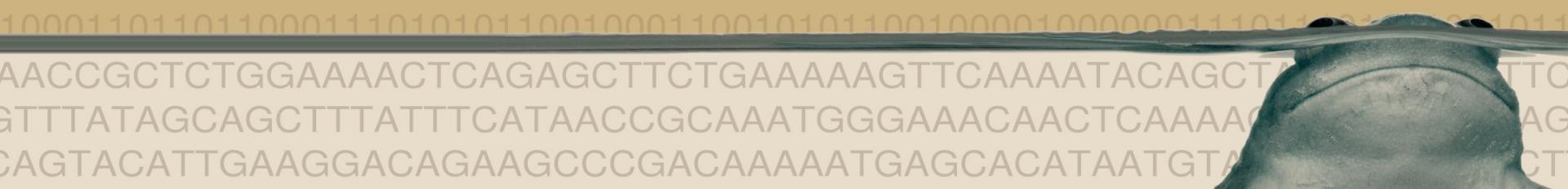


# *Xenopus* functional genomics: data availability and visualization

Simon J. van Heeringen

14<sup>th</sup> International *Xenopus* conference



# Support and more info

<http://simonvh.github.com/xenopus2012>

- Contains all presentations
- Links to databases, tools and materials

Mail me: [s.vanheeringen@ncmls.ru.nl](mailto:s.vanheeringen@ncmls.ru.nl)

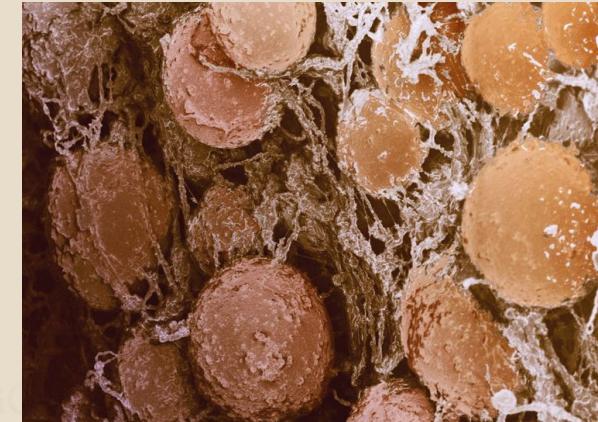
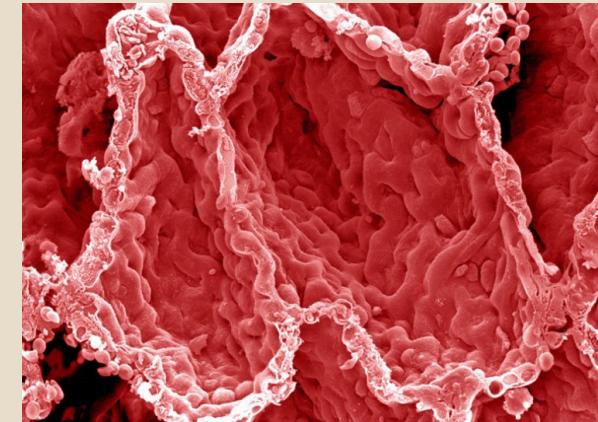
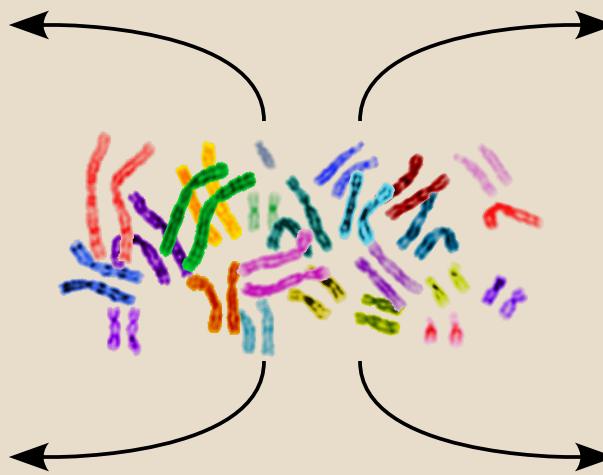
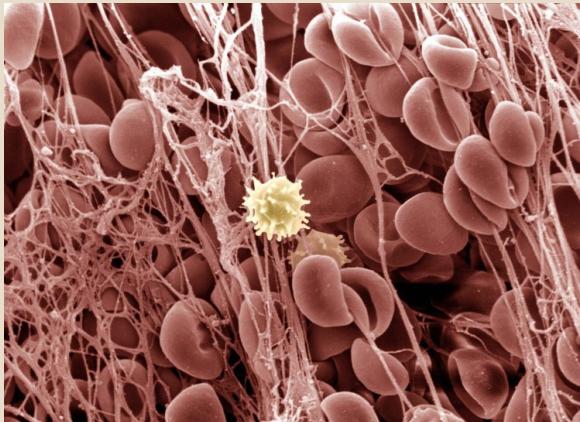
Our website:

<http://www.ncmls.nl/gertjanveenstra>

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# One genome, many functions



# Encyclopedia of DNA Elements

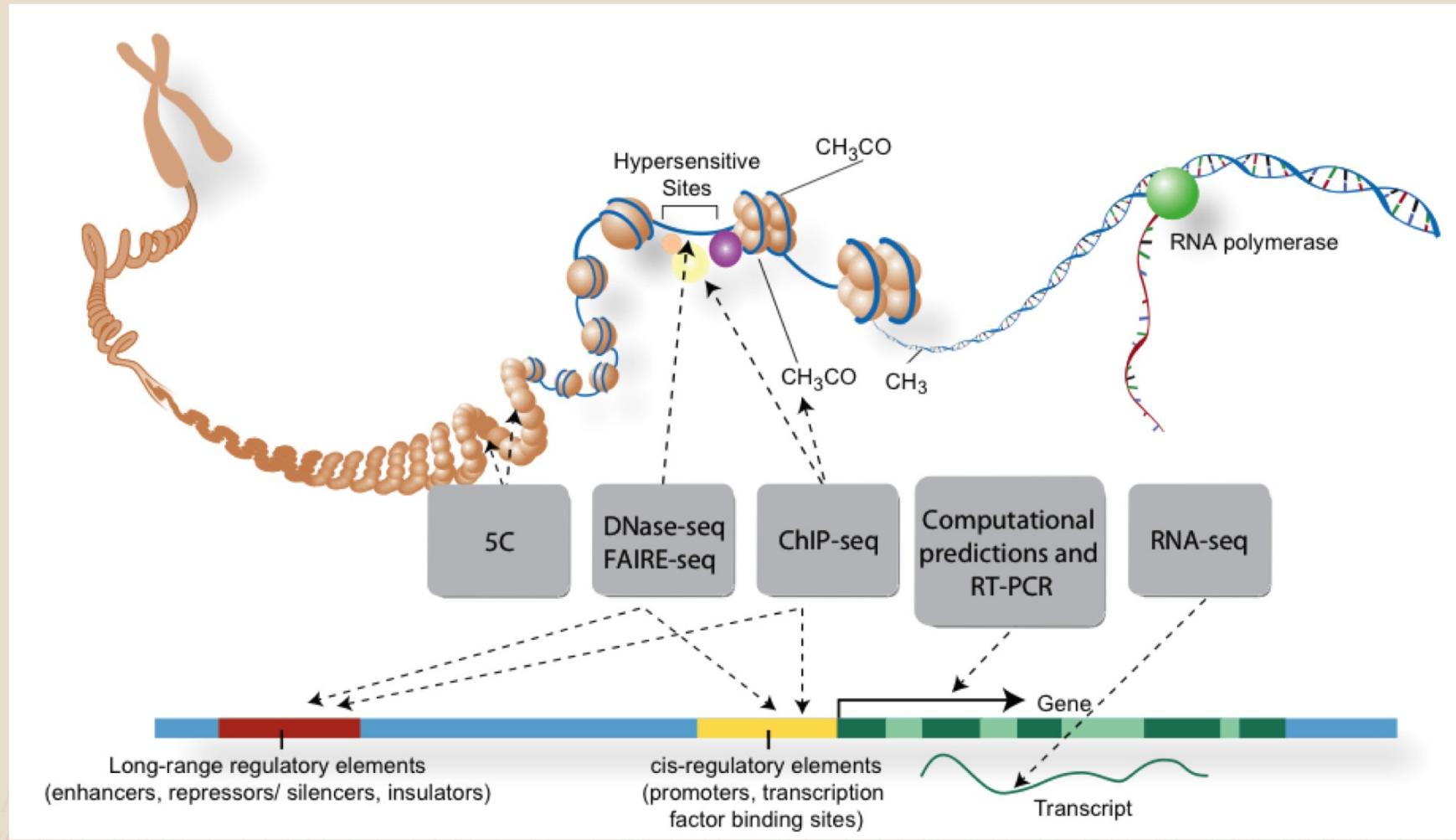


<http://www.nature.com/encode>

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# The functional genome



ENCODE, Darryl Leja (NHGRI), Ian Dunham (EBI)

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# Illumina HiSeq 2000

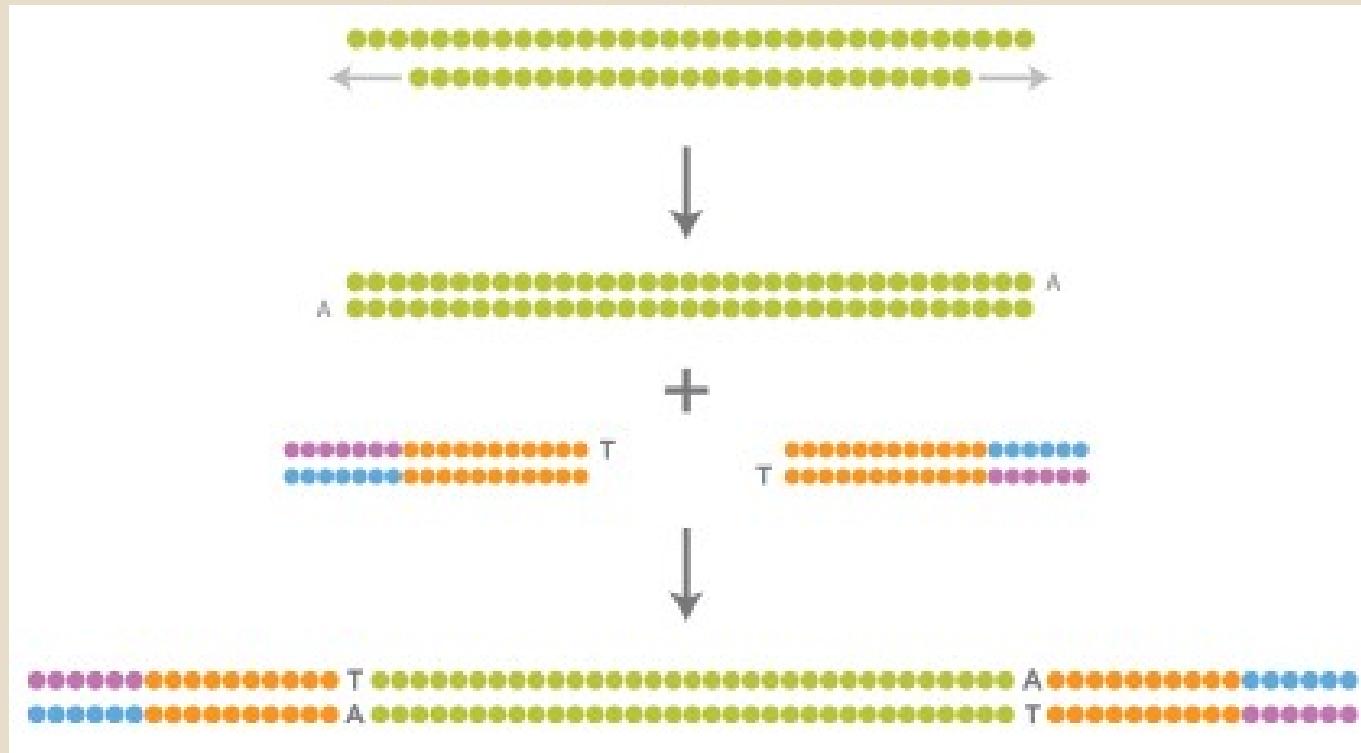


Illumina, Inc.

