2008 SABRE Participants

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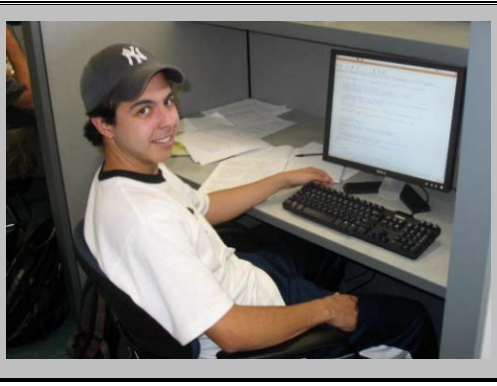
TAILORING SELECTION OF BINDERS AGAINST STREPTAVIDIN IN MICROFLUIDIC PHAGE DISPLAY

Phage display is a selection technique in which a specific peptide or protein is fused within a coat protein of a bacteriophage that results in the display of a fused protein on the surface of the virion. Due to its ability to select peptide ligands for a particular target, it has been widely used in the areas of discovering drugs and vaccines, new materials, and epitopes and is well known in the field of molecular biology. The conventional phage display has the disadvantages such as the use of a huge amount of target and uncontrolled, labor-extensive washing conditions. In our research, we employ streptavidin as our model target and tailor the stringency of the selection by decreasing the amount of the target and increasing the washing time while in search of the binding motif HPQ. By taking advantage of Continuous Trapping-Magnetic Activated Cell Sorter (CT-MACS) with the merits of handling a small amount of molecules and controlled washing which was recently developed in our lab, we demonstrate that the microfluidic device has the potential to be a powerful tool in peptide selection system. This newly developed microfluidic device aids in the rapid selection of peptides for a variety of targets.



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ASSESSING THE PERFORMANCE OF KALMAN FILTERING TECHNIQUES TO ESTIMATE THE PARAMETERS IN MODELS OF GENE EXPRESSION USING C CODE

Models of biological systems often have many unknown and unmeasurable parameters. The Kalman Filter (KF) algorithm can be a practical tool to estimate such parameters, so investigating its accuracy may turn out to be important for future experimentation in biology. The KF is a recursive algorithm that uses a model and time-series measurements from the process described by the model to give an estimate of a parameter. The measurements are assumed to be discrete-time, and they are characterized by their frequency (which sets the sampling time of the filter) and by their reliability, expressed as the amount of noise they contain. Using C code, we implemented a KF algorithm applied to a simple model of gene expression. Simulations show that there is an interval of sampling times in which the KF can estimate the parameters in the model with a high level of accuracy. Within this interval, there is an optimal sampling time where the accuracy is the highest. The estimates are accurate even for measurements with high noise levels. However, the first order KF algorithm we used could not estimate more than one parameter at a time and in some cases the estimates were not close to the real values. Further work will investigate higher order approaches, which are expected to fix these shortcomings.

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DEVELOPMENT AND OPTIMIZATION OF A LATERAL FLOW ASSAY FOR THE DETECTION OF BOTULINUM NEUROTOXIN SEROTYPE A

Clostridium Botulinum is a gram-positive bacteria whose different phenotypes give rise to seven different Botulism Neurotoxins, known as Serotypes A-G, throughout its development. *C. botulinum* can be found in contaminated water, in undercooked meat products, on the surface of low acid fruits and vegetables, and as spores in soil. These toxins are known to be the deadliest neurotoxin worldwide; a single gram of Botulism Neurotoxin Serotype A (BoNT/A) can kill ~1 million people if properly distributed. Botulism is an infection that attacks nerve terminals at myoneural junctions, thus blocking neuromuscular signals, preventing muscle contraction, and leading to paralysis. Current diagnosis methods include electromyograms (EMGs), lumbar punctures, serum and stool testing, and Computerized Axial Tomographies (CAT scans), all of which are long, tedious processes. The goals of this project are to isolate and identify peptides that will bind to the heavy chain of BoNT/A, and to construct a lateral flow assay that, upon exposure to a small liquid sample, will detect the toxin in less than half an hour. The development of this hand-held assay will prove to be a great benefit to both patients and doctors as it will severely reduce diagnosis time and cost.

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STUDY OF THE ROLE OF THE GENE OTX2 IN THE DEVELOPMENT OF RETINAL PIGMENT EPITHELIUM

The production of retinal pigment epithelium (RPE) from human embryonic stem cells (hESC) is a possible line of therapy for age-related macular degeneration (AMD) which is caused by the death of RPE. The primary objective of this research is to test the hypothesis that hESC differentiation to RPE follows the same pathway as in-vivo RPE development and requires the gene Otx2. This gene plays a co-regulatory role with the gene Mitf and is instrumental in the development of the eye and is a transcription factor of other genes namely;QNR71, TRP-1 and tyrosinase which are involved in differentiation of RPE. However, its role in hESC to RPE differentiation has yet to be tested. A Loss-of-function approach will be used to test the role of Otx2 in hESC to RPE differentiation. The main technique is RNA interference (RNAi), which is the sequence-specific, post-transcriptional gene silencing mediated by double-stranded RNA (dsRNA). We will construct lentivirus to deliver dsRNA to knockdown the expression of the Otx2 gene. A virus is used because it is the most effective way to insert nucleic acids into a cell. Following Otx2 knockdown, we will test the ability of hESC to differentiate into RPE. Differentiation will be assessed by pigmentation, immunocytochemistry and quantitative PCR. This research will help us understand how similar hESC derived RPE are compared to native RPE, a necessary first step in the development of a cell-based therapy for AMD.