2019 COURSE MANUAL

DRB 330qc: *Experimental Approaches in Stem Cell, Developmental & Regenerative Biology*

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The DRB "Bootcamp" course, DRB330qc, is designed to provide a survey of major topics, model systems and contemporary research methods in stem cell, developmental and regenerative biology. Students engage with and rotate in the laboratories of DRB/SCRB faculty across the Harvard campuses and affiliated hospitals. Each day of the course consists of an introductory lecture, followed by hands-on research activities and interactive discussions with faculty session leaders and laboratory assistants.

This manual contains detailed information for each day of bootcamp.

- To get the most out of the course please make some time to review the syllabus and recommended readings for each session. This will allow you to gain an understanding of the topic for the day, and fully engage in the lectures, experiments and discussion.
- Since we will be rotating between campuses, we have provided specific location information to help you navigate across campuses. Please double check to make sure you know where you are headed when you leave your house each morning!
- Lunch will be provided on all days of the course. Please be prepared to have breakfast and dinner on your own unless noted otherwise.
- Each session will start at 10 am. However, we ***strongly recommend*** that you ***arrive at 9:45am.*** Security for each building varies and those who arrive late may have to wait for a security escort and start the day later than expected.
- We have done our best to accurately describe the anticipated schedule for each session, however exact timing and/or activities may change due to unforeseen circumstances. Any changes will be announced during the lecture or via email, if necessary.

All students will be graded on a pass/fail basis.

- DRB330qc enrollees are expected to attend each laboratory session, and participate in class discussions and course wrap-up student presentations.
- Each student is requested to fill out a pre- and post- survey on the Canvas site at the end of each session, and at the end of the course. These surveys will help us understand how we can improve bootcamp and determine if we have met our goals with the course.

SCHEDULE AT A GLANCE

Date	Faculty	Title	Time	Location
Monday 1/6	Trista North & Mara Laslo	Introduction to DRB330 & why is developmental biology so cool? Followed by lunch	10:00 am – 12 pm	Longwood: Countway 505
Tues 1/7	Olivier Pourquie	Understanding musculo-skeletal axis development using chicken embryos and human iPSC models	**9:45 am** – 4:00 pm	Longwood: NRB 354
Wed 1/8	Jeff Macklis	Development and regeneration in the mammalian central nervous system: Cortical "projection neuron" molecular development, diversity, and regeneration	10:00 am – **4:15 pm**	Cambridge: Sherman Fairchild G62
Thurs 1/9	Jessica Whited	Techniques for studying axolotl limb regeneration	**9:45 am** – 4:00 pm	Cambridge: Sherman Fairchild lobby
Fri 1/10	Trista North & Wolfram Goessling	Zebrafish organogenesis – the hematovascular and hepatobiliary systems	**9:55 am** – 3:30 pm	Longwood: Karp Building, 1st floor
Mon 1/13	Eric Greer	Epigenetic regulation in *C. elegans* and *D. discoideum*	10:00 am – 4:00 pm	Longwood: Enders Building 1044
Tues 1/14	Ya-Chieh Hsu	Mammalian skin and hair follicle regeneration	10:00 am – 4:00 pm	Cambridge: Sherman Fairchild G62
Wed 1/15	Kristin White	Drosophila as a model system for examining neural stem cell fate decisions	10:00 am – 4:00 pm	Charlestown Navy Yard: Building 149
Thurs 1/16	April Craft & Jenna Galloway	Development and regeneration of the musculoskeletal system	**9:45 am** – 4:00 pm	Longwood: Enders Building 244
Thurs 1/16	DRB330 students / DRB Program	*DRB New Year's Party – Celebrating the course end and recent DRB PhD thesis awards*	7:00pm – 10:00pm	Longwood Towers, 20 Chapel St
Fri 1/17	DRB330 Students	*DRB330 Student Presentation Showcase*	10:00am – 1:00 pm	Longwood: Gordon Hall 311

Red indicates an early start time

MONDAY, JANUARY 6

Course Introduction

Faculty: Trista North & Mara Laslo

Location: Longwood, Countway Library 505

Objectives: Review of rationale and goals, expectations and structure of the 2019 DRB330 course and a short primer on major developmental biology concepts.

Schedule:

10:00 am – 12:00 pm	Lecture
11:30 am – 12:00 pm	Lunch

Tuesday, January 7, 2020

Understanding musculo-skeletal axis development using chicken embryos and human iPSC models

Faculty: Olivier Pourquié

Teaching Assistants: Charlene Guillot, Margarete Diaz Cuadros, Jyoti Rao

Location: LONGWOOD

A) Lecture: New Research Building Rm 354, 77 Avenue Louis Pasteur, Boston MA 02115
B) Lab: Hale Building for Transformative Medicine Rm 8032, 60 Fenwood Road, Boston MA 02115

Meet in NRB 354 **9:45 am (sharp)**.

