SURF PROPOSAL
INFO SESSION

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HIXON WRITING CENTER
Goals of Session

• Boil the SURF Proposal down to a manageable and writable project

• Review sample proposals to better understand what makes an effective or ineffective proposal

• Help you feel confident about (1) your next (or first) steps and (2) writing a successful proposal
Participant Gift Bag

- SURF Proposal Starter
- SURF Proposal Overview and Reviewer Questions
- Annotated Mock BBE SURF Proposal (used in session)
- 2 Sample Successful Proposals, not annotated (not used in session)
- PDF of Info Session Slides (emailed to all registrants after session)
- Sign-up for Small Group Workshop
**HIGH-RESOLUTION NUCLEOSOME MAPPING IN MAMMALIAN CELLS**

**INTRODUCTION**

The basic structural unit of eukaryotic chromatin is the nucleosome, which is composed of 147 base pairs of genomic DNA spooled around an octameric histone core [1]. A majority of our genome is tightly bound by nucleosomes, which can occlude the underlying regulatory DNA sequences and limit their accessibility to regulatory factors that must act on them [2]. Consequently, where nucleosomes are positioned along DNA can determine the transcriptional output of the genome. However, the extent to which nucleosome positioning influences gene expression is unknown.

Genome-wide nucleosome positioning has been most widely studied in yeast. Recently, the lab developed a novel chemical mapping method to determine nucleosome positions in budding yeast *S. cerevisiae* [3,4]. This mapping method relies on site-directed hydroxyl radical cleavage of nucleosomes carrying modified histones to determine the positions of nucleosomes in the genome. The resultant map defined nucleosome positions at single base pair resolution and revealed new aspects of *in vivo* nucleosome organization for the entire yeast genome that had not been observed using previous mapping technology. Though general features of nucleosome dynamics might be shared between yeast and mammals, the size and complexity of the mammalian genome present a challenge to accurately mapping its nucleosomes. Such mapping would pave the way for better understanding the role nucleosomes play in gene regulation in higher organisms.

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This mock SURF proposal was created for the purpose of the SURF Proposal Feedback Workshop. It may be shared or distributed during tutorials at the Hixon Writing Center.
Asking Questions

• Feel free to interrupt and ask me a question at any point in the presentation.

• Ask questions over mic or chat.

• Email lilien@caltech.edu.
What is the SURF proposal?
The SURF Proposal Is

• A chance to better understand and prepare for your SURF project

• 1-3 page plan for your research this summer:
  • what will you do?
  • how will you do it?
  • and why does it matter?
It Should Convince Your Reader

• You have a focused project that can be accomplished in 10 weeks or where significant progress can be made.

• Your proposed work is important and useful.

• You can write and think clearly and scientifically.

• You’re prepared for the work you’re proposing.
The SURF Proposal Is Not

• A personal statement or a job application letter.

• A research paper / article (no 2-column format)

• A place to discuss your previous research experiences in other labs/groups or your qualifications for SURF

Note: You can discuss any results or progress you’ve made with the same group).
Who will read your proposal?

**Mentor(s)**
Knows your project inside and out

**Outside Faculty**
Experts in your field (and, likely, in the big question you are studying) but not the nitty gritty details of your project
Anatomy of a SURF Proposal

- Introduction
- Objectives
- Approach
- Work Plan
- References
Introduction/Background

BOILED DOWN VERSION

• What is the background for your research?
• What is the challenge, gap, need, problem, etc?
• What is your solution?
• What is the significance of your work?
A

and

background and the significance

B

but

the conflict! get here quick

T

therefore

what your proposal is all about: proposed research
ABT is the DNA of Story

And

But

Therefore
Story Circle
and the Problem-Solution Narrative

Olson, Randy. 2015. Houston, We Have a Narrative. University of Chicago Press.
ST RONG PROPOSAL = STRONG STORY = STRONG ABT
INTRODUCTION

The basic structural unit of eukaryotic chromatin is the nucleosome, which is composed of 147 base pairs of genomic DNA spooled around an octomeric histone core [1]. A majority of our genome is tightly bound by nucleosomes, which can occlude the underlying regulatory DNA sequences and limit their accessibility to regulatory factors that must act on them [2]. Consequently, where nucleosomes are positioned along DNA can determine the transcriptional output of the genome. However, the extent to which nucleosome positioning influences gene expression is unknown.

