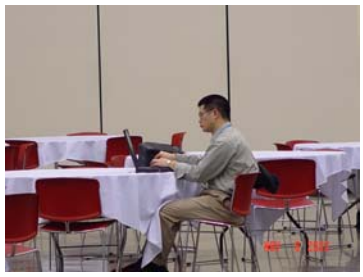
Nov 07, 2002

학회 5 일째를 맞이하고 있습니다만, 각 session 중에는 이미 발표가 마무리되어서 오늘 아침에는 많은 사람들이 떠나는 모습을 보이고 있습니다. ½는 이미 자신의 학교나 직장으로 돌아갔고 남아있는 사람들은 오늘과 내일의 발표에 관련된 사람들 뿐입니다. 시장과 학회장은 좀 북적여야 제맛인데 덜 붐비니까 재미는 좀 반감되네요. 썰렁한 남대문시장...보기 안좋잖아요.. 하지만 마지막까지 최선을 다하는 모습을 보이는 발표자나 청중들은 여전히 열정적인 모습입니다. 복도바닥에 앉아서 노트북을 펴놓은채 발표연습을 하는 사람들의 모습은 또 역시 이 학회에서나 볼 수 있는 아름다운 모습입니다. 눈에 띄는 점은 중국인 학자들과 인도 학자들이 두드러지게 많다는 점입니다. 인구를 떠올려보면 그리 놀라울 것은 없습니다만, 눈으로 직접 확인하고 이미 하나의 세력으로 자라나버린 그들을 보면서 부럽기도 하고 묘한 라이벌의식을 느끼기도 했습니다. 아...오늘은 영어를 한번 짚어야하지 않을까 싶습니다. 필자도 유학초기 영어때문에 고생을 했었습니다만, 여전히 native speaker 들과 속깊은 이야기를 나눌 만큼은 필자도 아님을 고백합니다.. 그러나. 국내의 대학원생들에게 영어의 중요성을 다시한번 강하게 강조하고 싶습니다. 자신의 연구를 만국공통어인 영어로 충분히 설명할 수 있어야 연구결과를 인정받을 수 있음은 이미 아실 것입니다. 필자의 견해로는 paper 와 talk 만이 연구자의 무기라는 생각이 듭니다. 이 점에 대해서는 필자도 돌아보고 반성하는 부분이 없지않습니다만, 이제 연구를 시작하려