*NOTE: Students should bring their Harvard ID to get into buildings!

Rationale:

Our understanding of human developmental biology is limited by the lack of access to live human embryos. As an alternative, developmental biologists use model organisms relevant to their research topic. Due to the similarities in axial patterning of chicken and human embryos, we study chicken musculo-skeletal axis development and apply our findings to recapitulate the same program using human iPSCs. In turn, directed differentiation of human iPSCs towards skeletal muscle raises novel questions that can be addressed *in vivo*. This lab is designed to showcase the complementarity of *in vivo* and *in vitro* approaches to understand the stepwise specification and differentiation of the vertebrate axis.

Learning objectives:

At the end of this Bootcamp session, you should be able to:

1. Understand the sequence of developmental transitions that give rise to the musculo-skeletal axis in amniotes.
2. Explain experimental techniques to study the specification and differentiation of muscle progenitor cells *in vivo* in chicken embryos and *in vitro* in human iPSCs.
3. Describe the strength of using complementary *in vivo* and *in vitro* models to understand human development.

Approach/methodology:

1. Targeting and visualization of paraxial mesoderm cells in chicken embryos by electroporation.
    a. Dissection of gastrulation stage chicken embryos
    b. Gene transfer by electroporation
    c. Visualization using fluorescence microscopy
    d. Observation of somitogenesis stage chicken embryos
2. *In vitro* differentiation of human iPSCs and observation of their stepwise acquisition of different musculo-skeletal fates.
    a. Dissociation and seeding of human iPSCs for mesodermal differentiation
    b. Observation of different stages of musculo-skeletal differentiation

c. Discussion of cell fate diversification from common early progenitors

Required Readings:

Required Reviews:

1. Hubaud A, Pourquié O. Signalling dynamics in vertebrate segmentation. Nat Rev Mol Cell Biol. 2014 Nov;15(11):709-21. doi: 10.1038/nrm3891. Review.
2. Domingos Henrique, Elsa Abranches, Laure Verrier, Kate G. Storey. Neuromesodermal progenitors and the making of the spinal cord. Development 2015 142: 2864-2875; doi: 10.1242/dev.119768

Recommended readings:

1. Margarete Diaz-Cuadros...Olivier Pourquie. In vitro characterization of the human segmentation clock; bioRxiv doi: https://doi.org/10.1101/461822
2. Bénazéraf B, Francois P, Baker RE, Denans N, Little CD, Pourquié O. A random cell motility gradient downstream of FGF controls elongation of an amniote embryo. Nature. 2010 Jul 8;466(7303):248-52. doi: 10.1038/nature09151.

Schedule:

9:45 – 10:00 am:	Meet in the lobby of NRB building
10:00 – 11:45 am:	Lecture: Somitogenesis and skeletal muscle development (NRB 354)
11:45 am – 12:30 pm:	Lunch and discussion with lab members (Hale 7004)
12:30 – 2:30 pm:	Lab Activity 1: Chicken embryo dissection and electroporation (Hale 8032)
2:30 – 4:00 pm:	Lab Activity 2: Human pluripotent stem cell culture and differentiation (Hale 8032)
4:00 pm:	Session end and depart Hale BTM

Wednesday, January 8, 2020

Development and regeneration in brain and spinal cord (of mouse)

Faculty: Jeff Macklis

Teaching Assistants: Ji-Yoon (Jiji) Kim (G2; MCO, GSAS); Yasuhiro Itoh (postdoc)

Location: CAMBRIDGE

A) Lecture: Sherman Fairchild Building, Rm G62, 7 Divinity Avenue, Cambridge, MA 02138
B) Lab: Bauer Lab Rm 103, 7 Divinity Avenue, Cambridge, MA 02138

Meet in Sherman Fairchild G62 at **10:00 am sharp**!

