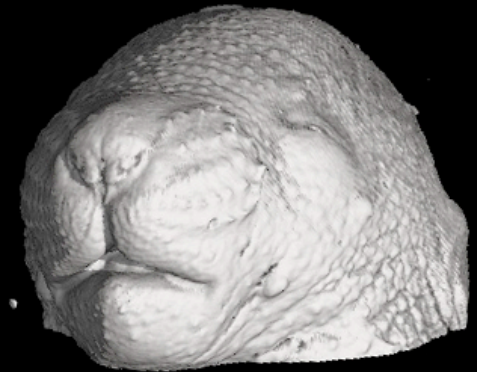
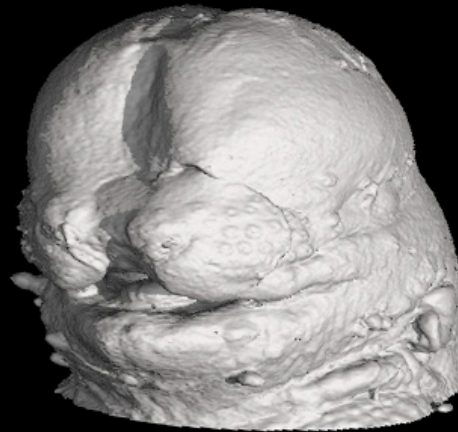
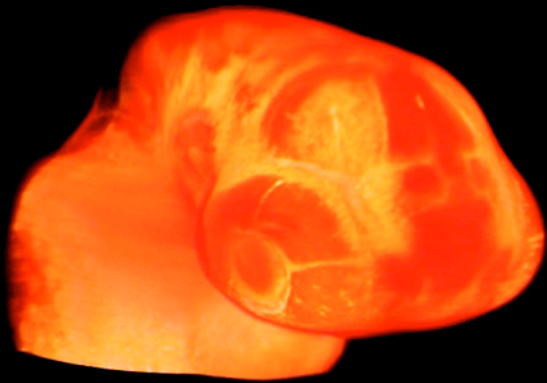


Multidimensional imaging of craniofacial development.



Scott Fraser

Departments of Molecular & Computational Biology,
Stem Cell and Regenerative Medicine, etc

USC

Biological Imaging Center

Caltech

 **FaceBase**

A Resource For Craniofacial Researchers

Multidimensional imaging of craniofacial development.

Volumetric Imaging of normal and mutant embryos

Tools for browsing, measuring, comparing

Multiplex in situ hybridization

Quantitative imaging of gene expression

Live specimen imaging

Two-photon light sheet

MRI microscopy

Transgenic quail

Transgenic zebrafish

Photoactivatable/convertible FP's

Binary system for isolating defined cell populations

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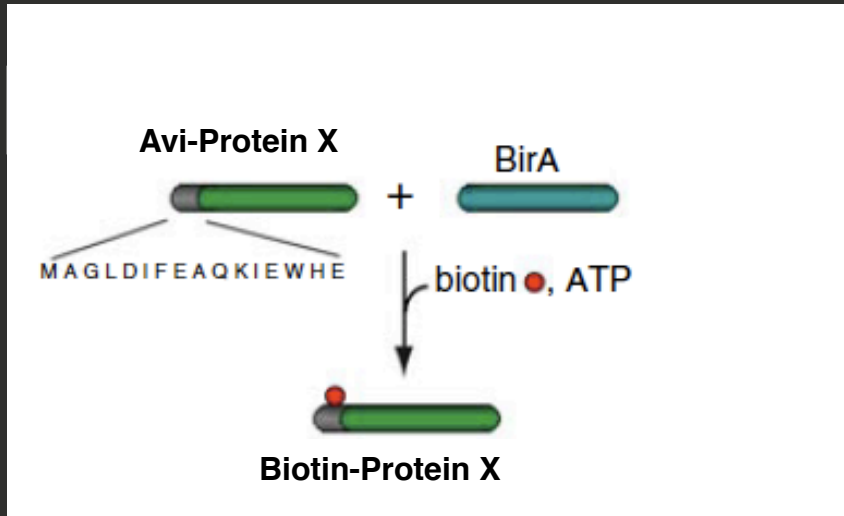
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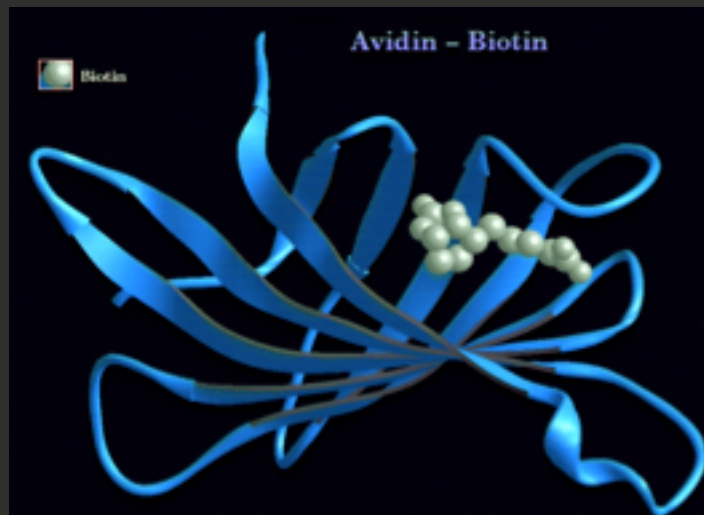
A Resource For Craniofacial Researchers

In vivo biotinylation using the biotin ligase, BirA



BirA

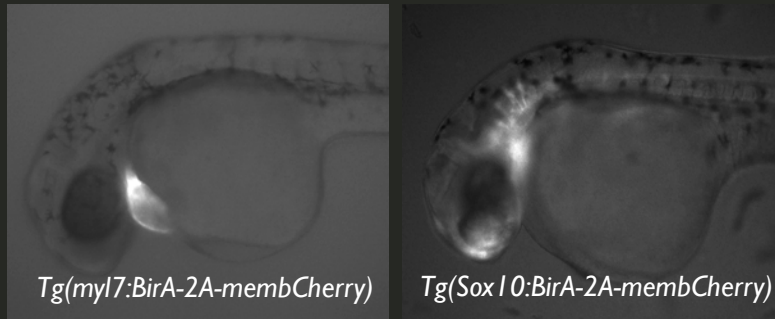
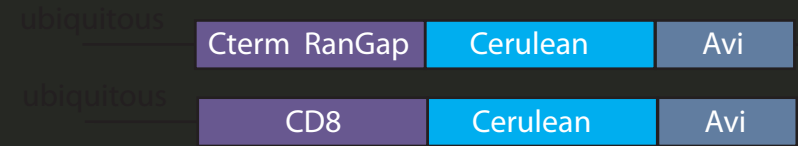
- Biotin holoenzyme synthetase
- Transfers biotin to a single lysine in Avi peptide sequence



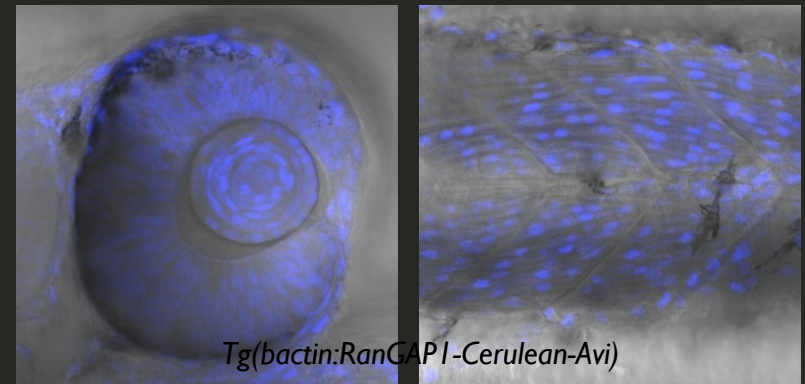
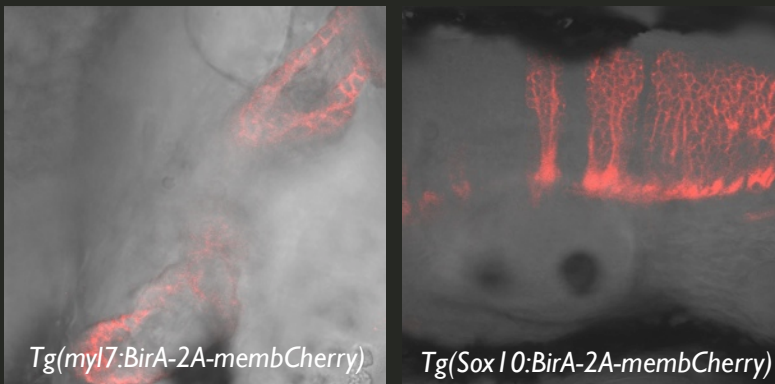
Biotin-Avidin:

- Strongest non-covalent interaction in nature (K_d of $\sim 10^{-14}$ mol/L)
- Used routinely for IHC and recently for CHIP and IP

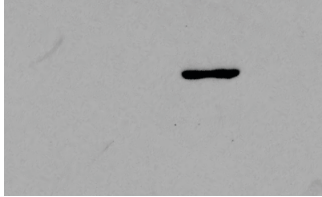
Binary approach: BirA and Avi-tagged target



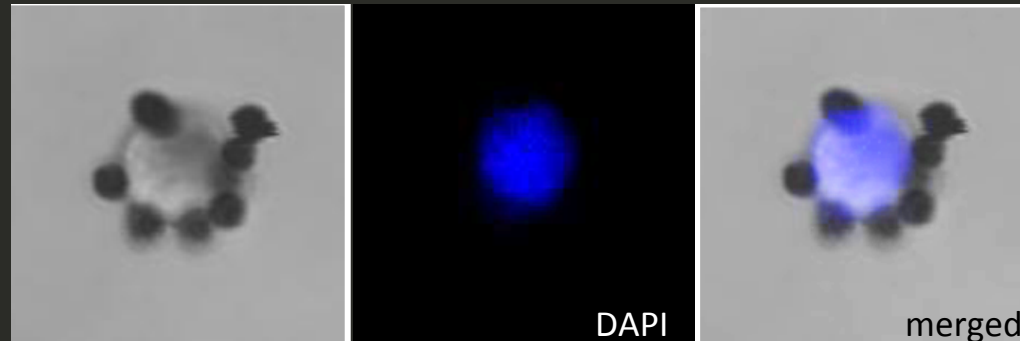
X



Specific isolation with streptavidin beads

	transgenic		wt	
Sox10:BirA	+	-	+	-
Avi RanGap	-	+	+	-
Streptavidin				

Selected nuclei isolated in a single affinity step using streptavidin-coated magnetic beads



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MRI Atlasing - 7T & 11.7T

Fixed, Golgi Stained (soft tissue), Gd soaked (Speed)

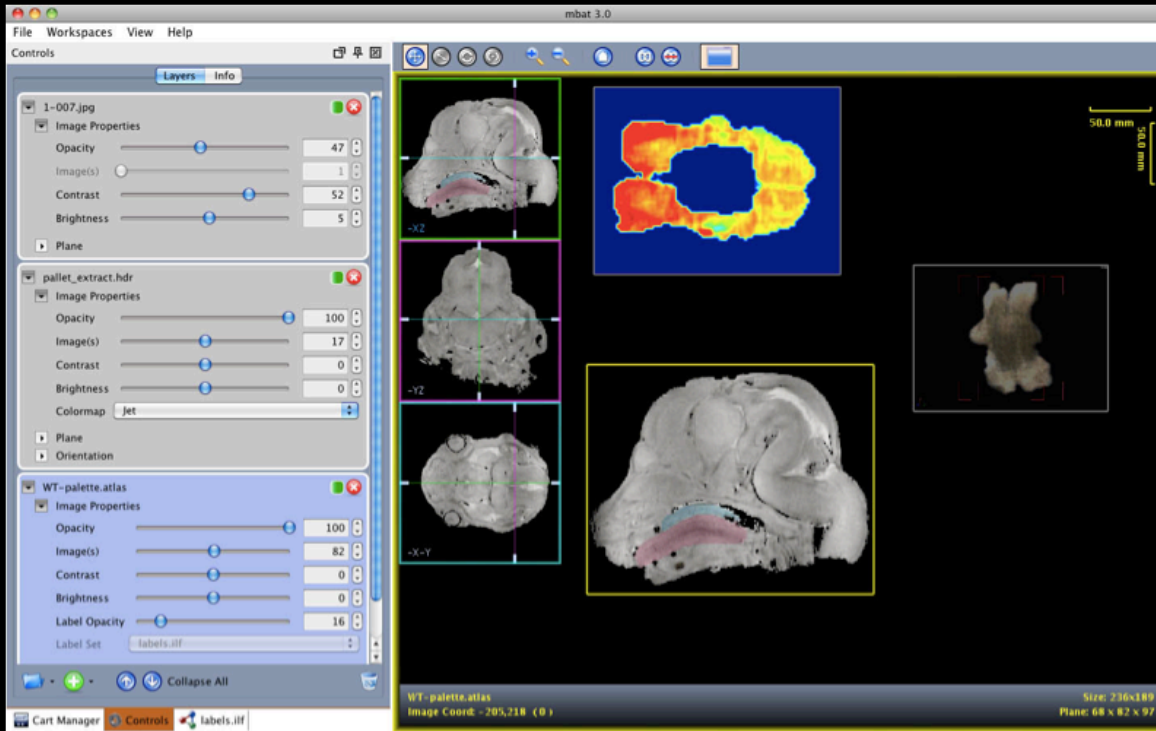
>100 on Hub

downloadable

variety of viewers

The screenshot shows a web browser window displaying the USC-CALTECH FACEBASE IMAGE CATALOG. The browser's address bar shows the URL <http://131.215.15.120/#>. The website header includes the text "USC-CALTECH FACEBASE IMAGE CATALOG" and navigation tabs for "home", "wildtype", and "TgflsCKO". The main content area features a sidebar on the left with the heading "About this site" and several paragraphs of text describing the catalog's purpose and data sources. The main content area displays MRI data for a "18.5 dpc WT" mouse, with the subject ID "hs04_17nov10". Three MRI slices are shown: a sagittal view, a frontal view, and a transverse view. Each slice has a corresponding slider below it for navigation. A "download image_data" link is visible in the top right corner of the main content area.

MRI Atlasing - Data viewed with MBAT (BIRN)



Problems:

Data not easily browsable

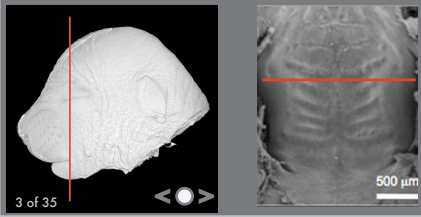
Viewers are powerful, but hard to adopt

Fantasy: A Multimodal Interface

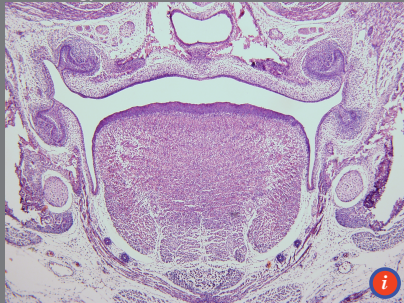


Active data

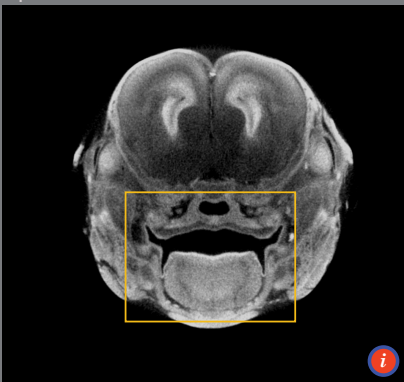
Stage: 18.5dpc Strain: C57Bl/6



Histology



μCT



Select related

stage (dpc)

- 12.5
- 13.5
- 14.5
- 15.5
- 16.5
- 17.5
- 18.5

stage (dpc)

- 12.5
- 13.5
- 14.5
- 15.5
- 16.5
- 17.5
- 18.5

Wildtype

Mutant

Mutation

Search

Micro-array

Find

Contrast

H&E

in situ hybridization

μCT

μMRI

OPT

Results

add selected to my collection

My collection

Fantasy: A Multimodal Interface w/ videos

FaceBase Mouse Image Data Explorer

Hello, Seth W Ruffins Log out

About Data **Tools** Resources Community Publications Biorepository Search FaceBase...

Active data Select related My collection

Stage: 3 of 3

Hist

μ CT

add selected to my collection

Fantasy: A Multimodal Interface w/ videos



Mouse Image Data Explorer

Hello, Seth W Ruffins

Log out

About

Data

Tools

Resources

Community

Publications

Biorepository

Search FaceBase...

Active data

Select related

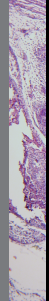
My collection

Stage:

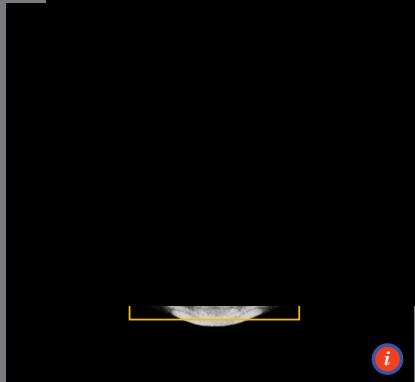


3 of

His



μ C



add selected to my collection

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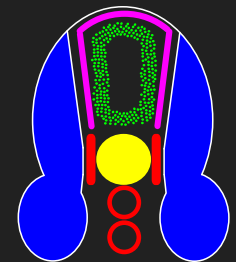
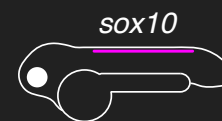
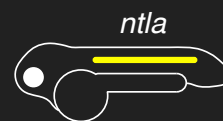
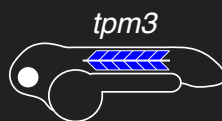
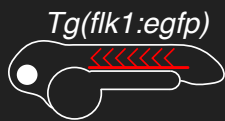
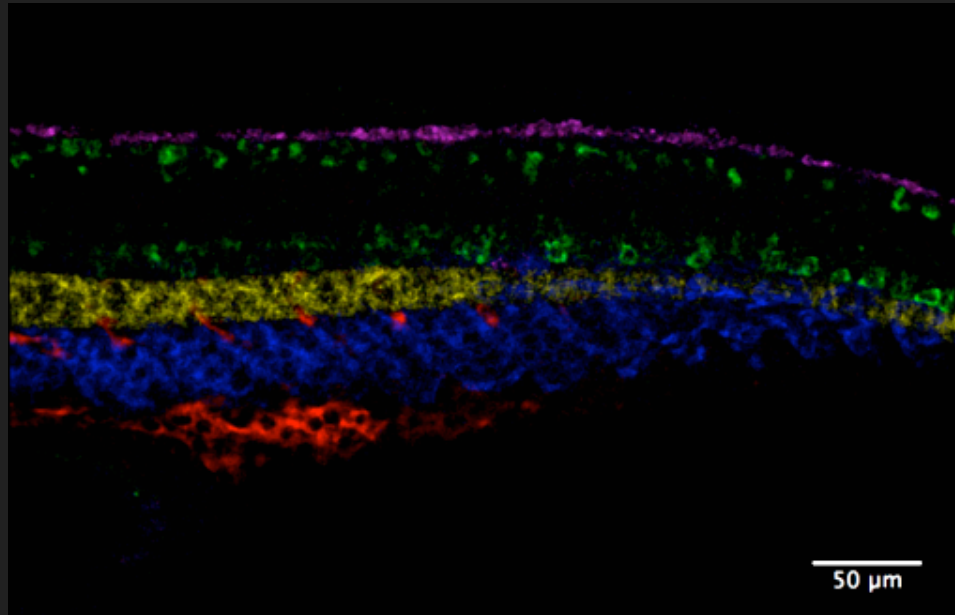
Photoactivatable/convertible FP's

Binary system for isolating defined cell populations

 **FaceBase**

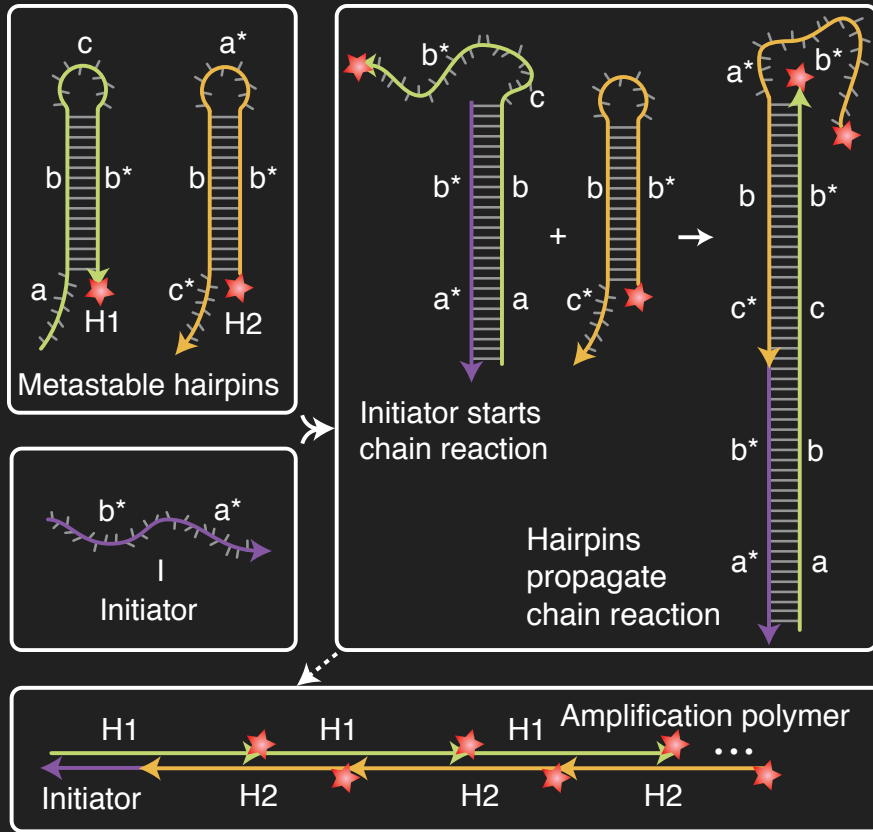
A Resource For Craniofacial Researchers

Five-Plex mRNA FISH in a fixed whole-mount zebrafish embryo



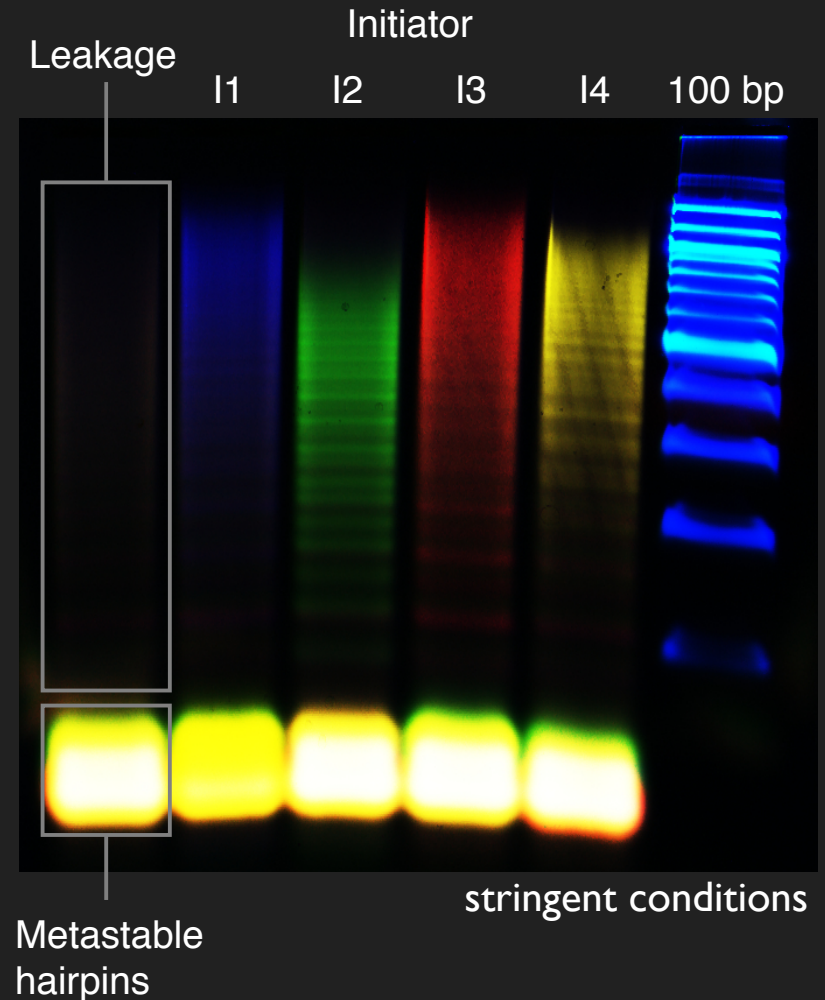
Hybridization Chain Reaction (HCR)

Hybridization Chain Reaction (HCR)

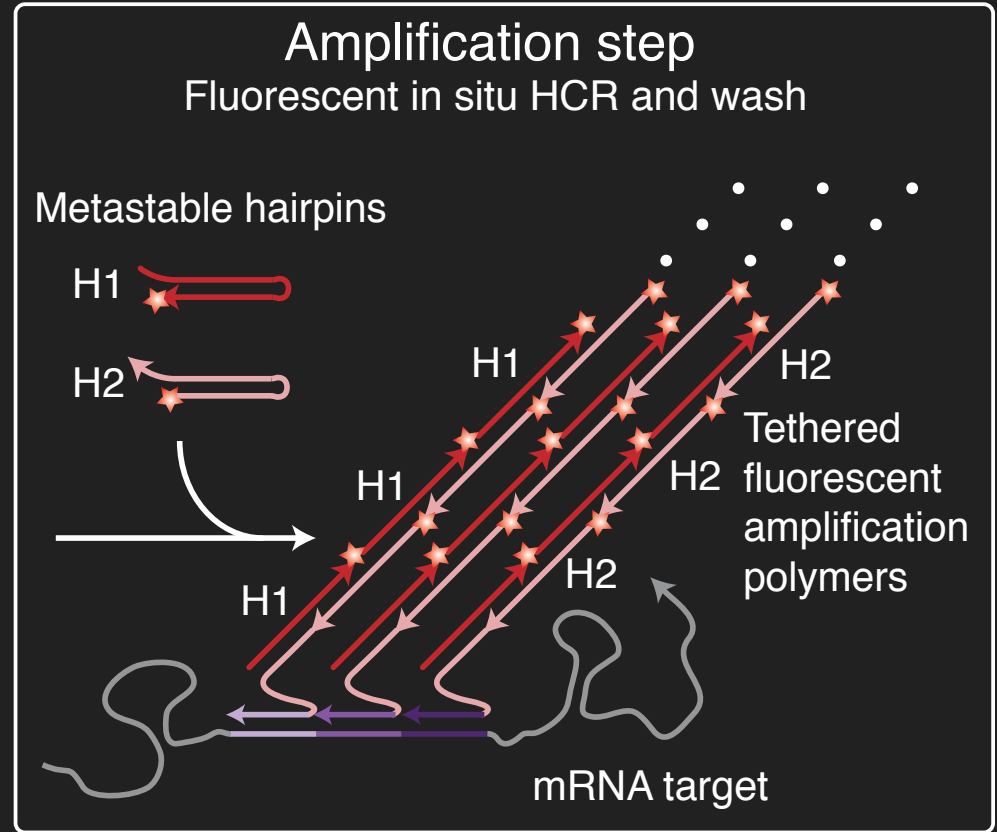
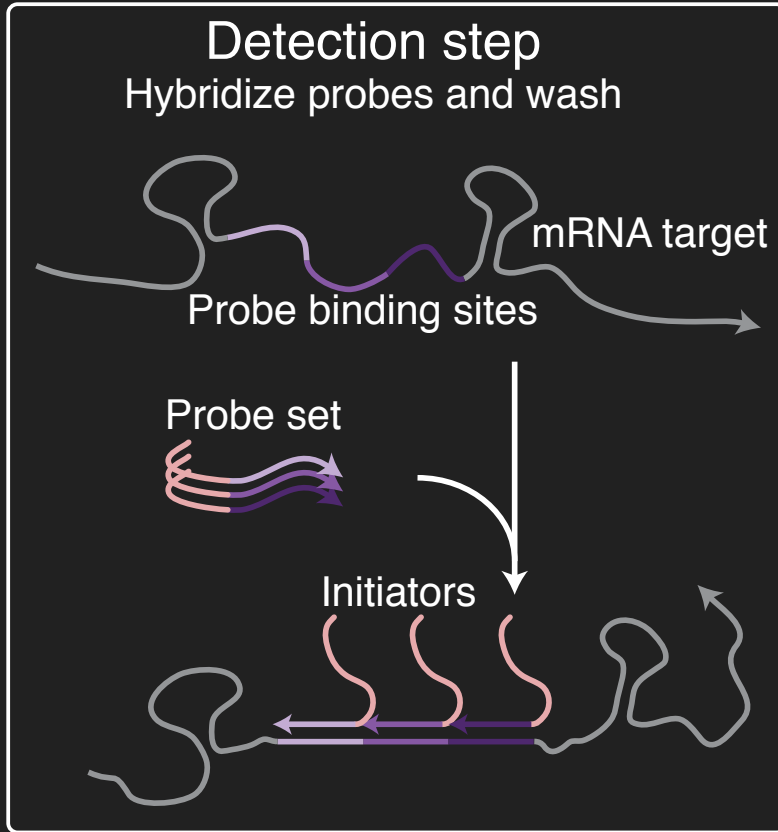


Dirks and Pierce, *Proc Natl Acad Sci USA*, 2004

Orthogonal HCR amplifiers

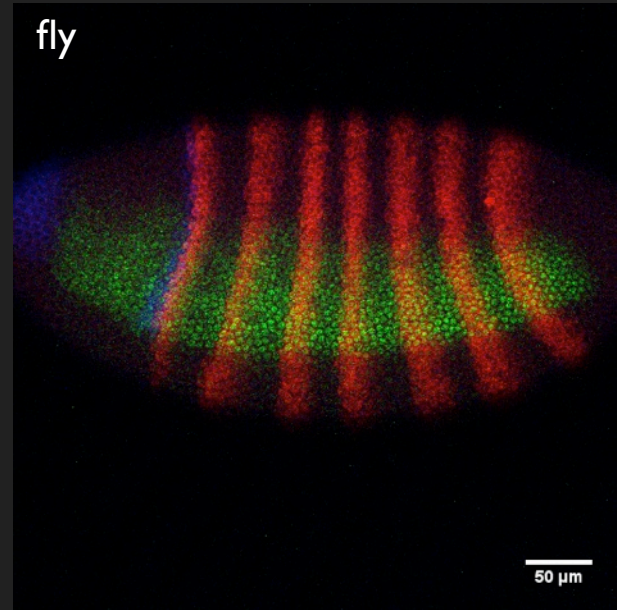


Multiplexed in situ hybridization using HCR

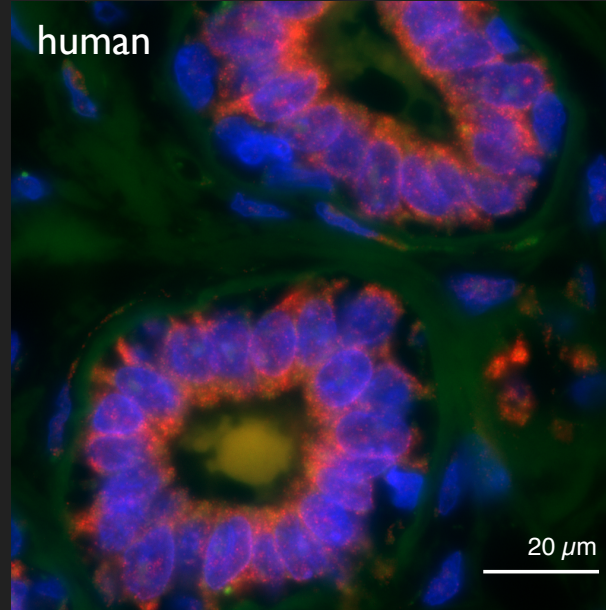


Adapting HCR for Diverse Systems

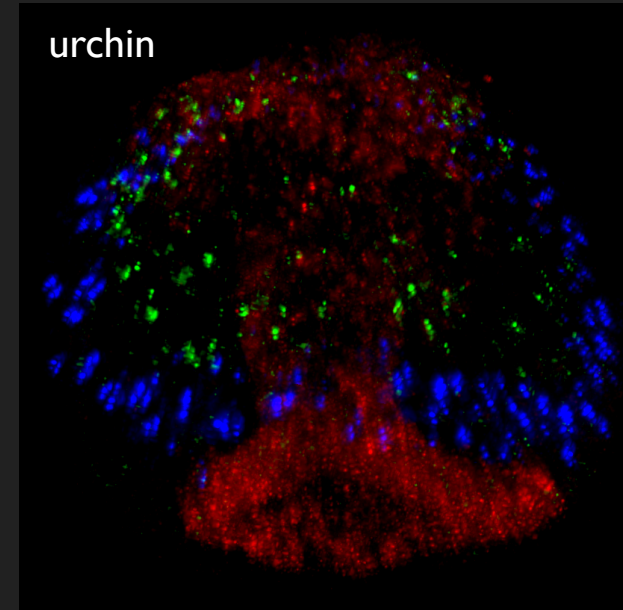
fly



human



urchin



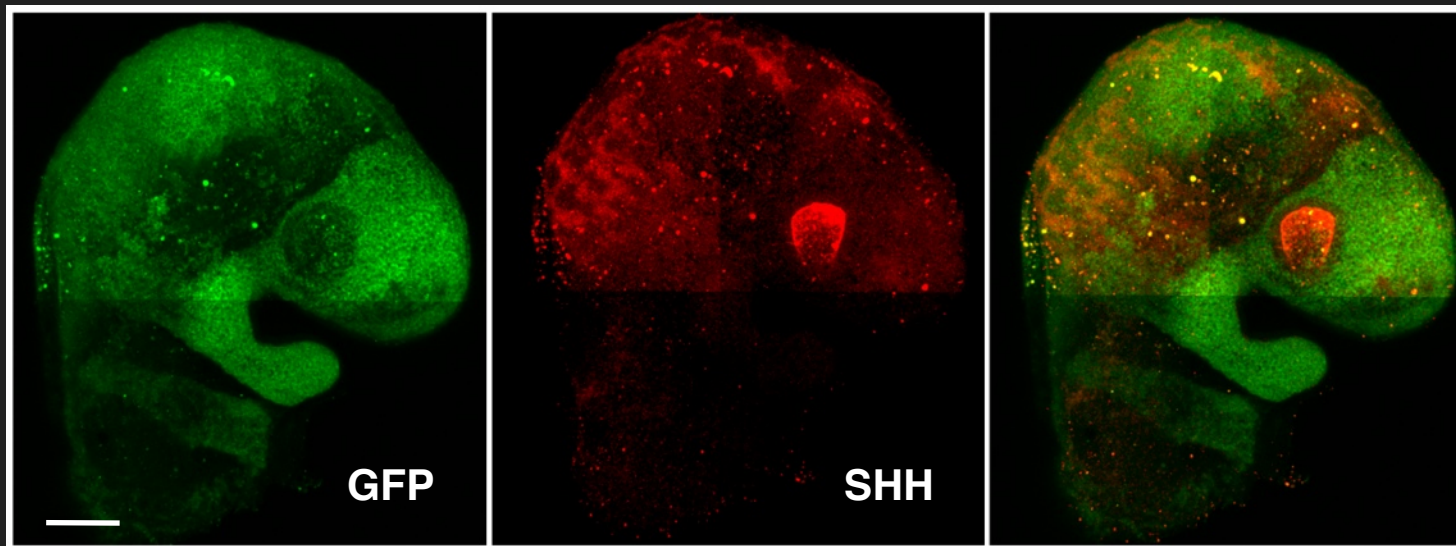
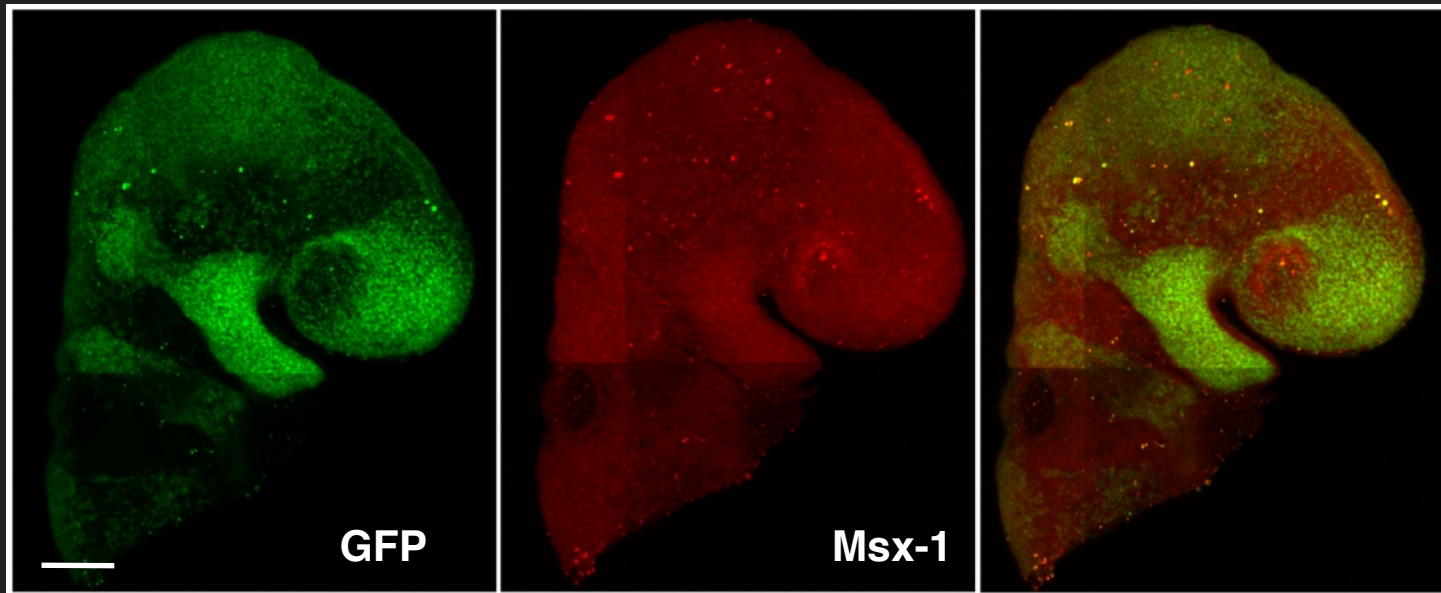
N. Husain, C. Calvert, H.M.T. Choi, *unpublished*

Problems:

Mouse much, much less reliable

Later stages less reliable

Tremendous work to optimize probes

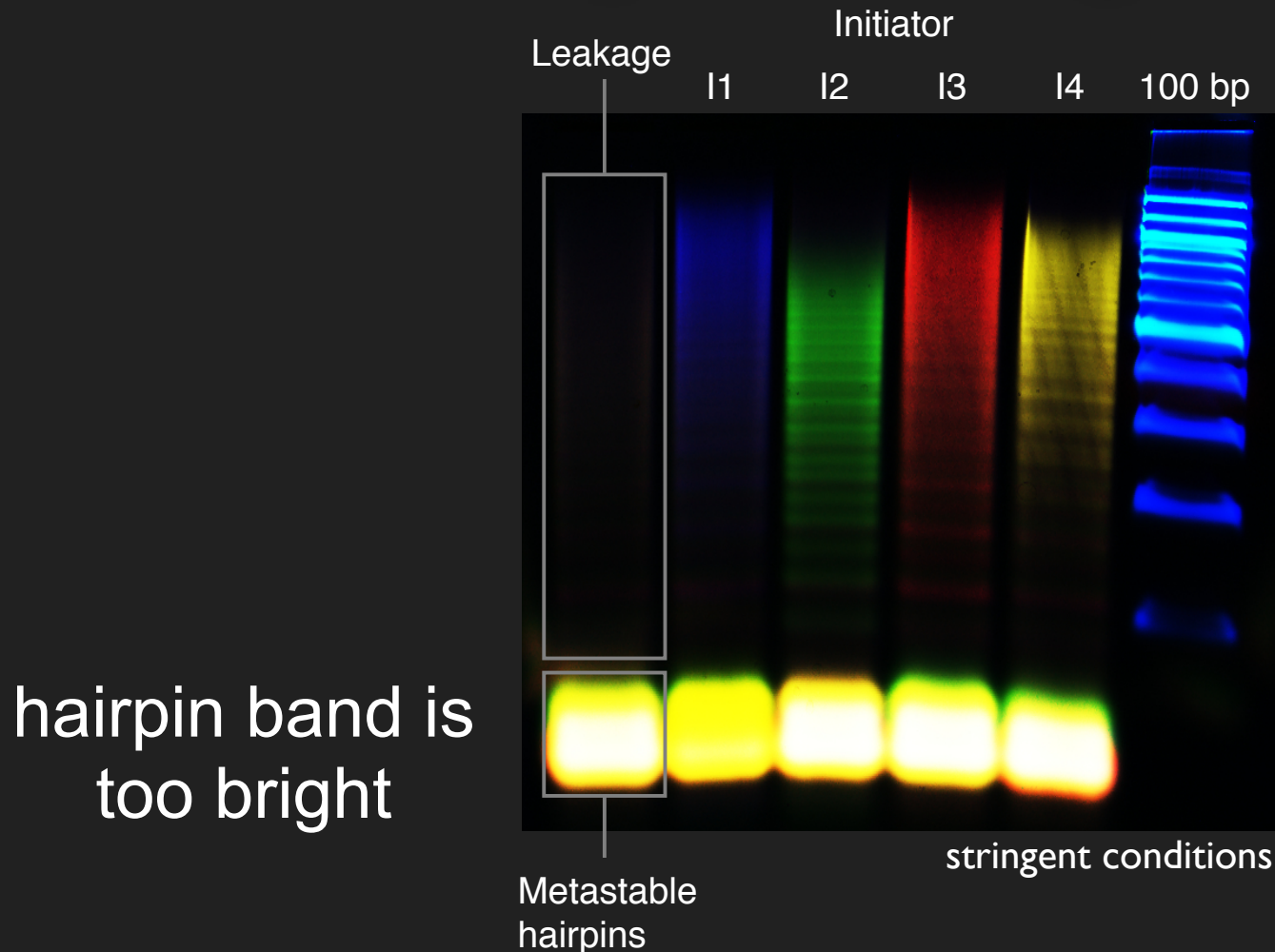


Alexa 546

Alexa 647

Merged

We finally studied the gel carefully

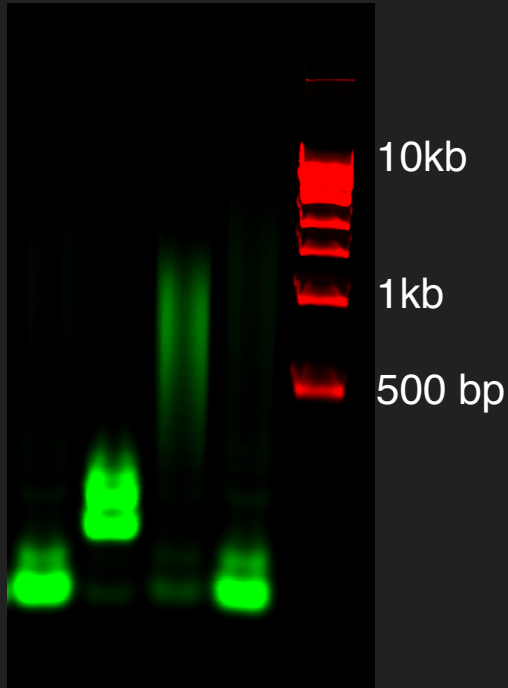


majority of hairpins not amplifying, making penetration & background & autofluorescence critical

Next-generation HCR: brighter, faster, cheaper

Published
RNA-HCR

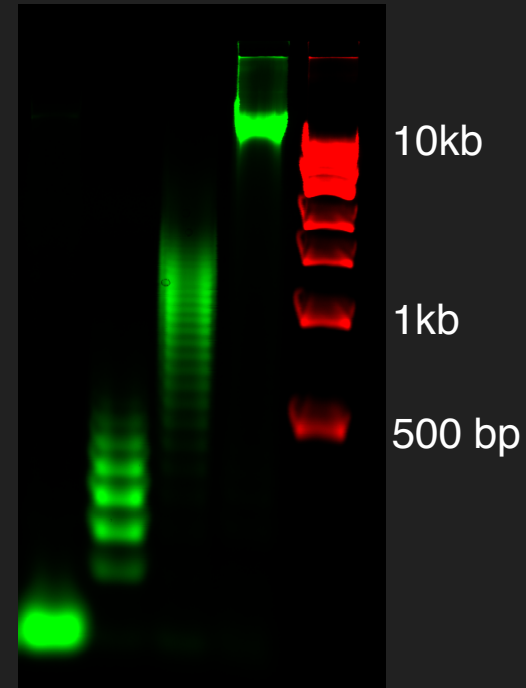
1xI 0.1xI 0.01xI



Dozens of hairpins per polymer

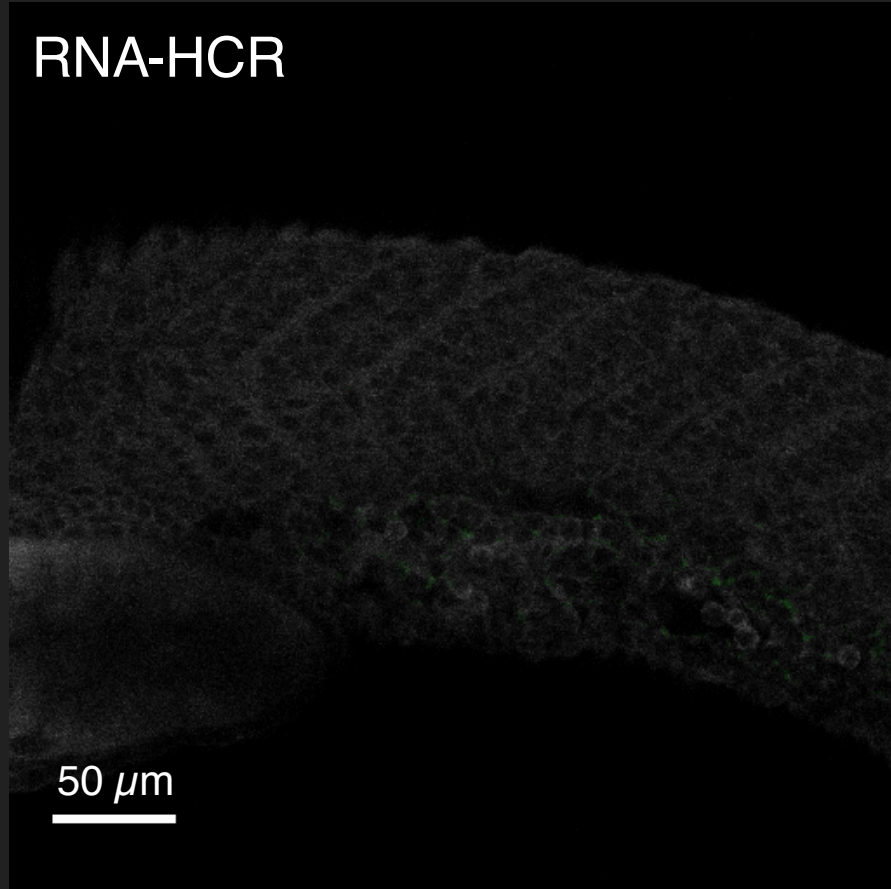
New
DNA-HCR

1xI 0.1xI 0.01xI

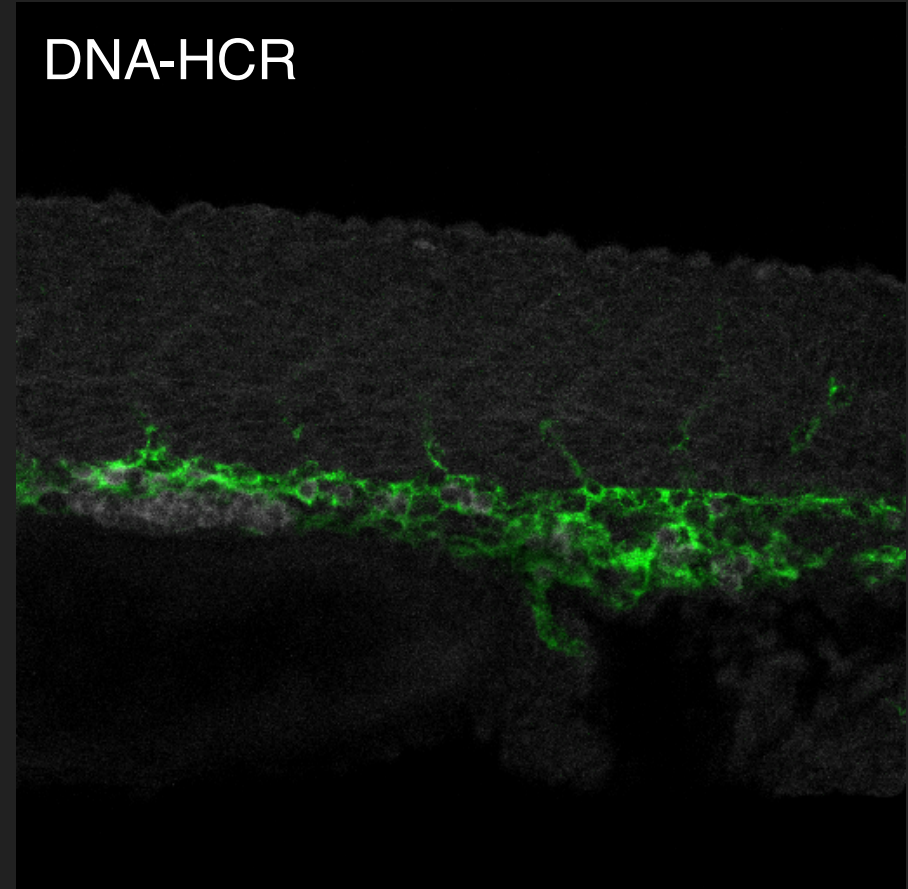


Hundreds of hairpins per polymer

Published technology



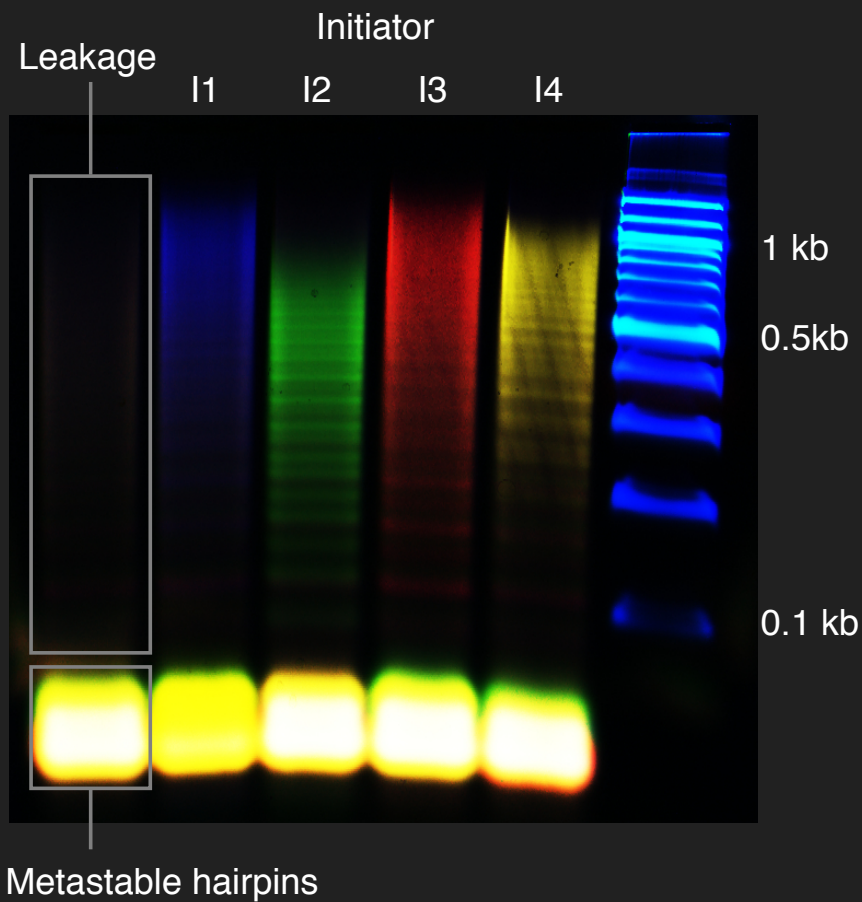
New technology



Microscope gain turned down to avoid saturating DNA-HCR

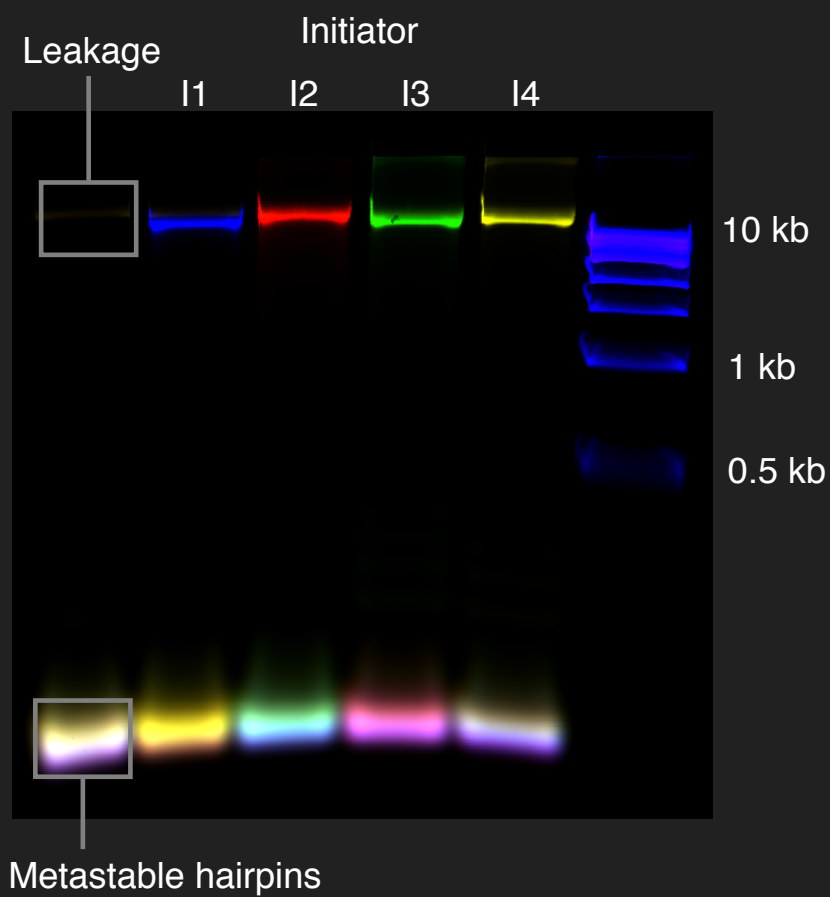
Published technology

RNA-HCR



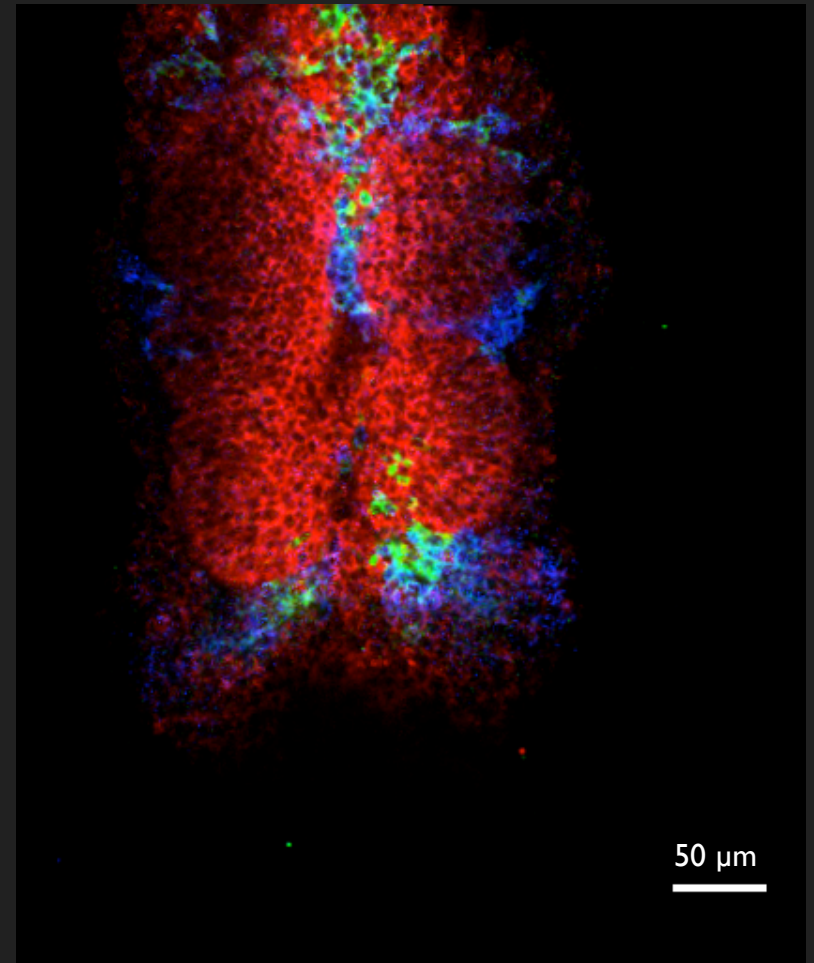
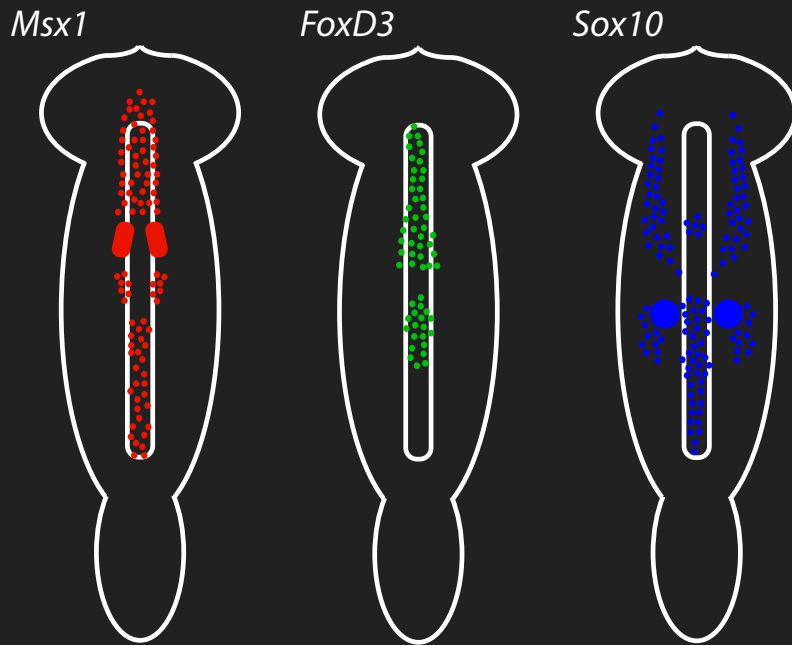
New technology

DNA-HCR



Orthogonal amplifiers designed using NUPACK (B.Wolfe)

DNA-HCR in a whole-mount chick embryo



Next-generation HCR: brighter, faster, cheaper

Published RNA-HCR Technology

- 2009: \$1800 per kit
- 2011: \$1100 per custom kit, \$600 per re-order kit

