

# Understanding non-coding DNA... and data sharing

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Dec 6<sup>th</sup> 2017

{

# GenAPpipes

- **RNA-Seq** Spliced alignment, QC, differential analysis, isoform analysis, ...
- **ChIP-Seq** Narrow/Wide peaks, Homer, GoSeq, other annotations, ...
- **RNA-Seq Denovo**, differential analysis, QC, transcript annotations, ...
- **DNA-Seq** Alignment, Realignment, MarkDup, Recalibration, SNV, CNV, SV, ...
- **Pacbio Denovo**, bacteria and genomes up to ~50Mb, annotations (in-progress)
  
- All pipelines are optimised for the hardware and schedulers at the different cluster sites (the configuration is adjustable)
- All pipelines include an HTML report with the references, explanations and details on the sequencing and analysis



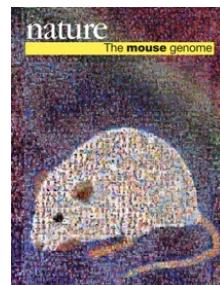
# GenAPpipes links

- Code + slides:
  - [https://bitbucket.org/mugqic/mugqic\\_pipelines](https://bitbucket.org/mugqic/mugqic_pipelines)
- Pipeline tutorial:
  - <http://www.computationalgenomics.ca/tutorials/>
- ChIP-seq simple example:
  - <https://github.com/bourqueg/Classes/blob/master/QLS600.md>

}

# Applications (I)

- *De novo* sequencing
  - From the human genome... To all model organisms... To all relevant organisms (e.g. extreme genomes)... To “all” organisms?



# Applications (II)

- Genome re-sequencing
  - Map genomic structural variations across individuals (to understand genetic disorders and also susceptibility factors)
  - Cancer genome sequencing
  - Agricultural crops