Approach/methodology:

1. Label corticospinal motor neurons by:
    a. Injection of fluorescent retrograde tracer into spinal cord of postnatal (P2) mouse pups
2. Transfect neural progenitors by:
    a. Injection and electroporation of DNA into lateral ventricle of ~E14.5 mouse embryos *in utero*
3. Review anatomical and cellular labeling methods via:
    a. Observation of cut immunofluorescence sections
    b. Observation of mouse brains prepared with surgical methods performed in first half lab
4. Determine effectiveness of corticospinal motor neuron and neural progenitor labeling with:
    a. Sectioning of previously prepared and labeled brains and spinal cords
    b. Fluorescent imaging of sections

Required readings (to skim):

1. Greig LC, Woodworth MB, Galazo MJ, Padmanabhan H, Macklis JD. Molecular logic of neocortical projection neuron specification, development and diversity. *Nat Rev Neurosci*. 2013; 14(11): 755-69.
2. Woodworth MB, Custo Greig L, Kriegstein AR, Macklis JD. SnapShot: Cortical Development. *Cell*, 2012; 151(4): 918-919.

Recommended readings:

Examples in Regeneration and Subcellular Analysis to skim Abstracts and Figures:

1. Wuttke, Markopoulos, Padmanabhan, Wheeler, Murthy, Macklis. Developmentally primed immature neocortical neurons maintain fidelity and establish appropriate long-distance functional connectivity after micro-transplantation. *Nat Neurosci*, 2018.
2. Poulopoulos*, Murphy*, Ozkan, Davis, Hatch, Kirchner, Macklis. Subcellular transcriptomes and proteomes of developing axon projections in cerebral cortex. *Nature*, 2019.

Optional Additional Papers in Molecular Development of Cerebral Cortex "Projection Neurons"/circuitry

1. Greig LC*, Woodworth MB*, Greppi C, Macklis JD. Ctip1 controls acquisition of sensory area identity and establishment of sensory input fields in the developing neocortex. *Neuron*. 2016 Apr 20;90(2):261-77.
2. Woodworth MB*, Greig LC*, Liu KX, Ippolito GC, Tucker HO, Macklis JD. Ctip1 regulates the balance between specification of distinct projection neuron subtypes in deep cortical layers. *Cell Rep*. 2016 May 3;15(5):999-1012.

3. Galazo MJ, Emsley JG, Macklis JD. Corticothalamic projection neuron development beyond subtype specification: Fog2 and intersectional controls regulate intraclass neuronal diversity. *Neuron*. 2016 Jul 6; 91: 90-106.

Schedule:

10:00 am – 10:50 am	SH G62 – J. Macklis overview lecture on mammalian neuronal organization, molecular development (/progenitor/stem cell biology), diversity, and axon growth, guidance, and connections and neuronal regeneration
10:50 am – 11:30 am	SH G62 – Experimental approach lectures by Ji-Yoon Kim and Yasuhiro Itoh
11:30 am – 11:50 am	Lunch break
12:00 pm – 2:00 pm	Mouse neonatal injection and *in utero* electroporation experiments
2:15 pm – 4:15 pm	Mouse brain tissue processing and microscopic imaging analysis

Thursday, January 9, 2020

Techniques for studying axolotl limb regeneration

Faculty: Jessica Whited

Teaching Assistants: Burcu Erdogan, Duygu Payzin-Dogru, Fallon Durant, Steve Blair, Madison Hurley, Hani Singer

Location: CAMBRIDGE

A) Lecture: Sherman Fairchild Building, Rm G62, 7 Divinity Avenue, Cambridge, MA 02138
B) Lab: Sherman Fairchild Building, 1st floor wet lab and basement aquatics facility, 7 Divinity Avenue, Cambridge, MA 02138

Meet in Sherman Fairchild Lobby at **9:45 am.**

Rationale:

The age-old question of why many salamanders can regenerate limbs with perfection well into adulthood has not been resolved. However, many modern approaches can now be brought to this problem and, combined with older techniques, promise to enable a deepened molecular understanding of the process. Understanding limb regeneration in these animals will offer critical insight into why mammals have little postnatal regenerative abilities in key organs, which may suggest possible therapeutic avenues in the future to address issues such as limb loss.

Learning objectives:

At the end of this Bootcamp session, you should be able to:

1. Appreciate the unresolved questions in limb regeneration and understand how modern approaches can be applied to these questions.
2. Describe how to generate transgenic axolotls. (If fresh eggs are not being laid at the time of the class, then we will use the most recent eggs in an embryo staging exercise.)
3. Visualize skeletal structures in any organism using a simple staining procedure.
4. Appreciate tissue transplantation techniques.

Approach/methodology:

1. Perform transgenesis with axolotl embryos.
    a. Collect and de-jelly axolotl embryos.
    b. Prepare DNA SMA::mCherry construct and DNA injection station.
    c. Injection of DNA.
2. Observe skeletal anatomy of axolotls.
    a. Alcian blue and alizarin red staining of axolotls.

