Pew Research Proposal Form
Union University

Cover Sheet

Name(s) of Applicant(s): **Esther Choi, Ph.D.**

Title of Proposed Project: **Inhibitory mechanisms of *Pseudomonas fluorescens* Culture Supernatants on Biofilm Formation of *Staphylococcus epidermidis* 1457**

Primary Discipline: **Biology** Secondary Discipline(s): **N/A**

Has this proposal been submitted to another agency, publication, or program (including for the Union University Research/Study Leave)? **No**

If so, which one(s)?

Location of proposed research: **Union University, Jackson, TN**

Desired start date: **November 2023**

Recommending Scholars and their disciplines:

External:
Sunga Choi, Ph.D.
Bioinformatics & Biosystems, Assistant Professor
Seongnam-campus of Korea Polytechnics, South Korea
sachoi@kopo.ac.kr

Union:
Willam Thierfelder, Ph.D.
Associate Professor of Biology and Director of Edward P. Hammons Center for Scientific Studies
Union University
Jackson, TN, 38305
731-661-5252
wthierfelder@uu.edu

In consultation together, we recommend the approval of the proposal as an acceptable project and affirm that the applicant has the professional wherewithal to accomplish the project satisfactorily.

Chair of your department _____ Date: 9/25/2023

Dean of your school: _____ Date: 9/25/23

PEW RESEARCH PROPOSAL

(Use the space necessary to present your proposal clearly to the committee)

1. **Title:** Inhibitory mechanism of *Pseudomonas fluorescens* culture supernatant on *Staphylococcus epidermidis* biofilm formation

2. **Statement of the end product(s)**:
   Results from this work will be presented at the Kentucky-Tennessee regional meeting of the American Association of Microbiology (ASM) in 2023 or 2024. The results could be published in a peer-reviewed microbiology journal.

3. **A description of the project and its major goals:**

   Research question
   What are the active compounds of *Pseudomonas fluorescens* culture supernatant (CS), and what are their inhibitory mechanism(s) on *Staphylococcus epidermidis* biofilm formation?

Staphylococcus epidermidis is a skin colonizer and a major cause of nosocomial infections that can lead to systemic infections. Turning the harmless commensal form of the bacteria to a pathogenic *S. epidermidis* mainly lies in its ability to form microbial communities, called biofilms. Biofilms are composed of extracellular substances such as polysaccharides, proteins, nucleic acids, and lipids, causing microorganisms to tightly adhere to each other. The biofilms on medical devices are hard to control with conventional antibiotics because of the poor penetration of the drugs. Furthermore, they are not well detected by immune cells. There have been many endeavors for preventing and combating biofilm formation and accumulation, one of which is to utilize bacterial culture supernatant (CS) as probiotics or inhibitors. My preliminary experiments showed that *Pseudomonas fluorescens*, one of our gut microbes, had robust anti-biofilm activity against *S. epidermidis* strain 1457 (BMC research Note, under revision). I hypothesized that *P. fluorescens* produces small molecule inhibitors that specifically interfere with production of the matrix components of *S. epidermidis* 1457 biofilms. To elucidate its inhibitory mechanisms and develop it as a biofilm inhibitor, my research aims to achieve the following.

Research goals

1) Determining the active compound that exhibits anti-biofilm activity in *P. fluorescens* CS
   - A biosensor strain that detects bacterial small molecules like N-acyl homoserine lactones (AHL) will be purchased from ATCC (American Type Culture Collection)
   - This will be used for testing the presence of AHL in *Pseudomonas fluorescens* culture supernatants
   Research goal 1 will be performed by Union undergraduate or graduate research students.

2) Determining the different gene expression of *Staphylococcus epidermidis* 1457 when treated with *P. fluorescens* CS
   -Total RNA will be prepared from *S. epidermidis* untreated or treated with *P. fluorescens* CS and an RNA-sequencing (RNA-seq) will be performed by a sequencing company for measuring the transcription levels of all genes in the organism
   -Genes that are significantly different between the samples will be determined.
   Research goal 2 will be carried out as a collaboration with Dr. Sunga Choi (an external recommending scholar for this application and one of the co-authors of my recent papers)

3) Grouping genes into different categories based on their functions and analysis of the RNA-seq data. Confirm the different gene expression levels determined by RNA-seq with quantitative reverse transcription-polymerase chain reaction (qRT-PCR)
   Research goal 3 will be performed by Union undergraduate or graduate research students.