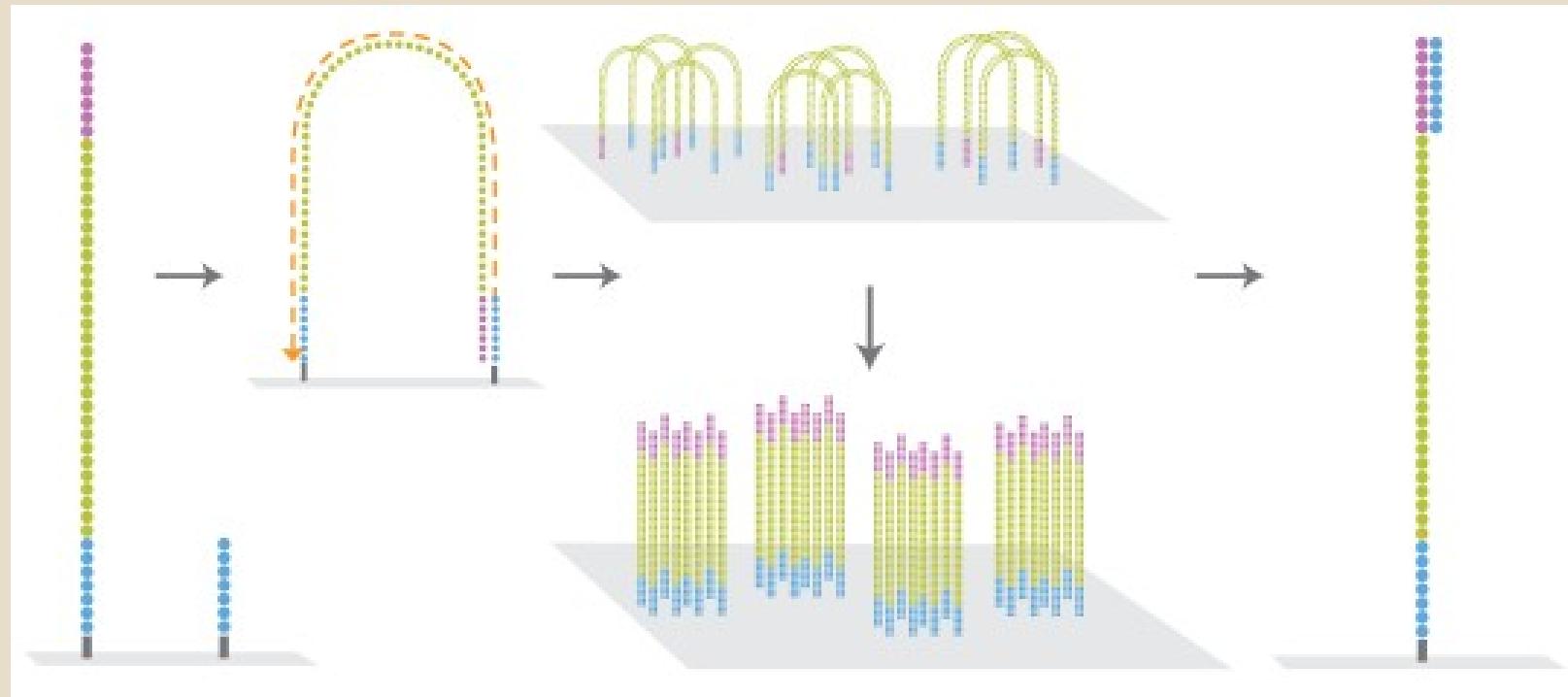
Radboud University Nijmegen



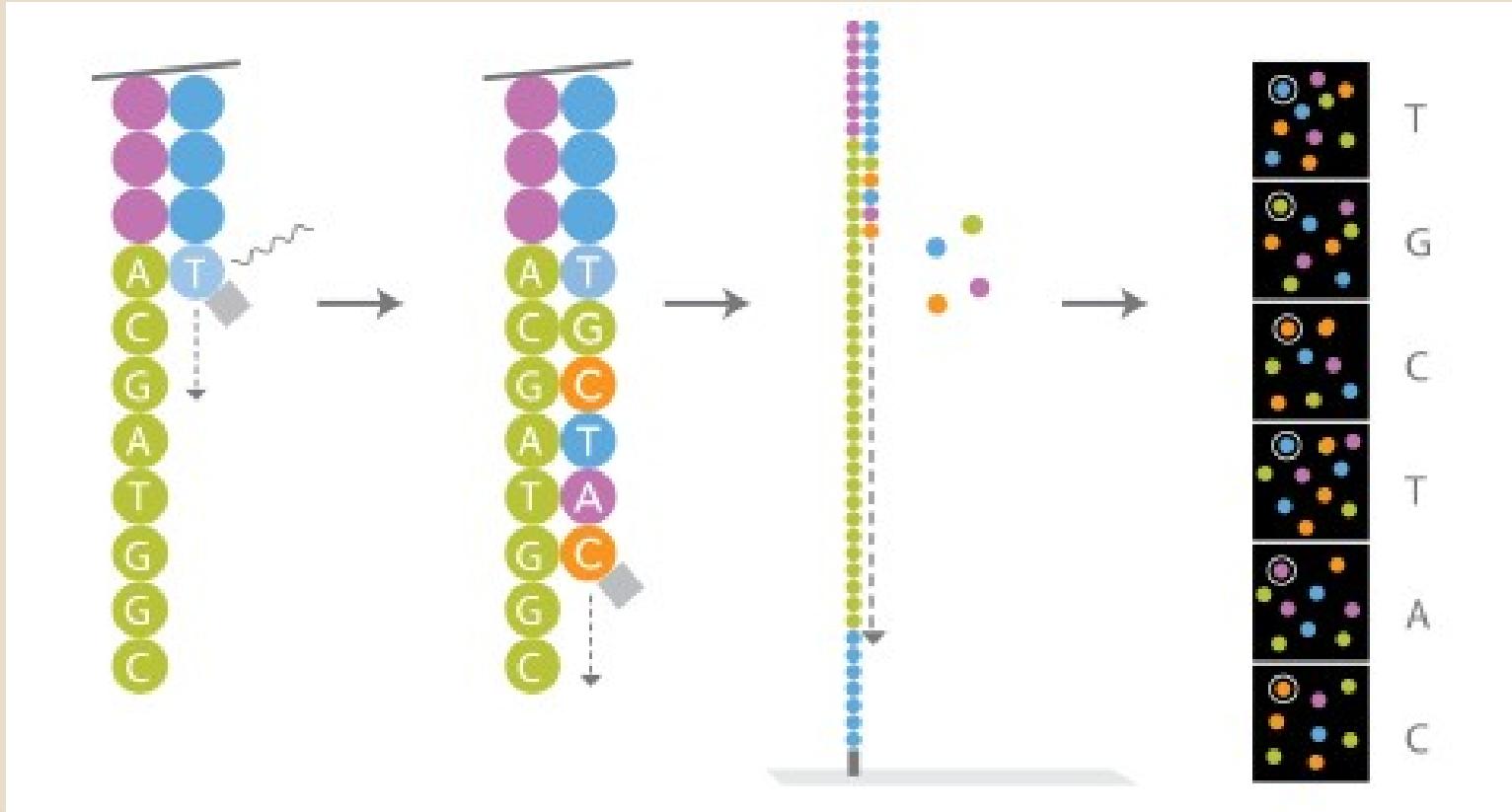
# Adapter ligation



# Cluster generation



# Sequencing

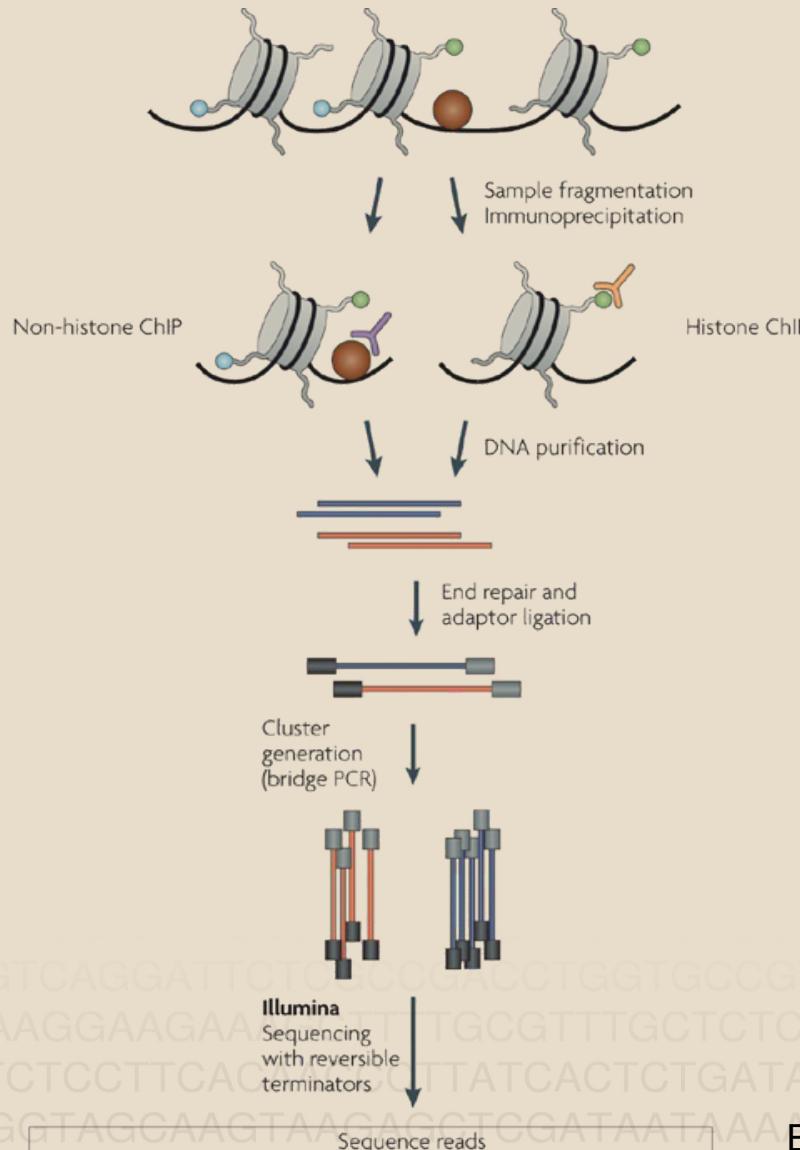


# Applications of sequencing

- RNA-seq: gene expression, small RNA, non-coding RNA
- ChIP-seq: histone modifications, transcription factors
- Bisulphite seq / MethylCap: DNA methylation
- DNase-seq / FAIRE-seq: “open chromatin”
- CAGE-seq / TSS-seq: transcription start sites



# ChIP-sequencing



- ChIP
- Sample prep
- Sequencing
- Mapping
- Analysis
  - Peak calling
  - Location
  - Motifs
  - ...