3. Electroporation of either control (GFP) or gene of interest into limb blastemas.

Required Reading:

1. Haas, B. J., and **Whited, J. L.** 2017. Advances in decoding axolotl limb regeneration. Review. *Trends in Genetics*, 33(8)553-565. PMID: 28648452.
2. Payzin-Dogru, D., and **Whited, J. L.** 2018. An integrative framework for salamander and mouse limb regeneration. Review. *Int'l Journal of Developmental Biology*, 62:(6-7-8). PMID: 29943379.

Schedule:

There will be four stations: (1) embryo de-jellying; (2) embryo injection; (3) skeletal staining; (4) electroporation

9:45 – 10:00 am:	Meet in the lobby of Sherman Fairchild (7 Divinity Avenue)
10:00 am – 1 pm:	Orientation in lab & preparatory work & rotation through 2 stations
1:00 – 2:15 pm:	Lecture and discussion over lunch (SH G62)
2:15 – 2:30 pm:	Break
2:30 – 4:00 pm:	Students rotate through remaining station

Friday, January 10, 2020

Zebrafish organogenesis – the hematovascular and hepatobiliary systems

Faculty: Trista North and Wolfram Goessling

Student Teaching Assistants: Rebecca Soto, Olivia Weeks, Scott Freeburg, Bess Miller

Location: LONGWOOD

A) Lecture: Karp Family Research Building 05-201, 1 Blackfan Circle, Boston MA 02115
B) Lab: HMS New Research Building, 33 Avenue Louis Pasteur, Boston MA 02115

Meet in the 1st floor of the Karp building at the security desk by **10:00am (sharp).**

Students will be escorted to and from each site due to security restrictions*.

*NOTE: A Harvard ID is REQUIRED for access to each of the buildings

Rationale:

The development of functional organ systems, including the genetic networks controlling these processes, is remarkably well conserved across vertebrate species. Zebrafish provide a unique model system in which one can utilize the many advantages of invertebrate models (fast generation time, large cohort/clutch sizes, rapid mutagenesis, ex vivo development) to evaluate the formation of cell types and tissues present only in vertebrate species. Recent advances in gene modifications, cell labeling approaches and chemical genetics, have propelled the Zebrafish model forward as an exceptional system for identifying and evaluating the impact of novel regulatory pathways on embryonic development in vivo. These studies have led to the identification of causal mutations in several human disease states, characterization of novel rational adjuvants and therapeutics for tissue regeneration and cancer, and highlighted the strong conservation of both cellular and genetic function between fish, mice and humans. The lab is designed to familiarize the student with zebrafish development in comparison with that of other vertebrates, and illustrate some the current methodologies available in the Zebrafish system for studying vertebrate organogenesis, with a focus on the development of the hematovascular and hepatobiliary systems.

Approaches:

1. Analysis of alterations in blood and vascular formation

    A. Light microscopy for developmental abnormalities (blood)

    B. Fluorescence microscopy of blood and vascular development

    C. Analysis of whole mount in situ for blood stem cells after chemical modulation

    D. O-dianisidine staining for blood defects in mutant lines

2. Characterization of modification of liver development and homeostasis

    A. Light microscopy for developmental abnormalities (liver)

    B. Fluorescence microscopy of endodermal lines for altered development

    C. Analysis of whole mount situ hybridization for hepatocytes after chemical modulation

    D. Immunohistochemical analysis after hepatic mutation/injury

Objectives:

1) To become familiar with the utility and approaches of the zebrafish model for the study of vertebrate organogenesis

2) To understand various methodologies available to assess tissue specific development using zebrafish embryos

3) To appreciate the alternative means of modifying tissue repair in vivo in the zebrafish model

Required Readings:

Blood:

Frame J, Lim SE, **North TE**. Hematopoietic stem cell development: using the zebrafish to identify extrinsic and intrinsic mechanisms regulating hematopoiesis. **Methods in Cell Biology.** 2017. 138:165-192.

Endoderm:

Cox AG and Goessling W. The lure of zebrafish in liver research: regulation of hepatic growth in development and regeneration. **Curr Opin Genet Dev**. 2015. 32:153-161.

