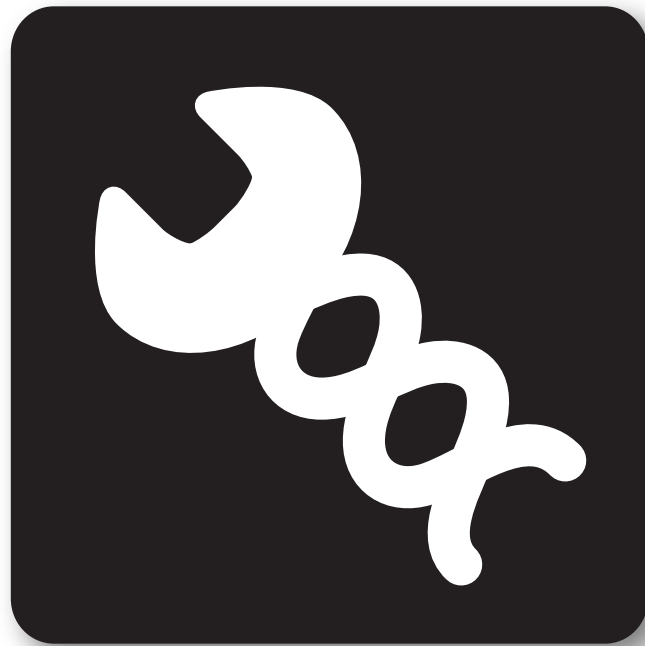


The TransgeneOme Project



Mihail Sarov

Max Planck Institute of Cell Biology and Genetics



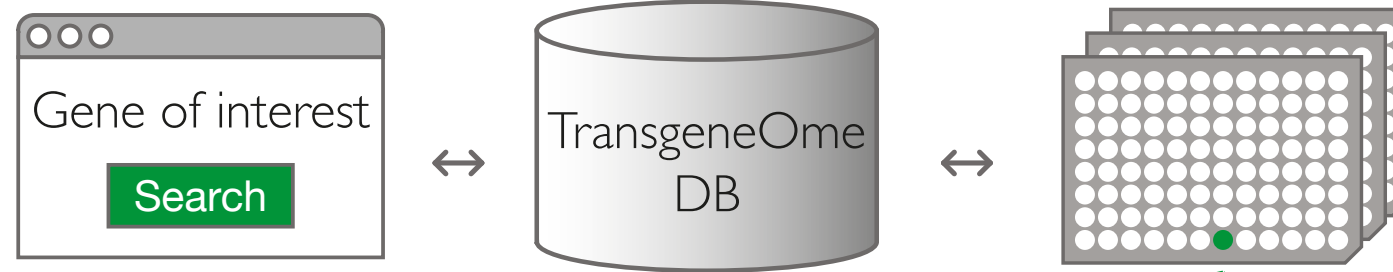
<http://transgeneome.mpi-cbg.de>



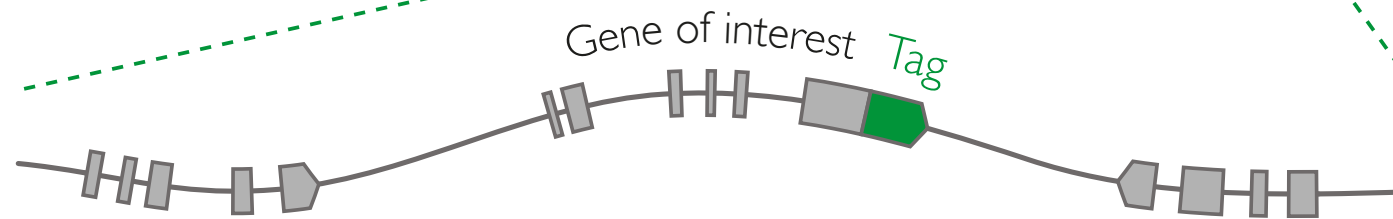
@TransgeneOme

The one page summary

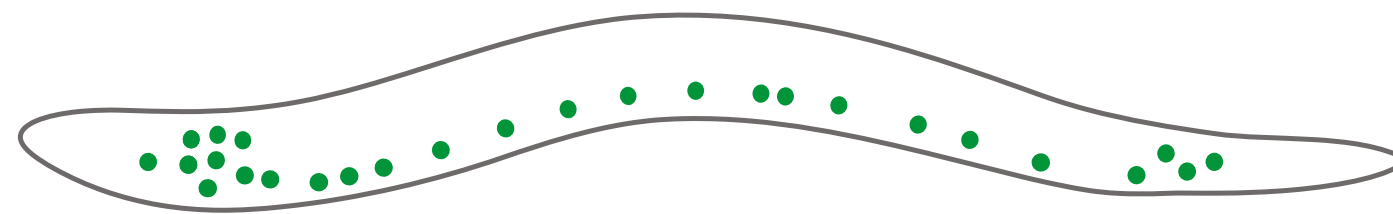
Genome-wide collection of tagged gDNA transgenes



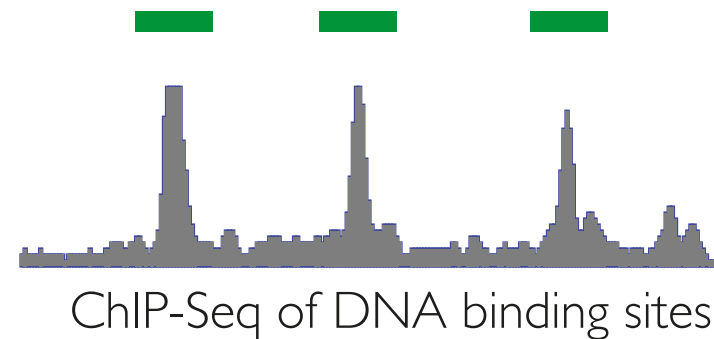
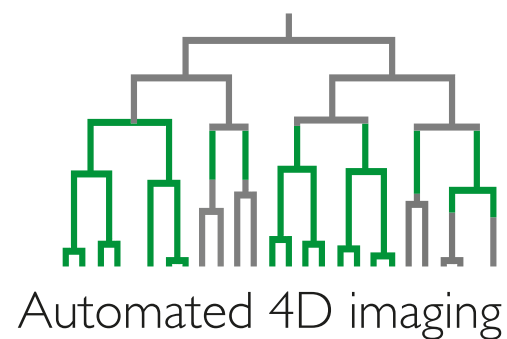
Stable integration into the genome



Tagged protein expression under endogenous in vivo regulatory control



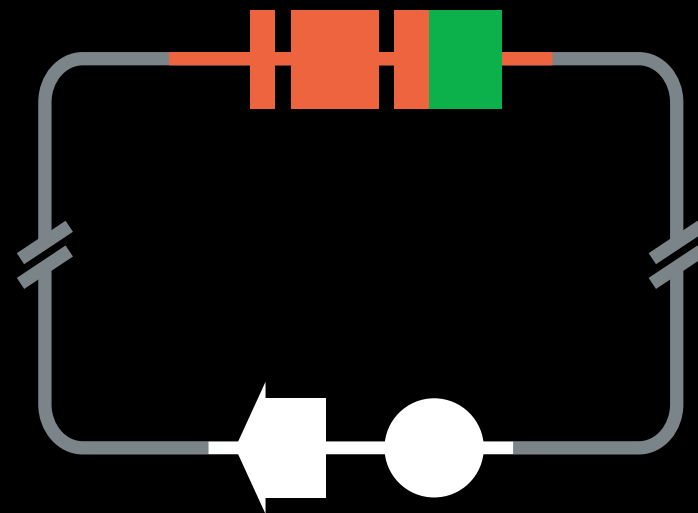
Digital protein localization patterns at cell/tissue and molecular level