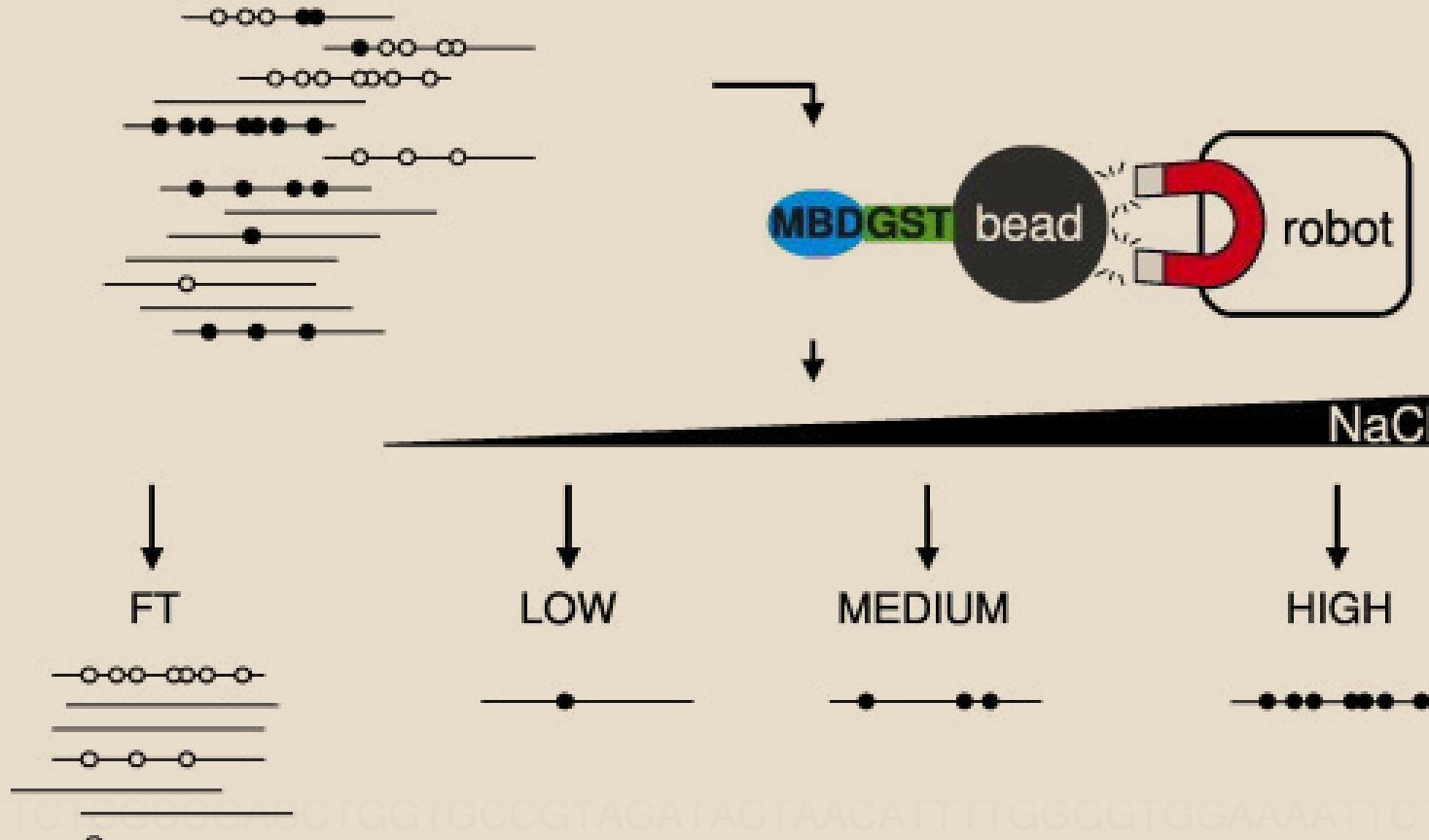
Based on Park, 2009

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# MethylCap

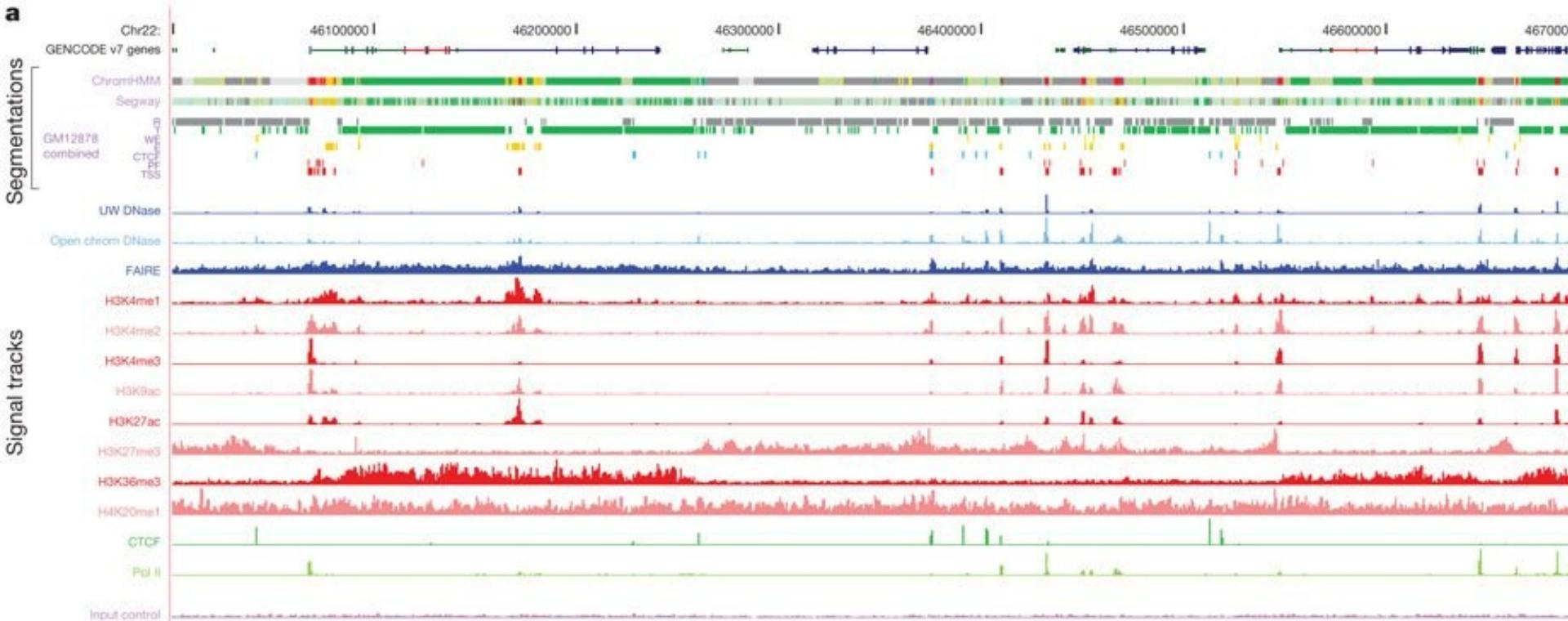
Sonicated gDNA



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# A genome-wide view of regulation



# ChIP-seq workflow

Data type

```
TAACGTGAACCCCTCATCTTCCTCACAGATTG  
TAGTTCTCACTTCAAGTTATCCAGCAACCTGGAA  
TTTGAACATCATGTTCCCTGCATGTTGGTGCTTG  
GACTGCTAATATCCTTATCATTACAAAAGGGTAC  
GCTTTTACATTGGACCCTTAATAAATGACTAG  
TCCTTATCCTATGCTCTTACCCCCATATTACTGC  
CAGAACAGGAATGAGGGGTCTCTAAATGGCTGATA  
CTGCTAAATGTCAATAACTATAATAGCTATGATT  
TGTGGTATTTATCAAATACATGTTAAACAAATG  
TCCCTATCTTAAAATCCAGTGCACAAAGAATTG
```

raw  
reads

aligned  
data

visualization,  
peak  
calling



# File formats

```
TAACGTGAACCCCTCATCTTCCTTACAGATTG  
TAGTTCTCACTTCAAGTTATCCAGCAACCTTGGAA  
TTTGAACATCATGTTCTGCATGTTTGGTGTGTTG  
GACTGCTAATATCCTTATCATTACAAAAGGGTAC  
GCTTTTACATTGGACCCTTAATAATGACTAG  
TCCTTATCCTATGCTCTTACCCCCATTACTGC  
CAGAACAGGAATGAGGGGTCTCAAATGGCTGATA  
CTGCTAAATGTCAATAACTATAATAGCTATGATT  
TGTGGTATTTATCAAATACATGTTAAACAAATG  
TCCCTATCTTAAAATCCAGTGCACAAAGAATTG
```

Data type

raw  
reads

File formats

FASTQ  
SAM / BAM



aligned  
data

BED  
SAM / BAM

visualization,  
peak  
calling

BED  
WIG

# File formats

- All (except BAM) are text-based

- FASTQ

```
@SOLEXAWS1:2:6362AAAXX:1:24:1990:6915
AATGATGCCACGATCGGCCGAAATCAGCTGAAAATGTTA
+
GG=GGGHHHFGBDGGF<DFCGGEDEG8GGGGGG@8GDGD
@SOLEXAWS1:2:6362AAAXX:1:80:5526:11071
AATGATGCCACGATCGGCCGAAATCAGCTGAAAATGTTA
+
IGIGGIIDIFIIHIIIIIIHBIHHHHIIIGH
```