Schedule:

9:45 am – 10:00 am:	Meet in the first-floor lobby of the Karp building to be escorted to North lab
10:00 am – 10:15 am:	Introduction to Zebrafish development (Karp 5th floor conference room (05-201)
10:15 am – 10:45 am:	Lecture (30 mins) - Hematovascular development (Karp 05-201)
10:45 am – 12:15 pm:	Lab Activity 1 (1.5 hours)- Blood Specification and Production (Karp 05-004)
12:15 pm – 12:30 pm:	Walk to New Research Building (NRB)
12:30 pm – 1:30 pm:	Lunch and Career Discussion with Trista and Wolfram (NRB 457)
1:30 pm – 2:00 pm:	Lecture (30 mins) - Hepatobiliary development (NRB 457)
2:00 pm – 3:30 pm:	Lab Activity 2 (1.5 hours) – Liver Development and Repair, NRB-458)
3:30 pm:	Session end and depart NRB

Monday, January 13, 2020

Epigenetic Regulation in *C. elegans* and *D. discoideum*

Faculty: Eric Greer

Teaching Assistants: Konstantinos Boulias and Simon Wang

Location: LONGWOOD

A) Lecture: Enders Building Rm 1044, Boston Children's Hospital, 320 Longwood Ave, Boston MA 02115
B) Lab: Enders Building Rm 1010, Boston Children's Hospital, 320 Longwood Ave, Boston MA 02115

Meet at Enders Building, BCH, 320 Longwood Ave Rm 1044 at **10:00 am.**

Rationale:

Caenorhabditis elegans provides a powerful model organism which is easy to manipulate both genetically and environmentally. *C. elegans* short generation time, 3 days from hatching to reproductive maturity, make it ideal for examining transgenerational biology. This coupled with the fact that most basic biological processes are conserved in *C. elegans* (several were initially identified in the nematode!) make it a good model system to understand.

Dictyostelium discoideum undergoes rapid physiological and morphological changes without changes to its genetic material making it an ideal model organism for studying epigenetics. *Dictyostelium* have clear selfish and altruistic cells and is also one of the rare organisms which can exist in unicellular and multicellular states. Therefore, we are trying to develop *Dictyostelium* as a new epigenetic model organism to study the involvement of epigenetics in the evolution of cellular altruism and multicellularity.

Learning objectives:

At the end of this Bootcamp session, you should be able to:

1. Explain the history and anatomy of *C. elegans* and *Dictyostelium discoideum* and the experimental tools available in these model organisms.
2. Understand how both models are used to decipher the molecular mechanisms underlying transgenerational epigenetic inheritance and the evolution of multicellularity.

Approach/methodology:

1. Characterize *C. elegans* and *Dictyostelium* as model organisms and describe the role of each in the field of epigenetics.
    a. Lecture & paper discussion.
2. Perform *C. elegans* husbandry.
    a. Make a pick.
    b. Observe basic worm anatomy and growth and identify different developmental stages and phenotypes.
    c. Set up mating experiments (if time allows).
3. Inject *C. elegans* to learn how to create transgenic worm lines.
4. Examine *C. elegans* fluorescent strains with different reporter lines.
5. Practice *Dictyostelium* husbandry and experiment.

a. Observe *Dictyostelium* life stages.
b. Perform *Dictyostelium* chemotaxis assays.

Required reading:

1. Moore RS, Kaletsky R, and Murphy CT Piwi/PRG-1 Argonaute and TGF-ß Mediate Transgenerational Learned Pathogenic Avoidance *Cell* 2019 177(7):1827-1841

Recommended reading:

2. Gaydos LJ, Wang W, and Strome S Gene repression. H3K27me and PRC2 transmit a memory of repression across generations and during development *Science* 2014 345(6203):1515-8
3. Heard E and Martienssen RA Transgenerational epigenetic inheritance: myths and mechanisms Cell 2014 157(1): 95-109

Schedule:

10:00 am – 11:30 am:	Lecture
11:30 am – 12:00 pm:	Set up *Dictyostelium* chemotaxis assays
12:00 pm – 12:30 pm:	Student led paper discussion
12:30 pm – 1:30 pm:	Lunch and brainstorming
1:30 pm – 4:00 pm:	Lab activities. Students will break up into three groups and rotate through different modules.
4:00 pm	End of session; attend DRB faculty student seminar if possible (NRB 350)

Tuesday, January 14, 2020

Mammalian skin and hair follicle regeneration

Faculty: Ya-Chieh Hsu

Teaching Assistants: Sekyu Choi, Megan He, Yulia Shwartz, Bing Zhang

Location: CAMBRIDGE

A) Lecture: Sherman Fairchild Rm G62, 7 Divinity Avenue, Cambridge MA 02138
B) Lab: Sherman Fairchild 2nd floor, 7 Divinity Avenue, Cambridge MA 02138

Meet at Sherman Fairchild G62 (7 Divinity Ave, Cambridge) at **10:00am**

Rationale:

Skin, the largest organ we have, protect us from insults, dehydration, and infection. It is the most accessible organ and is also one of the very few organs in mammals that undergo regeneration and repair throughout our life. Using cultured epidermal cells to treat burn patients has been a long-standing success in regenerative medicine. The skin stem cells and their niches are spatially defined. The skin is also sensitive to systemic changes of the body, for example stress and aging. Together, the mammalian skin thus represents an excellent paradigm to study questions in Developmental Biology and Regeneration.

