diagram The northern blot is used to study the expression patterns of a specific type of RNA molecule as relative comparison among a set of different samples of RNA. It is essentially a combination of denaturing RNA gel electrophoresis , and a blot. In this process RNA is separated based on

size and is then transferred to a membrane that is then probed with a labeled complement of a sequence of interest. The results may be visualized through a variety of ways depending on the label used; however, most result in the revelation of bands representing the sizes of the RNA detected in sample. The intensity of these bands is related to the amount of the target RNA in the samples analyzed. The procedure is commonly used to study when and how much gene expression is occurring by measuring how much of that RNA is present in different samples. It is one of the most basic tools for determining at what time, and under what conditions, certain genes are expressed in living tissues. Western blot In western blotting , proteins are first separated by size, in a thin gel sandwiched between two glass plates in a technique known as SDS-PAGE. The proteins in the gel are then transferred to a polyvinylidene fluoride PVDF , nitrocellulose, nylon, or other support membrane. This membrane can then be probed with solutions of antibodies. Antibodies that specifically bind to the protein of interest can then be visualized by a variety of techniques, including colored products, chemiluminescence , or autoradiography. Often, the antibodies are labeled with enzymes. When a chemiluminescent substrate is exposed to the enzyme it allows detection. Using western blotting techniques allows not only detection but also quantitative analysis. Analogous methods to western blotting can be used to directly stain specific proteins in live cells or tissue sections. Eastern blot The eastern blotting technique is used to detect post-translational modification of proteins. Proteins blotted on to the PVDF or nitrocellulose membrane are probed for modifications using specific substrates.

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