4. **Brief examination of scholarly literature or context of the activity within my discipline.**
   One of the major causes of hospital-related infections is *Staphylococcus epidermidis*. It is normally a harmless microorganism that lives on human skin. However, *S. epidermidis* can form microbial aggregates called biofilms and infect hospitalized patients (Ziebuhr et al. 2006). They are often associated with hip implants and catheters, causing systemic infections (Oliveira *et al.* 2018). In the United States, over five million central venous catheters are placed every year, and of those placed, over 50% become infected with a biofilm (Nobile and Johnson 2015). *S. epidermidis* biofilms are notoriously resistant to many antibiotics, including methicillin, and require up to 1000-fold higher concentration for eradication (Ziebuhr et al. 2006).

   Biofilm formation occurs when microorganisms attach and accumulate on a surface. Microorganisms in biofilm withstand environmental stresses more effectively than their free-floating counterparts (Fey

and Olson 2010). This is because they form a thick bio-matrix, called an extracellular polymeric substance (EPS) made up of polysaccharides, proteins, and lipids. EPS allows microorganisms to avoid immune defense and physically block antibiotics from reaching the microbes encased in the biofilm (Ghosh et al. 2020)

S. epidermidis forms biofilm through four stages, including adherence, accumulation, maturation, and detachment which are achieved through transcriptional regulation (Fey and Olson 2010). Our preliminary data demonstrated that the CS of *P. fluorescens* showed specific inhibition on maturation stages (18 hrs after incubation) of *S. epidermidis* biofilm (Choi et al. 2022). Quantitative RT-qPCR analysis revealed gene regulation at the transcriptional level. The treatment of *P. fluorescens* CS downregulated the *ica* genes and upregulated *tcaR* in *S. epidermidis*. Ica proteins are involved in producing polysaccharide intracellular adhesion (PIA) in a biofilm matrix, a major component of *S. epidermidis* biofilm (Vuong et al. 2004). It was found that the PIA-positive strain caused infection at significantly higher levels than PIA-negative *S. epidermidis,* resulting in bacteremia (bacteria in blood) in hospitals (Rupp et al. 1999). Our result confirms the importance of PIA in *S. epidermidis* biofilm formation, providing an excellent tool to study *S. epidermidis*-mediated infection in medical devices. TcaR is a repressor that inhibits the *ica* gene expression (Hoang et al. 2019), indicating that the CS treatment resulted in higher level of TcaR repressors, which blocked *ica* genes from expression.

Since our results showed almost complete inhibition of *S. epidermidis* biofilm (95% in our data), we hypothesized that the anti-biofilm activity of the CS globally affects *S. epidermidis* gene expression in addition to the two genes in which we've observed differences. This proposal aims to detect differential gene expression of *S. epidermidis* before and after the CS treatment at transcriptome level. The CS is believed to affect many different functional categories of genes, such as stress-response genes and biofilm-associated genes (Freitas et al. 2018, Huang et al. 2014, Schaeffer et al. 2015, Wang et al. 2007). To characterize a global picture of transcriptional activity, *S. epidermidis* treated and untreated with *P. fluorescens* CS will be compared through RNA-seq. This technique can be used to compare specific pairs of samples and provides profiles of thousands of genes at once (Liu et al. 2020). We will extract total RNA from *S. epidermidis* samples and copy it into stable double-stranded copy DNA (cDNA), which is then sequenced using a platform established by a sequencing company. Quantitative results can provide the expression levels for the genes of interest.

Next, the results from transcriptome analysis will be validated using qRT-PCR. The same total RNA samples analyzed by RNA-seq will first be converted to cDNA. For PCR, specific primers will be designed and obtained to amplify several reference genes and the targets. This validation step will allow us to confidently and conclusively interpret and conclude the RNA-seq data.

This proposal also aims to detect small molecule inhibitors present in *P. fluorescens* CS. Targeting only biofilm formation without interfering with bacterial growth is an important property in developing biofilm inhibitors. This is because those interfering with cell growth often induce selective pressure on drug-resistant strains, resulting in survival of highly virulent pathogens (Szczuka et al. 2017). Several bacterial CSs have been shown to contain inhibitory components against *S. epidermidis* (Qin et al. 2009, Xie et al. 2019). *P. fluorescens* is abundant in plant roots, often associated with food spoilage, and was recently recognized as part of the human microbiome (Patel et al. 2013). Our lab found the CS of *P. fluorescens* specifically inhibited maturation stages of *S. epidermidis* biofilm while not affecting planktonic growth. The CS was previously shown to inhibit *Shewanella baltica* by producing various quorum-sensing (QS) molecules such as acyl-L-homoserine lactones (AHLs) and the autoinducer-2 (AI-2) (Zhao et al. 2016). QS molecules are produced and released from microorganisms to sense their surroundings and modulate their gene expression for competitive survival (Pena et al. 2019). Given the potential of coexistence of *P. fluorescens* with *S. epidermidis* in human and competition for environmental resources (Patel et al. 2013), I hypothesize that *P. fluorescens* produces bioactive agents that are effective against *S. epidermidis* biofilm formation in addition to other known bioactive substances (Scales et al. 2014) To confirm my hypothesis, a biosensor strain that was engineered to detect AHLs will be obtained from ATCC (American Type Culture Collection). *P. fluorescens* CS will be incubated with a bioassay strain that illuminates in the presence of AHLs. Several purified AHL compounds with a various number of carbon acyl chains are already in our stock as references, and the remainder will be purchased from Sigma as positive controls.

P. fluorescens is one of few CSs reported to specifically inhibit staphylococcal biofilms without affecting bacterial growth (Xie et al. 2019). Identifying active compounds in the CS and elucidating the detailed mechanism of their anti-biofilm activity through two goals proposed here will provide important parameters for the CS to be developed into a biofilm inhibitor. Combinations of specific inhibitors from the CS and antibiotics against staphylococci may provide an effective measure to control staphylococcal biofilm EPS within medical devices, providing better control against *S. epidermidis*-mediated hospital-related infections.

References

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Schaeffer, C. R.; Woods, K. M.; Longo, G. M.; Kiedrowski, M. R.; Paharik, A. E.; Buttner, H.; Christner, M.; Boissy, R. J.; Horswill, A. R.; Rohde, H.; Fey, P. D., Accumulation-associated protein enhances *Staphylococcus epidermidis* biofilm formation under dynamic conditions and is required for infection in a rat catheter model. *Infect Immun* 2015, 83, (1), 214-26.

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Vuong, C.; Voyich, J. M.; Fischer, E. R.; Braughton, K. R.; Whitney, A. R.; DeLeo, F. R.; Otto, M., Polysaccharide intercellular adhesin (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system. *Cell Microbiol* 2004, 6, (3), 269-75.

Wang, C.; Li, M.; Dong, D.; Wang, J.; Ren, J.; Otto, M.; Gao, Q., Role of ClpP in biofilm formation and virulence of *Staphylococcus epidermidis*. *Microbes Infect* 2007, 9, (11), 1376-83.

Xie, T. T.; Zeng, H.; Ren, X. P.; Wang, N.; Chen, Z. J.; Zhang, Y.; Chen, W., Antibiofilm activity of three Actinomycete strains against *Staphylococcus epidermidis*. *Lett Appl Microbiol* 2019, 68, (1), 73-80.

Zhao, A.; Zhu, J.; Ye, X.; Ge, Y.; Li, J., Inhibition of biofilm development and spoilage potential of *Shewanella baltica* by quorum sensing signal in cell-free supernatant from *Pseudomonas fluorescens*. *Int J Food Microbiol* 2016, 230, 73-80.

Ziebuhr, W.; Hennig, S.; Eckart, M.; Kranzler, H.; Batzilla, C.; Kozitskaya, S., Nosocomial infections by *Staphylococcus epidermidis*: how a commensal bacterium turns into a pathogen. *Int J Antimicrob Agents* 2006, 28 Suppl 1, S14-20.

5. **An essay (500 - 1000 words) describing how the Christian faith relates to my understanding of my discipline and how it relates to this scholarly endeavor.**

I believe we are made to glorify God, and God put a desire in me to share the Good News with others. I study and teach biology so that I may use it as a tool to talk about God's creation and His love for us. That is one way I worship and glorify Him.

As a scientist, I have had the privilege to observe His fingerprints on living things in many ways. In my previous research, I have labeled two different proteins with distinct fluorescent dyes (red and green) to track their localization in a human cell. The images captured in real-time with a microscope showed me how two proteins located in a compartment of a cell and transiently interacted with each other (shown in yellow speckles) to perform a certain biological function. I also photobleached their colocalization to determine how quickly their interactions and functions could become restored. While I spent several hours in a dark room studying the localization and interactions of proteins, I felt like I was in space looking at the various sizes and colors of stars. It looked like the same law that operates the entire universe also applied to the smallest unit of a living organism, a cell.
I often tell God how awesome and wonderful God He is while I am performing experiments. I see just a glimpse of His perfect and intellectual design all around us. New discoveries and findings in nature

give me a greater and deeper appreciation of what He has done for us. I hope and pray I deliver the excitement and wonders of God in my classes.

The research proposed here addresses a critical issue in contemporary medicine—antibiotic resistance. According to the National Institute of Health, approximately 80% of medical device-associated infections are linked to bacteria that form biofilms. *Staphylococcus epidermidis* is one of these bacteria that is frequently encountered, and we are witnessing a continuous increase in its multi-drug resistant strains affecting various medical devices. This includes contact lenses, prosthetic implants, pacemakers, ventilators, urinary and venous catheters. A recent retrospective study demonstrated that biofilm-forming bacteria played a significant role in the exceptionally high incidence (61.5%) of ventilator-associated pneumonia among COVID-19 patients undergoing mechanical ventilation, ultimately resulting in respiratory failure (Wicky et al. 2023).

In addition to their prevalence, biofilm-associated infections often require surgical removal of the infected implant, followed by extended courses of antibiotic therapy (Oliveira et al. 2018). This approach places a substantial physical burden on patients who are already vulnerable due to their weakened immunity. Furthermore, this form of treatment carries significant financial burdens, with estimated costs exceeding $50,000 per occurrence (Sculco et al. 1995). Notably, bacteremia resulting from *S. epidermidis*-infected catheters alone is estimated to incur annual costs of approximately $2 billion in the United States (Otto, 2009). Due to the significant challenges posed by biofilm-mediated device-associated infections, various prevention methods, including the use of silver-coated medical devices and mechanical biofilm removal, have been developed. However, these methods have proven to be limited.

Given the significance of my research topic, I frequently find myself drawing considerable attention in conferences. This underscores the potential of my research to serve as a powerful tool for educating others and raising scientific awareness, not only among fellow researchers, but also within both the broader community and our Christian community.

During the COVID-19 pandemic, we witnessed distinct and polarized groups of individuals who held biased opinions on vaccines and mitigation measures, often disregarding scientific data. It is my aspiration to contribute to society by starting with my students here at Union. I am to instill in them the ability to think critically like scientists. This includes equipping them with the skills to proficiently read and interpret scientific data, draw logical conclusions, and devise optimal solutions

that they can carry forward. My ultimate goal is to empower my students to excel in their respective professional fields and, more importantly, to become the best individuals they can possibly be. I have a deep desire to be an instrument used by God to guide them in aligning their pursuit of knowledge in science with their faith in Christ. Through this holistic approach, they can make meaningful contributions to their communities, our society, and this nation.

My research projects serve as invaluable tools for achieving my educational objectives. Working with microorganisms is particularly advantageous in teaching-oriented institutions like Union. Bacteria and fungi offer distinct benefits, with their shorter generation times (1-2 days) and ease of cultivation, enabling the generation of data with statistical significance. Notably, the culture media and reagents required are more cost-effective compared to those for mammalian cell systems. These advantages have afforded me the opportunity to work with a larger number of students, and the ability to foster discussions not only about science but also life, faith, and the futures they envision under God's providence.

With God's blessing, my research students and I have been highly productive in our research endeavors, culminating in the recent publication of papers to peer-reviewed journals (Choi et al. 2022 and 2023). This achievement highlights the meaningful impact that projects in my laboratory have had on students' lives in various ways.

As I look to expand my research project, I propose initiatives that are not only intriguing but also cost-efficient, labor-effective, and time-effective. Exploring different gene regulations within RNA-sequencing data will create opportunities for numerous students to delve into the study of bacterial adaptation to environmental stress, with potential implications in the field of medicine. My Korean collaborator and I possess experience with this cutting-edge technology (Shepard et al. 2011) and are confident in our ability to successfully complete this project within the allotted time frame. This project will provide me with a valuable scientific tool to further my commitment to excellence-driven, Christ-centered, people-focused, and future-directed teaching and mentoring.

References

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6. **A time frame for the completion and a plan for the dissemination of the project.**

Dec. 2023-Aug. 2024	RNA-sequencing analysis of wild-type *S. epidermidis* and *S. epidermidis* treated with *P. fluorescens* culture supernatants
Oct. 2023-Aug.2024	AHL detection using a biosensor strain
Nov. 2024	Kentucky-Tennessee regional meeting of the American Association of Microbiology (ASM) in 2023
Apr. 2025	Present results at PEW Research Luncheon
May- Aug. 2025	Write and submit a manuscript to a peer-reviewed journal

7. **A brief budget** (For example, funds may be used for purchasing equipment, travel, conference attendance, etc. If requesting funds as a stipend, please justify the need for a stipend to complete the project.)

Purchasing a strain:	$500
RNA-seq and reagents:	$3,000
Conference attendance:	$1,000
Total:	$4,500

8. **A current *curriculum vitae*.**

Please see attached.

9. **Two letters of recommendation should be submitted directly to chair of the Research Committee attesting to the worth of the project, the candidate's scholarly competence, and his/her ability to complete the project with distinction in a timely fashion.**

The letters of recommendation have been requested to Drs. William Thierfelder (internal) and Sunga Choi (external).

Euna (Esther) Choi , Ph.D.

Curriculum Vitae

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1050 Union University Dr.
Department of Biology
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TEACHING EXPERIENCE

2016-present Associate Professor of Biology, Union University
Course: Microbiology, Microbiology laboratory,
Clinical microbiology (pharmacy program),
Survey microbiology (pre-nursing program),
Developmental biology, RNA biology, Seminars

2015 Guest instructor
School of Biological Sciences, University of Nebraska, Lincoln
Course: Molecular Genetics

2008-2009 Graduate teaching assistant,
Department of Molecular Genetics and Microbiology, University of California at Irvine
Course: Medical Microbiology

RESEARCH EXPERIENCE

Nov. 2013-July 2016 Post-doctoral Research Associate
School of Biological Sciences
The University of Nebraska, Lincoln
Project: Regulation of nonsense-mediated decay in *Saccharomyces cerevisiae*

Aug. 2009- Aug.2013 Assistant Specialist
Department of Microbiology and Molecular Genetics
The University of California, Irvine
Project: Alternative polyadenylation and cancer

Nov. 2007- July. 2009 Post-doctoral Research Associate
Department of Microbiology and Molecular Genetics

The University of California, Irvine
Project: Combinatorial regulation of 5'splice site activation and ccharacterizing pre-mRNA splicing error rates in cancer cells

Sep. 2005 – Oct. 2007 Post-doctoral Research Associate
Department of Cell Biology
The Scripps Research Institute
Project: Screening inhibitors of importin-beta mediated nuclear import

Aug. 2004 – Aug. 2005 Post-doctoral Research Associate
Department of Microbiology and Immunology
The University of Illinois at Chicago, USA
Project: Studying the mechanism of Woodchuck Hepatitis Virus post-transcriptional element (WPRE) in gene therapeutic vector

EDUCATION

2005 **Ph. D.** in Microbiology and Immunology
The University of Illinois, Chicago
Thesis title: Post-transcriptional regulatory mechanisms of bovine leukemia virus and woodchuck hepatitis virus
Advisor: Prof. Thomas J. Hope

1996 **MS** in Genetic Engineering, Hallym University, South Korea
Project: Analysis of Hepatitis B Virus Surface protein pre-S region to develop a new HBV vaccine
Advisor: Prof. Soo-young Choi
Institute Korea Research Institute of Bioscience and Biotechnology (KRIBB), KIST, South Korea

1994 **BS** in Genetic Engineering, Hallym University, South Korea

PUBILICATIONS

- Choi E, Murray B, Choi S. Biofilm and Cancer: Interactions and Future Directions for Cancer Therapy. Int J Mol Sci. 2023 Aug 16;24(16):12836. doi: 10.3390/ijms241612836. PMID: 37629016; PMCID: PMC10454087.

- Choi, E., Wells, B., Mirabella, G. *et al.* Anti-biofilm activity of *Pseudomonas fluorescens* culture supernatants on biofilm formation of *Staphylococcus epidermidis* 1457. *BMC Res Notes* 15, 370 2022. https://doi.org/10.1186/s13104-022-06257-z

- Choi SA, Lee Y., Kim K., Choi E, Jeon B. Dual Function of Secreted APE1/Ref-1 in TNBC Tumorigenesis: An Apoptotic Initiator and a Regulator of Chronic Inflammatory Signaling. International Journal of Molecular Sciences, 2022, 23, 9021.

- Choi S, Kim KM, Choi E APE1/Ref-1 with reducing activity induces mesenchymal-to-epithelial transition in TNF-α-stimulated breast cancer cells. Integr Cancer Sci Therap, 2022, 9: DOI: 10.15761/ICST.1000365.