Learning objectives:

At the end of this Bootcamp session, you should be able to:

1. Understand developmental biology and stem cell biology of the skin.
2. Understand the progression of hair cycle and gain appreciation of how one can use the naturally occurring hair cycle as a paradigm to study tissue regeneration.
3. Describe tools and approaches used to study skin regeneration based on your own practical experience.
4. Understand approaches to study interactions between endocrine glands, peripheral nervous system, and tissue regeneration

Approach/methodology:

1. Perform techniques for inducing and visualizing skin regeneration including:
    a. Wounding, suturing, intradermal injection, OCT embedding of mouse skin, and cryosectioning.
2. Test neuronal and endocrine influences on tissue regeneration and stress responses, respectively.
    a. Conduct adrenalectomy to remove a major endocrine gland crucial for stress response.
    b. Conduct denervation to test neuronal influences on tissue regeneration.
3. Appreciate the wide variety of cell types in the skin, including epidermis, dermis, hair follicles, adipocytes, immune cells, and nerve fibers using immunofluorescence.
4. Solve “real life” projects by applying your knowledge gained in class.

Required reading:

1. Bing Zhang, Pai-Chi Tsai, Oliver Chung, Benjamin Boumard, Carolina N. Perdigoto, Elena Ezhkova, and Ya-Chieh Hsu (2016) Hair Follicles' Transit Amplifying Cells Govern Concurrent Dermal Adipocyte Production through Sonic Hedgehog. ***Genes & Development***, 30(20):2325-2338.

Recommended reading:

1. Ya-Chieh Hsu, Lishi Li, and Elaine Fuchs (2014) Transit-Amplifying Cells Orchestrate Stem Cell Activity and Tissue Regeneration. *Cell* 157: 935-949
2. Ya-Chieh Hsu, Lishi Li, and Elaine Fuchs (2014) Emerging Interactions between Skin Stem Cells and Their Niches. *Nature Medicine* 20:847-856

Schedule:

10:00 am – 11:30 am:	Lecture (Sherman Fairchild G62)
11:30 am – 1:00 pm:	Lunch and brainstorming (Sherman Fairchild G62)
1:00 pm – 2:00 pm:	Laboratory module 1: Techniques to study skin stem cells
2:00 pm – 3:00 pm:	Laboratory module 2: Adrenalectomy and denervation surgery
3:00 pm – 4:00 pm:	Laboratory module 3: Case studies: solving phenotype puzzles

Wednesday, January 15, 2020

Drosophila as a model system for examining neural stem cell fate decisions

Faculty: Kristin White

Teaching Assistants: Kate Harding, Katerina Heath

Location: CHARLESTOWN

C) Lecture: CBRC tea room 3.230, MGH Charlestown Navy Yard, Building 149, 14 13th Street, Charlestown, MA 02129
D) Lab: CBRC fly lab 3.207, CBRC confocal core 3.226, MGH Charlestown Navy Yard, Building 149, 14 13th Street, Charlestown, MA 02129

Meet in CNY149 guard desk in the lobby **9:45am (sharp).**

*NOTE: Students should bring their Harvard ID to get into buildings!

To facilitate presentations at the end of the day, students should bring laptops

Rationale:

This session is focused on demonstrating the power of Drosophila as a model system to understand stem cell fate decisions, in particular programmed cell death. We will review the current understanding of the role of cell death in nervous system development, and how it is regulated. We will also discuss the value of genetic interaction screens and their design, including underlying assumptions. In the laboratory exercise, we will use nervous system dissections and fluorescent microscopy to examine the role of epigenetic regulators in modifying the Sox2 phenotype, both in terms of neural stem cell viability and organismal lethality. By the end of this session, we will have conveyed the value of genetic approaches, and demonstrated the strengths of the Drosophila system in examining stem cell fate decisions.

Learning objectives:

At the end of this Bootcamp session, you should be able to:

1. Appreciate the contribution of cell death to normal development.
2. Understand how genetic interaction screens can help identify mechanisms of gene function.
3. Consider the importance of experimental controls.
4. Learn to use publicly available databases (FlyBase) to understand gene functions.
5. Brainstorm possible approaches to follow up on genetic interactions identified in an enhancer/suppressor screen.