Genome-wide nucleosome positioning has been most widely studied in yeast. Recently, the __________ lab developed a novel chemical mapping method to determine nucleosome positions in budding yeast S. cerevisiae [3,4]. This mapping method relies on site-directed hydroxyl radical cleavage of nucleosomes carrying modified histones to determine the positions of nucleosomes in the genome. The resultant map defined nucleosome positions at single base pair resolution and revealed new aspects of in vivo nucleosome organization for the entire yeast genome that had not been observed using previous mapping technology. Though general features of nucleosome dynamics might be shared between yeast and mammals, the size and complexity of the mammalian genome present a challenge to accurately mapping its nucleosomes. Such mapping would pave the way for better understanding the role nucleosomes play in gene regulation in higher organisms.
To date, three genome-wide nucleosome maps in mammalian cells have been reported [5,6,7]. However, due to their low-resolution, these maps are inadequate for studying the dynamic features of nucleosome positioning. To dramatically improve the resolution and accuracy of nucleosome mapping for mammalian cells, we propose to extend the chemical mapping method into mouse cells and construct the first high-resolution nucleosome map in the mammalian genome.

The first single base pair resolution mouse nucleosome map will advance our understanding of the dynamic interplay between nucleosome positioning and gene expression in higher organisms. Specifically, my SURF project will establish a genetic toolkit and a chemical mapping method that will allow researchers to interrogate the epigenomic function of nucleosomes in mammals.
INTRODUCTION

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INTRODUCTION

The basic structural unit of eukaryotic chromatin is the nucleosome, which is composed of 147 base pairs of genomic DNA spooled around an octomeric histone core [1]. A majority of our genome is tightly bound by nucleosomes, which can occlude the underlying regulatory DNA sequences and limit their accessibility to regulatory factors that must act on them [2]. Consequently, where nucleosomes are positioned along DNA can determine the transcriptional output of the genome. **However, a high-resolution mammalian nucleosome map has not been generated. We propose to use a chemical mapping method into mouse cells and construct the first high-resolution nucleosome map in the mammalian genome.**
STRONG ABT = STRONG STORY = STRONG PROPOSAL
Avoid AAA and DHY

ABT = and, but, therefore

AAA = and, and, and
never introduces problem

DHY = despite, however, yet
introduces too many problems
Objectives

BOILED DOWN VERSION

What do you plan to do in the 10 weeks of SURF?
Objectives

The overall aim of this SURF project is to determine genome-wide nucleosome positions in mammalian cells at single base pair resolution. The specific aims are to:

1. Engineer mutant H4S47C mouse embryonic stem (ES) (AB2.2 cell line) for chemical mapping.
   
   (a) The chemical mapping approach requires introducing a unique cysteine into histone H4 at position 47 (H4S47C) to covalently attach a sulfhydryl-reactive copper-chelating label. This label enables the chemical cleavage of DNA at the nucleosome center. The criteria for success is our ability to show H4S47C mouse ES cells have sufficient levels of H4S47C and are functionally equivalent to wild type cells.

2. Establish and optimize the chemical mapping protocol for H4S47C mouse ES cells to demonstrate feasibility of chemically mapping nucleosomes in mammals.
   
   (a) This protocol is based on the lab’s previously established protocol in yeast [4]. The success of this aim will be determined by our ability to (1) generate the desired cuts at nucleosome centers with limited non-specificity and (2) obtain sufficient amount of DNA for downstream analysis.