하거나 과정 중에 있는 학생들은 꼭 다시한번 되새겨 보시기 바랍니다. 영어가 필요하다는 것은 몸으로 느낄 때는 이미 늦어버릴 때가 참 많습니다. 노인네같이 이야기한다고 거부감을 표시하는 독자도 계시겠지만 사실은 사실이니 군더더기를 더 붙이지 않아도 당연히 이렇게 이야기할 수 밖에 없네요. 미국에 와서 자신이 먹고 싶은 음식 하나 제대로 주문하지 못하는데 학회에서 다른 사람을 영어로 어떻게 설득할 수 있겠습니까? 때론 그들 중에는 자신의 이론과 배치되는 이론을 믿고 있는 사람들도 있을 텐데 말입니다. 영어는 기본이 되어야 한다는 생각이 참 많이 듭니다. 영어는 습관이어야 합니다. 미국 유학시절 처음 미국에 온 한국학생 중에 영어가 잘 안되던 사람들을 몇몇 보았습니다. 그러나 오랫만에 이곳에서 만난 그들은 이미 미국인들과 어울려 영어로 토론을 하고 질의응답을 하는 수준에 있었습니다. 그들이 영어를 따로 공부할 시간이 과연 학위 중에 있었을까요? 당근 없었습니다. 살기 위해서 영어로 매일 이야기를 하고 때로는 미팅을 위해 밤새워 영어로 글을 써서 달달달달 외웠을 지도 모릅니다. 매일 아침 미국학생들과 수업을 들어야 하고 가족들을 위해 물건을 사러 가면서 때로는 망신도 당하고 면박도 당하면서 습관화되었을 것입니다. 영어를 따로 공부하겠다고 두꺼운 toefl 이나 toeic 책을 들고 다니지만 말고 생활에서 익숙해지시기 바랍니다. 여기와서 보시면 발음을 이상하게 하는 외국사람들 참 많습니다. 그러나 의사소통이 다 잘되고 서로 웃고떠들며 이해하기도 합니다. 발음을 미국인처럼 잘 하면 좋겠지만 그게 아니어도 상관이 없습니다. 다만 익숙해져야 한다는 것입니다. 익숙해지면 자연스러워지고 자연스러워지면 설령 내 말을 이해못하는 미국인에게 영어로 두번 세번 다시 설명해 줄 수 있는 여유가 생길 것입니다. 당황하고 부끄러워하고 X 팔려서 안으로 숨어 들어가면 영어는 늘지 않습니다. 필자가 처음 미국에 왔을 때 이야기를 하나 하죠. Grocery store 에 가서 물건을 몇개 집어들고 계산대에 갔습니다. 계산하는 점원이 필자에게 paper or plastic?이라고 물었죠. 필자는 그것이 cash 로 계산할래, 아니면 credit card 로 계산할래? 라는 소리로 알아들어서 (사실은 혼자 때려맞추고는) cash 로 한다고 대답했습니다. 어이없어 하면 여러번 묻는 그 종업원에게 끝까지 cash 로 한다고 우겼던 기억이 납니다. 지금 생각해 보면 실소를 금할 수 없는 헤프닝입니다만 그 단어 중에 어느 하나 필자가 모르는 단어가 있었겠습니까? Paper 는 paper 고 plastic 은 plastic 인데 말입니다. 그래도 필자는 이해하지 못했었고 대화에서 role 을 하지 못했었습니다. 모든 미국인의 생활에 우리가 익숙할 필요는 물론 없습니다만 적어도 국제학회에서 이야기를 나눌 수 있을 정도의 영어실력과 방법만은 알고 있어야 미래를 기약하고 현재를 도약의 발판으로 삼을 수 있을 것입니다. 빨리 귀국해서 영어를 좀 더 해야겠다는 생각이 드네요. 도데체 영어를 언제까지 이렇게 잡고 늘어져야 하는 걸까요? 젠장…

오늘 필자가 이렇게 엉뚱한 이야기만 늘어놓는 것은 오늘 강의들이 필자의 연구분야와 동떨어졌으며 매우 specific 하고 깊이가 있어서 재미가 없었기 때문입니다. 사실 박사라는 학위는 자기 분야 안에서만 의미가 있는 것이지 다른 모든 분야까지 아는 것은 아니니 말입니다. 필자가 재미가 없었으니 필자의 글은 당연히 흥미가 떨어질 것이고 독자들은 읽다가 손을 놓게 될 것입니다. 하지만, 그래도 그냥 가기는 섭하니 오늘 강연들을 간단하게 정리해보겠습니다.

[324] - Advances in Genomics

Chair: Ryan T Gill, University of Colorado

Vice Chair: Matthew P DeLisa, University of Texas-Austin

Session Schedule

8:30 AM	Normalization-Hybridization : A Novel Approach to cDNA Microarray Assays **Abstract:** A mathematical model of cDNA microarray hybridization that incorporates the dynamics of diffusion of labeled strands to the surface and reaction with immobilized strands has been derived. Due to inherent differences in the mRNA concentrations of rare and abundant sequences, the fluorescence intensity for spots corresponding to these sequences differs by three orders of magnitude. This may lead to inaccurate estimations of fluorescence measurement because of detector saturation for high fluorescence values and interference due to background fluorescence for low intensity values. It would be advantageous to have the absolute fluorescence intensity in a narrow range of values while maintaining the relative fluorescence intensity corresponding to the two fluorophores at each spot. We present a strategy that exploits the nonlinear nature of DNA hybridization kinetics to achieve this goal. The modified protocol (called Normalization-Hybridization) uses labeled, melted double stranded cDNA in solution instead of single strands. Such a strategy allows for the normalization of single strand concentration corresponding to rare and abundant sequences because of renaturation in the solution phase. We describe model simulations and laboratory experiments carried out for demonstrating the validity of this approach, and present quantitative results for the absolute intensity and relative fluorescence levels for sequences present at defined concentrations corresponding to rare and abundant transcripts in a typical E. coli cell growing in defined medium.
8:50 AM	Operon Prediction Using Genomic and Transcriptomic Data **Abstract:** The elucidation of operons, the smallest unit of transcription in prokaryotes, is the first step towards understanding the regulatory network at the whole genome level. Sequence information, in particular the distance between open reading frames, has been used to predict if adjacent genes belong to an operon in prokaryotes. Here we predict operons in Archeaoglubos fulgidus using E. coli as a training set. While appreciably successful, these predictions need to be validated and refined experimentally. We use microarray data from experimental conditions in which a large number of genes were perturbed to validate the sequence-based predictions. The correlation between expression ratios of adjacent genes was used in a Bayesian classification scheme to determine whether they belong to the same operon. The genes whose expression levels changed significantly across the various experimental conditions, allowed a refinement of the sequence-based predictions. However, for a portion of gene pairs, the set of array experiments considered did not contain sufficient information to determine whether they are in the same transcriptional unit. Altering experimental design and conditions may help clarify whether the sequence predictions were accurate for these genes.
9:10 AM	High Resolution Primer Separation in Microfabricated devices for application in Multiplex Genotyping Reactions **Abstract:** Over the past decade there has been tremendous emphasis in