# BED format

- Defined by the UCSC  
<http://genome.ucsc.edu/FAQ/FAQformat.html>
- Basic BED  
chrom start end

scaffold_1	100203	100238
scaffold_1	100213	100248

# BED format

- Defined by the UCSC  
<http://genome.ucsc.edu/FAQ/FAQformat.html>
- Basic BED with strand information  
chrom start end name score strand

scaffold_1	100203	100238	0	0	+
scaffold_1	100213	100248	0	0	-



# Wiggle format

- .wig files
- For visualization
  - Lossy!
  - Don't use for analysis!
- Similar to BED:

scaffold\_1    100203    100213    123.0



# Wiggle format

- .wig files
- For visualization
  - Lossy!
  - Don't use for analysis!
- Similar to BED:

scaffold\_1 100203 100213 123.0



- Several other wig formats, for data compression, see UCSC

# SAM / BAM format

- Can contain both raw sequence and quality information as well as alignment
- SAM is text-based
- BAM is a binary version of SAM
  - Not human-readable
  - Much smaller filesize
  - Can be indexed for faster analysis
- <http://samtools.sourceforge.net/>

# ChIP-seq workflow

Data type

```
TAACGTGAACCCCTCATCTTCCTCACAGATTG  
TAGTTCTCACTTCAAGTTATCCAGCAACCTGGAA  
TTTGAACATCATGTTCCCTGCATGTTGGTGCTTG  
GACTGCTAATATCCTTATCATTACAAAAGGGTAC  
GCTTTTACATTGGACCCTTAATAAATGACTAG  
TCCTTATCCTATGCTCTTACCCCCATATTACTGC  
CAGAACAGGAATGAGGGGTCTCTAAATGGCTGATA  
CTGCTAAATGTCAATAACTATAATAGCTATGATT  
TGTGGTATTTATCAAATACATGTTAAACAAATG  
TCCCTATCTTAAAATCCAGTGCACAAAGAATTG
```

raw  
reads

aligned  
data

visualization,  
peak  
calling



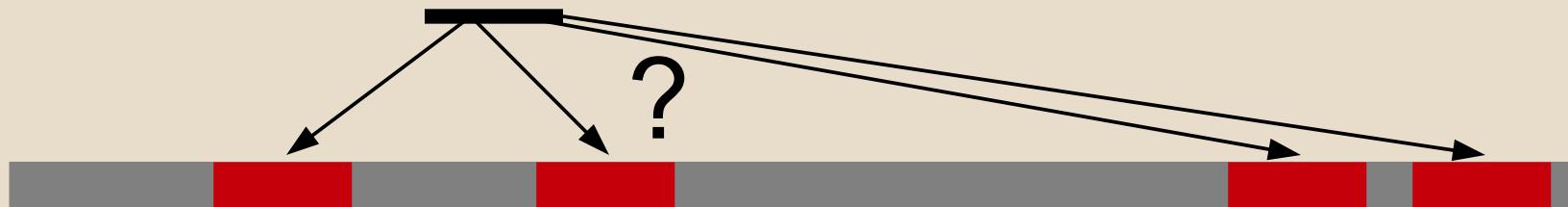
# Mapping to reference genome

- A time- and memory-intensive process
- Lots of options:
  - ELAND (Illumina)
  - BWA <http://bio-bwa.sourceforge.net/>
  - Bowtie <http://bowtie-bio.sourceforge.net/>
- However, for ChIP-seq it doesn't really matter which you use.



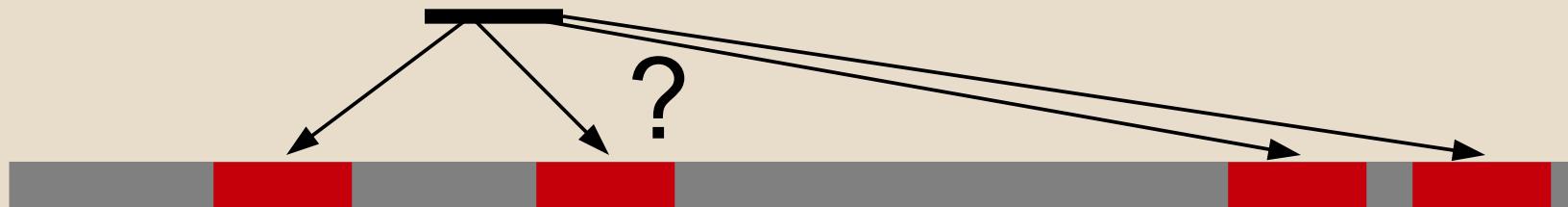
# Repeats and duplicates

- Repeats



# Repeats and duplicates

- Repeats



- PCR duplicates



# Alignment & post-processing

- Different mappers behave differently
  - BWA randomly assigns a repeat read to one location
  - ELAND cannot map to repeats
- Post-processing (at least in our lab)
  - Remove reads in repeats
  - Remove all duplicates
    - Except in RNA-seq



# Which reference genome?

- JGI 4.1, 4.2, 7.1, ...?
- Raw data can always be remapped
  - As long as you keep unmapped reads, no data is lost
  - FASTQ, SAM, BAM can be remapped
- Aligned data is dependent on a specific reference
  - BED, WIG



# Veenstra lab data

- Blastula, gastrula, neurula, tadpole stages
  - H3K4me3 (associated with activation)
  - H3K27me3 (associated with repression)
  - RNAPII (active transcription)
  - RNAseq (expression)
- Blastula and gastrula stages
  - H3K4me1 (enhancers)
  - MethylCap (DNA methylation)
- Gastrula
  - TBP (near Transcription Start Site)

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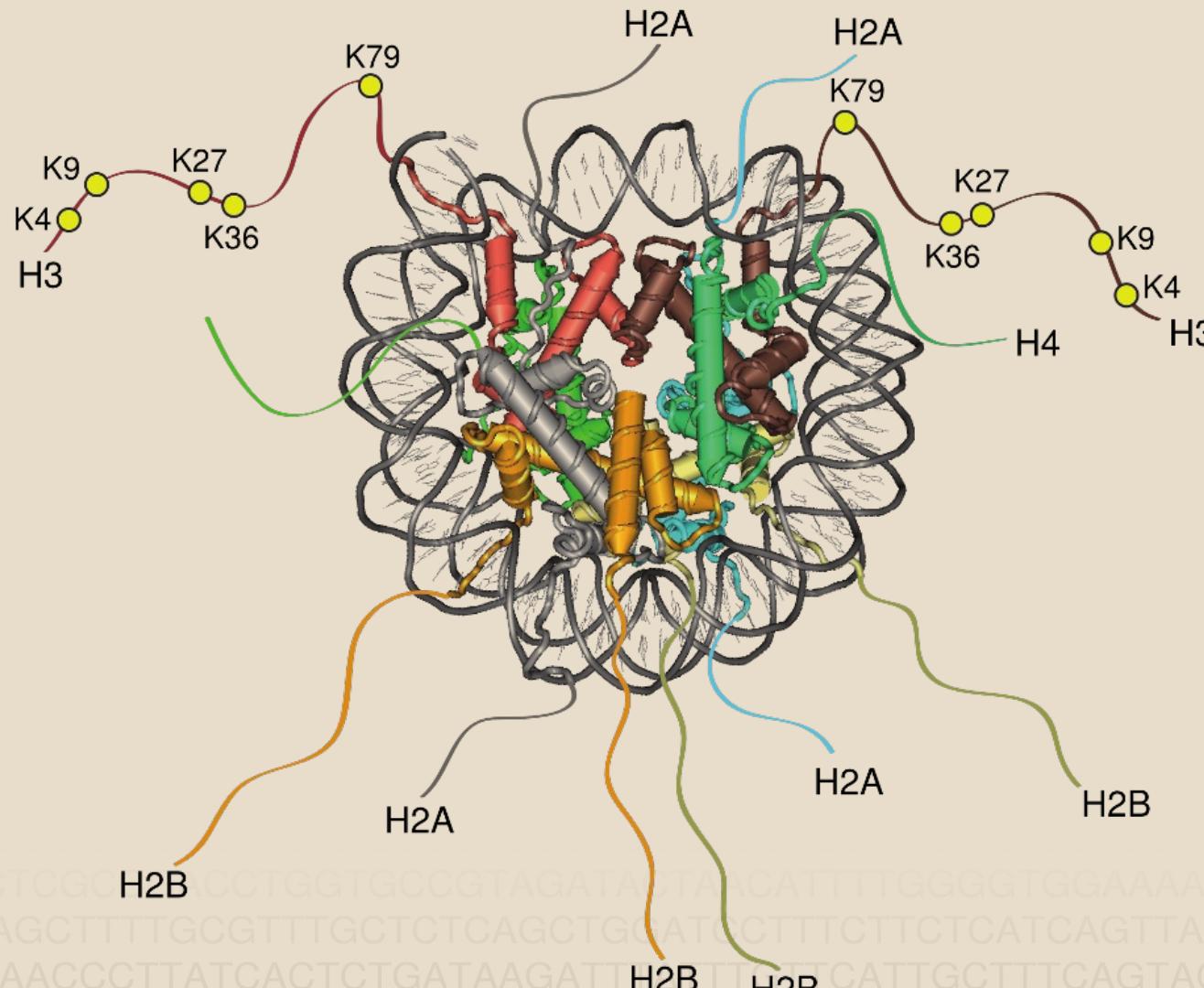
# Veenstra lab data

- Blastula, gastrula, neurula, tadpole stages
  - H3K4me3 (associated with activation)
  - H3K27me3 (associated with repression)
  - RNAPII (active transcription)
  - RNAseq (expression)
- Blastula and gastrula stages
  - H3K4me1 (enhancers)
  - MethylCap (DNA methylation)
- Gastrula
  - TBP (near Transcription Start Site)

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# Histone modifications

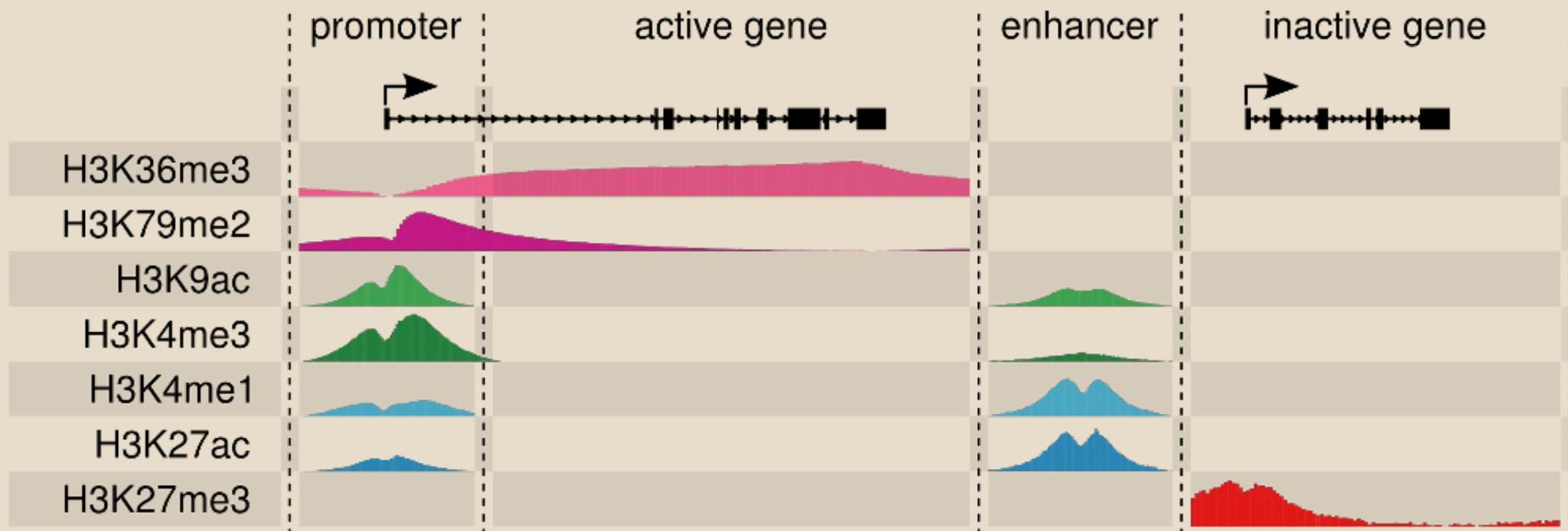


Bogdanovic, 2012

Radboud University Nijmegen



# Histone modifications



# How to visualize this data?

- Gene profile browser
- Xenbase
- UCSC Genome Browser



# Quick gene-based overview

- <http://www.ncmls.nl/gertjanveenstra/>
  - Resources → Epigenome gene profile browser

The screenshot shows a web browser window with the following details:

- Address Bar:** Web 131.174.221.43/gertjanveenstra/geneprofile.htm
- Toolbar:** Includes back, forward, search, and other standard browser icons.
- Page Header:**
  - Veenstra Lab** logo featuring a stylized bar chart.
  - Text: Embryonic gene regulation - Dept. Molecular Developmental Biology
  - Navigation menu: Home, Research, People, Positions, Publications, Resources, Contact info.
- Section Title:** **Epigenome gene profile browser**
- Text:** Type gene symbol (exact and case-sensitive, unforgiving). Visit [Xenbase](#) pages for official gene symbols.
- Form:** Gene input field with placeholder "Gene" and a "Submit" button.
- Footer:**
  - Radboud University Nijmegen logo.
  - Radboud University Nijmegen text: Radboud University Nijmegen, Faculty of Science, Nijmegen Centre for Molecular Life Sciences.
  - Radboud University Nijmegen text in red: Radboud University Nijmegen.



# Quick gene-based overview

- <http://www.ncmls.nl/gertjanveenstra/>
  - Resources → Epigenome gene profile browser
- Images are automatically generated
  - scale, size, annotation might be not optimal
- H3K4me3 and H3K27me3 only
- Exact gene name (lower case)!
- Static image, no browsing



# Xenbase

- Integrated with all other Xenbase resources
- H3K4me3 and H3K27me3 stage 12 ChIP-seq data (JGI 4.1 only; Akkers, 2009)
  - Header: Methylation Data
- Xenbase is working on supporting (custom) wig files



# Genome browser tracks

- UCSC Genome browser
  - <http://genome.ucsc.edu/>
  - Only *X. trop* JGI 4.1 tracks
- Genome Browser mirror at MRC NIMR (Mike Gilchrist)
  - <http://genomes.nimr.mrc.ac.uk>
  - *X.trop* JGI 4.1 & 7.1
  - *X.laevis* 2011 assembly
    - No public tracks as of now



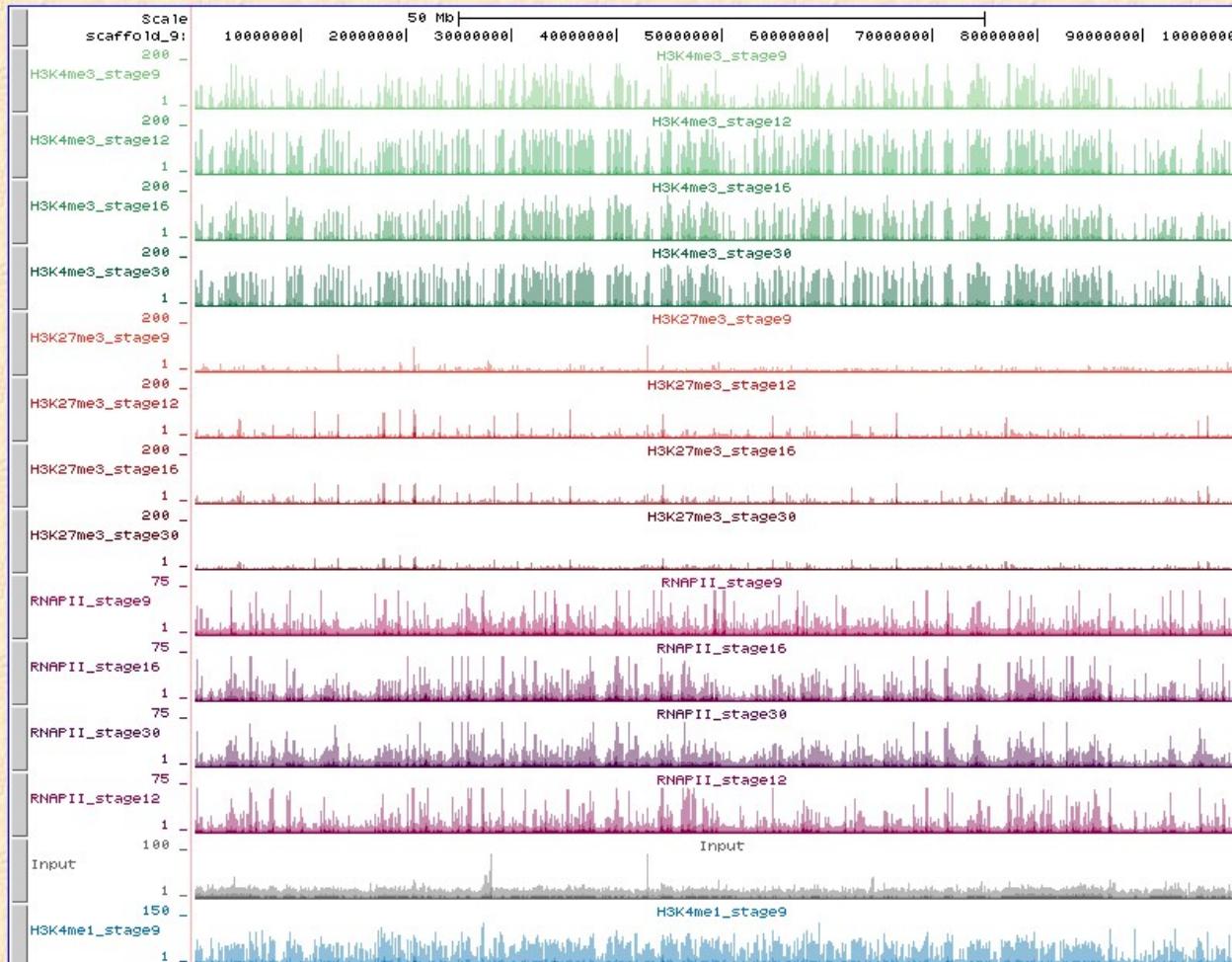
# Genome Browser

Home Genomes Tables DNA PDF/PS Session Help

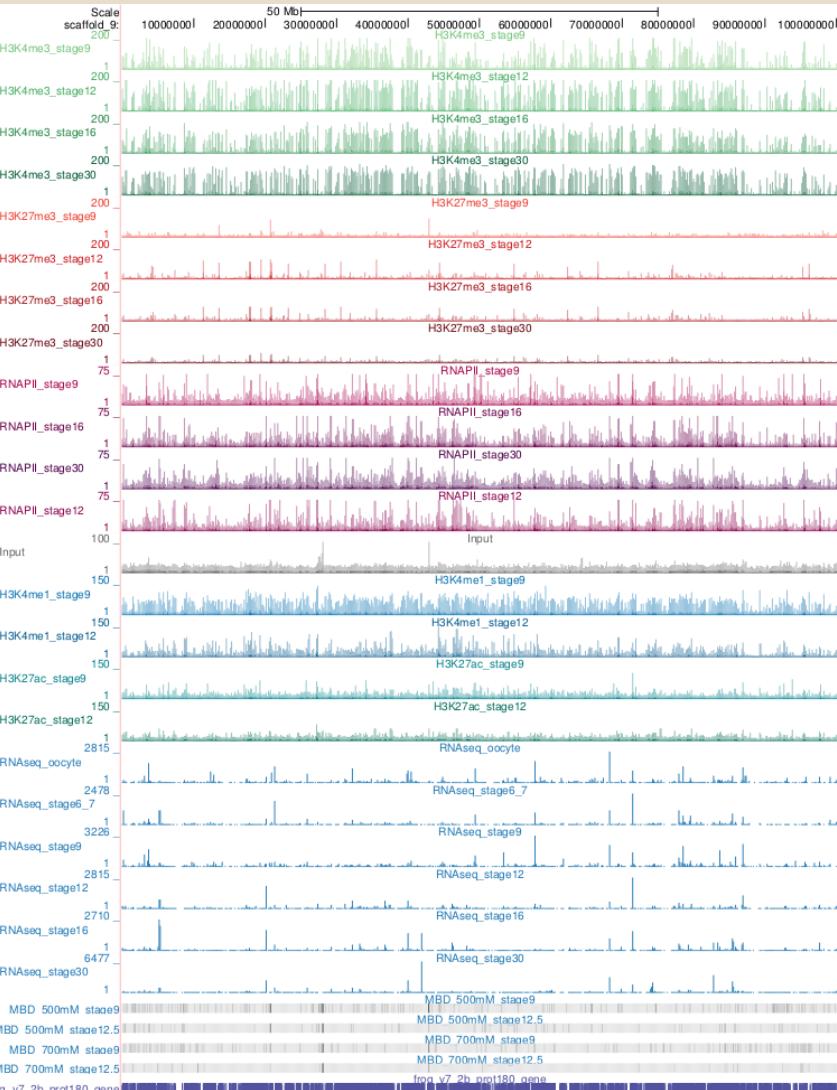
## UCSC Genome Browser on X. tropicalis Aug. 2011 xenTro3beta Assembly

move <<< << < > >> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x

position/search scaffold\_9:1-100,021,500 jump clear size 100,021,500 bp. configure



# Genome Browser



Genome backbone;  
base position

Annotation tracks  
Genes  
ChIP-seq profiles  
GC%  
Repeats  
Conservation  
Etc..

# Adding tracks

← → ⌂ ⌂ Web genomes.nimr.mrc.ac.uk/cgi-bin/hgGateway ★  Search with Google

Home Genomes Blat Tables Session FAQ Help

## X. tropicalis (*Xenopus tropicalis*) Genome Browser Gateway

The UCSC Genome Browser was created by the [Genome Bioinformatics Group of UC Santa Cruz](#).  
Software Copyright (c) The Regents of the University of California. All rights reserved.

clade genome assembly position or search term

[Click here to reset](#) the browser user interface settings to their defaults.

### About the X. tropicalis Nov. 2011 (xenTro7\_1) assembly ([sequences](#))

## X. tropicalis



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# add custom tracks

← → ⌂ Web genomes.nimr.mrc.ac.uk/cgi-bin/hgGateway ★ Search with Google

Home Genomes Blat Tables Session FAQ Help

## X. tropicalis (*Xenopus tropicalis*) Genome Browser Gateway

The UCSC Genome Browser was created by the [Genome Bioinformatics Group of UC Santa Cruz](#).  
Software Copyright (c) The Regents of the University of California. All rights reserved.

clade genome assembly position or search term  
Vertebrate ▾ X. tropicalis ▾ Nov. 2011 ▾ scaffold\_1:113,526,568-113,6 ▾ submit

[Click here to reset](#) the browser user interface settings to their defaults.

[add custom tracks](#) [configure tracks and display](#) [clear position](#)

### About the X. tropicalis Nov. 2011 (xenTro7\_1) assembly ([sequences](#))

## X. tropicalis



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# add custom tracks

Web genomes.nimr.mrc.ac.uk/cgi-bin/hgCustom

Home Genomes Genome Browser Blat Tables Session FAQ Help

## Add Custom Tracks

clade  genome  assembly  [xenTro7\_1]

Display your own data as custom annotation tracks in the browser. Data must be formatted in [BED](#), [bigBed](#), [bedGraph](#), [GFF](#), [GTF](#), [WIG](#), [bigWig](#), [MAF](#), [BAM](#), [BED detail](#), [Personal Genome SNP](#), [VCF](#), or [PSL](#) formats. To configure the display, set [track](#) and [browser](#) line attributes as described in the [User's Guide](#). URLs for data in the bigBed, bigWig, BAM and VCF formats must be embedded in a track line in the box below. Publicly available custom tracks are listed [here](#). Examples are [here](#).

Paste URLs or data:  Or upload:

Optional track documentation: Or upload:

Click [here](#) for an HTML document template that may be used for Genome Browser track descriptions.



# add custom tracks

← → 🔍 Web genomes.nimr.mrc.ac.uk/cgi-bin/hgCustom ⭐ Search with Google

Home Genomes Genome Browser Blat Tables Session FAQ Help

## Add Custom Tracks

clade  genome  assembly  [xenTro7\_1]

Display your own data as custom annotation tracks in the browser. Data must be formatted in [BED](#), [bigBed](#), [bedGraph](#), [GFF](#), [GTF](#), [WIG](#), [bigWig](#), [MAF](#), [BAM](#), [BED detail](#), [Personal Genome SNP](#), [VCF](#), or [PSL](#) formats. To configure the display, set [track](#) and [browser](#) line attributes as described in the [User's Guide](#). URLs for data in the bigBed, bigWig, BAM and VCF formats must be embedded in a track line in the box below. Publicly available custom tracks are listed [here](#). Examples are [here](#).

Paste URLs or data:      Or upload:  Choose...

Optional track documentation: Or upload:  Choose...

Click [here](#) for an HTML document template that may be used for Genome Browser track descriptions.



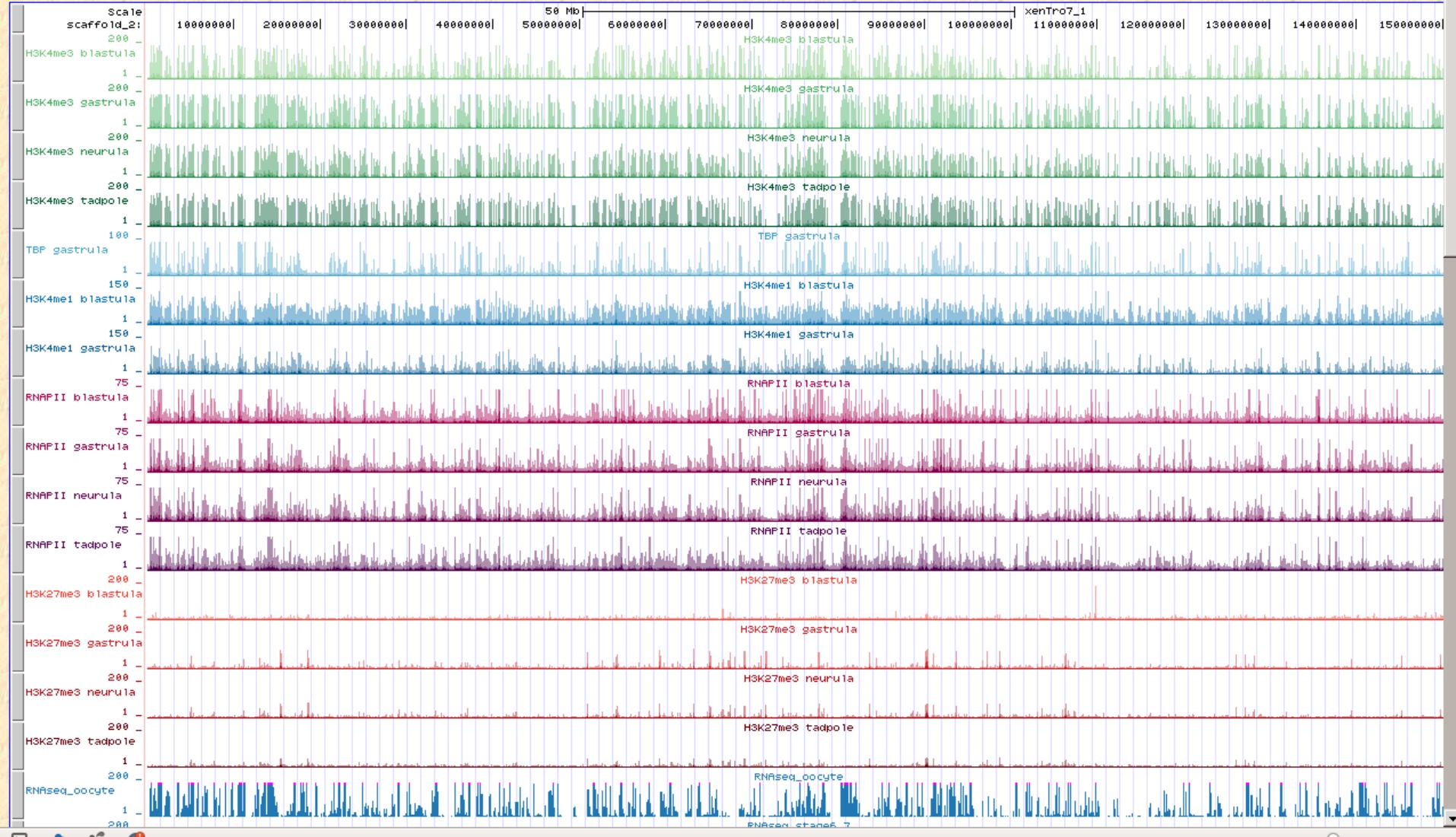
Tracks from:

<http://131.174.221.43/gertjanveenstra/genomedata.asp>

## UCSC Genome Browser on X. tropicalis Nov. 2011 Assembly (xenTro7\_1)

move &lt;&lt;&lt; &lt;&lt; &lt; &gt; &gt;&gt;&gt; zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x

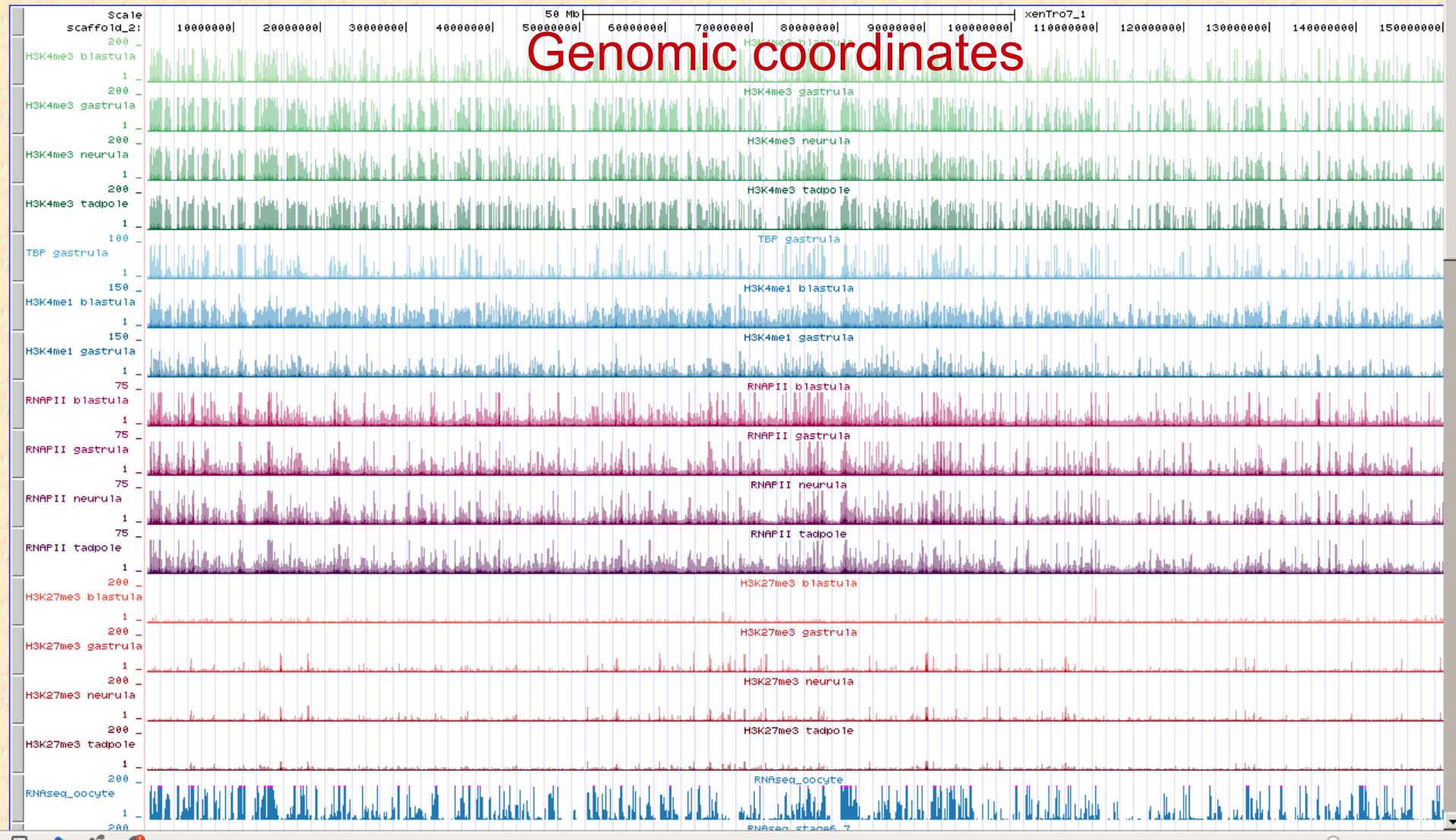
position/search scaffold\_2:1-150,913,983 jump clear size 150,913,983 bp. configure



## UCSC Genome Browser on X. tropicalis Nov. 2011 Assembly (xenTro7\_1)

move &lt;&lt;&lt; &lt;&lt; &lt; &gt; &gt;&gt;&gt; zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x

position/search scaffold\_2:1-150,913,983 jump clear size 150,913,983 bp. configure

**Genomic coordinates**

# Your favorite gene

- Search by gene name is (not yet) available for JGI 7.1
- Get coordinates from Xenbase!



Opera Postvak IN - Outlo... X. tropicalis scaffold... Xenopus laevis sta... Veenstra- NCMLS t Summary [speci... +

Web www.xenbase.org/gene/showgene.do Search with Google

# Xenbase

[2.8.1]

Blast Frogs Genes Expression Literature Genome Browsers Anatomy & Development Community Reagents & Protocols Stockcentre FTP

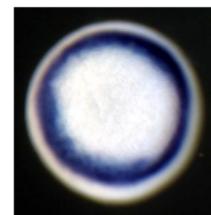
Search Genes For t Search Submit Data Give Feedback

Summary Expression (409) Gene Literature (409) Nucleotides (229) Proteins (32) Interactants (632) Wiki

XB-GENEPAGE-478788

## Gene Symbol: t

Gene Name: T, brachyury homolog



◀ ▶ 1/3

Synonyms: Xbra, X-bra, bra, Xbrachyury, brachyury, ntl [Add Xenopus synonyms](#)

Gene Function: T-box transcription factor

Interactants: [gsc](#)(131), [chrd](#)(92), [nodal](#)(61), [wnt8a](#)(61), [myod1](#)(58), [otx2](#)(45), [bmp4](#)(38) [View All](#)

Molecules	XB-GENE-478789 tropicalis	XB-GENE-865212 laevis a	XB-GENE-6255736 laevis b
Gene	<a href="#">JGI XT Genome 7.1</a> <a href="#">JGI XT Genome 4.1</a> <a href="#">Ensembl 4.1</a> <a href="#">Entrez Gene</a>	<a href="#">Entrez Gene</a>	<a href="#">Entrez Gene</a>
mRNA	<a href="#">Refseq</a>	<a href="#">Refseq</a>	<a href="#">Refseq</a>
Protein	<a href="#">Refseq</a>	<a href="#">Refseq</a> <a href="#">UniProtKB</a>	<a href="#">Refseq</a>
Genomic	tropicalis		
Genome Browser	<p>scaffold_5:72053579..72062232 72060k Xenbase 7.1 Gene Models t Xenbase 4.1 Gene Models t Xenbase JGI Ensembl UCSC</p>		
Synteny	<a href="#">Metazome</a>		
Expression	tropicalis	laevis a	laevis b
UniGene	Str.55517 [+]	XI.514	XI.7970
Unigene EST Profile	<a href="#">EST Profile</a>	<a href="#">EST Profile</a>	<a href="#">EST Profile</a>
GEO data		<a href="#">GEO</a>	<a href="#">GEO</a>
Images	238 expression image(s) for tropicalis and laevis		
Phenotype	tropicalis	laevis a	laevis b

Opera Postvak IN - Outlo... X. tropicalis scaffold... Xenopus laevis sta... Veenstra- NCMLS t Summary [speci... +

Web www.xenbase.org/gene/showgene.do Search with Google

**Xenbase**

[2.8.1]

Blast Frogs Genes Expression Literature Genome Browsers Anatomy & Development Community Reagents & Protocols Stockcentre FTP

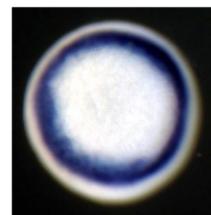
Search Genes For t Search Submit Data Give Feedback

Summary Expression (409) Gene Literature (409) Nucleotides (229) Proteins (32) Interactants (632) Wiki

XB-GENE PAGE-478788

## Gene Symbol: t

Gene Name: T, brachyury homolog



◀ ▶ 1/3

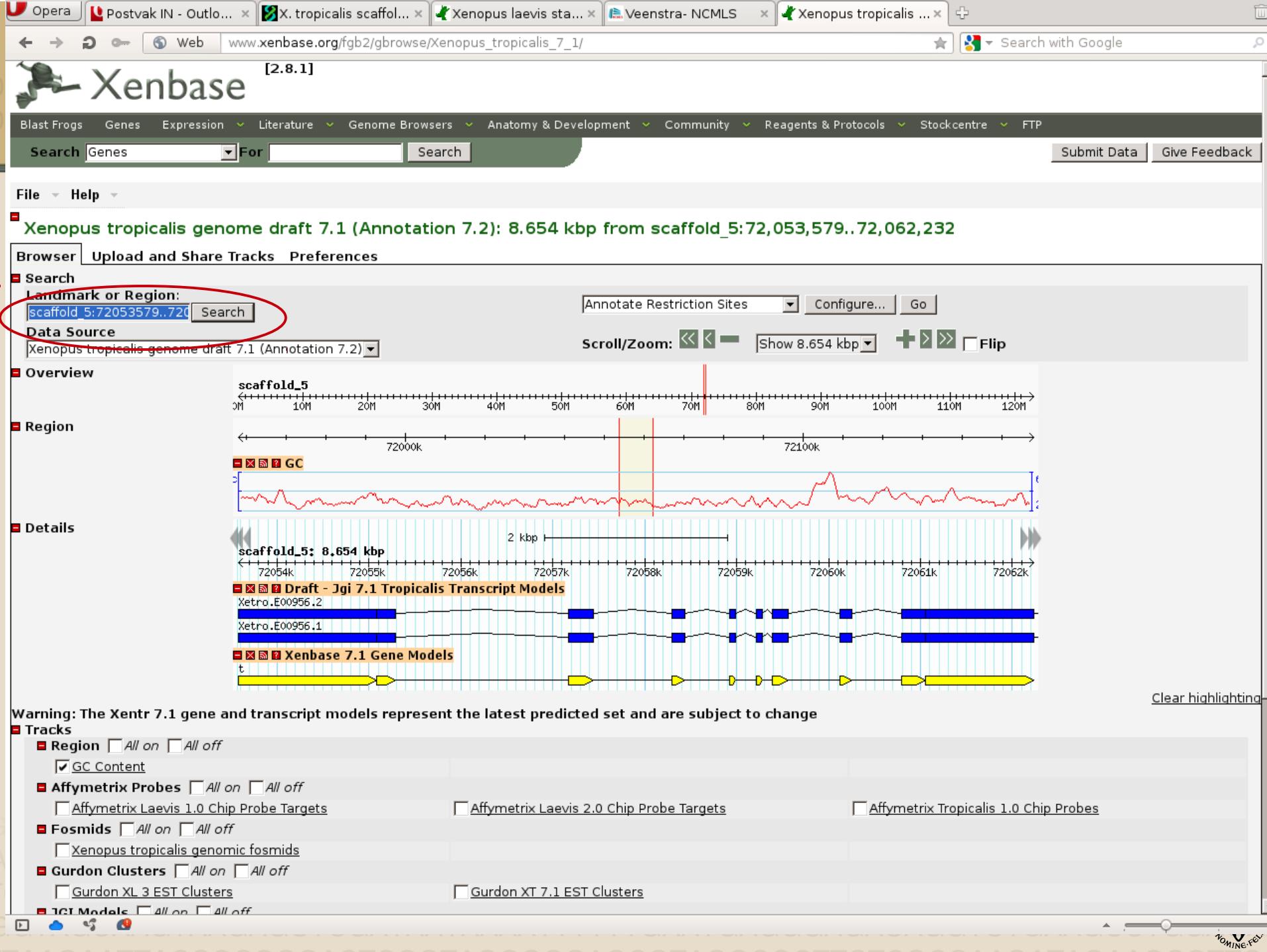
Synonyms: Xbra, X-bra, bra, Xbrachyury, brachyury, ntl [Add Xenopus synonyms](#)

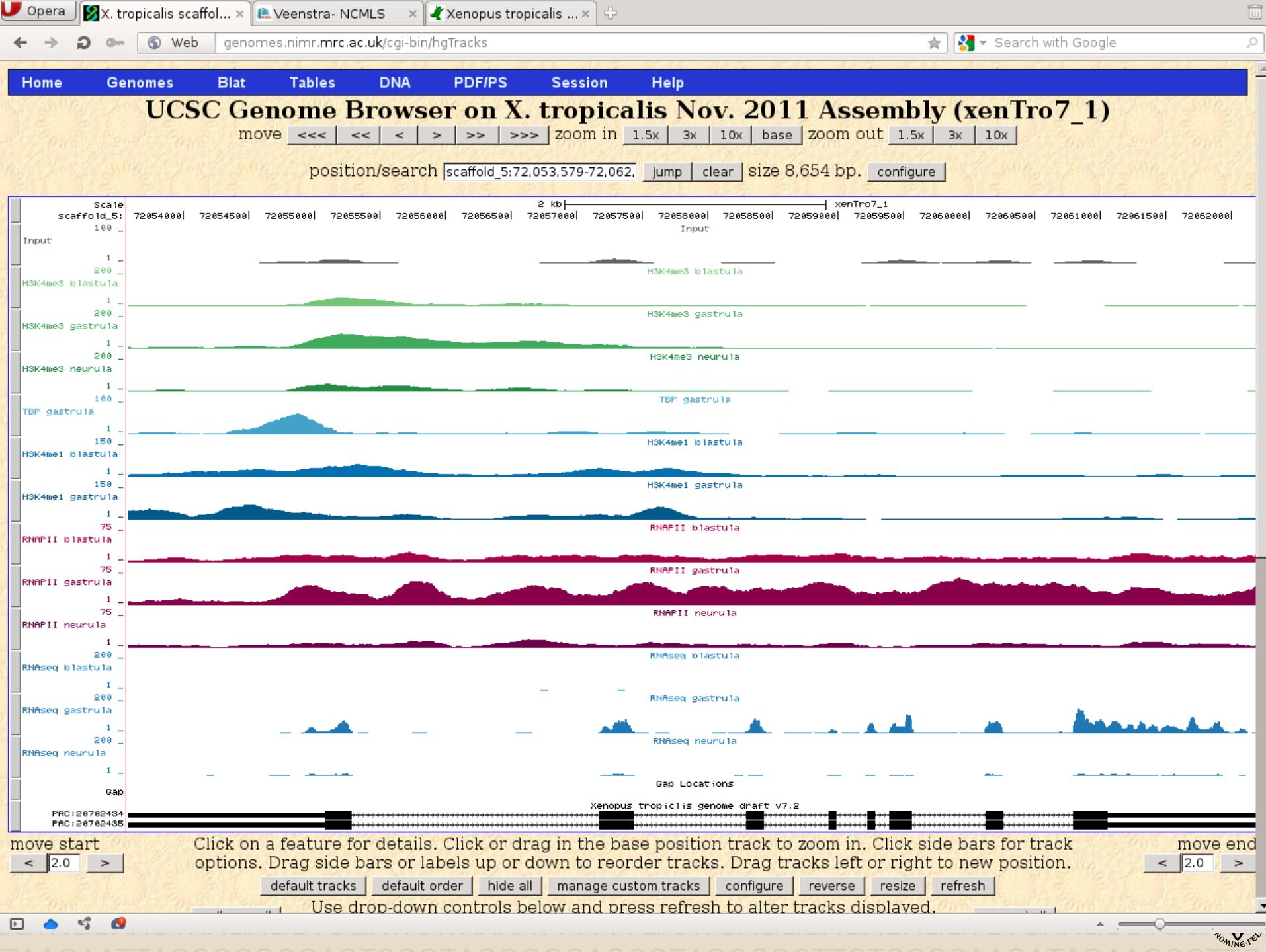
Gene Function: T-box transcription factor

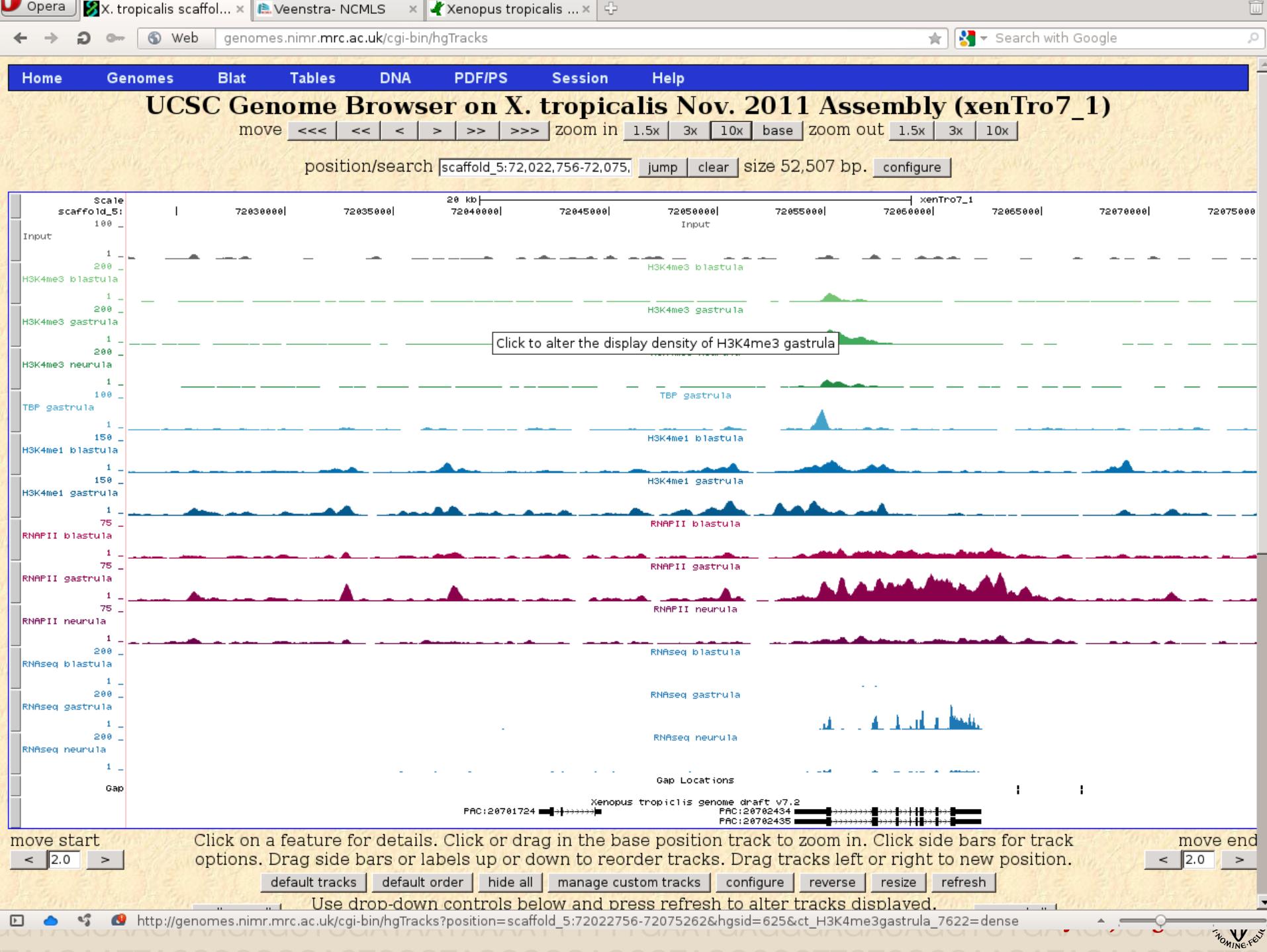
Interactants: [gsc](#)(131), [chrd](#)(92), [nodal](#)(61), [wnt8a](#)(61), [myod1](#)(58), [otx2](#)(45), [bmp4](#)(38) [View All](#)

JGI 7.1 coordinates      JGI 4.1 coordinates

Molecules	XB-GENE-478789 tropicalis	XB-GENE-865212 laevis a	XB-GENE-6255736 laevis b
Gene	<a href="#">JGI XT Genome 7.1</a> <a href="#">JGI XT Genome 4.1</a> <a href="#">Ensembl</a> <a href="#">Entrez Gene</a>	<a href="#">JGI XT Genome 7.1</a> <a href="#">JGI XT Genome 4.1</a> <a href="#">Ensembl</a> <a href="#">Entrez Gene</a>	<a href="#">JGI XT Genome 7.1</a> <a href="#">JGI XT Genome 4.1</a> <a href="#">Ensembl</a> <a href="#">Entrez Gene</a>
mRNA	<a href="#">Refseq</a>	<a href="#">Refseq</a>	<a href="#">Refseq</a>
Protein	<a href="#">RefSeq</a>	<a href="#">RefSeq</a>	<a href="#">RefSeq</a>
Genomic	tropicalis	tropicalis	tropicalis
Genome Browser	 scaffold_5:72053579..72062232 72050k Xenbase 7.1 Gene Models t Xenbase 4.1 Gene Models t Xenbase JGI Ensembl UCSC	 scaffold_432:292557..299806 Xenbase 7.1 Gene Models t Xenbase 4.1 Gene Models t Xenbase JGI Ensembl UCSC	 scaffold_5:72053579..72062232 72050k Xenbase 7.1 Gene Models t Xenbase 4.1 Gene Models t Xenbase JGI Ensembl UCSC
Synteny	<a href="#">Metazome</a>		
Expression	tropicalis	laevis a	laevis b
UniGene	Str.55517 [+]	XI.514	XI.7970
Unigene EST Profile	<a href="#">EST Profile</a>	<a href="#">EST Profile</a>	<a href="#">EST Profile</a>
GEO data		<a href="#">GEO</a>	<a href="#">GEO</a>
Images	238 expression image(s) for tropicalis and laevis		
Phenotype	tropicalis	laevis a	laevis b







# Other resources

- All published sequencing data should be available in raw and processed format
- See NCBI GEO for public datatets

<http://www.ncbi.nlm.nih.gov/geo/>



**NCBI**

**GEO**  
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**Gene Expression Omnibus:** a public functional genomics data repository supporting [MIAME-compliant](#) data submissions. Array- and sequence-based data are accepted. Tools are provided to help users query and download experiments and curated gene expression profiles. [More information »](#)

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**Site contents**

**Public data**

Platforms	10,482
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**Results: 3**

**1: GSE30146 record: HEB and E2A function as SMAD/FOXH1 cofactors [ *Xenopus (Silurana) tropicalis* ]**

**Summary:** (Submitter supplied) Nodal signaling, mediated through SMAD transcription factors, is necessary for pluripotency maintenance and endoderm commitment. We have identified a new motif, termed SMAD Complex Associated (SCA) that is bound by SMAD2/3/4 and FOXH1 in human embryonic stem cells (hESCs) and derived endoderm. We demonstrate that two bHLH proteins - HEB and E2A - bind the SCA motif at regions overlapping SMAD2/3 and FOXH1. Further, we show that HEB and E2A associate with SMAD2/3 and FOXH1, suggesting they form a complex at critical target regions. This association is biologically important, as E2A is critical for mesendoderm specification, gastrulation, and Nodal signal transduction in *Xenopus* tropicalis embryos. Taken together, E2A is a novel Nodal signaling cofactor that associates with SMAD2/3 and FOXH1 and is necessary for mesendoderm differentiation.

**Type:** Genome binding/occupancy profiling by high throughput sequencing

**Supplementary Files:** BED, TXT download...

**Samples:** 1 related Platform

**2: GSE21482 record: Nucleotide composition-linked divergence of vertebrate core promoter architecture [ *Xenopus (Silurana) tropicalis* ]**

**Summary:** (Submitter supplied) Transcription initiation involves the recruitment of basal transcription factors to the core promoter. A variety of core promoter elements exists, however for most of these motifs the distribution across species is unknown. Here we report on the comparison of human and amphibian promoter sequences. We have used oligo-capping in combination with deep sequencing to determine transcription start sites in *Xenopus* tropicalis. To systematically predict regulatory elements we have developed a de novo motif finding pipeline using an ensemble of computational tools. A comprehensive comparison of human and amphibian promoter sequences revealed both similarities and differences in core promoter architecture. Some of the differences stem from a highly divergent nucleotide composition of *Xenopus* and human promoters. more...

**Type:** Genome binding/occupancy profiling by high throughput sequencing; Expression profiling by high throughput sequencing

**Supplementary Files:** BED, TXT download...

**Samples:** 3 related Platform

**3: GSE14025 record: A Hierarchy of H3K4me3 and H3K27me3 Acquisition in Spatial Gene Regulation in Xenopus Embryos [ *Xenopus (Silurana) tropicalis* ]**

**Summary:** (Submitter supplied) Epigenetic mechanisms set apart the active and inactive regions in the genome of multicellular organisms to produce distinct cell fates during embryogenesis. Here, we report on the epigenetic and transcriptome genome-

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```
("xenopus"[MeSH Terms] OR "xenopus laevis"[MeSH Terms] OR ("Xenopus"[Organism] OR "Xenopus"[Organism]) OR Xenopus[All Fields]) AND chip-seq[All Fields]
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# Other pointers

- UCSC Genome Browser Tutorials:  
<http://www.openhelix.com/ucsc>
- Publications
  - Akkers *et al.*, Dev Cell, 2009
  - Bogdanovic *et al.*, Genome Research, 201
  - Van Heeringen *et al.*, Genome Research, 2011
- The epigenome in early development
  - Bogdanovic *et al.*, Genesis, 2012





# NCMLS

# Radboud University, Nijmegen

Robert Akkers  
Ozren Bogdanovic  
Ulrike Jacobi  
Gert Jan Veenstra



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