1000 Genomes Project



The Cancer Genome Atlas

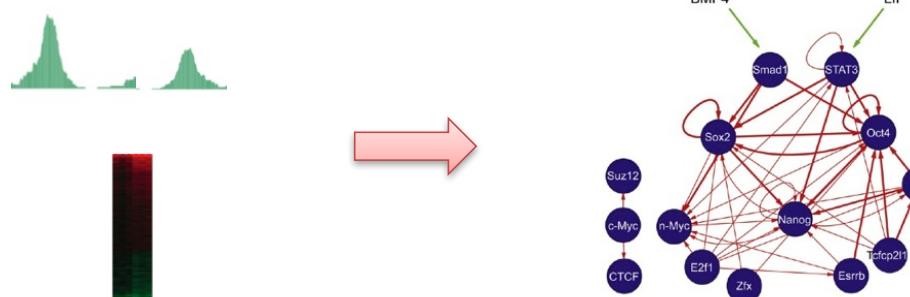


## Complete Resequencing of 40 Genomes Reveals Domestication Events and Genes in Silkworm (*Bombyx*)

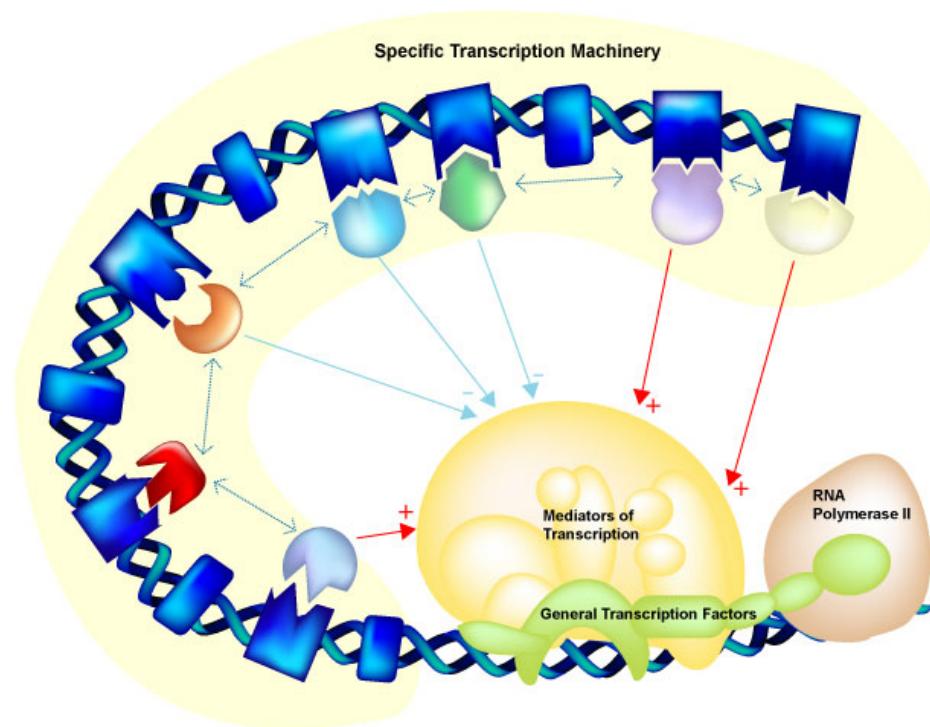
Qingyou Xia,<sup>1,2\*</sup> Yiran Guo,<sup>3\*</sup> Ze Zhang,<sup>1,2\*</sup> Dong Li,<sup>1,2,4</sup> Zhaoling Xuan,<sup>3\*</sup> Zhu Li,<sup>3\*</sup> Fangyin Dai,<sup>4</sup> Yingrui Li,<sup>3</sup> Daojun Cheng,<sup>3</sup> Ruiqiang Li,<sup>3</sup> Tingcai Cheng,<sup>3,5</sup> Tan Jiang,<sup>3</sup> Celine Beiques,<sup>3,7</sup> Xun Xu,<sup>2</sup> Chun Liu,<sup>2</sup> Xinguo Zha,<sup>3</sup> Wei Fan,<sup>2</sup> Ying Lin,<sup>3</sup> Yihong Shen,<sup>1</sup> Lan Jiang,<sup>3</sup> Jef Huygen,<sup>6</sup> Ines Heidmann,<sup>6</sup> Ming Tang,<sup>3</sup> Ping Xiao,<sup>3</sup> Jun Xu,<sup>3</sup> Cheng Yu,<sup>3</sup> Guoqi Wang,<sup>3</sup> Jun Li,<sup>3</sup> Shiqing Liu,<sup>3</sup> Ningyu Li,<sup>3</sup> Yan Zhou,<sup>3</sup> Haizhu Liu,<sup>3</sup> Jing Zhao,<sup>3</sup> Chen Ye,<sup>2</sup> Zhouhe Du,<sup>3</sup> Guojing Pan,<sup>1</sup> Aichun Zhao,<sup>3</sup> Huijing Shi,<sup>3,7</sup> Wei Zeng,<sup>3</sup> Ping Wu,<sup>3</sup> Chunfeng Li,<sup>3</sup> Minhuai Pan,<sup>3</sup> Jingling Lu,<sup>3</sup> Xuyang Yin,<sup>3</sup> Dawei Li,<sup>3</sup> Juan Wang,<sup>3</sup> Huihong Zheng,<sup>3</sup> Wen Wang,<sup>3</sup> Xueying Zhang,<sup>3</sup> Songgang Li,<sup>3</sup> Huanning Yang,<sup>3</sup> Cheng Lu,<sup>3</sup> Rasmus Nielsen,<sup>4,5</sup> Zeyang Zhou,<sup>3,6</sup> Jian Wang,<sup>3</sup> Zhonghua Xiang,<sup>3,1†</sup> Jun Wang<sup>3,4,‡</sup>

## Applications (III)

- Quantitative biology of complex systems
  - New high-throughput technologies in functional genomics: ChIP-Seq, RNA-Seq, ChIA-PET, RIP-Seq, ...
  - From single-gene measurements, to thousands of probes on arrays, to profiles covering all 3B bases of the genome