	obtaining rapid and reproducible results in human genetic analysis. In this regard, single nucleotide polymorphism (SNP) detection is becoming increasingly important. Microarrays [1] and microfluidic devices are the two most popular ways of achieving this on a microscale. Microaarays have the advantage of high throughput parallel processing. Although typical reaction based systems lack this ability, multiplexed genotyping systems such as Fluorescent Primer extension reaction (FluPE)[2]can be used to increase the throughput in microfluidic devices. FluPE comprises of a series of reactions followed by a separation. Of key importance to the device function is the high-resolution separation of these products using gel electrophoresis. Issues such as gel concentration, band dispersion in the gel, and sample loading have to be addressed. We have performed high-resolution FluPE separations at extremely short distances. FluPE is able to reproducibly detect a rare nucleotide variant when it appears as one part in 500 with a major variant. Multiple nucleotide sites can be simultaneously interrogated with uniquely labeled primers of different lengths. We have separated the products of H19 Flupe assay, which has a base pair difference as low as 3 bases at a read length of 30 bases. Complete separation is obtained at distances shorter than 15mm and in times less than 20 minutes. On a conventional slab based sequencing machines, a separation like this typically takes about 10 hours. Minimizing dispersion of the ssDNA in the gel is an important factor in obtaining good resolution separation at such short base lengths. For this we have used a higher percentage (19%) cross-linked polyacrylamide gel for the sieving media, electrode loading for sample loading and a low electric field for separation. Such optimized separation systems can be powerful tools for obtaining high throughput results from multiplexed genotyping systems. References: 1 Pease, A.C., Solas, D.,Sullivan E.J., Cronin, M.T., Holmes, C.P. and Fodo, S.P.A. "Light-generated oligonucleotide arrays for rapid DNA sequence analysis" Proc.Natl.Acad.Sci.USA. 91,5022-5026.1994 2 Fahy, E., Nazarbaghi,R., Zomorrodi,M., Herrnstadt,C., Parker, W.D., Davis, R.E., and Ghosh,S.S. "Mulitplex fluorescence-based primer extension method for quantitative mutation analysis of mitochondrial DNA and its diagnostic application for Alzheimer's disease". Nucleic Acids Research. 25, 3102-9.1997
9:30 AM	Performance of a Mass-spectrometric Method for the Analysis of Gene Expression **Abstract:** Sequence-specific quantification of nucleic acids, primarily for the analysis of messenger RNA (mRNA) transcripts in gene expression studies, is central to functional genomics. As computational methods for making biological inferences from gene expression data become more sophisticated, the demand for high-quality measurements of low-abundance transcripts originating from tightly-regulated genes increases. Furthermore, a method of gene expression analysis that yields exact quantities of mRNA species of interest without relying on housekeeping genes or other estimators would be useful. We have developed a new method for quantifying gene expression denoted as the Mass-spectrometric Analysis of Gene Expression (MAGE).MAGE relies on novel conjugates of DNA oligonucleotide 25-50mers, each unique sequence of which is joined via photolabile linker to an N-substituted glycine oligomer (peptoid) of unique mass. Deuterated bromoacetic acid submonomer is incorporated into some of the peptoids to allow the creation of two chemically identical probe conjugates of different molecular weights for each nucleic acid sequence of interest. These probes, along with 3' adjacent biotin-labeled oligonucleotides of equal length, are used to interrogate a target mixture of cDNA. Following hybridization, the two adjacent probes are ligated to enhance the specificity of the identification, and to

	enable the use of a biotin-affinity column for removal of confounding peptoid tags. The resulting mixture is exposed to longwave ultraviolet light to release the peptoid tags, which are quantified using MALDI-TOF mass spectrometry using the isotopically labeled peptoids as internal standards. The chemistry used to create the peptoid-oligonucleotide conjugates will be described, as will the subsequent performance of the conjugates in a gene expression assay. The MAGE methodology unambiguously quantifies one or more nucleic acids simultaneously in multiple samples and could be particularly suited to quantifying those genes that express in low abundance (i.e., yield small numbers of mRNA molecules). An assay that makes use of mass spectrometry for transduction is desirable because in recent years mass spectrometer sensitivity has reached the zeptomolar region and below, and dynamic ranges in excess of six orders of magnitude; these capabilities could relax the dynamic range restrictions and decrease nucleic acid material requirements inherent in current methods for studying gene expression. Further, MAGE relies on solution-phase hybridization for target recognition, a practice thought to contribute to the greater reliability of many single-transcript methods (e.g., RNase protection assay) as compared to high-throughput methods (e.g., cDNA microarrays).

[328] - Microscale Separations in Biotechnology

Session Schedule

2:00 PM	Bioseparation for Biochips **Abstract:** Biochip technology has generated the ability to rapidly detect pathogenic bacteria compared to conventional methods. Detection of some pathogens, notably Listeria monocytogenes, takes up to a week, which is costly, both economically and in terms of food safety. Biochips offer detection times in hours, not in days. However, sample pre-processing is required in order to remove interfering substances, ideally leaving only the microorganisms. Biological samples, especially food products, are complex substances containing carbohydrates, proteins, lipids, and salts, which interfere the selectivity of the binding sites on the chips. This paper describes the pre-processing of food samples by various chromatographic resins, including cationic, anionic ion exchangers, hydrophobic, bifunctional and reverse-phase resins. Among these, Amberlite 35, which is a strong cationic ion exchanger, gave the highest adsorption of aqueous proteins.
2:18 PM	Microfluidic Separations Using Spin-on Polymer Membranes **Abstract:** Significant advances have been made recently in the microfluidic transport and detection of biological molecules. However, many devices begin with product streams which have already undergone purification, or conduct sample separations on mixtures containing only a few components. In the future, biological microdevices will consist of cell culture chambers from which desired products must then be transported and isolated from a much more complex mixture for further processing and analysis. Such devices will therefore require a wide range of separation capability. Current membrane-based microfluidic processes often involve the adhesion of commercial membranes onto prefabricated microfluidic devices. Such processes may be incompatible with standard microfluidic fabrication procedures and can introduce additional contaminants into the system. We have developed an alternative procedure for incorporating polymeric membranes into microfluidic devices using standard