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- **Choi EA**, Yao C., Weng L, Xie X, Wan J, Xing Y, Moresco JJ, Tu PG, Yates JR 3rd, Shi Y. (2013) Overlapping and distinct functions of CstF64 and CstF64T in mammalian 3'processing. RNA. 2013 Dec;19(12):1781-90

- **Choi EA**, Shepard P, Busch A, Hertel KJ. (2011) Efficient internal exon recognition depends on near equal contributions from the 3' and 5' splice sites , *Nucleic Acids Res.* Jul 27

- **Choi EA,** Shepard P, Lu J, Flanagan L, Hertel KJ and Shi Y. (2011) Complex and dynamic landscape of RNA polyadenylation revealed by PAS-Seq. *RNA*, Apr;17(4):761-72.

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- **Choi, E.A.,** Hope, T. J. (2005) Mutational analysis of Bovine Leukemia virus Rex; Identification of a dominant-negative inhibitor, *Journal of Virology.* Vol.79, 7172-7181

- Park, J. H., **Choi, E.A.**, Cho, E.W., Lee, Y,J., Park, J. M., Na, S.Y., Kim, K.L. (2000) Detection of cellular receptors specific for the hepatitis B virus preS surface protein on cell line s of extrahepatic origin. *Biochem. Biophys. Res. Commun.* 277, 246-254

- Lee, D.G., **Choi, E. A.**, Kim, K.L., Ham, K. S. (1996) Identification of HBV pre S1 binding protein on HepG2 cells. *Asia Pacific Journal of Mol. Biol. and biotech.* 4:37-42

FUNDING

2016-2021	Recipient for Union professional development
2016-2021	Union research grant for undergraduates
2020	Union research grant for master students

MEMBERSHIP

- American Society of Microbiology

UNIVERSITY COMMITTEE SERCIVE

2021-	Member---Internal review board (IRB)
2017-2021	Member and a secretary--Intercultural competence committee

RESEARCH TALK or POSTER PRESENTATION

- Inhibition of *Staphylococcus epidermidis* biofilm using cell-free supernatant of *Pseudomonas fluorescents,* American society of Microbiology, 2020

- Environmental and genetic factors promoting *Pseudomonas fluorescence* biofilm formation, American society of Microbiology, 2019

- Biofilm formation and virulence factors of Candida albicans in JEG-3 cell line, Association of southeastern biologists meeting, American society of Microbiology, 2019

- Effect of cosmetic preservatives on the growth, biofilm formation and the gene expression of *Staphylococcus epidermidis*1457. American society of Microbiology, 2019

- Global regulation of mRNA alternative polyadenylation by an oncogenic signaling pathway, Cold spring harbor meeting, New York, 2011.

- Competing 5'splice sites accelerates the intron removal, Cold spring harbor meeting, New York, 2009.

- The stabilizing effect of the woodchuck hepatitis posttranscriptional regulatory element for stimulating gene expression. American Society of Gene Therapy, Washington, D.C, 2003.

- The role of the woodchuck hepatitis posttranscriptional regulatory element in stabilizing ARE-containing messages. RNA. Madison, WI, 2002.

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- The woodchuck hepatitis posttranscriptional regulatory element enhances transgene expression by modulating the 3'end metabolism of mRNAs. Seattle, WA, American Society of Gene Therapy, 2001.

SCIENTIFIC TECHNIQUES

- Fluorescent Microscopy
- Fluorescent staining fixed cells and slide preparation
- FACS
- ELISA
- Primary and stem cell culture
- Working with mice to produce monoclonal Antibody
- Immunoprecipitation and Western blot
- Mammalian cell lines culture
- Receptor ligand assays
- CAT (chloroamphenicol acetyltransferase) assay
- Virus preparation
- Plasmid cloning
- Protein, DNA, RNA extraction
- Nucleic acid preparation
- Site-directed mutagenesis, PCR, oligonucleotide primer design, DNA sequencing
- Real time qPCR

- Designing siRNA and transfection
- Mammalian cell culture, transfections (DNA, RNA), transduction, and metabolic labeling
- Southern blot
- Northern blot (radiolabelling DNA and RNA)
- In vitro transcription
- RNase H assay (similar to RNase protection assay)
- Running sequencing gel
- Recombinant protein expression in bacteria and protein purification
- In vitro nuclear import assay
- Mass culture of HeLa suspension cells and cytosol preparation
- Culture different yeast cell lines and enzymatic assay using yeast extract (eg, aconitase assay of iron regulatory protein)

REFERENCES

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