Approach/methodology:

1. Understand how genetic interaction screens can be used to determine possible mechanisms of action of a gene of interest.
    a. Students will assess the effect of gene knock down on organismal lethality caused by overexpression of the gene of interest (Drosophila Sox2)

b. Students will use nervous system dissection and microcopy to understand how changes in ectopic stem cell survival are correlated with organismal viability.

Required reading:

1. Harding K, **White K**. Drosophila as a Model for Developmental Biology: Stem Cell-Fate Decisions in the Developing Nervous System. J. Dev. Biol. 2018, 6, 25.

Schedule:

9:45 am:	Meet in the first-floor lobby of Building 149, 13th St., in the Charlestown Navy yard, to be escorted to White lab
10:00 am – 10:45 am:	Lecture-Programmed cell death in nervous system development and introduction to lab exercises (CBRC tea room-3.230)
10:45 am – 11:15 am:	Introduction to CNS dissections (Fly room)
11:15 am – 12:30 pm:	Lab activity – ALL: Larval CNS dissections and mounting slides
12:30 pm – 1:00 pm:	Return to conference room for lunch and informal discussion
1:00 pm – 2:00 pm:	Lab Activity Group will be split- probably working in teams of 2-3 Group 1 Assaying genetic interactions through changes in viability Group 2 Nervous system microscopy
2:00 pm – 3:00 pm	Lab activity-groups switch
3:00 pm – 3:30 pm:	CBRC tea room- Prepare presentations of data including understanding the general functions of the genes tested by the group and thoughts about follow up
3:30 pm – 4:00 pm:	Presentations and discussion

Thursday, January 15, 2020

Development and regeneration of the musculoskeletal system

Faculty: April Craft & Jenna Galloway

Teaching Assistants: Galloway lab: Xubo Niu, Marie Noedl, Luke O'Connor
Craft lab: Mireia Alemany Ribes, Rosie Raftery, Jimmy Cai, Steve Pregizer

Location:

A) Lecture: Enders Research building 2nd floor, room 244, Boston Children's Hospital, 320 Longwood Ave, Boston 02115
B) Lab: Enders Research building 2nd floor, Rm 270 and 212, Boston Children's Hospital, 320 Longwood Ave, Boston 02115

Meet in the lobby of the Enders Research Building at 9:45 am!

NOTE: Students should bring their Harvard ID to get into buildings!

Rationale:

Interdisciplinary approaches to studying musculoskeletal biology can broaden our understanding of biological mechanisms and accelerate our ability to treat human disease. Because of its strong conservation with higher vertebrate developmental programs, the zebrafish provides a unique opportunity to perform high-throughput screens to identify new regulators of the musculoskeletal system. Embryonic stem cells have remarkable power to generate any and all cell types found in our body. However, this approach requires a foundation of developmental biology and precise experimental techniques. Clinical translation of these discoveries is possible due to the serum-free directed differentiation approach that results in the pure populations of target cells. This session will introduce the techniques available to study the musculoskeletal system in the zebrafish and embryonic stem cells, with a particular emphasis on tendon and cartilage tissues. Here we will show how hits from a zebrafish high-throughput chemical screen can be identified and applied to embryonic stem cell models with the goal of generating end stage cartilage and tendon tissues to repair damaged tissues in a large animal model.

Learning objectives:

At the end of this Bootcamp session, you should be able to:

3. Describe why the zebrafish is a good model for understanding musculoskeletal development and regeneration.
4. Identify phenotypic changes to musculoskeletal tissue formation in a chemical screen.
5. Test candidate hit chemicals and their targeted pathways in ESC/iPSC directed differentiation cultures.
6. Understand the limitations of current cell and tissue based surgical approaches for the treatment of cartilage and tendon injuries, and the challenges associated with translation of cell and tissue-based therapies into the clinic.

Approach/methodology:

1. Characterize zebrafish development and musculoskeletal tissue formation via observation.
2. Identify hit compounds via genetic and chemical screening.
3. Analyze lineage fate of ESCs.
    a. Directed differentiation of ESCs towards cartilage and tendon.

b. Observe differentiated ESCs with fluorescence microscopy and flow cytometry.

4. Discuss the opportunities, promises, and challenges of clinical translation of human pluripotent stem cell-derived tissues.