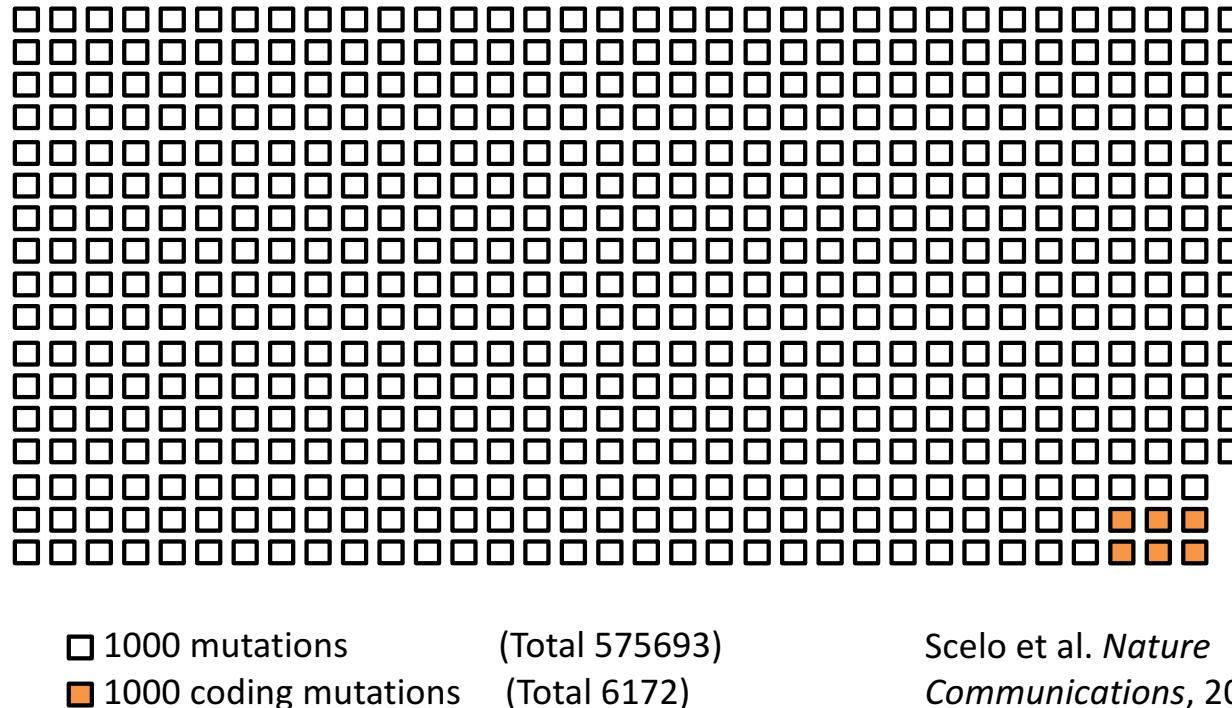


# Transcription regulation



[www.genwaybio.com](http://www.genwaybio.com)

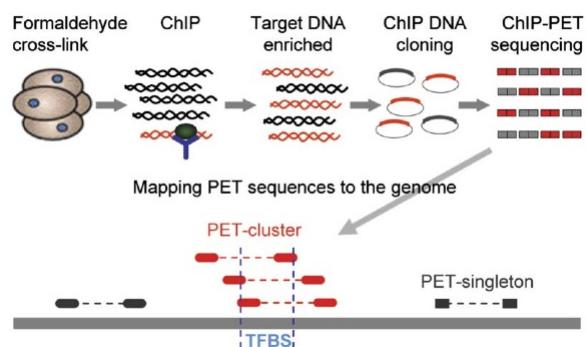
# Somatic mutations in 100 kidney tumors