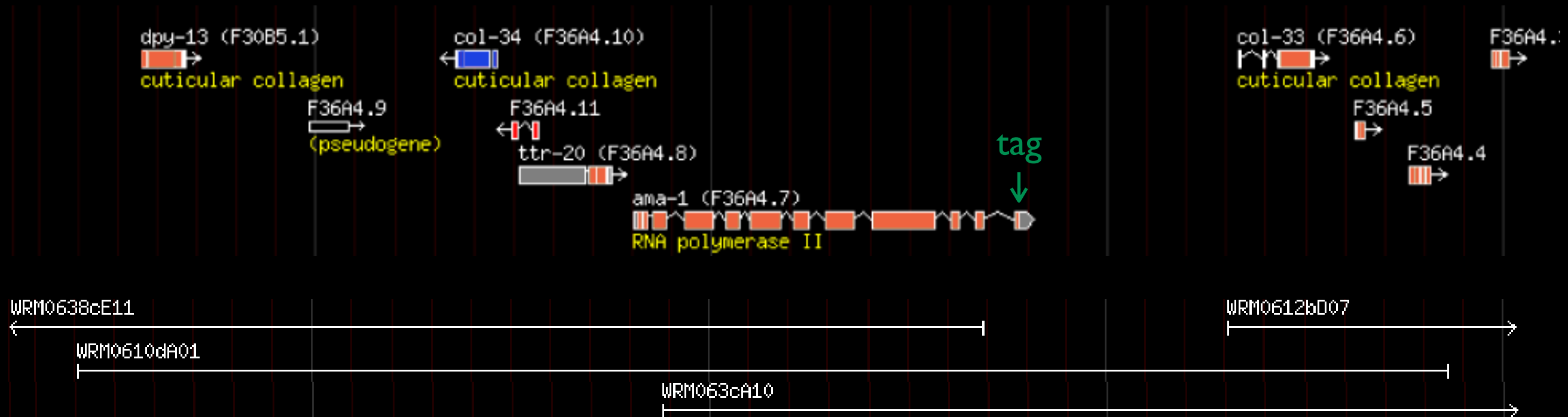
Part I

Engineering the TransgeneOme

gDNA fosmids as transgenes



← 10 kbp →

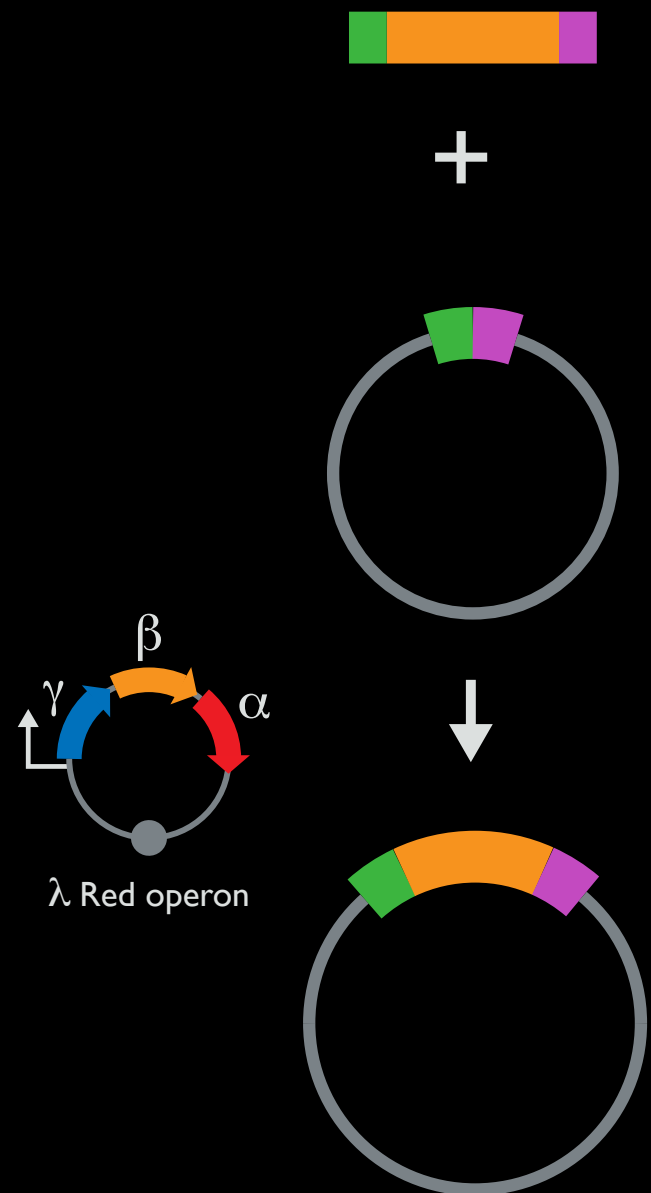


Recombineering

Homologous **recombination** mediated gene **engineering** in E. coli



Francis Stewart



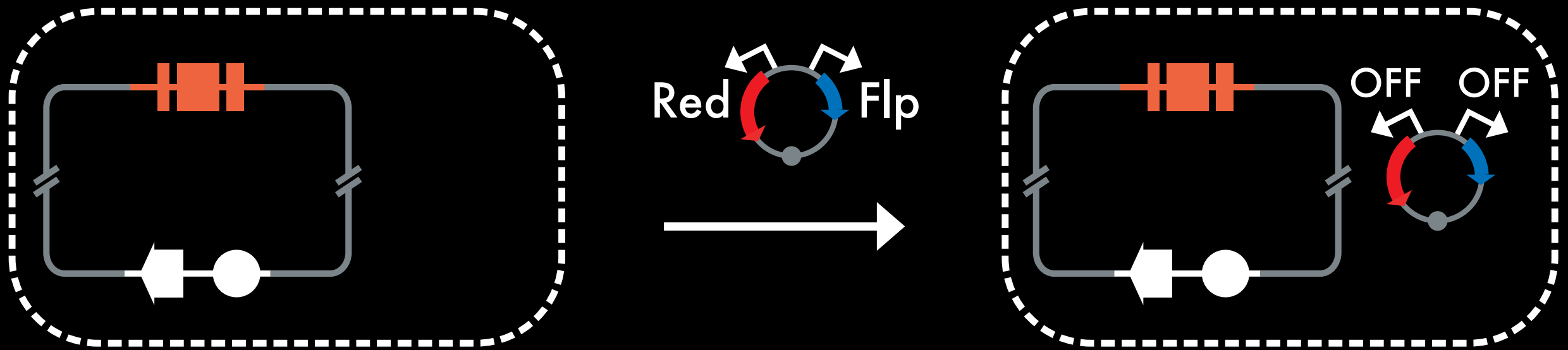
No size limitations

Not dependent on the presence of specific sequence

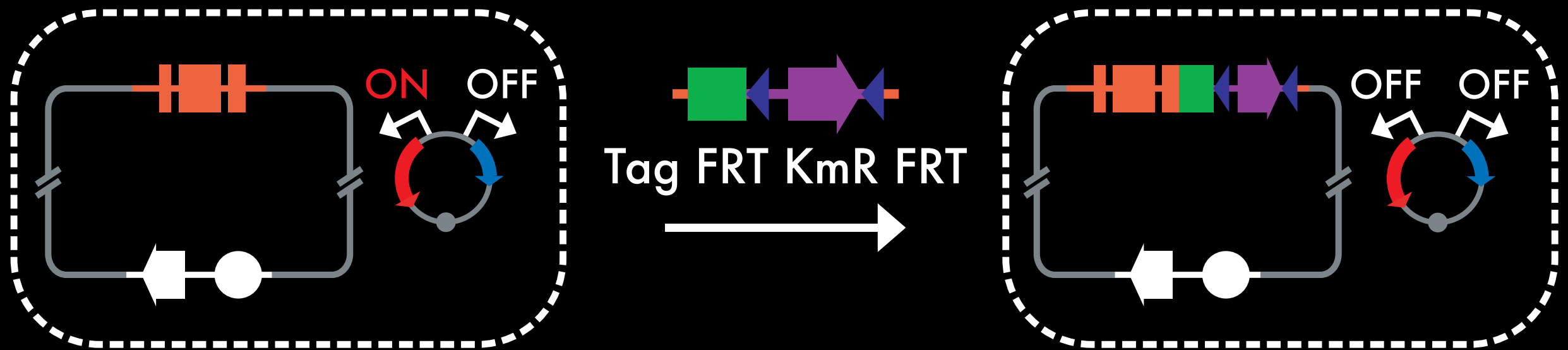
High efficiency: requires **short** homologies (>30bp)