New DNA-HCR Technology

- 2013: \$350 per kit

Kit: Probe set, amplifier, buffers (scale for 20 zebrafish experiments, with 10 fish per experiment)

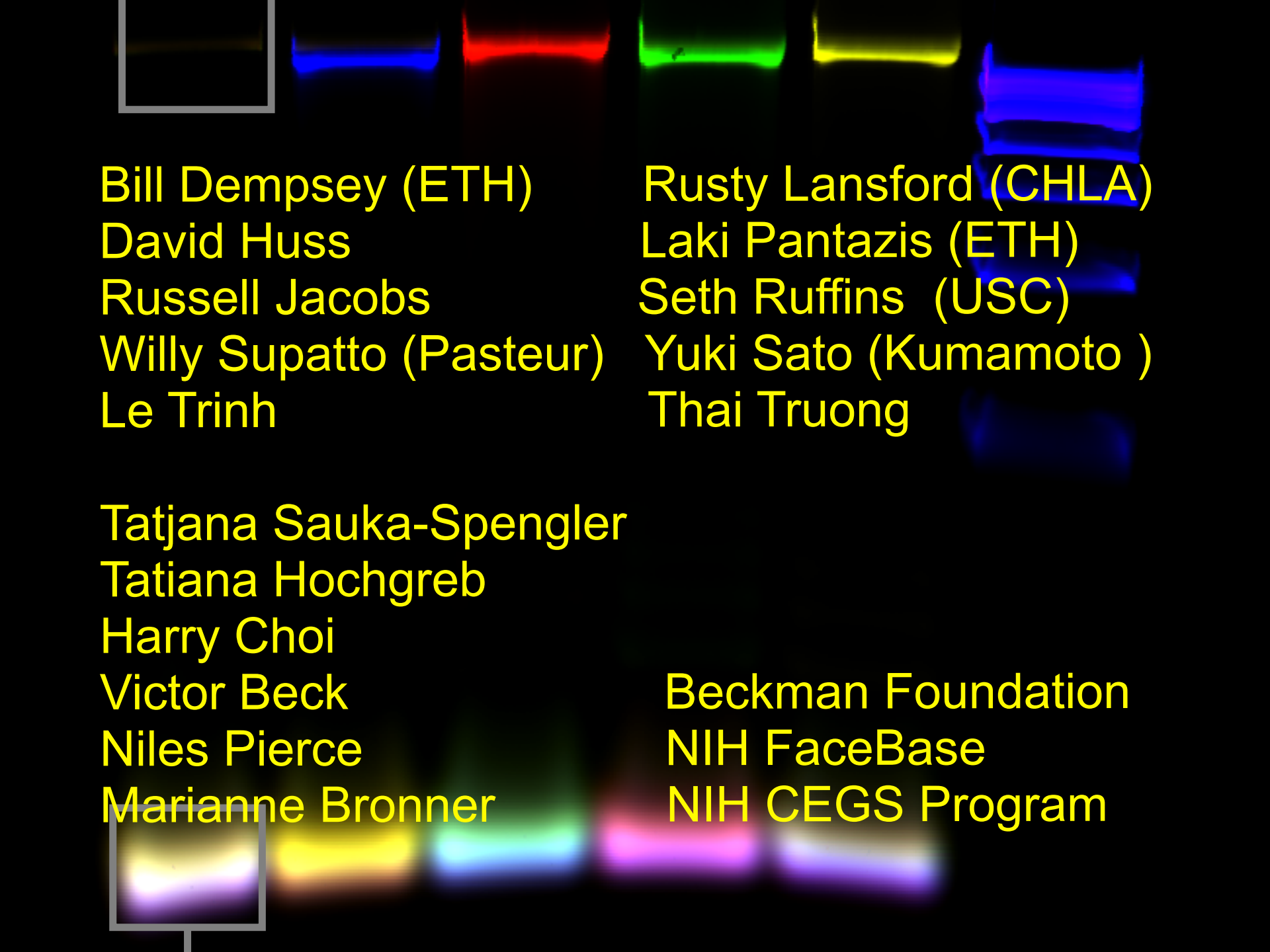
Multidimensional imaging of craniofacial development.

Coming year:

Create molecular atlas of zebrafish, mouse, avian
Transfer to Hub (help with viewing, aligning tools)

Curate MRI data sets to make accessible
organization, thumbnails, downloads

Create instructional text and videos for rendering,
instructional text and videos for analysis



Bill Dempsey (ETH)
David Huss
Russell Jacobs
Willy Supatto (Pasteur)
Le Trinh

Rusty Lansford (CHLA)
Laki Pantazis (ETH)
Seth Ruffins (USC)
Yuki Sato (Kumamoto)
Thai Truong

Tatjana Sauka-Spengler
Tatiana Hochgreb
Harry Choi
Victor Beck
Niles Pierce
Marianne Bronner

Beckman Foundation
NIH FaceBase
NIH CEGS Program