Required Reading:

1. **Craft AM**, Ahmed N, Rockel JS, Baht GS, Alman BA, Kandel RA, Grigoriadis AE, Keller GM. Specification of chondrocytes and cartilage tissues from embryonic stem cells. *Development*. 2013 Jun;140(12):2597-610. PubMed PMID: 23715552.
2. Chen, JW and **Galloway, JL**. (2014). The development of zebrafish tendon and ligament progenitors. *Development*, 141: 2035-2045.

Recommended Reading:

1. Gardner OFW, Juneja S, Whetstone H, Nartiss Y, Sieker J, Veillette C, Keller GM, **Craft AM**. Effective repair of joint cartilage using human pluripotent stem cell-derived tissue. *eCM*. 2019 Nov 5; 38:215-227. DOI: .10.22203/eCM.v038a15. www.ecmjournal.org/papers/vol038/vol038a15.php
2. Xu C, Tabebordbar M, Iovino S, Ciarlo C, Castiglioni A, Price E, Liu M, Barton ER, Kahn CR, Wagers AJ, Zon LI. A zebrafish embryo culture system defines factors that promote vertebrate myogenesis across species. Cell 2013 Nov 7, 155 (4):909-921.

Recommended Reviews:

1. Camarero-Espinosa S, Rothen-Rutishauser B, Foster EJ, Weder C. Articular cartilage: from formation to tissue engineering. Biomater Sci. 2016 May 26;4(5):734-67. doi: 10.1039/c6bm00068a. *Pages 734-746 only* (through section 4.1: cell sources and culture conditions)
2. Huang AH, Lu HH, and Schweitzer, R. (2015) Molecular Regulation of Tendon Cell Fate During Development. Journal of Orthopaedic Research, 33(6): 800-812.

Schedule:

10:00 am – 10:30 am:	Introductory Lecture (EN244): Musculoskeletal Development and Genetic Approaches in the Zebrafish
10:30 am – 12:00 pm:	Lab activities – Students will analyze zebrafish embryos using bright field and fluorescent microscopy (1) Analysis of zebrafish development time course (2) Analysis of musculoskeletal tissue phenotypes in a chemical screen
12:00 pm – 1:00 pm:	Working Lunch (EN244): Discussion of Results & Intro Lecture on Directed Differentiation of Pluripotent Stem Cells into Cartilage and Tendon Lineages
1:00 pm – 2:45 pm:	Lab activities – Students will test the hypothesis that chemicals that induce cartilage or tendon fates in zebrafish embryos have conserved roles in stem cell cultures (1) Evaluate fate of mESC-derived cells following chemical treatment using microscopy (2) Flow cytometry analysis of mESC cultures treated with chemicals
2:45 pm – 3:15 pm:	On-screen FACS analysis, Discussion of results/conclusions, Intro to clinical translation
3:15 pm – 4:00 pm	Lab activity: Repairing cartilage defects in a porcine knee explant

Friday, January 16, 2020

Student Presentation Showcase

To synthesize and apply what was learned during the bootcamp sessions, as well as practice communication skills, students will be asked to complete a brief final assignment.

Working alone or in pairs, students will propose experiments to a) extend ongoing investigations or b) address a related question of interest using methods and models covered in the course.

Each presenter/ presenting pair will have 5 mins to **outline** their *objective*, *hypothesis*, and *proposed approach*, and *potential limitations* followed by and/or including up to 5 mins of interactive Q/A from classmates (*10 minute total maximum* presentation and discussion time)

The presenter may choose to take questions throughout or hold them until the end of the talk.

Two presentation formatting options are available:

1) Chalk talk, using prepared notes and white board
2) Slide presentation, using 5 slides or less (images/diagrams must be student made)

On the next page is a rubric for your presentation (modified from *Entering Research*).

Rubric:

	0	1	2	3
Introduction (background, context, objective)	absent	Background info presented lacked the content needed to understand the scientific basis of hypothesis	Relevant background info & broader significance of the research was presented, but poorly organized	Relevant background info & broader significance of the research was presented & organized such that they hypothesis logically followed
Hypothesis (testable, a prediction)	absent	A statement was made, but it was neither a hypothesis nor a research question	A hypothesis statement was made, but it was neither concise nor followed logically from background info	A clear and concise hypothesis was made that followed logically from the background info
Research methods (proposed approach)	absent	Experiments were described, but were not connected to the stated hypothesis; expected results not explained or connected to hypothesis	Either experiments or expected results were well-explained and connected to the stated hypothesis, but not both	Both experiments and expected results were well-explained and directly connected back to the hypothesis
Potential limitations	absent	Limitations were mentioned	Limitations were mentioned and discussed with the group	Limitations were mentioned & alternative experiments or ideas proposed, then discussed with group

NOTES: