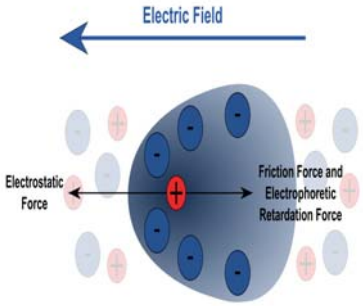
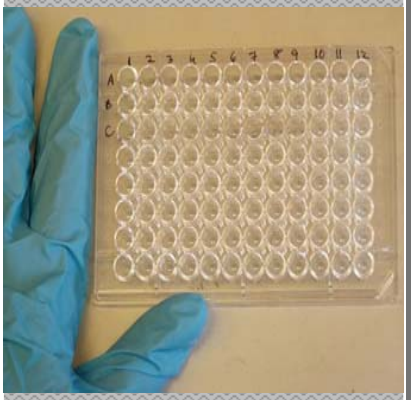




EMGEN Newsletter

Vol. 6, Issue 5

IN THIS ISSUE:

Eastern Mediterranean Health Genomics and Biotechnology Network (EMGEN) was created in 2004 with collaboration of representatives of selected centers of excellence in (health related) molecular biology, biotechnology & genomics in the Eastern Mediterranean region by recommendations and efforts of WHO/EMRO. Sponsored by Iran Biotechnology Development Council.

Address:
Biotechnology building, #69, Pasteur Ave., Pasteur Institute of Iran
Tehran, Iran, 13164
Tel: +98-21-66954324
Fax: +98-21-66465132
E-mail: emhgbn@gmail.com, emgen@pasteur.ac.ir
Websites: www.emgen.net
www.emhgbn.net

Prepared by: Monireh Ramandi
Page design: Mahdi Aalikhani
Assistant editor: Mahdi Aalikhani
Editor: Dr. Soroush Sardari

LAB ON A CHIP

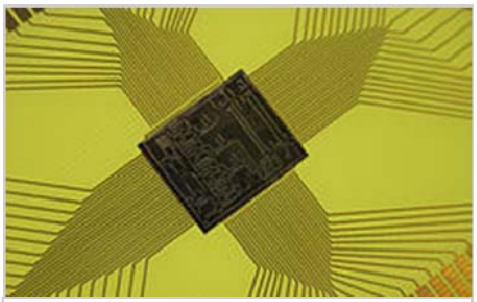


Figure 1: Microelectromechanical systems chip (lab on a chip).

A lab-on-a-chip (LOC) is a small apparatus that carry out single or a number of examines (e.g. DNA sequencing or biological recognition) on a solitary chip which is typically completed in a lab. LOC studies mostly emphases on humanoid disease recognition and DNA examination. Sometimes, it focuses on the biochemical productions. Shrinking the biochemical processes which generally handled in a lab has many benefits, for example cost reduction, coordination, quickness and sensibility. The expansion of the LOC mostly depends on two central knowledges: microfluidics and molecular biology. Microfluidic knowledge utilized in LOC apparatus let the construction of lots of microchannels, each gauging sheer micrometers, on a distinct chip that fits easily in the hand. The microchannels let working with trace amounts of liquids such as a few picoliters in addition to the running of biochemical responses in a slight size. Obviously, to empower all of these processes, LOC apparatus are not only an assembly of microchannels. They furthermore need combined pumps, electrodes, regulators, electrical fields and electronics to turn into a complete LOC analytical system. The core of the LOC vision is to mix hundreds of biochemical processes onto a particular chip that could be completed by using just a droplet of blood acquired from the patient with the intention of getting an exact analysis of possible illnesses. Right now, we are very far from this, but scientists are trying to achieve this goal as soon as possible.