High fidelity: requires **perfect** match

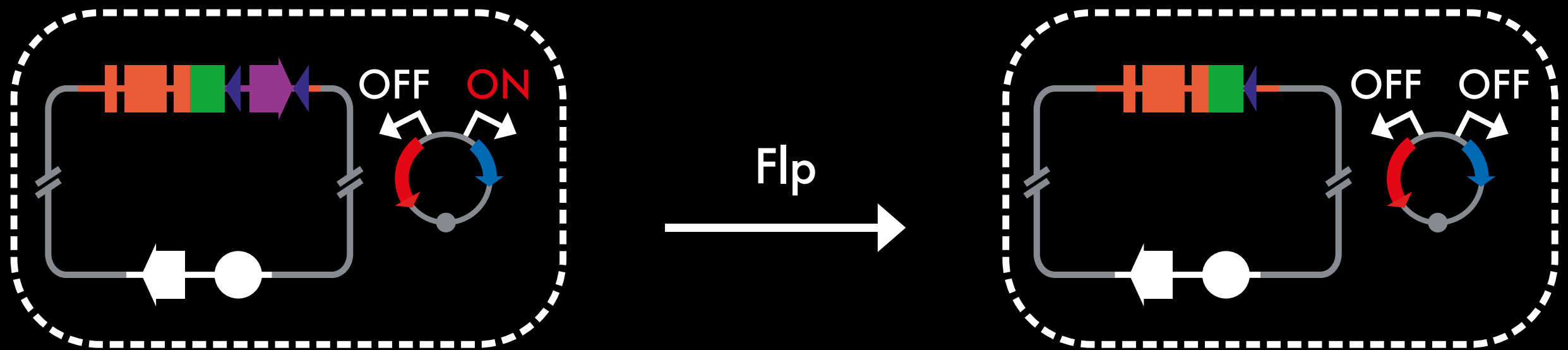
I. Make recombineering proficient



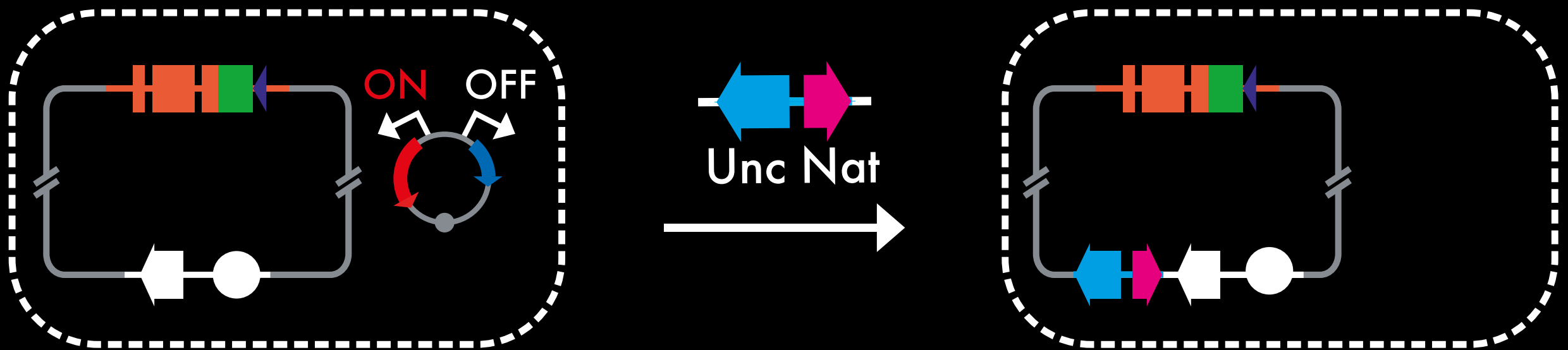
2. Insert tagging cassette



3. Flp out the selection marker



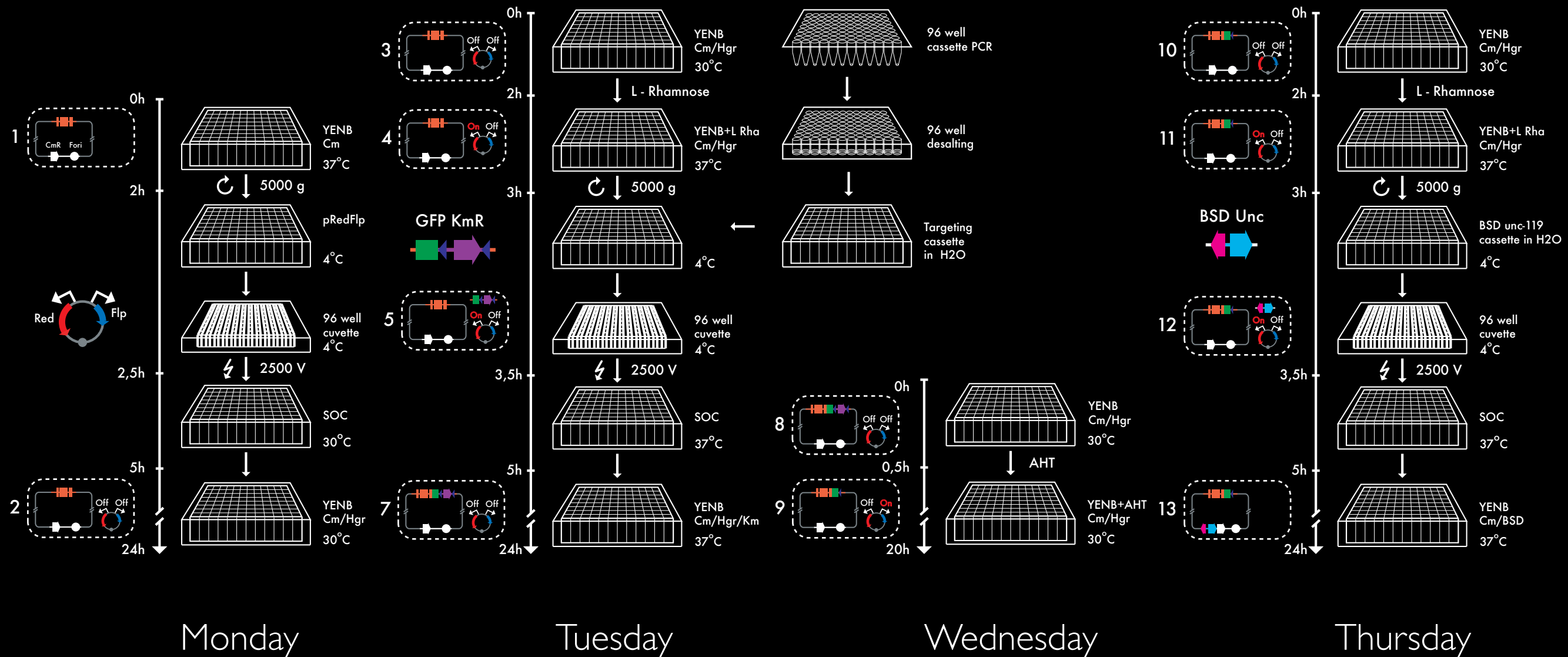
4. Retrofit the fosmid vector



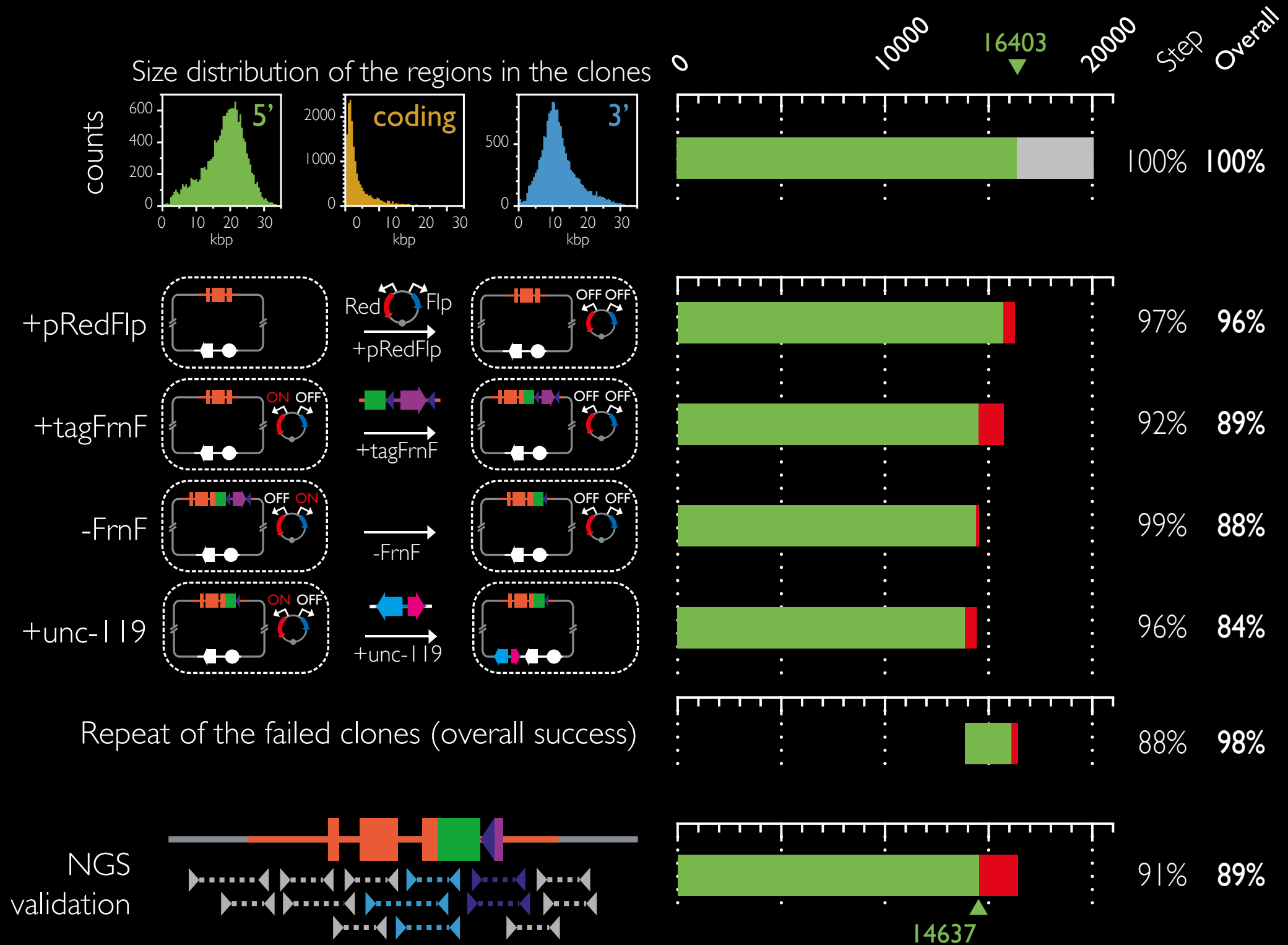
Tagging a genome



Tony Hyman



Tagging a genome



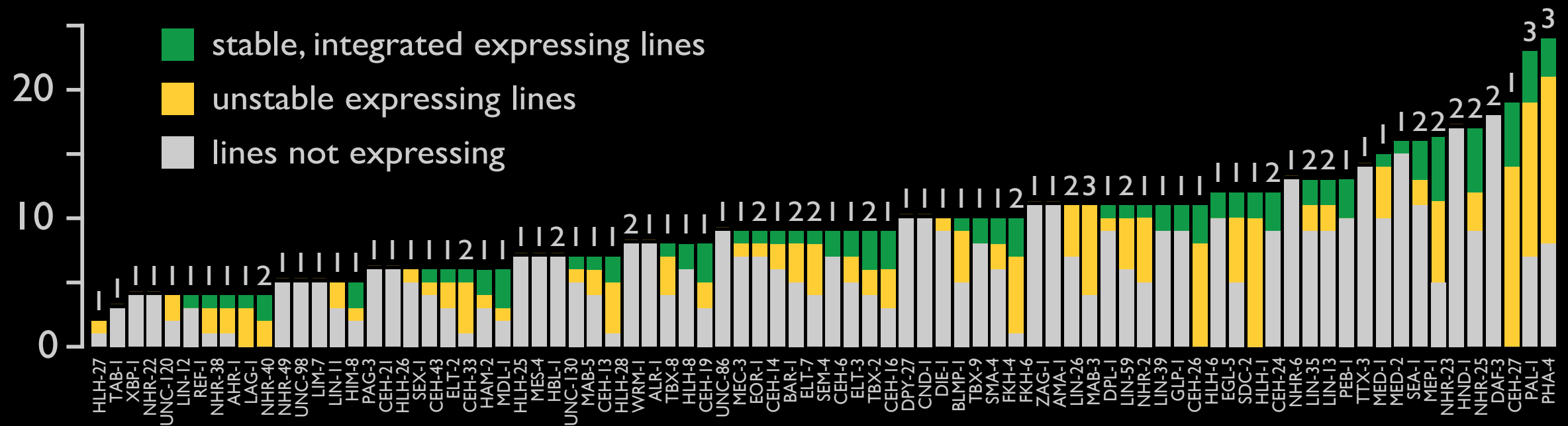
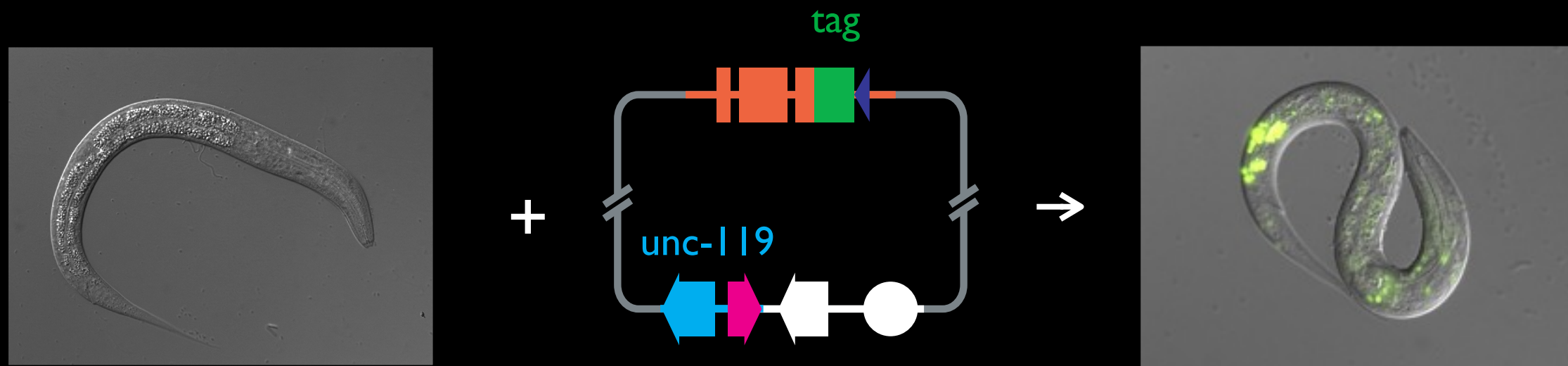
Part II

Strategies for transgenesis

Strategies for making lines

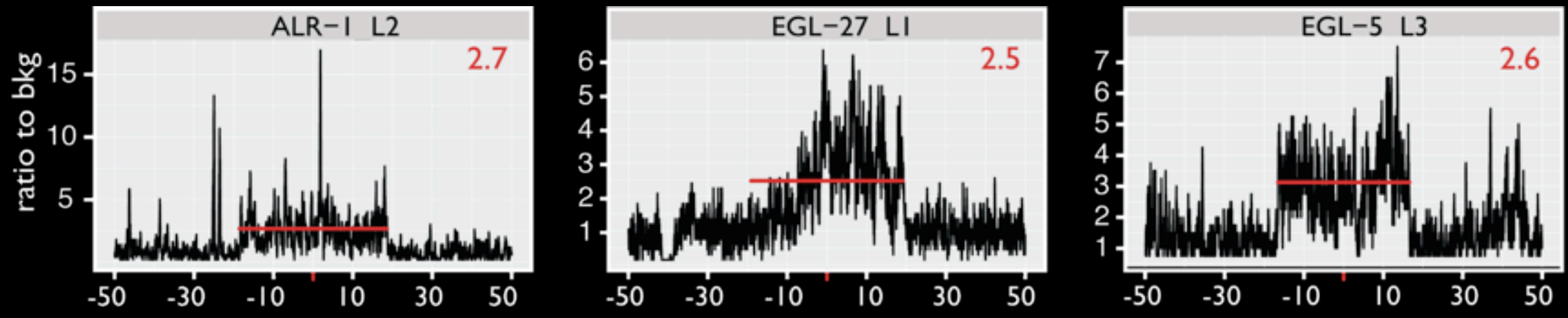
- ▶ Microinjection
- ▶ Bombardment
- ▶ MosSci (subclone into pCFJ vectors first)
- ▶ Mos based transposition (add the MiniMos)
- ▶ Targeting (MosTic, Zn Fingers, Talens, CRIPRs)