	semiconductor processing techniques. This technique enables the casting of thin polymer membranes without the need for additional adhesives. Ultrafiltration membranes were spin cast onto silicon substrates from several commonly used polymers in compatible solvents. Cast membranes were initially characterized by SEM. Additionally, fluid reservoirs fabricated in polydimethylsiloxane elastomer were attached to membrane containing devices and flow tests were conducted to assess membrane integrity and rejection characteristics. Preliminary results show that membranes produced by this method display excellent adhesion to the substrate without the need for additional adhesives and have good rejection characteristics enabling their use for a range of separation problems.
2:36 PM	The Fabrication of Microfluidic Capillary Electrochromatography Devices with Monolithic Stationary Phases **Abstract:** In this project a novel separation system using capillary electrochromatography with a monolithic polymer stationary phase was developed for the extraction and analysis of toxic compounds used in drug-facilitated sexual assault. Capillary electrochromatography (CEC) is a hybrid of two popular analytical separation instruments, high performance liquid chromatography (HPLC) and capillary electrophoresis (CE). HPLC is a pressurized separation system that is based upon the ability of a compound to partition in and out of packed stationary phase. CE is an open-tube system that exploits the ability of a compound to exhibit dissimilar behavior under the influence of electrical fields. CEC uses the same types of stationary phases utilized in HPLC but requires no pressure pump as the column flow is produced using electro-driven by high voltage electroosmosis. The advantage of this system is that the electroosmotic mobility generates a more uniform flow profile while the added phase enables the separation of molecules with identical charge. Traditional CEC stationary phases consist of chemically modified silica particles, which must be packed into the separation capillary. Unfortunately such systems are prone to failure through bubble formation and loss of electrical continuity. Monolith polymer stationary phases can be produced in situ though a simple three-step preparation process: surface modification, polymerization, and washing. This stationary phase is polymerized in a temperature controlled UV-light box in less than two hours. A polymethacrylate matrix is combined with small molecules called porogens, which intercalate between the rigid polymer backbone creating fissures allowing the passage of mobile phase. The stationary phase for this drug application exhibit hydrophobic exchange properties with negative functionalities due to the addition of specific surface molecules. Microscale capillary columns will be used as models for the production of microfluidic systems based on these technologies. These miniature devices will provide a convenient and disposable method for the analysis of non-reactive analytes. The trend towards analytical CE micromachining has brought about many examples of glass and polymer wafers for open channel microfluidic separations. These same procedures are implemented with the addition of an embedded monolithic polymer to yield a CEC device. Due to the wide flexibility offered through the UV-light initiation, the channels produced in these devices are easily be modified to permit the inclusion of monoliths. The entire microfluidic channel is filled with monomer solution while only a specified desired length is subjected to UV-light. The micro analytical systems are fabricated using photolithography. A wet-chemical etching procedure is performed on a borosilicate wafer using hydrogen fluoride (HF) as the primary etchant. Access holes are drilled in the cover wafer for placement of electrodes and the two plates are bonded using thermal diffusion.

	These new technologies will be applied to the complex analysis problems surrounding forensic drug analysis. For example, there is a great concern regarding gamma-hydroxybutyric acid (GHB) and its chemically related drug analogs in drug-facilitated sexual assault. The ultimate goal of this project is to achieve a separation of GHB, gamma-butyrolactone (GBL), 1,4-butanediol, gamma-valerolactone (GVL), and the neurotransmitter gamma-aminobuytric acid (GABA). Monolithic polymer columns and microfluidic devices should prove to be sensitive and specific enough to preconcentrate and detect GHB and its analogs at very low levels. The proposed monolithic CEC devices can be effective for many other clinical and forensic applications such as therapeutic drug monitoring and screening for drugs of abuse. This project will produce pioneering work through the combination of analytical, polymer, and engineering technologies providing a unique and valuable tool for separation science laboratories.
2:54 PM	Microdevice-based Measurements of Diffusion, Dispersion, and Separation Resolution in Gel Electrophoresis **Abstract:** Although microfabricated devices have the potential to offer a portable low-cost alternative to conventional DNA analysis equipment, a major problem with the microdevice format is the requirement that electrophoretic separations be performed over much shorter distances than those encountered in conventional macroscale systems. In this paper, we show how the increased resolving power offered by crosslinked poly-acrylamide gels, along with improved sample injection techniques, can enhance separation performance in microfabricated electrophoresis systems. Using these techniques, we are able to perform high-resolution gel electrophoresis of single-stranded DNA fragments under denaturing conditions in microfabricated devices incorporating integrated on-chip electrodes, heaters, and temperature sensors. These separations are performed over distances of 1.5 centimeters or less using low electric fields (~ 15 V/cm). We compare our results with separation of identical samples in an automated DNA sequencer, and find them to be in agreement with theoretical predictions. Further progress toward improving separation resolution can be made through a more detailed understanding of diffusion and dispersion phenomena in the gel matrix. Unfortunately, it has thus far proven difficult to obtain extensive measurements of these quantities due in large part to the lack of a convenient experimental platform. We demonstrate the use of our microfabricated gel electrophoresis devices to measure diffusion and dispersion of single-stranded DNA fragments in crosslinked and uncrosslinked polyacrylamide gels. The microdevice format allows a complete set of diffusion and dispersion data to be collected in approximately one hour, as opposed to experiment times lasting several days using conventional sequencing equipment. These results are compared with corresponding data obtained in a macroscale DNA sequencer, and the effects of gel composition and initiation chemistry are explored. We also examine the influence of polymer concentration and operating temperature, and compare the performance of crosslinked and uncrosslinked matrices. We find that crosslinked polyacrylamide gels yield significantly lower diffusion and dispersion coefficients than linear polyacrylamide. These results suggest that crosslinked polyacrylamide gels may be better candidates than conventional linear gels for use in microfabricated systems.
3:12 PM	A Synthesis Framework for Microscale Electrophoretic Separation Systems. **Abstract:** Micro-scale electrophoretic separation systems provide a highly effective, versatile and inexpensive method for separating a wide variety of chemical components [1]. Particularly useful applications include separation of biological molecules, chemical sensing, and mobile drug delivery systems. Micro-scale electrophoretic separation systems are an integral part of 'lab-on-a-chip' technology. Current work in the area of design of microfluidic separation systems

	has made use of two different methodologies. The first [3,5] iteratively fabricate and test a multitude of different design schemes based on heuristic rules and understanding. The second approach [2,4] is based on developing piecewise phenomenological models for specific channel geometries such as for straight channel sections and channels with turns. From a design point of view, it is desired to bridge the gap between current analytical models and practical chip design. The basic idea of our work is to incorporate the phenomenological description of electrophoretic separation into a generalized optimization framework by combining available, closed-form algebraic models. We will demonstrate how the framework enables a systematic approach to the design of electrophoretic chips that does not require pre-specification of device geometry or layout. The optimization formulation addresses device performance through metrics like separation resolution or separation time, and is subject to fabrication and operation constraints on quantities such as voltage or device size. The synthesis result is an optimal arrangement of connected channel sections that satisfy the overall specifications. This approach will be demonstrated for the separation of two and three component species and incorporate all relevant design constraints. Specifically, the following three cases will be presented; (a) two species with differing mobilities, (b) three species with differing mobilities (c) three species, two with similar mobilities and the third with a differing mobility.
3:30 PM	Using a Pulsed Electric Field for Detecting Electrophoretic Mobility Differences of Fluorescent Species in a Microchannel
3:48 PM	Separation and Selective Collection of DNA Fragments in Microfabricated Electrophoresis Devices
4:06 PM	A Microfluidic Device for Subcellular Organelle Separation
4:24 PM	Miniaturized Flow Fractionation Device assisted by the Alternating E-Field Pulse for Biomolecules Separation
4:42 PM	A Novel Gel Loading Method for Improved Microchip Capillary Electrophoresis

Abstract 가 있는 발표는 필자가 참석한 것이므로 당연히 slide 에 대한 사진이 있습니다. 필요하신 분은 연락바랍니다.

오후 4 시 30 분부터는 Bioengineering 분야의 poster 발표가 있었습니다. oral 발표(여기에서는

talk 라고 말합니다.)가 많아서인지 상대적으로 poster 발표는 그 수가 적었습니다. 또한, 두드러지는 특징은 논문의 완성도가 떨어진다는 점, 발표자의 연구경력이 짧다는 점, 아시아국가에서 온 연구자들의 발표수가 많다는 점입니다. 세번째의 경우 영어에 대한 자신감의 결여가 결정적인 이유가 될 것입니다. 더구나 싱가폴대학에서 온 팀은 다른 발표자와는 달리 poster 만 붙여놓고 사라졌습니다. 국내 학회 poster 발표에서 흔히 볼 수 있는 광경이라 필자에게는 그리 낯설지 않았습니다만, 미국연구자들은 상당히 의아해 하더군요. 다행히 국내에서 참가한 서울대팀은 끝까지 자리를

지키고 있어서 보기 좋았습니다. Bioengineering 분야 국내 poster 발표 참가자는 서울대팀이 유일했습니다. 내년에는 필자가 속한 연구팀이 발표할 예정입니다.

내일은 필자의 연구분야인 Plant Cell Culture 분야 talks 가 있습니다. 기대가 크군요. 그리고 필자는 내일 Seattle 로 이동을 하므로 제때에 글을 올릴 수 있을 지 모르겠습니다. 하지만 언제나 그랬듯이 최선을 다하겠습니다. 하루쯤 늦어진다고 등돌리기 없깁니다.

지금까지 Indianapolis 에서 윤성용이었습니다.