Lab-on-a-chip applications:

1) Lab-on-a-chip and Molecular Biology

For DNA/RNA intensification and recognition, LOC enables high outcomes in terms of recognition quickness while conserving the same delicacy. Since the DNA intensification by PCR depends on thermal rounds, the capability to execute fast thermal changes at the microscale clarifies why LOC converted to the fastest way of doing PCR. For DNA and RNA sequencing, LOC delivered a novel domain of chances. The first humanoid genome sequencing took years and required the effort of hundreds of scholars to finish the job.Nowadays, by applying LOC to mix a collection of DNA probes, we are capable to sequence genomes very quicker. Additionally, nanopore knowledge, which yet requires to be improved, holds huge possibility in the forthcoming to

being quicker for genome sequencing than real LOC utilizing a collection of DNA probes. All the bimolecular processes which are completed by the LOC display huge possibility for ultra-fast bacteria and virus recognition, likewise for ailment biomarker recognition (DNA and RNA). Furthermore, the LOC has vast prospects for immunoassays, which can be finished very quicker than utilizing macroscopic skills. Also, in the molecular segregation arena, LOC show more effective segregation than the routine methods.

2) Lab-on-a-chip and Proteomics

In the field of proteomics LOC offers the chance to execute protein studies while mixing all the stages within an identical chip: extraction from the cell, segregation by electrophoresis, ingestion and examination by mass spectrometry. These combined procedures display the capability to significantly reduce protein examination from hours with macroscopic procedures to a few minutes with LOC. It demonstrates huge possibilities for protein crystallization as well. By LOC, scholars are capable to control the situation concurrently and in the fastest way which allowing the crystallization of a candidate protein. The most vital issue is the likelihood to significantly parallelize crystallization situation and examine their configurations using X-ray diffraction.

3) Lab-on-a-chip and Cell Biology

Since microchannels have a similar size as cells, LOC shortly turned its attention on cell biology. LOC exhibits the capability to regulate cells at the single-cell stage while dealing with a great quantity of cells in seconds. At the microscale level flow alteration can be very quick and decreases only in tens of milliseconds. Appling fast visual sensors can identify and separate a target cell with high output. There are numerous requests for LOC in cell biology, comprising micro patch pin, control of stem cell separation, high-speed flow cytometry and cell arranging.

4) Lab-on-a-chip and Chemistry

The capability to execute quick heating and cooling at the microscale permits greater efficacy in some chemical reactions. Then, more studies have been done by utilizing LOC as microsized and extremely parallelized micro chemical apparatuses. LOC apparatuses can similarly be at attention when treating with hazardous and flammable mixtures, since they have lower risks of treating with lesser amounts at a time.

Advantages of lab-on-a-chip compared to conventional technologies

- Low cost: LOC techniques will decline the price of the examination via reducing the price of data calculation. The combination will allow several tests to be done on a chip, reducing to a small price the cost of each separate examination.
- High parallelization: Due to its capability for mixing microchannels, LOC will allow hundreds of examines to be done concurrently on a chip. This will allow medics to aim definite diseases through the time of a deliberation till prescribe more rapidly and successfully the best suitable antibiotic or antiviral.
- User-friendliness and compactness: LOC permits the combination of a large quantity of processes in a skimpy size. Finally, a tiny chip attached to a machine as little as a laptop will permit examines similar to those done in full analytical labs. Diagnostics by LOC will need very less handling and difficult processes and usually they can be done by a nurse.
- Decline of human fault: As it will intensely decrease human action, automatic diagnoses completed by the LOC will significantly decrease the risk of human fault.
- Quicker reply time and analysis: At the micrometric size, dispersion of compounds, stream switch and distribution of heat is quicker.
- Low size samples: Since LOC techniques just need a small size of the samples (e.g. blood), this technique will decline the price of examination by decreasing the need of costly elements. It will allow the recognition of a great number of diseases without demanding great amounts of blood from patients.
- Real time examination and improved sensitivity: Because of rapid reactivity at the microscale, the atmosphere of a biochemical reaction can be examined in real time in the LOC, yielding more precise outcomes.
- Operative: Because of their low expense, automation and low energy expenditure, LOC policies will furthermore be capable to be utilized in outdoor locations for air and water observing sans the necessity of human action.
- Health for all the communities: LOC will decrease diagnostic prices, the need of medical personals and the price of structures. Consequently, LOC technique will make novel medication more available to communities at lower charges.