Stable fosmid transformation by bombardment

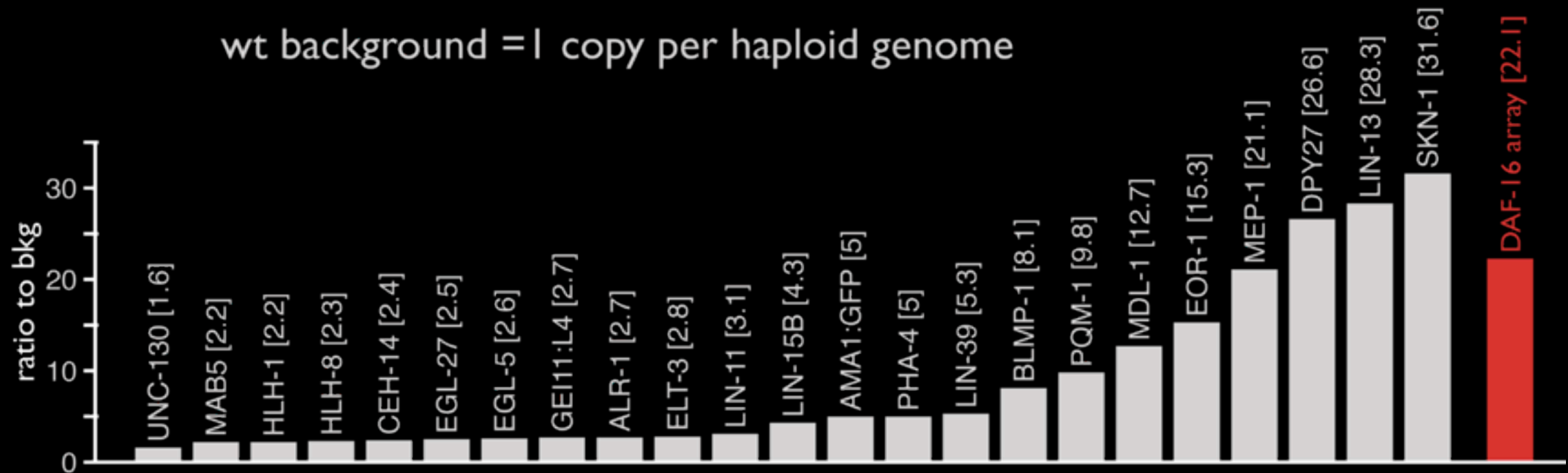


FAQs and detailed protocols at <http://transgeneome.mpi-cbg.de>

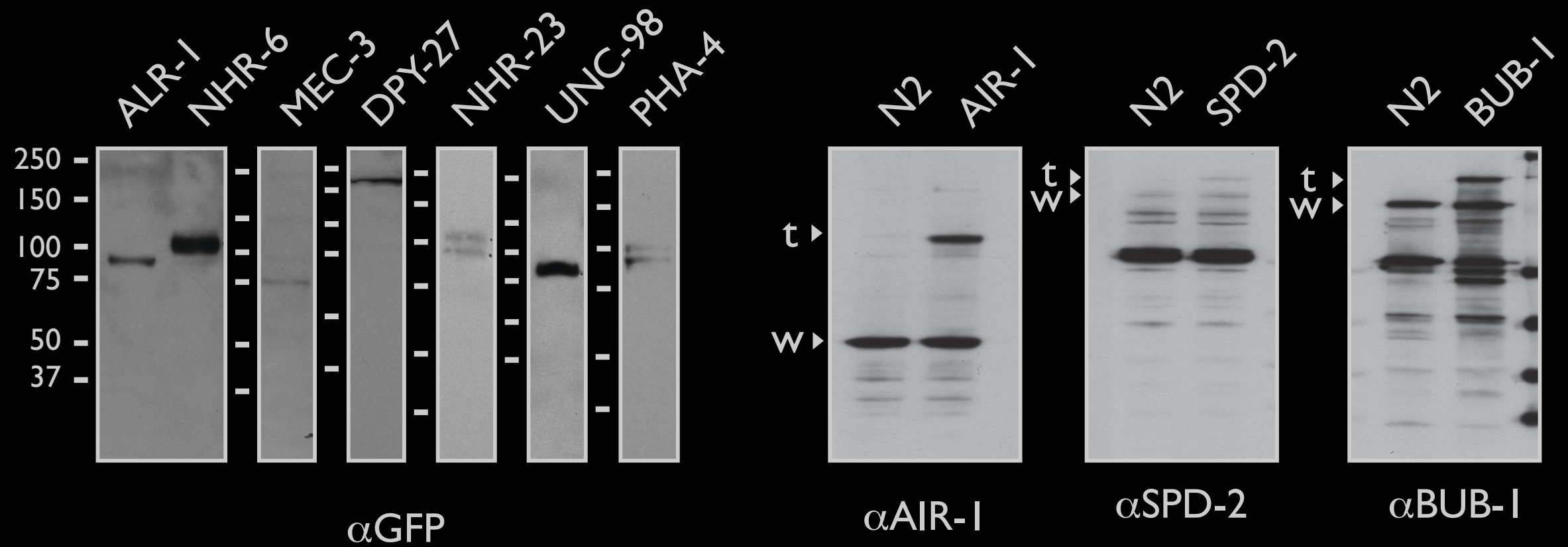
Most constructs integrate at a low copy



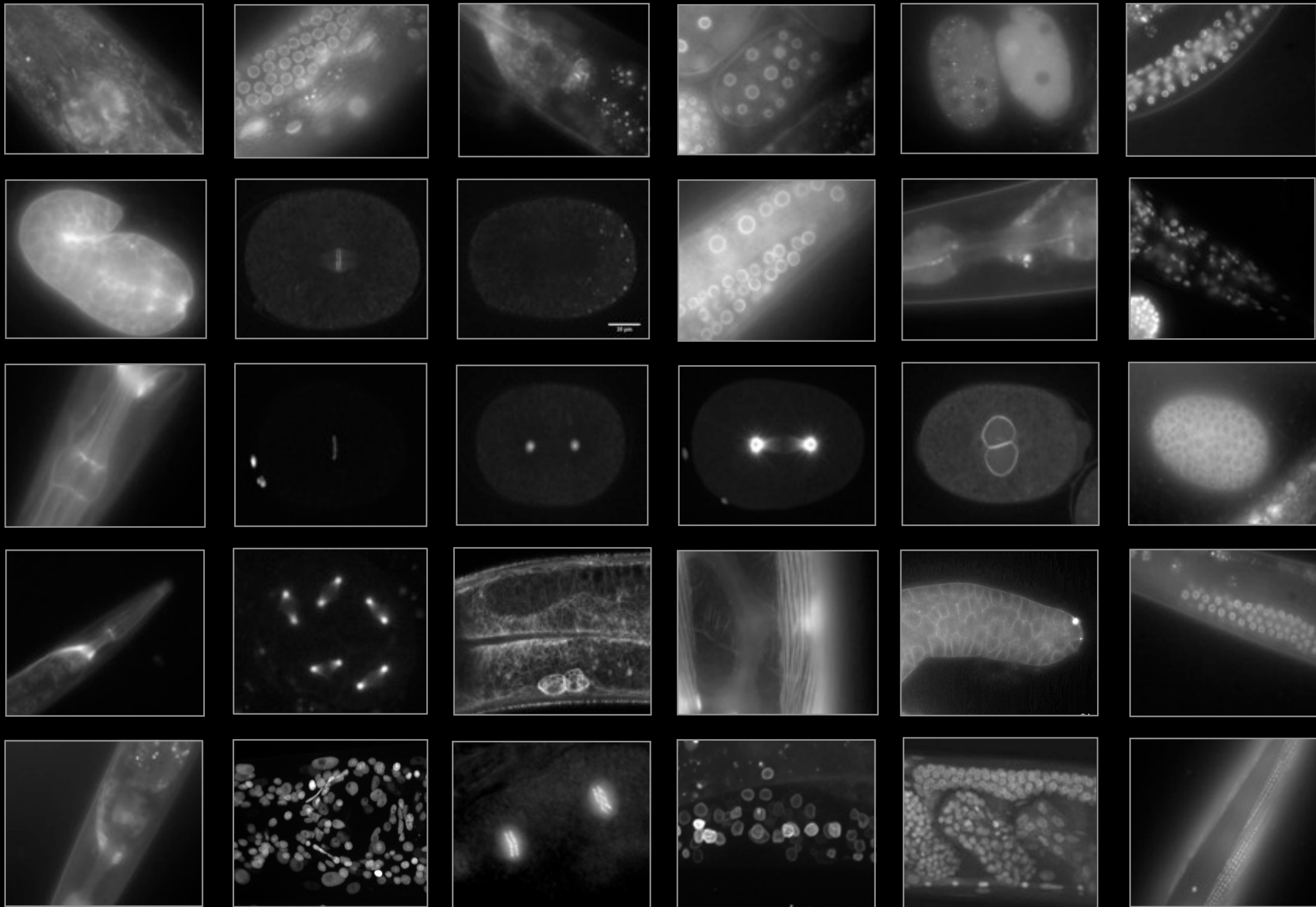
wt background = 1 copy per haploid genome



Correct regulation is maintained at protein level



Faithful localization patterns*



*Including germline and early embryonic expression

Potential pitfalls and possible solutions

- ▶ Endogenous expression levels might be too low for detection
 - use high magnification scope for screening
 - use an ex array for overexpression
- ▶ The tag may affect function
 - try N terminal tagging or tag another isoform
- ▶ No rescue or no expression
 - validate the transgene by sequencing
 - avoid fosmid fragmentation
 - consider possible toxicity

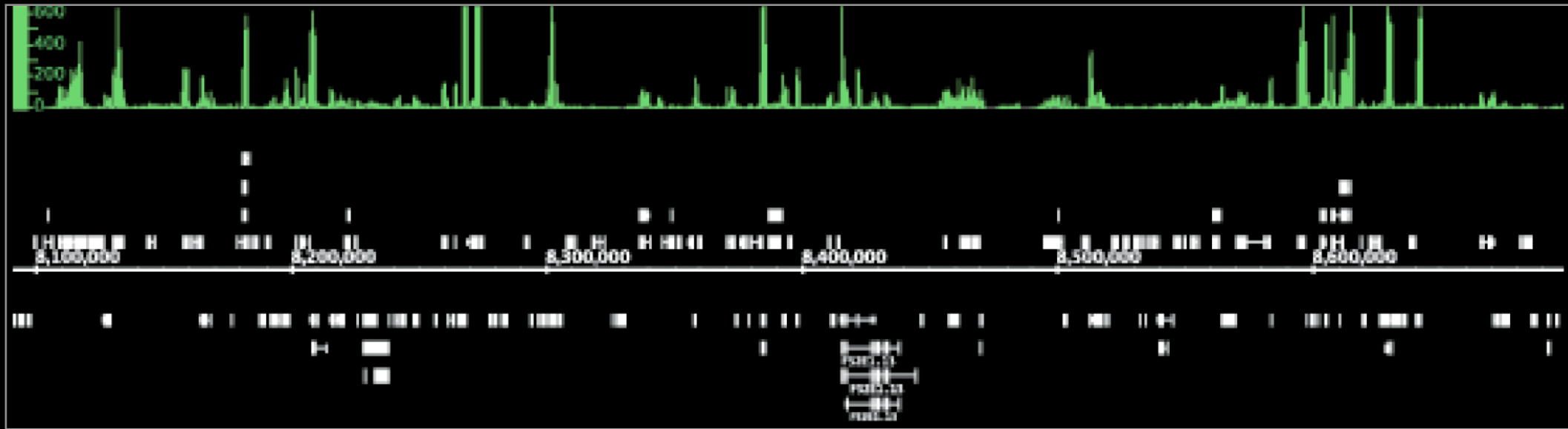
FAQs and detailed protocols at <http://transgeneome.mpi-cbg.de>

Part III

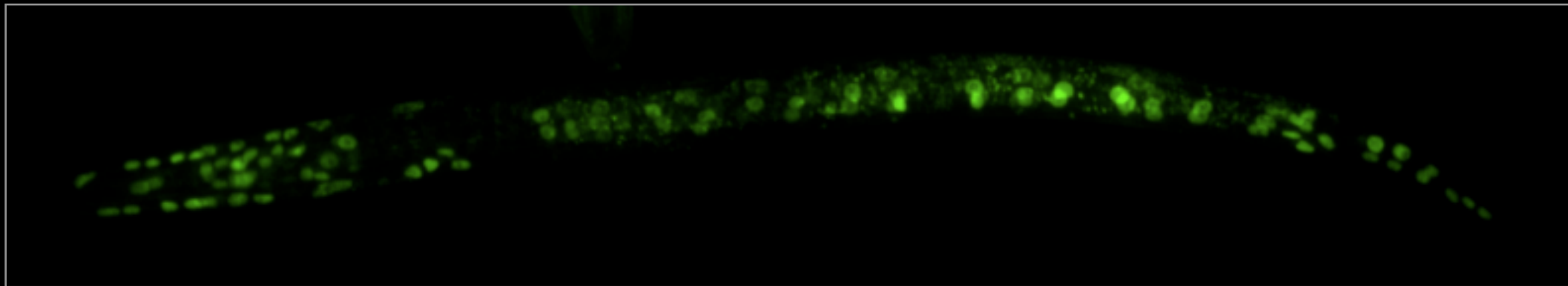
Application Examples

Transcription factor function discovery

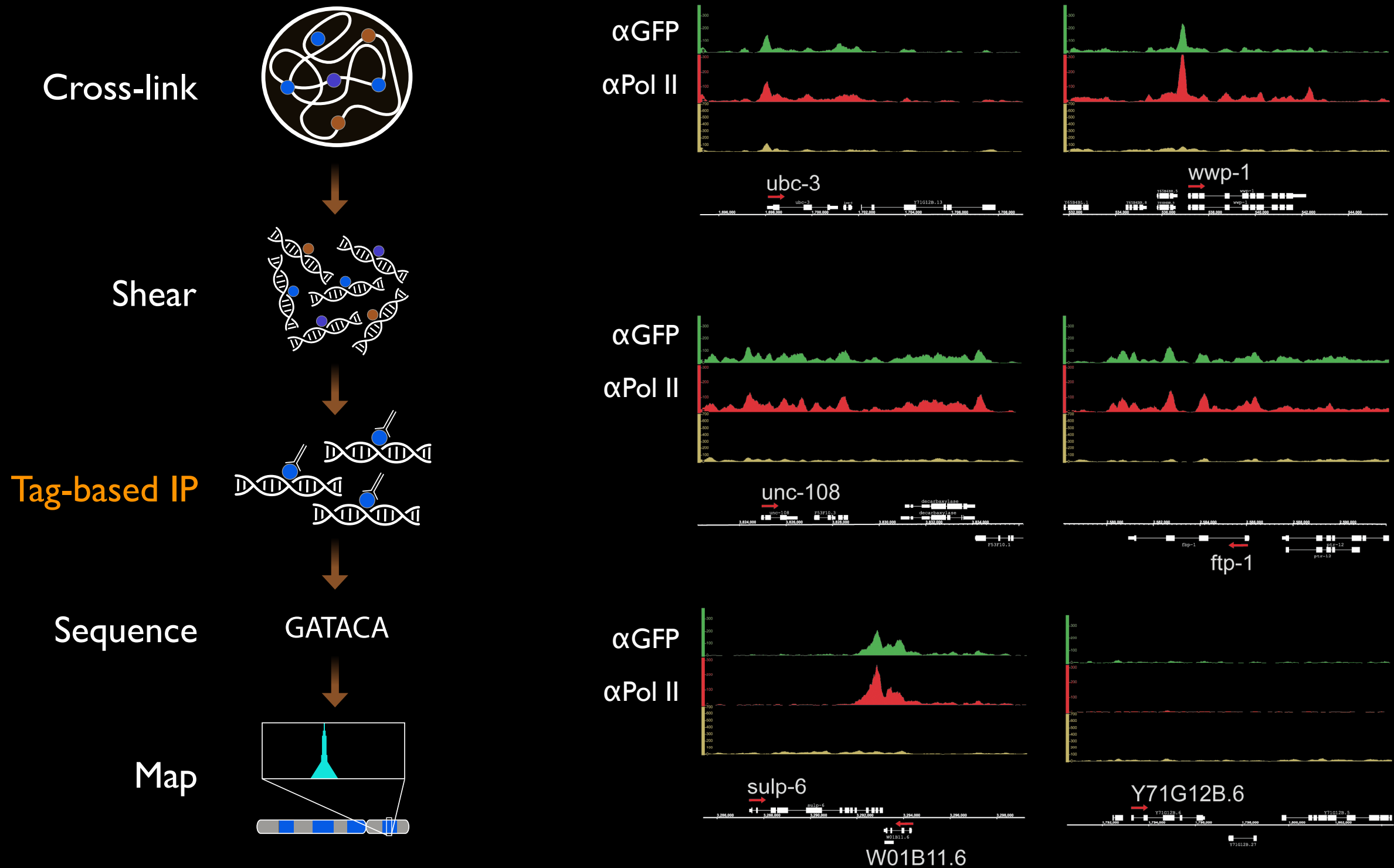
In vivo localization at molecular level (ChIP Seq)



In vivo localization at cellular level (live fluorescence)

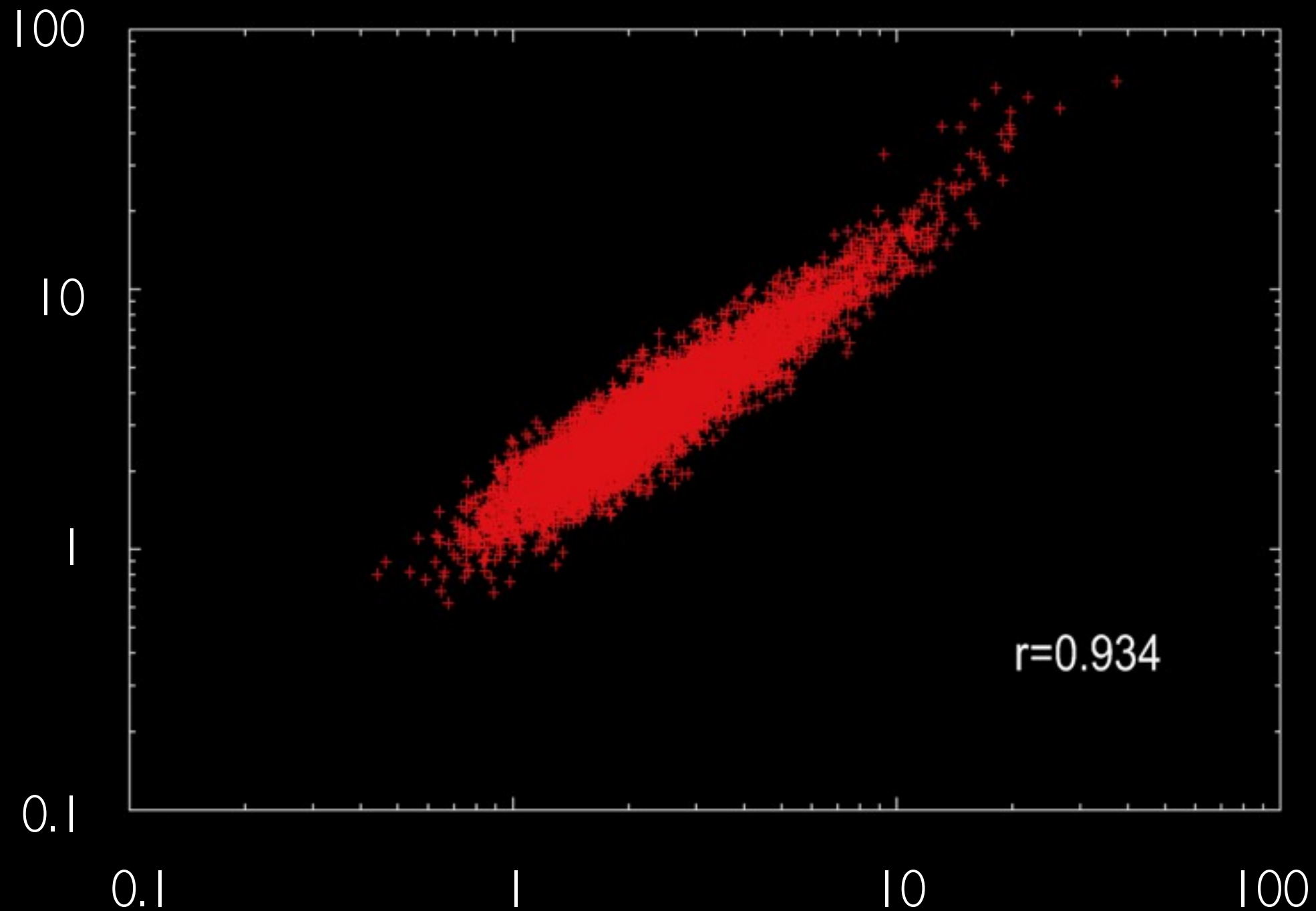


ChIP/Seq mapping of DNA binding sites



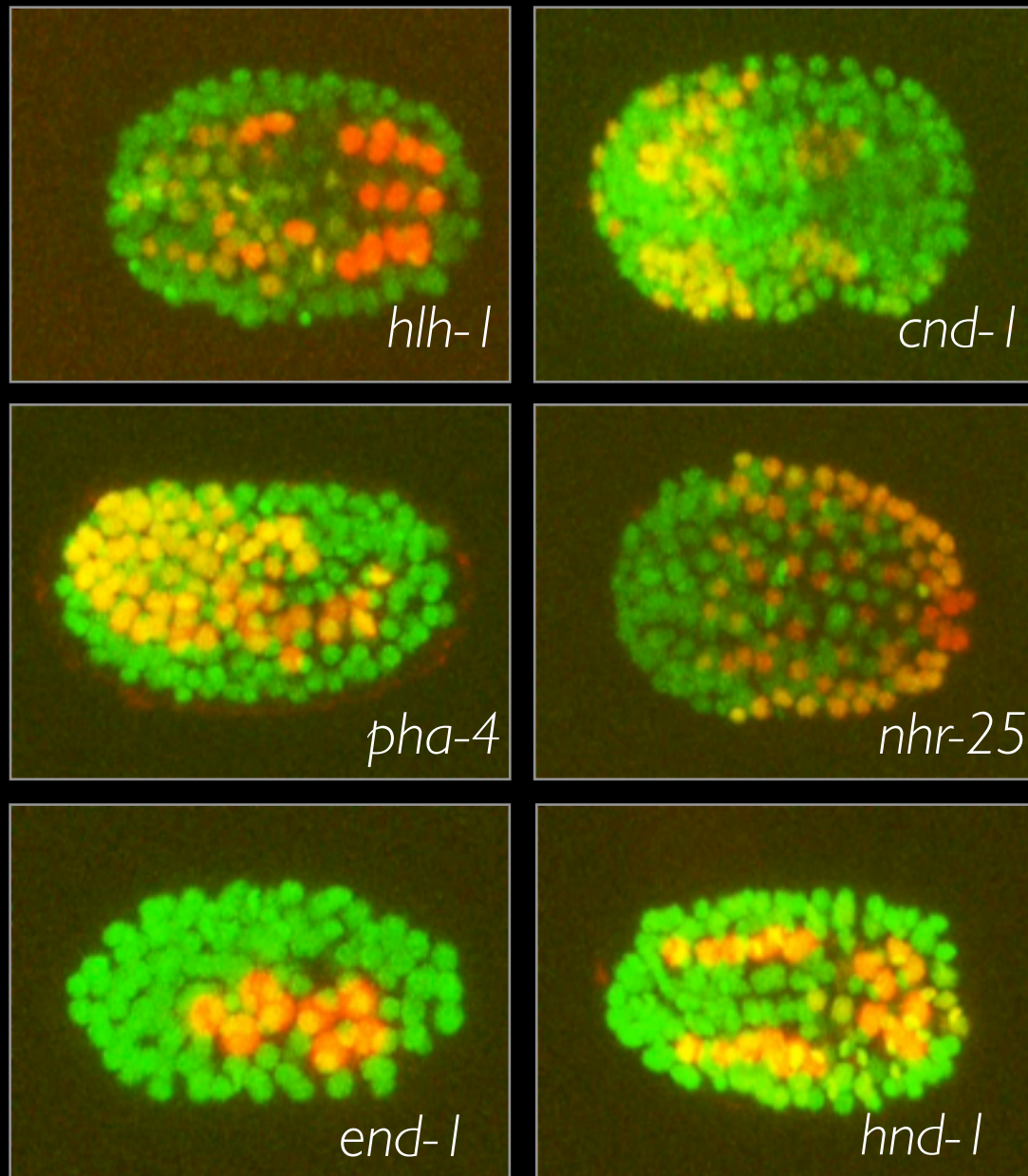
Zhong et al. Genome-wide identification of binding sites defines distinct functions for *Caenorhabditis elegans* PHA-4/FOXA in development and environmental response. PLoS Genet (2010)

ChIP/Seq mapping of DNA binding sites

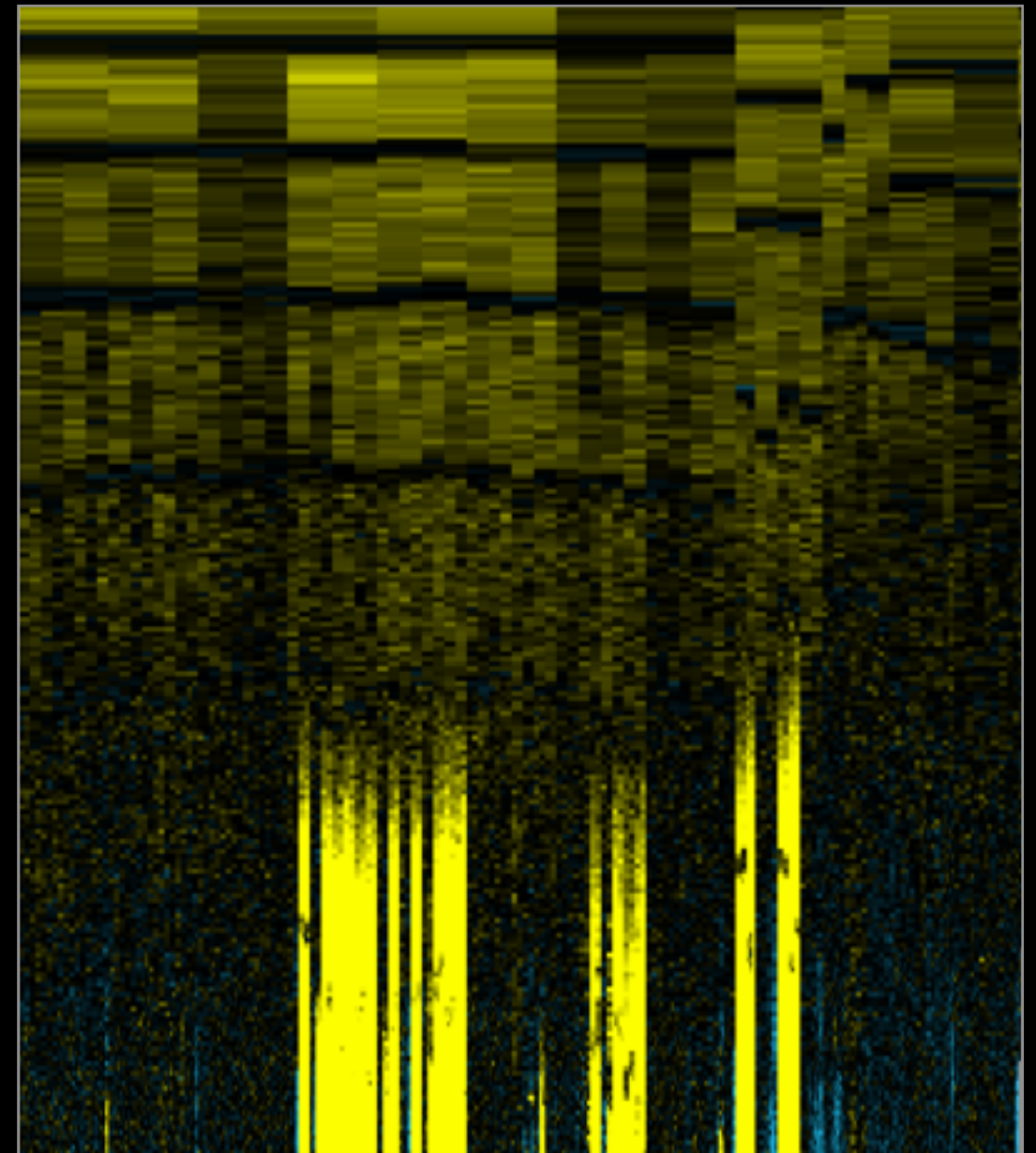


4D localization during embryonic development

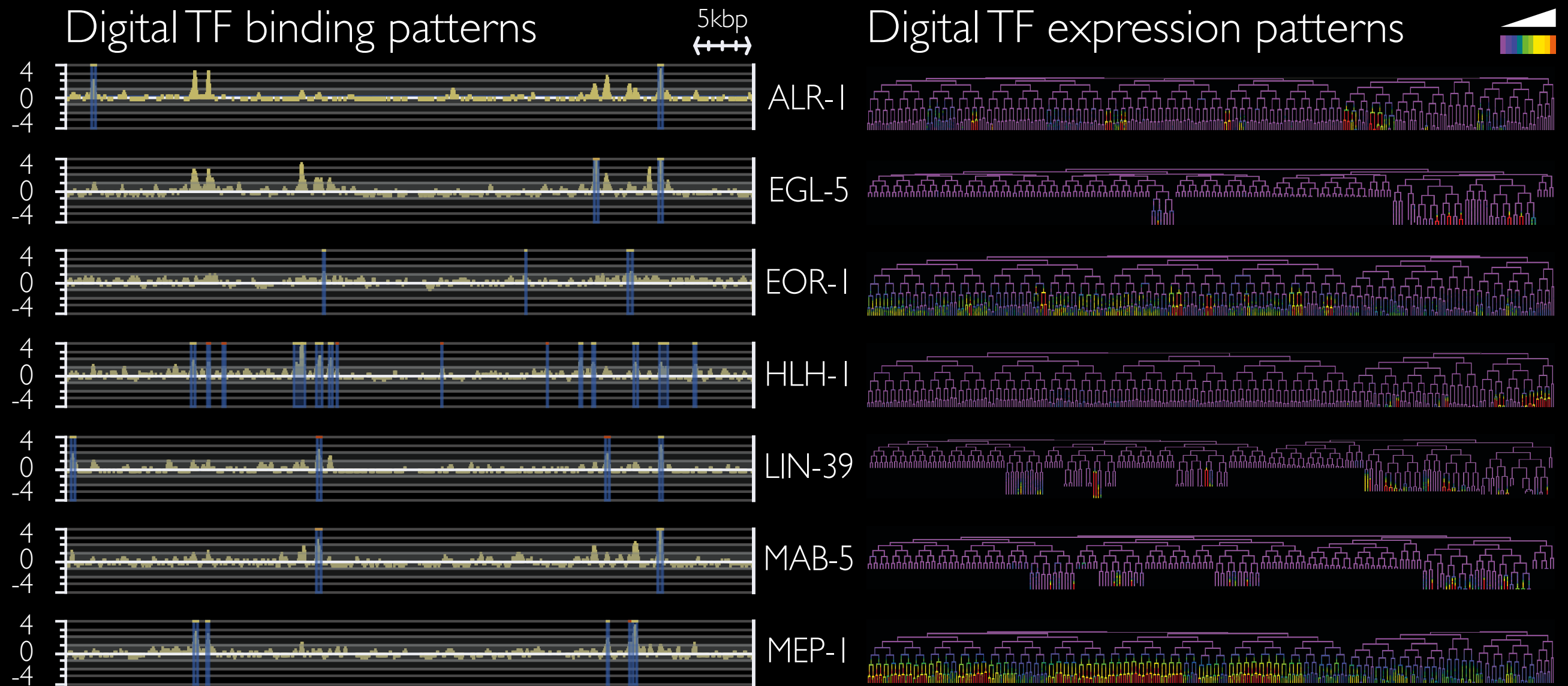
space



time



High resolution maps of TF function (ModENCODE)

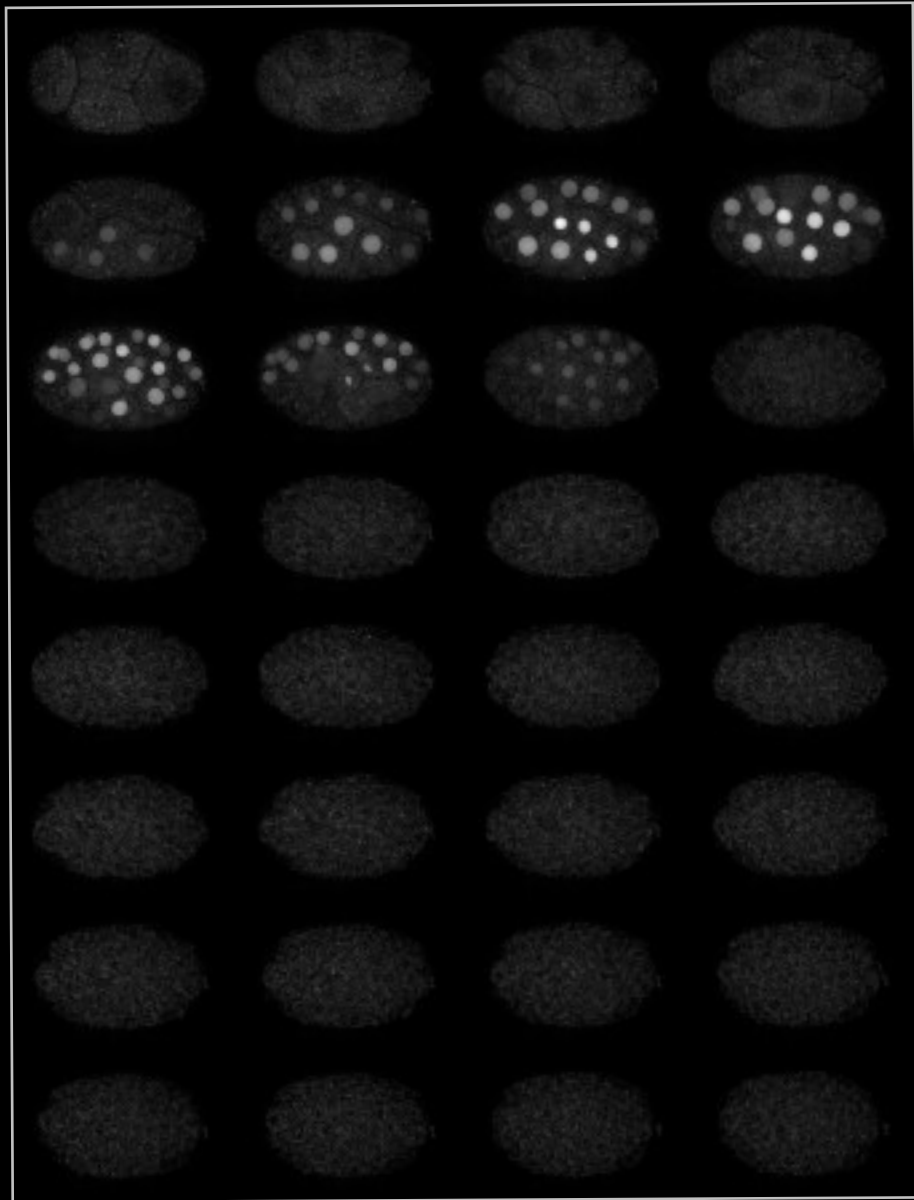


www.modmine.org

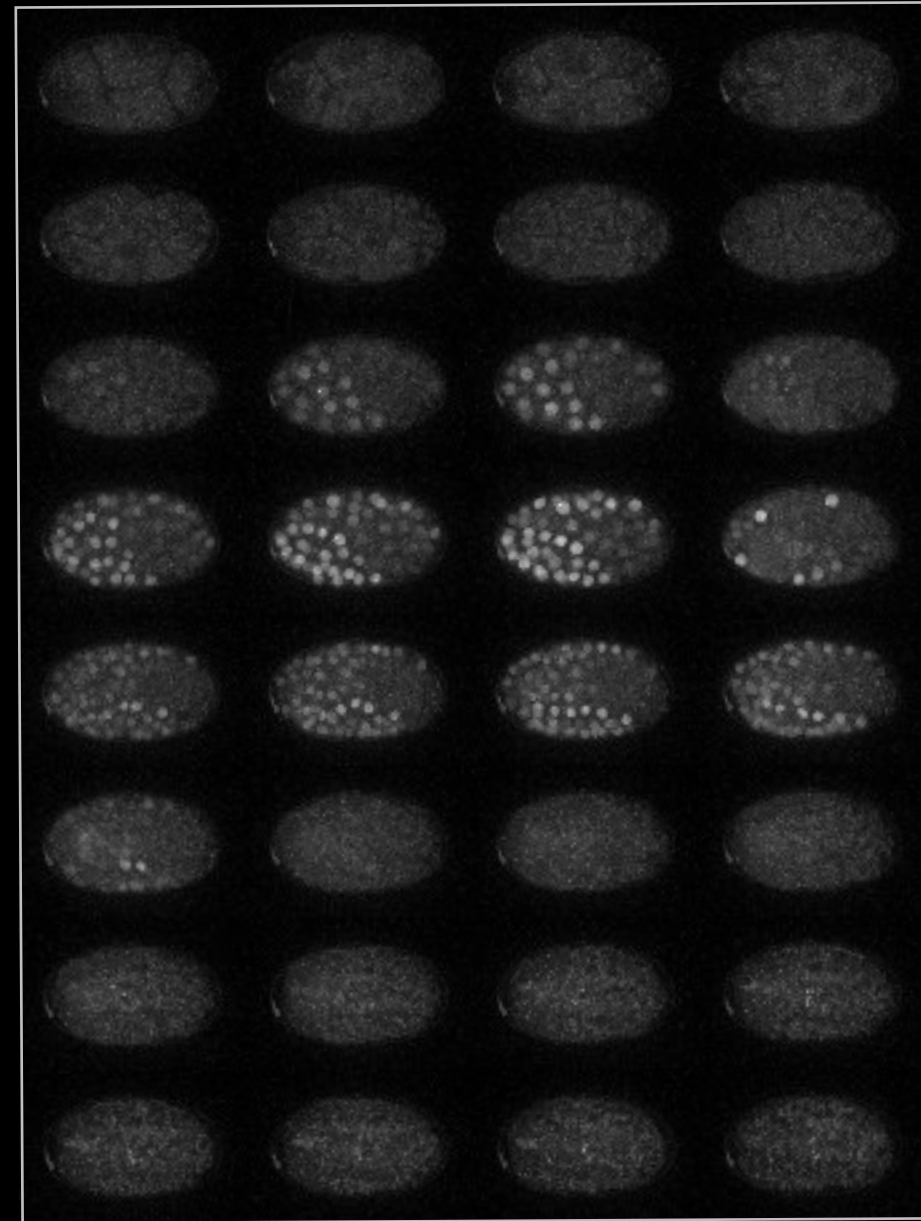
<http://epic.gs.washington.edu>

Rapid expression dynamics

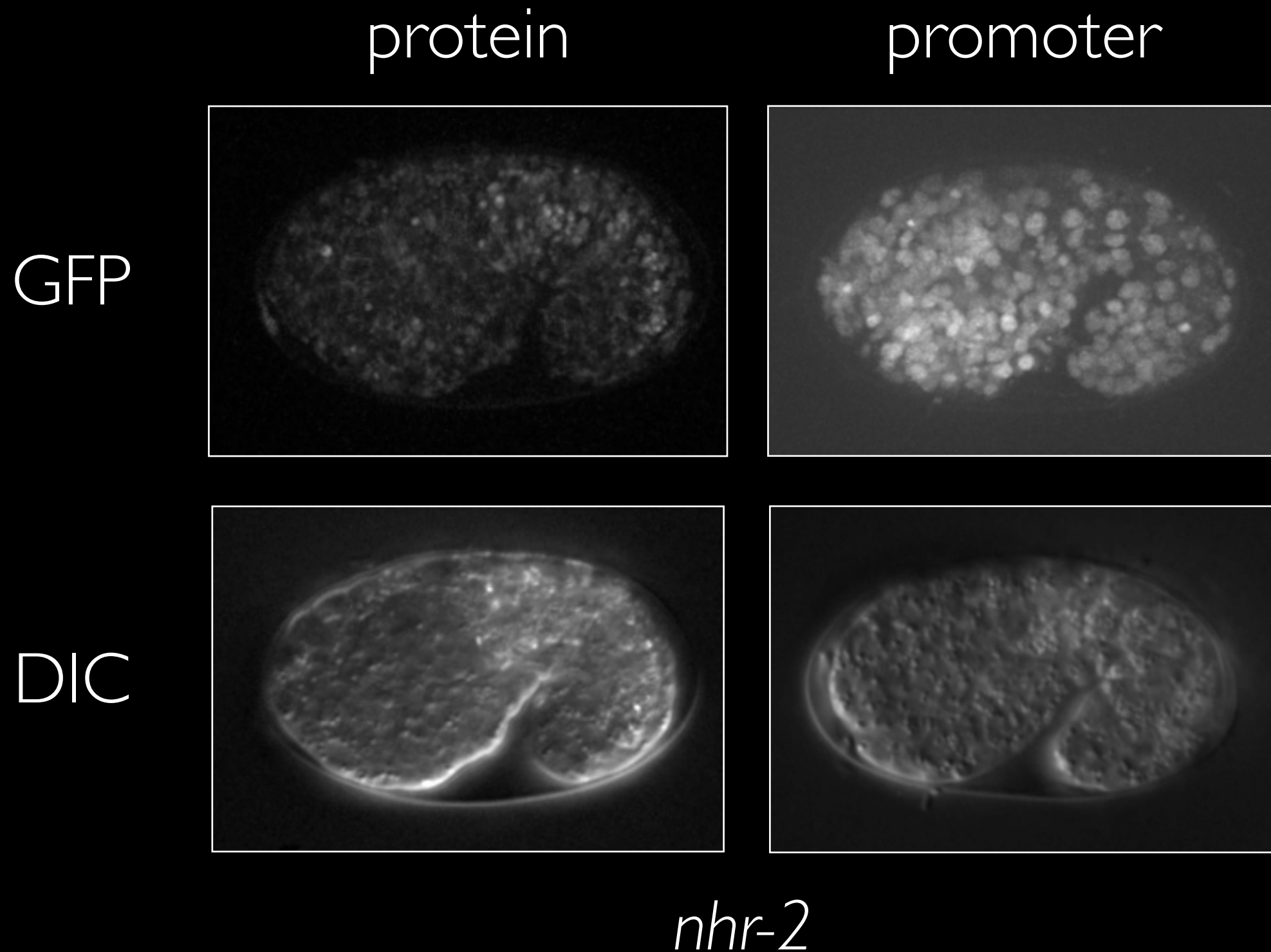
FKH-4



NHR-2

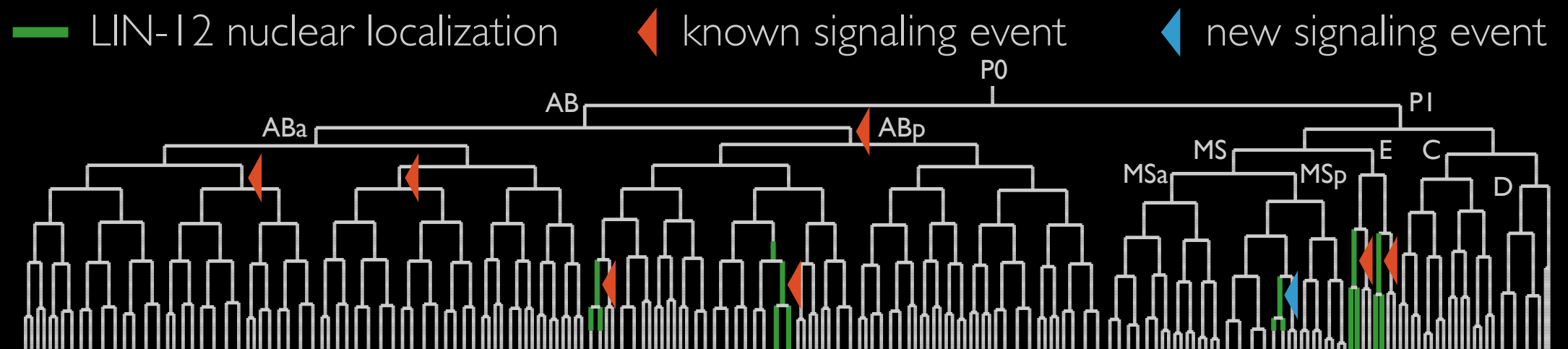
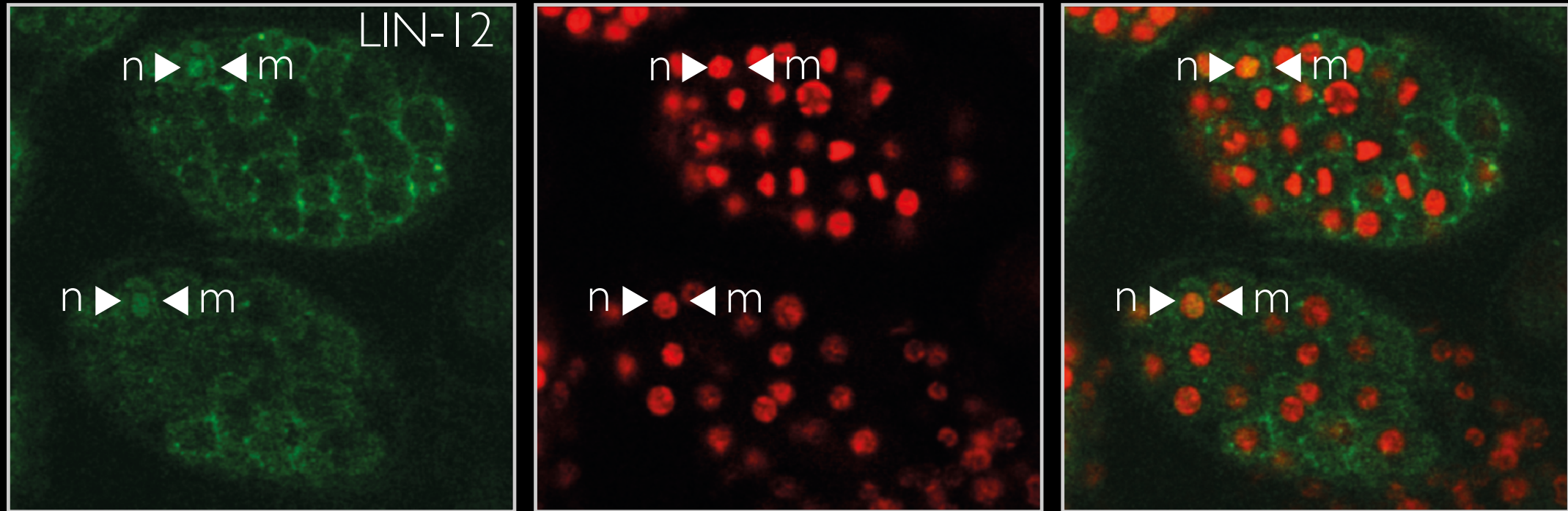


Promoter::GFP reporter misses the dynamics

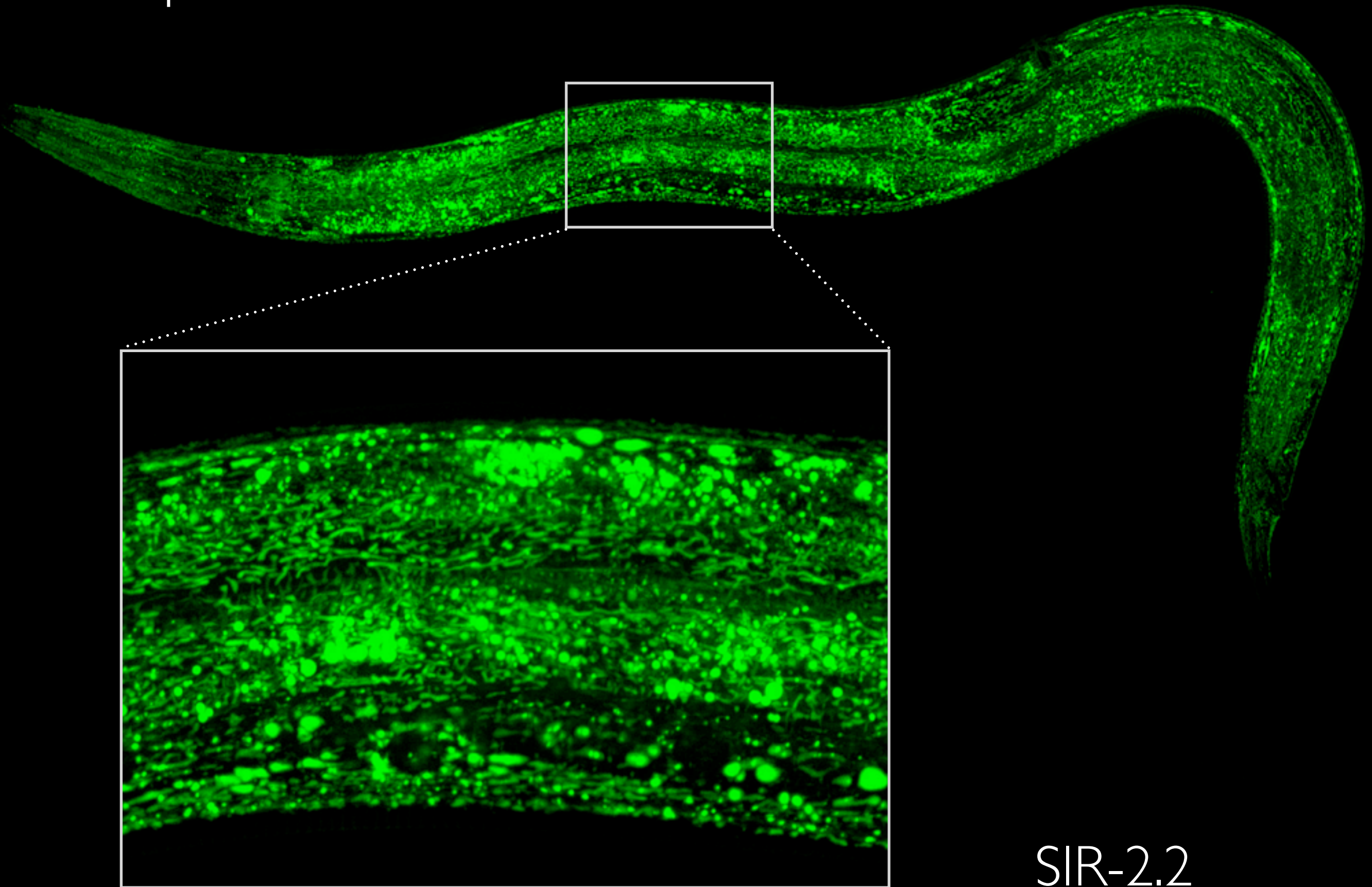


Dynamic expression patterns

in vivo relocation of LIN-12 (Notch)



New patterns



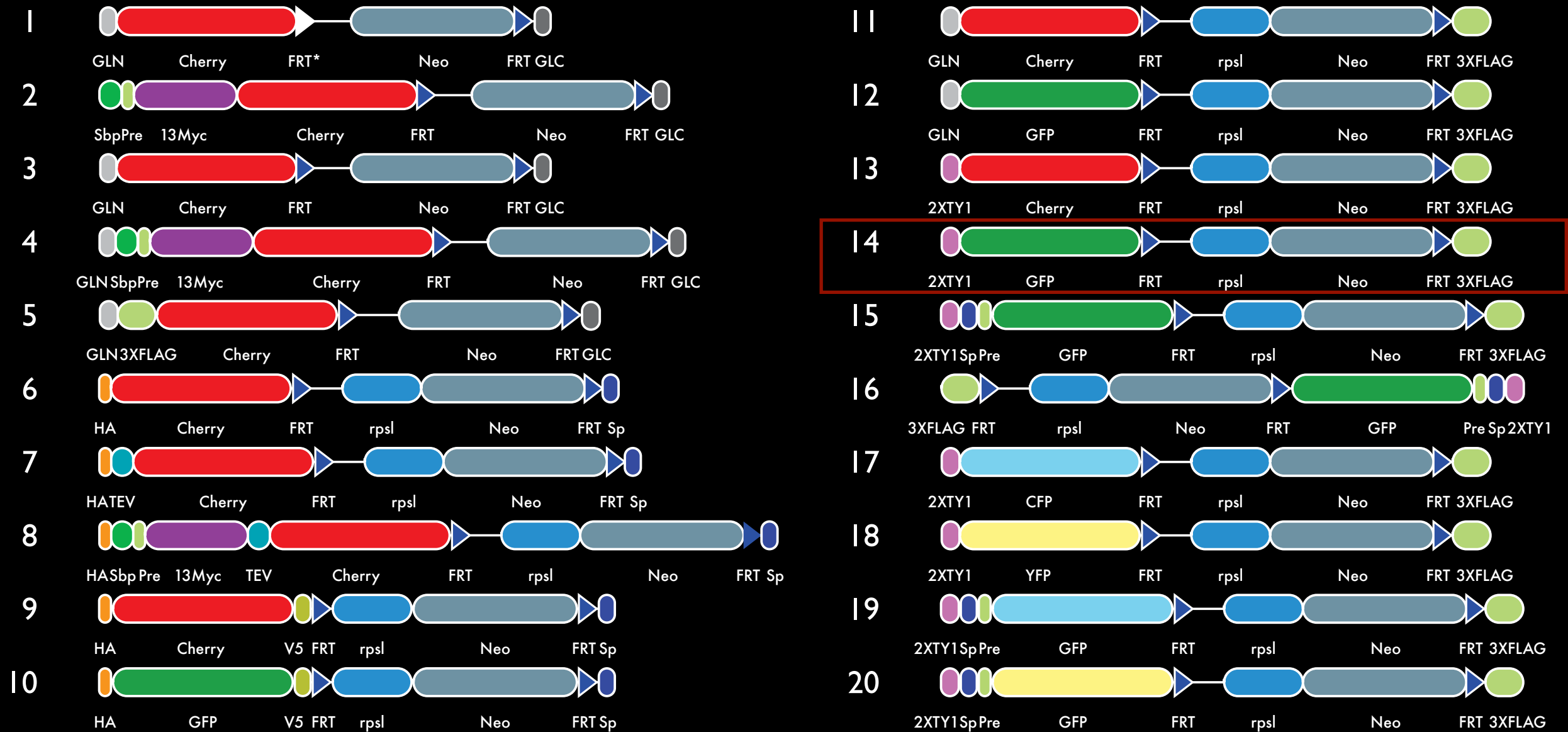
SIR-2.2

Further applications

- ▶ Protein-protein, Protein-RNA interactions
- ▶ Fine tuned reporters in genetic/drug screens
- ▶ Structure function studies (mutations, indels)*

*poster 901C Madhu Sengupta, Peter Boag

More localization and purification tags



Affinity epitopes: GLN, GLC, TYI, SBP, 13Myc, HA, Sp, 3xFlag

Fluorescent tags: Cherry, eGFP, eYFP, eCFP

Last bit

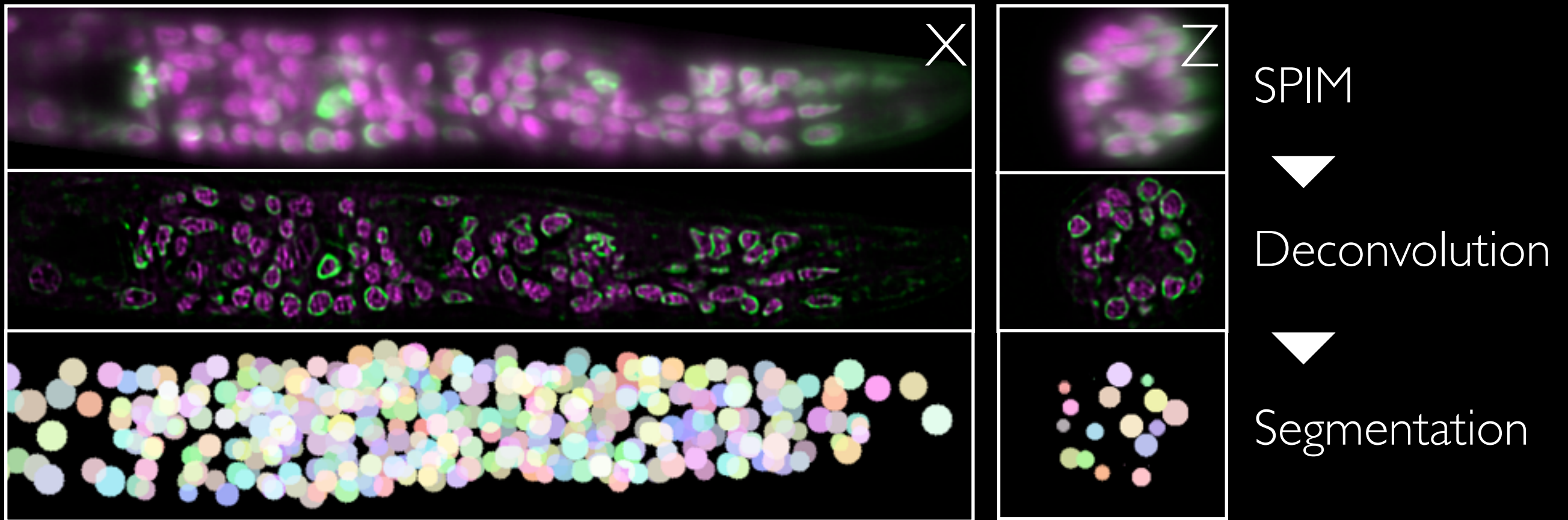
Tag your proteins and contribute to
localizing the proteome

What's next?



Stephan Preibisch, Gene Myers

from worm lines



to a high resolution protein localization atlas

Join us and contribute



Henrik Bringmann
Lionel Pintard
Adam Antebi

Brian D. Ackley

Michael Hengartner

Shi-Ding Cai

Alfred Fisher

Bob Goldstein

Oliver Hobert

Mei Ding Allison Abbott

Monica Colaiacovo

Iva Greenwald

Shai Shaham

Madeleine Erard Garcia

William Kelly

Ben-Zvi

Victor Ambros

WILLIAM SCHAFFER

Shai Shaham

Madeleine Erard Garcia

John Murray

Alex Parker

Cori Barymann

LIMOR BRODAY

Bruce Wigham

COLLAICWO

The TransgeneOme web app

The screenshot displays the TransgeneOme web application interface. The browser address bar shows the URL: transgeneome-srv1.mpi-cbg.de:8081/transgenomics/public/clone.html?wellid=13987746. The page title is "Clone: 42358680504058166 E11".

The interface includes a navigation menu on the left with options: Updates, Search (by Gene, by Pattern), Your Data (Genes, Constructs, Preps, Transformations, Lines, Patterns), Score, Strains, Tools, Admin, and Report Bugs.

The main content area displays the following information:

- Clone: 42358680504058166 E11**
- WRM0621C_F11(pRedfp-Hgr)(air-2)(18749):2XTY1-eGFP(Cele)-3XFLAGd(FRT):unc-119-Nat
- Explore in Wormbase: [cele-1844 \[5916072..5949298\] \(-\)](#)
- Genes in pCC1fos vector

Name	Length (bp)	Start (clone)	End (clone)	Strand (clone)	Start (genome)	End (genome)	Strand (genome)
B0207.7	1586	12852	14437	+	5945759	5947344	-
gpa-14	3119	14657	17775	-	5942421	5945539	+
air-2	1405	21313	23761	-	5937479	5938883	+
B0207.6	1408	24294	25701	+	5935539	5936946	-
B0207.5	14176	25822	39997	-	5921243	5935418	+

Molecules

Name	Type	Length (bp)	Export
pRedfp-Hgr	helper_plasmid	11325	Fasta GenBank GFF
wTRG3.1_42358680504058166_E11_air-2_WRM0621C_F11:2XTY1-eGFP(Cele)-3XFLAGd[18823..20198]	genomic_construct	45168	Fasta GenBank GFF

Preparations

Name: wTRG3.1_in_42358680504058166_E11_01
Add Preparation

Strains

Name: No strains are available for this construct.
Add Strain

Tag junction sequencing

Type	Status	Trace
Tag L	exact	download

PREDICTED GC GGGT CCT GATT GGT AT G CACT TCG T GATT CCG A GACT T G C T C T T T T T C A A T C T T T T G C T G C T T C A G C T C T A A T T T T G C C T C C A T C A T T C C C T
REAL GC GGGT CCT GATT GGT AT G CACT TCG T GATT CCG A GACT T G C T C T T T T T C A A T C T T T T G C T G C T T C A G C T C T A A T T T T G C C T C C A T C A T T C C C T

MATCHES: 125 | MISMATCHES: 0 | N: 0 | GAPS: 0 | GAP EXT.: 0

Click here ► <http://transgeneome.mpi-cbg.de>

The TransgeneOme web app

The screenshot displays the TransgeneOme web application interface. The browser address bar shows the URL: transgeneome-srv1.mpi-cbg.de:8081/transgenomics/user/geneLocalization.html?id=135201. The page title is "Localization". The user is logged in as "sarov (admin)".

The main content area shows "Composite Annotations (all strains and imaging data)" for the gene **ztf-4** (Gene ID: Gene:WBGene00020399). Below this, there are three diagrams illustrating localization by developmental stage: **embryo**, **L3 larva**, and **adult hermaphrodite**. The L3 larva and adult hermaphrodite diagrams show blue highlights indicating localization in the head region.

Below the diagrams is a table of "Imaging Data" for strain OP322. The table has columns for Strain, Image, Life Stage, Contributed by, Annotations by, Anatomy Annotations, Subcellular Annotations, Actions, and Construct/Tag.

Strain	Image	Life Stage	Contributed by	Annotations by	Anatomy Annotations	Subcellular Annotations	Actions	Construct/Tag
OP322		L3 larva	TransgeneOmics Facility 2011-02-06				Annotate	1777975531725265 E07 2XTYI-eGFP(Cele)-3XFLAG
		L3 larva	TransgeneOmics Facility 2011-02-06				Annotate	
		L3 larva	TransgeneOmics Facility 2011-02-06				Annotate	
		L3 larva	TransgeneOmics Facility 2011-02-06				Annotate	
		L3 larva	TransgeneOmics Facility 2011-02-06				Annotate	
		L3 larva	TransgeneOmics Facility 2011-02-06	Mihal Sarov	bodywall gonadal preanodum head midbody	nucleus	Annotate	
		embryo	TransgeneOmics Facility 2011-03-02				Annotate	
		L3 larva	TransgeneOmics Facility 2011-03-02				Annotate	
		L3 larva	TransgeneOmics Facility 2011-03-02				Annotate	

Click here ► <http://transgeneome.mpi-cbg.de>

The TransgeneOme web app

The screenshot displays the TransgeneOme web application interface. The browser address bar shows the URL: `transgeneome-srv1.mpi-cbg.de:8081/transgenomics/user/browsePatterns.html`. The page title is "Browse Patterns".

The interface features a navigation sidebar on the left with options like "Updates", "Search", "Your Data", "Genes", "Constructs", "Preps", "Transformations", "Lines", and "Patterns".

The main content area is titled "Browse Patterns" and is divided into three columns:

- Life Stage:** Includes categories like "embryo", "postembryonic", "adult", and "larva", with sub-categories for "dauer larva", "L1 larva", "L2 larva", "L3 larva", and "L4 larva".
- Anatomic Localization:** Includes categories like "Cell and Anatomy", "Anatomy", "Cell", "Function", and "Organ system", with sub-categories like "alimentary system", "coelomic system", "epithelial system", "excretory secretory system", "muscular system", and "nervous system".
- Subcellular Localization:** Includes categories like "mitochondrion", "mitosome", "monolayer-surrounded lipid", "monomer", "nucleomorph", "nucleus", "PHA granule", "pinulosome", "plastid", "platelet dense subular netwo", and "spore body".

Below the filters, there are checkboxes for "Include child terms" and a "Search" button. A table below the filters shows gene entries:

Gene ID	Gene Name	Image 1	Image 2
OP124	977887491443534 H03		
	zrf-4		

At the bottom of the page, there is a large inset image showing a merged microscopy image of a C. elegans worm. The image is divided into three panels: a grayscale channel, a fluorescence channel, and a merged channel showing green fluorescent spots. The text "135201 | OP322" is visible at the bottom of the inset image.

Click here ► <http://transgeneome.mpi-cbg.de>



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Gene Myers

Stephan Preibisch

Francis Stewart

Jun Fu, Youming Zhang

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<http://transgeneome.mpi-cbg.de>



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