- Note: Aim 2 is dependent on the success of Aim 1.
BOILED DOWN VERSION

What will you do in your 10 weeks of SURF in order to meet your objectives?
Goldilocks Problem
Help Your Reader Understand

**Protocol**

I will construct mU6-driven H4-shRNA. First, I will set up the following reaction in a microcentrifuge tube on ice:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>T4 DNA Ligase BU (TDC)</td>
<td>2 µl</td>
</tr>
<tr>
<td>HindIII cut PB-mU6::PGK-Puro vector</td>
<td>20 ng, 100 µl</td>
</tr>
<tr>
<td>H4-shRNA</td>
<td>20 ng, 100 µl</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>10 µl</td>
</tr>
<tr>
<td>T4 DNA Ligase</td>
<td>1 unit</td>
</tr>
</tbody>
</table>

(T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.)

**Methods**

To construct mU6-driven H4-shRNA, the oligos will be phosphorylated, annealed, and ligated into a BbsI and XhoI digested PB-mU6::PGK-Puro vector. H4-Sh1 sense and H4-Sh1 antisense, H4-Sh2 and H4-Sh2, and H4-Sh3 sense and H4-Sh3 antisense to target all mouse H4 genes. To express H4S47C in the presence of H4-shRNA, we will synthesize a codon-modified, RNAi-resistant H4S47C cDNA expression vector PB-CAG-H4S47C::PGK-Hygro.

**Approach**

We will analyze the sequences of 13 mouse H4 genes, identify two common regions shared by all H4 genes in mouse, and design shRNA constructs to knockdown all H4 genes in the mouse genome. Concurrently, we will synthesize an RNAi-resistant H4S47C transgene vector that will express the H4S47C transgene in the mouse ES cells.
The chemical mapping strategy relies on the substitution of wild type histone H4 with H4S47C, in which serine 47 has been replaced with a cysteine [3,4]. The unique cysteine in H4S47C symmetrically flanks the nucleosome center axis and is in close proximity to the DNA backbone (Fig. 1A). Covalent linkage of a sulphydryl binding copper-chelating label (phenanthroline-iodoacetamide) to the cysteine anchors a copper ion to the DNA at the same position—symmetric around the center axis (Fig. 1A). With the addition of hydrogen peroxide, the copper becomes a site of hydroxyl radical production, and a localized hydroxyl radical reaction cleaves the DNA precisely at the center (Fig. 1B). H4S47C-targeted nucleosome cleavage generates a characteristic DNA ladder in the presence of the copper chelator (Fig. 1C). Each step in the DNA ladder in the agarose gel represents the center-to-center distance between two adjacent nucleosomes.

Specific Aim 1: The first step in developing a chemical mapping strategy for mouse ES cells is to substitute multiple endogenous histone H4 with H4S47C. The mouse genome encodes 13 histone H4 genes, each of which encodes for identical H4 proteins. However, it is impossible to replace all 26 alleles of mouse H4 genes with the engineered H4S47C through gene targeting. Chemical mapping experiments in fission yeast S. pombe, however, showed that substitution of only two of the three H4 genes with H4S47C produced comparable levels of chemical cleavage to the strain with all three H4 genes replaced [8]. Therefore, we will design a strategy to replace a majority of endogenous H4 proteins with mutant H4S47C by a combination of RNAi knockdown and cDNA expression.

To determine whether chemical mapping strategy is feasible in mouse ES cells, we will analyze the sequences of 13 mouse H4 genes, identify two common regions shared by all H4 genes in mouse, and design shRNA constructs to knockdown all H4 genes in the mouse genome (Fig. 2A, box). Concurrently, we will synthesize an RNAi-resistant H4S47C transgene vector that will express the H4S47C transgene in the mouse ES cells.
To test whether H4-shRNA constructs can efficiently target endogenous wild type H4 and not the synthetic H4S47C, we will transiently transfect each H4-shRNA with Flag-tagged-H4 and Flag-tagged H4S47C into HEK 293T cells and test for loss of Flag-tagged-H4 protein expression by Western blot analysis. After confirming that our shRNA constructs are specific only for endogenous H4, we will use a PiggyBac transgenic approach to simultaneously express the H4-shRNA and the H4S47C into mouse ES cells (A. Fox, Personal Communication, Dec 30, 2013). After sequential drug selection with Hygromycin (for PB-CAG-H4S47C vector) and Puromycin (for U6-shRNA-H4 vector), we will establish several stable ES cell clones (Fig. 2) and analyze the clones for functional equivalence to wild type cells. First, we plan to analyze expression levels of synthesized H4S47C and endogenous H4 by RT-PCR and select the cell lines with high levels of H4S47C and low levels of endogenous H4 for further validation and characterization. The criteria for selection include choosing mutant H4S47C ES cells with total H4 protein levels comparable to levels in the wild type ES cells. Second, we will select H4S47C-expressing ES cells that demonstrate similar growth patterns to wild type cells and express comparable levels of core pluripotency protein factors Oct4, Nanog, and Sox2.