Limitations of lab-on-a-chip compared to conventional technologies

- Industrialization: Most LOC tools are not yet capable of employment. Concerning its main submission, the ultra-complex analysis, at this time it is not definite which usage will come to be the regular utilization?
- Signal/noise ratio: In some submissions shrinking the tools upsurges the signal/noise ratio and consequently, LOC delivers worse outcomes than conventional techniques.
- Morals and humanoid behavior: In the absence of rules, real time examination and the extensive availability of LOC may cause worries about the inexpert diagnosing. Furthermore, the DNA sequencing capability of LOC may allow everyone to sequence the DNA of others by a droplet of sputum.
- LOC requires an additional apparatus to work: Albeit LOC machines can be tiny and powerful, they need particular apparatuses (e.g. electronics or stream regulators) to work correctly. In the absence of an accurate structure to inoculate, separate and regulate the setting of samples, the LOC is unusable. Additional apparatuses raise the ultimate size and the price of the complete machine.

References:

1. Erickson D. and et al. (2014). Smartphone technology can be transformative to the deployment of lab-on-chip diagnostics. *Lab on a Chip*, 14(17): 3159-3164.
2. Gallegos D. and et al. (2013). Label-free biodetection using a smartphone. *Lab on a Chip*, 13(11): 2124-2132.
3. Abgrall P. and Gue A.M. (2007). Lab-on-chip technologies: making a microfluidic network and coupling it into a complete microsystem-a review. *Journal of Micromechanics and Microengineering*, 17 (5): R15.
4. https://en.wikipedia.org/wiki/Lab-on-a-chip

DNA VACCINATION

DNA inoculation is a method for defending an animal beside sickness by inoculating it with modified DNA, thus cells directly secrete an antigen, causing a defensive immunological answer. Numerous DNA vaccines have been generated for veterinary application, and there are increasing studies utilizing vaccines for bacterial, parasitic, and viral infections, in addition to numerous cancer varieties. While just one DNA vaccine has been accepted for human usage, these may have numerous possible benefits upon normal vaccines, comprising the capability to stimulate a broader spectrum of immune responses.

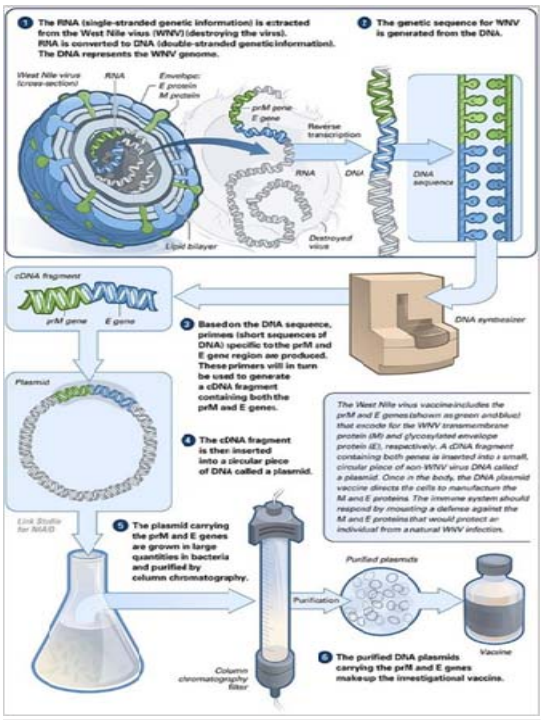


Figure 1: DNA vaccine production.

Current usage

None DNA vaccines have been accepted for humanoid usage in the US till yet. Up until now, scarce trials have suggested a good outcome in regard of the efficacy of DNA vaccines versus ailments, while tempting, it needs to be decisively confirmed in humans. As of June 2015 just one DNA vaccine has been accepted for humanoid usage, the single-dose Japanese encephalitis vaccine named IMOJEV, introduced in 2010. Nevertheless, a veterinary DNA vaccine to defend horses from West Nile virus has been accepted. In August 2007, an initial research in DNA inoculation versus MS was described as being effectual.

Advantages of DNA vaccines

Inoculation with no danger of infection; antigen presentation by both MHC class I and class II; capable to separate T-cells; immune answer centralized solitary on the desired antigen; simplicity of expansion and manufacture; constancy of vaccine for storing and delivering; economical; eliminate the necessity for peptide production and refinement of recombinant proteins; long-term durability of immunogen.

Disadvantages of DNA vaccines

Restricted to protein immunogens; danger of influencing genes regulating cell growth; probability of causing antibody synthesis against DNA; probably of endurance to the antigen (protein) produced; likelihood of abnormal processing of bacterial and parasite proteins.

Vector design

DNA vaccines provoke the greatest immune answer when extremely powerful expression carriers are utilized. Accordingly, plasmids with a powerful viral promoter to motivate *in vivo* expression of the target gene can be applied. Intron A can be added to increase mRNA constancy and hereafter upsurge protein expression. Such plasmids moreover comprise a powerful polyadenylation/transcriptional ending indicator, e.g. bovine growth hormone or rabbit beta-globulin polyadenylation structures. Multicistronic carriers are occasionally made to produce more than one immunogen, or to produce an immunogen and an immunostimulatory protein.

Mechanism of plasmids

When the plasmid imports itself into the nucleus of the cell, it begins to encode for a gene causing the creation of a peptide strand of an exotic antigen. The cell on its external exhibits the exotic antigen with both histocompatibility complex (MHC) classes I and II particles. The antigen-presenting cell (APC) then moves to the lymph nodes and renders the antigen peptide and stimulant molecule activated by T-cell causes the beginning of the immune answer.

Vaccine insert strategy

Immunogens can be directed to different cellular parts to enhance antibody or cytotoxic T-cell activations. Discharged or plasma membrane-bound antigens are more impressive to bringing antibody answers than cytosolic antigens, while cytotoxic T-cell activation can be enhanced by directing antigens for cytoplasmic destruction and following entry to the MHC class I pathway. This is typically done by the adding of N-terminal ubiquitin indications.

Delivery methods

DNA vaccines have been used in animal tissues by dissimilar procedures. The two most common procedures are inoculation of DNA in saline by a typical hypodermic needle, and gene gun transfer. Inoculation in saline is ordinarily done intramuscularly in skeletal muscle, or intradermally, with DNA being transported to the extracellular areas. This can be aided by electroporation; by momentarily injuring muscle fibers with myotoxins (e.g. bupivacaine); or by applying hypertonic dilutions of saline or sucrose. Immune replies to this technique of distribution can be affected by numerous elements, comprising needle form, needle placement, speed of inoculation, size of inoculation, muscle type, and age, gender and physiological circumstance of the vaccinated animal. Gene gun transfer, the other frequently used technique, ballistically speeds up the injection of plasmid that has been mixed with gold or tungsten microparticles, utilizing squeezed helium as an accelerator.

Another transfer approach is spraying the bare DNA on mucosal areas, as well as the nasal and lung mucosa, and local directing the plasmid to the eye and vaginal mucosa. Mucosal transport has moreover been attained by cationic liposome-DNA particles, recyclable microspheres, weakened *Salomenalla*, *Shigella* or *Listeria* carriers for oral direction to the intestinal mucosa, and recombinant adenovirus carriers. Another carrier is a hybrid transporter made of bacteria cell and artificial polymers. An *E. coli* inner core and poly beta-amino ester covering work perfectly to enhance the plasmid transport by addressing hurdles related to antigen-presenting cell which comprises cellular absorption, phagosomal abscond and intracellular load doping. Tried on mice, the hybrid carrier was found to prompt immune response.

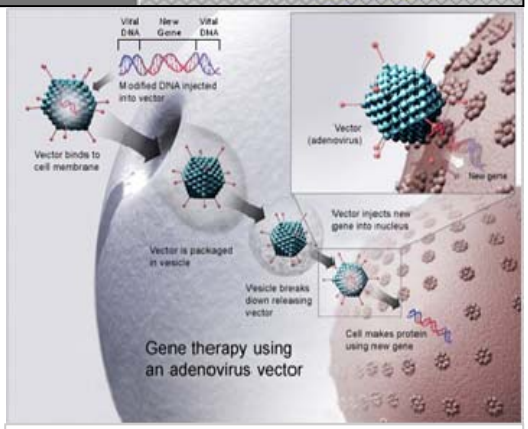


Figure 2: DNA vaccine and gene therapy techniques are similar.

The technique of transport defines the dose of DNA necessary to increase an operational immune answer. Saline inoculations necessitate flexible quantities of DNA, from 10 μg^{-1} mg, while gene gun transfers need 100 to 1000 times less DNA than intramuscular saline inoculation to increase an actual immune answer. Usually, 0.2 µg – 20 µg are necessary, while amounts as low as 16 ng were tested. These amounts differ from classes to classes, with mice, for instance, needing about 10 times less DNA than mammals. Saline inoculations need more DNA since the DNA is transported to the extracellular areas of the target tissue, where it has to vanquish physical barriers before it is absorbed by the cells, while gene gun transfers bombard DNA straightly into the cells, causing less "wastage". Alternative method to DNA inoculation is expression library immunization (ELI). By this method, possibly all the genes from a pathogen can be transported instantly, which may be valuable for pathogens which are hard to weaken or culture. ELI can be applied to recognize which of the pathogen's genes produce a defensive answer. This has been verified with *Mycoplasma pulmonis*, a murine lung pathogen with a comparatively small genome, and it is released that even incomplete ELIs can bring protection from succeeding incidence.

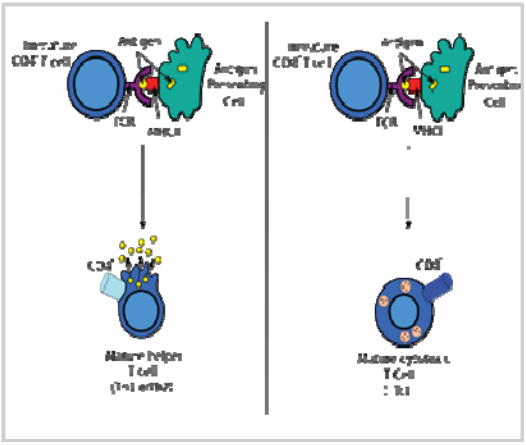


Figure 3: Antigen presenting via APC cells.

Immune response raised by DNA vaccines

1) T helper cell (TH) answers

DNA inoculation is talented to increase a variety of TH replies, comprising lympho-proliferation and the genesis of a range of cytokine profiles. A primary benefit of DNA inoculations is the simplicity with which they can be operated to distinguish the type of T-cell help in the direction of a TH1 or TH2 answer.

2) Cytotoxic T-cell answers

One of the best benefits of DNA inoculations is that they are capable to activate cytotoxic T lymphocytes (CTL) deprived of the intrinsic danger related with live inoculations. CTL answers can be increased versus immune-dominant and immune-recessive CTL epitopes, in addition to subdominant CTL epitopes, in a way which seems to simulate normal infection.

3) Humoral (antibody) answer

Antibody answers provoked by DNA inoculations are under the impression of numerous changeable, for example kind of antigen used; position of used antigen; quantity, the incidence and dose of injections; placement and technique of antigen transfer, and etc.

References:

1. Alarcon J.B., Waine G.W. and McManus D.P. (1999). DNA vaccines: technology and application as anti-parasite and anti-microbial agents. *Advances in parasitology*, 42: 343-410.
2. Robinson H.L. and Pertmer T.M. (2000). DNA vaccines for viral infections: basic studies and applications. *Advances in virus research*, 55: 1-74.
3. Widera G. and et al. (2000). Increased DNA vaccine delivery and immunogenicity by electroporation in vivo. *The Journal of Immunology*, 164(9): 4635-4640.
4. Stüve O., Eagar T.N., Frohman E.M. and Cravens P.D., (2007). DNA plasmid vaccination for multiple sclerosis. *Archives of neurology*, 64(10): 1385-1386.
5. https://en.wikipedia.org/wiki/DNA_vaccination

STEM CELLS FEEL THE FORCE

All cells have a similar genetic code; it is not important they are liver or blood cells. Nevertheless, these cells are in contact with very dissimilar kinds of atmospheres and situations. Scientists recognize that cells reply to external stimulants by altering their structure and their gene expression to be better appropriate for their precise surroundings and to be capable to perform their particular duties. So far the exact mechanisms of this behavior are not understood. To evaluate how skin cells reply to stimulants, scholars utilized a singular mechanical method to subject skin stem cells to analogous mechanical stimulants that they would face in the tissues. The examination of the gene regulation of these stem cells by next generation sequencing showed that hundreds of genes decreased their activity, while very few genes amplified their products. This caused an extensive suppression of the transcriptional activity of the cell, which shows that fewer DNA is copied to mRNA to produce proteins. For a stem cell to mature, it requires to express a huge amount of genes to obtain its particular construction and utility. Because of the mechanical forces, utilized stem cells would not mature in the attendance of a stimulant. By investigating the results and the cellular process of the DNA reorganizations, scientists realized that the mechanical stimulants were being detected at the nuclear cover, a configuration that environs the DNA and detaches it from the other parts of the cell. A crucial molecule in this process was a protein named emerin, which attaches the nucleus and DNA to the cytoskeleton, the supporting construction of the cell. This was very exciting, as emerin is altered in an ailment named Emery-Dreifus muscular dystrophy, which brings a collapse of mechanically important tissues, e.g. the skeletal muscle, heart, and skin. As the mechanical possessions of tissues alter with age, an additional goal is to recognize how aged stem cells feel stimulants.

Reference: https://www.sciencedaily.com/releases/2016/07/160712110249.htm

RAT STUDY SHOWS GUT MICROBES PLAY A ROLE IN COLON CANCER SUSCEPTIBILITY

The tiny creatures that live in our intestines have more benefits than digest nutrition. A novel research in mice provided some evidences that the intricate combination of germs lived in the intestine, recognized as intestinal

microbiota, may affect an individual's probability of emerging colon tumor. Former investigations in hominids have presented that the tumor is related to alterations in intestine microbiota. In a recent examination, scholars utilized mice to discover the probable association among tumor and germs in the intestine. They inserted embryos from a phylum of mice genetically modified to produce colon tumor into the wombs of 3 other strains, each with different intestinal microbiota. Following, the microbiota of the newborns, which naturally produce tumors in ages 2 to 4 months, simulated that of their successor mothers. The scientists searched for tumors when the newborns had attained 6 months of age and realized that LEW strain mice produced meaningfully less tumors than the other strains. Actually, 2 mice with the LEW strain intestinal microbiota did not produce colon tumors anyways. Additionally, scientists discovered more tumors in the F344 strain that had greater amounts of *Peptococcaceae* and *Akkermansia muciniphila* microbes in their intestine. Generally, the outcomes of this research offer a different vision about the role of the intestinal microbiota as an intermediate and a forecaster of tumors in this mouse model.

Reference: https://www.sciencedaily.com/releases/2016/07/160713100901.htm

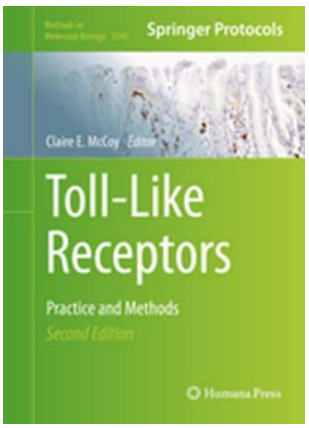
DISCOVERING THE MUTATIONS BEHIND DRUG RESISTANCE

In a recent exploration, scientists have established a quick and cheap method to discover the gene alterations related to medicine resistance. They utilized baker's yeast to examine the method in the presence of methotrexate resistance and recognized 10 alterations that can offer resistance to the medicine, proposing novel clues that could assist experts make the medicine more effective. The technique they established is a "back-to-the-future" tactic that merges an old-style examination method for recognizing sources of medicine resistance with newer methods of "greatly parallel" and "deep" genomic sequencing, accompanied by advanced arithmetical algorithms. This approach is an important progress over current methods to comprehend the genetic alterations related to medicine resistance. The method`s achievement in recognizing methotrexate resistance alterations validates its possible applications for realizing not only the appliances related to resistance for this key chemotherapy factor, however possibly for numerous other medicines as well.

Reference: https://www.sciencedaily.com/releases/2016/07/160713100905.htm

Book Alert

TOLL-LIKE RECEPTORS



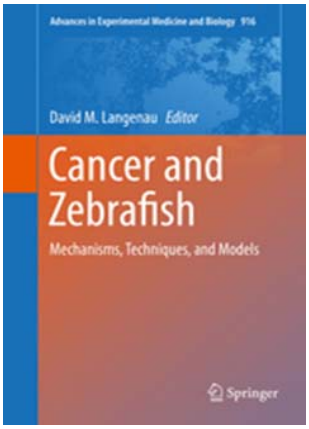
Publisher: Springer international publishing

Authors: Claire E. McCoy

Publication Date: 2016

ISBN: 978-1-4939-3335-8

CANCER AND ZEBRAFISH



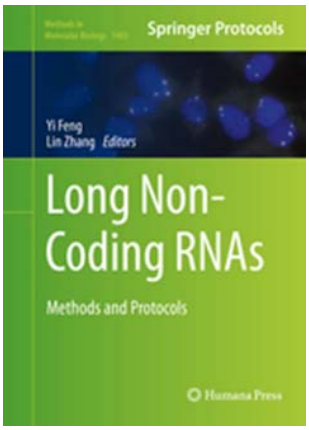
Publisher: Springer international publishing

Authors: David M. Langenau

Publication Date: 2016

ISBN: 978-3-319-30654-4

LONG NON-CODING RNAS



Publisher: Springer international publishing

Authors: Yi Feng and Lin Zhang

Publication Date: 2016

ISBN: 978-1-4939-3378-5

NEW BIOTECHNOLOGY

Scope: Science of biotechnology and its surrounding political, business and financial milieu.

Impact Factor: 3.199

ISSN: 1871-6784

COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY: PART D: GENOMICS AND PROTEOMICS

Scope: Comprehensive approaches to comparative biochemistry and physiology comprising " -omics", e.g., genomics, functional genomics (transcriptomics), proteomics, metabolomics, and underlying bioinformatics.

Impact Factor: 2.254

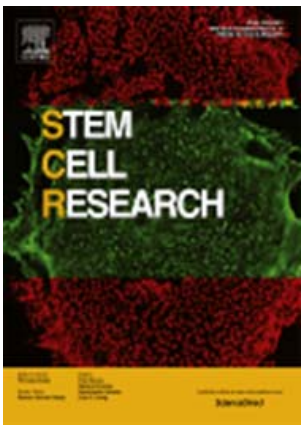
ISSN: 1744-117X

STEM CELL RESEARCH

Scope: Biology and applications of stem cell research, including embryonic stem cells, tissue-specific stem cells, cancer stem cells, developmental studies, stem cell genomes, and translational research.

Impact Factor: 3.892

ISSN: 1873-5061



Announcements

http://www.biologicalproduction.com/

http://www.icbbb.org/

http://icnnn.org/

GAS CHROMATOGRAPHY

Chromatography is a term for introducing a set of lab practices for the segregation of combinations. Chromatography is a physical process of segregation that allocates constituents to two distinct phases, one static and the other ambulatory, moving in an apparent rout. The combination is dissolved in a liquid titled the ambulatory phase, which transmits it into a construction, holding another substantial termed the static phase. The dissimilar components of the combination move at diverse speeds, forcing them to distinct. The parting is grounded on disparity separating among the ambulatory and static phases. Insensible variances in a mixture's partition constant cause disparity separating on the static phase and accordingly shifting the departure. Chromatography can be preliminary or analytic. The aim of preliminary chromatography is to distinct the constituents of a mixture for later use (and is therefore a type of purification). Analytic chromatography is completed usually with lesser quantities of substances and is for gauging the comparative extents of analytes in a combination.

Reference: https://en.wikipedia.org/wiki/Chromatography

ELECTROPHORESIS

Electrophoresis is the movement of discrete elements relevant to a liquid under the effect of a spatially identical electric arena. This electrokinetic aspect was detected for the first time in 1807 by F.F. Reuss, who saw that the use of a continuous electric arena evinced argil elements discrete in water to transfer. It is produced by the attendance of an charged interaction among the element superficial and the adjacent liquid. It is the fundamental of several diagnostic methods applied in chemistry for splitting particles by size, charge, or binding tendency. Electrophoresis of positively charged atoms is titled cataphoresis, whereas electrophoresis of negatively charged atoms is titled anaphoresis. Electrophoresis is a practice utilized in the labs to distinct macromolecules according to the size.

The method produces a negative charge so proteins travel on the way to the positive charge end. This method is applied for both DNA and RNA examination. Polyacrylamide gel electrophoresis (PAGE) has a stronger lucidity than agarose and is more appropriate for computable examinations.

In this practice DNA foot-printing can show how proteins attach to DNA. It can be utilized to distinct proteins by size, density and clarity. Moreover, it can be utilized for plasmid examination, which improves our knowledge regarding the microbe`s specifications.

Reference: https://en.wikipedia.org/wiki/Electrophoresis

ELISA

The Enzyme-Linked Immunosorbent Assay (ELISA) is an examination that utilizes antibodies and dye alteration to recognize a material. ELISA is a general setup of "wet-lab" kind analytic biochemistry examination that applies a solid-phase enzyme immunoassay (EIA) to identify the attendance of a material, generally an antigen, in a watery or moist specimen. The ELISA has been applied as an analytic instrument in medicine and herb pathology, in addition to an modality control screening in numerous activities. Antigens from the specimen are connected to a superficial. Afterward, a definite antibody is utilized on the superficial, thus it can connect to the antigen. This antibody is attached to an enzyme, and, in the last phase, a material comprising the enzyme`s substrate is subjoined. The following reaction creates a visible indication, most usually a color alteration in the substrate. Executing an ELISA needs at least one antibody with specificity for a specific antigen. The specimen with an unidentified quantity of antigen is fixed on a hard surface. After that antigen is fixed, the recognition antibody is subjoined, creating a complex with the antigen. The recognition antibody can be covalently attached to an enzyme, or can itself be identified by an inferior antibody that is attached to an enzyme. At the end of every stage, the dish is rinsed with a moderate cleanser dilution to eliminate any antibodies that are not attached. Next to the last rinsing stage, the dish is advanced by the addition of an enzymatic substrate to create a detectable indication, which specifies the amount of antigen in the specimen.

Reference: https://en.wikipedia.org/wiki/ELISA