Toward Specific Aim 2, we plan to adapt the chemical mapping protocol to the engineered H4S47C cells. This protocol is based on the previously established protocol in S. cerevisiae [3,4]. We will need to make significant modifications in cell permeabilization methods and the concentrations and incubation times with the copper-chelating label. By optimizing these conditions, we aim to show by DNA agarose gel that H4S47C-targeted nucleosome cleavage in ES cells can generate characteristic DNA ladder in the presence of the copper chelator. Following the mapping protocol, we will use the Qiagen PB system to purify the DNA fragments representing the center-to-center distance between two adjacent nucleosomes and quantify the sample by NanoDrop.
Work Plan

BOILED DOWN VERSION

What week(s) will you do what you said you’d do in the approach section?
Work Plan

If you are performing multiple tasks sequentially:

**Pre-SURF - Week 1** | (1) Design RNAi-resistant H4S47C transgene vector and shRNA constructs for H4 knockdown (2) Prepare and expand mouse ES cell cultures

**Week 2 - 4** | Sequential drug selection with Hygromycin (for PB-CAG-H4S47C) and Puromycin (for U6-shRNA-H4) to establish stable ES cell clones.

**Week 5 - 7** | Evaluate clones via functional assays: RT-PCR, Western blot, and growth curves to test for normal cell behavior and gene/protein expression in mutant ES cell clones.

**Week 8 - 10** | Optimize chemical mapping protocol for mouse ES cells and purify nucleosomal DNA

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Writer organizes major activities and demonstrates (in a perfect world) the project can be completed in 10 weeks

This work plan calls back what was discussed in the approach.
# Work Plan

If you are performing multiple tasks in parallel:

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Work Cited / References

BOILED DOWN VERSION

What references can you bring in to support your ideas, definitions, and plans and to demonstrate your knowledge of the field/relevant literature?
Work Cited / References

- Choose a scientific citation style (ACS, Nature, Science)
- Be consistent
- Use in-text citations (not just bibliography)
- More references = stronger proposal
- Learn a citation management system (Zotero, Endnote, Mendeley, Cite This For Me)
Work Cited / References


Before You Start Writing

• Discuss project with mentor
  Set up a Zoom call with Grad student/post-doc/staff mentor and, if possible, with the faculty (PI) mentor

• READ, READ, READ!
  Published papers
  Previous proposals
  Internal documents
Key Questions to Consider

Example Qs for mentor:

- What is the big picture?
- Specific objectives?
- How much flexibility or independence in designing the research, experiments, testing, etc?
- Where are we in the project?
- Technical hurdles to overcome in the experiment, test, design, etc?
- Mentor’s role and involvement?
HWC SURF Proposal Starter

- Draft an outline / fill out the **starter** with specifics (as much as you can)

- Find out what you don’t know

- Bring to research mentor or HWC Writing Specialist or Peer Tutor to discuss
Small Group Workshops

Mar 4 - noon
Mar 5 - noon
Mar 11 - noon
Mar 12 - noon
Small Group Workshops

• Peer feedback on your draft
• Info Session participants only
• Facilitated by STEM Writing Specialist and Peer Tutor

You:
• Have a complete draft by your workshop date.
• Read 2-3 SURF proposal drafts.
• Sign-up emailed after session.
Individual Appointments

Meet with Writing Specialist or Peer Tutor on access.caltech.edu
> Writing Center Scheduling >
schedule with open times
THANK YOU FOR ATTENDING!