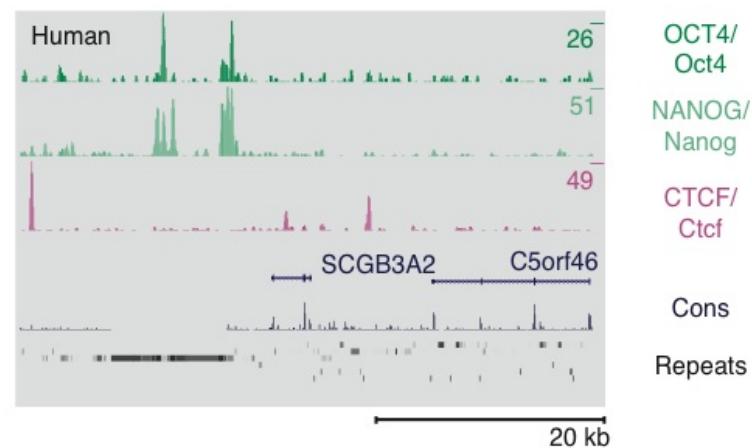




# ChIP-Seq : Genome-wide binding profiles

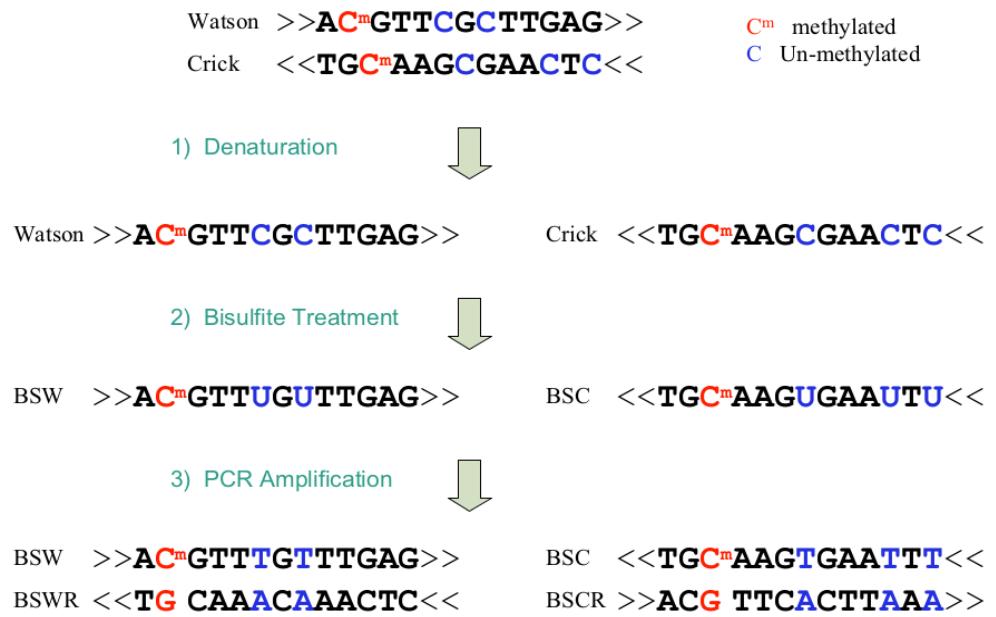


Wei et al., *Cell*, 2006



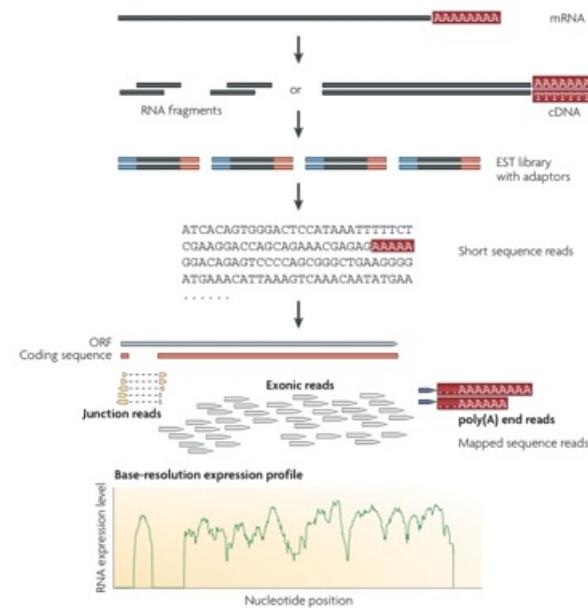
Kunarso et al., *Nat. Genet.*, 2010

# Methylation sequencing



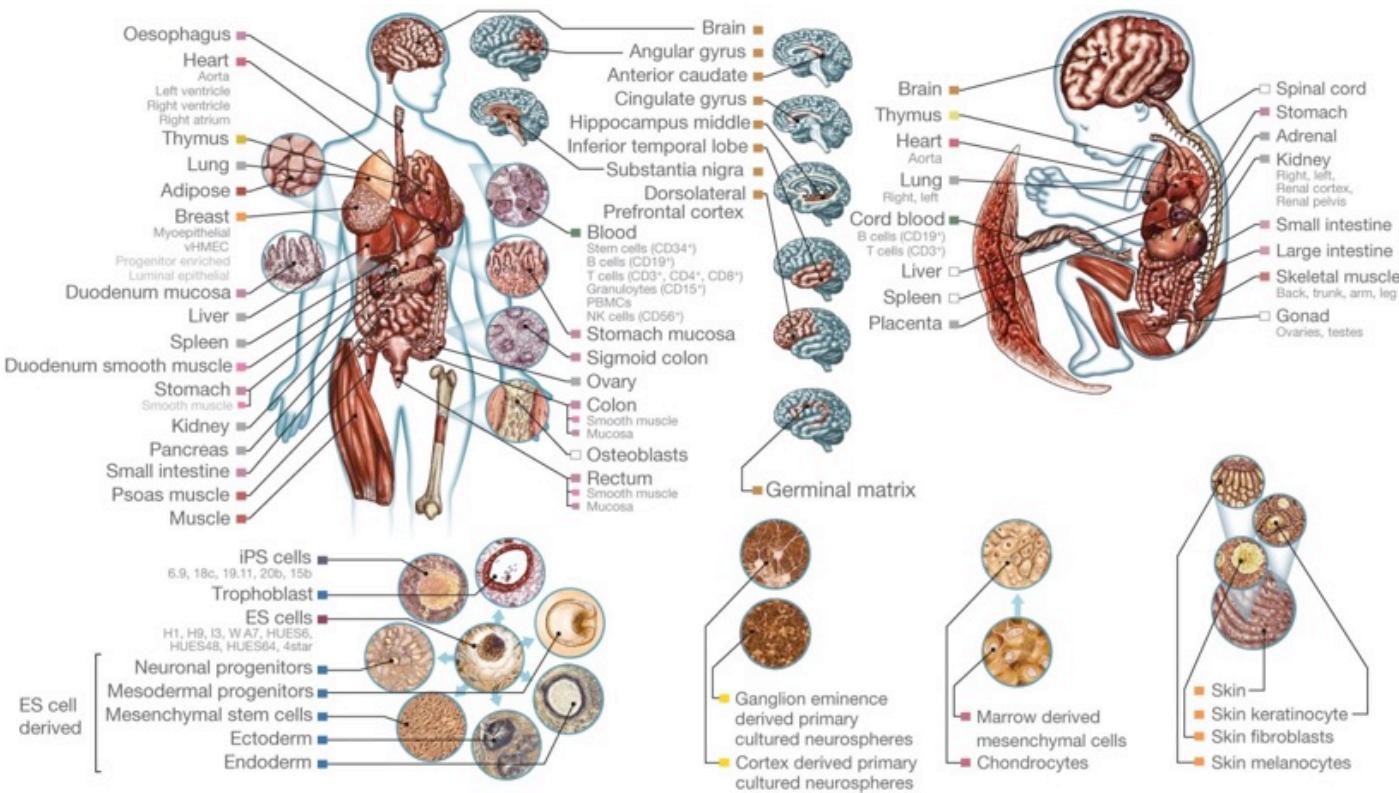
Xi and Li, *BMC Bioinformatics*, 2009

# RNA-Seq: digital expression and much more



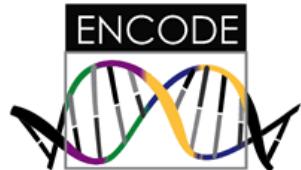
Wang et al. *Nat. Rev. Genet.*, 2009

# One genome... Many epigenomes



Roadmap Epigenomics Consortium *et al.* *Nature* **518**, 317-330 (2015) doi:10.1038/nature14248

# Epigenome mapping consortiums



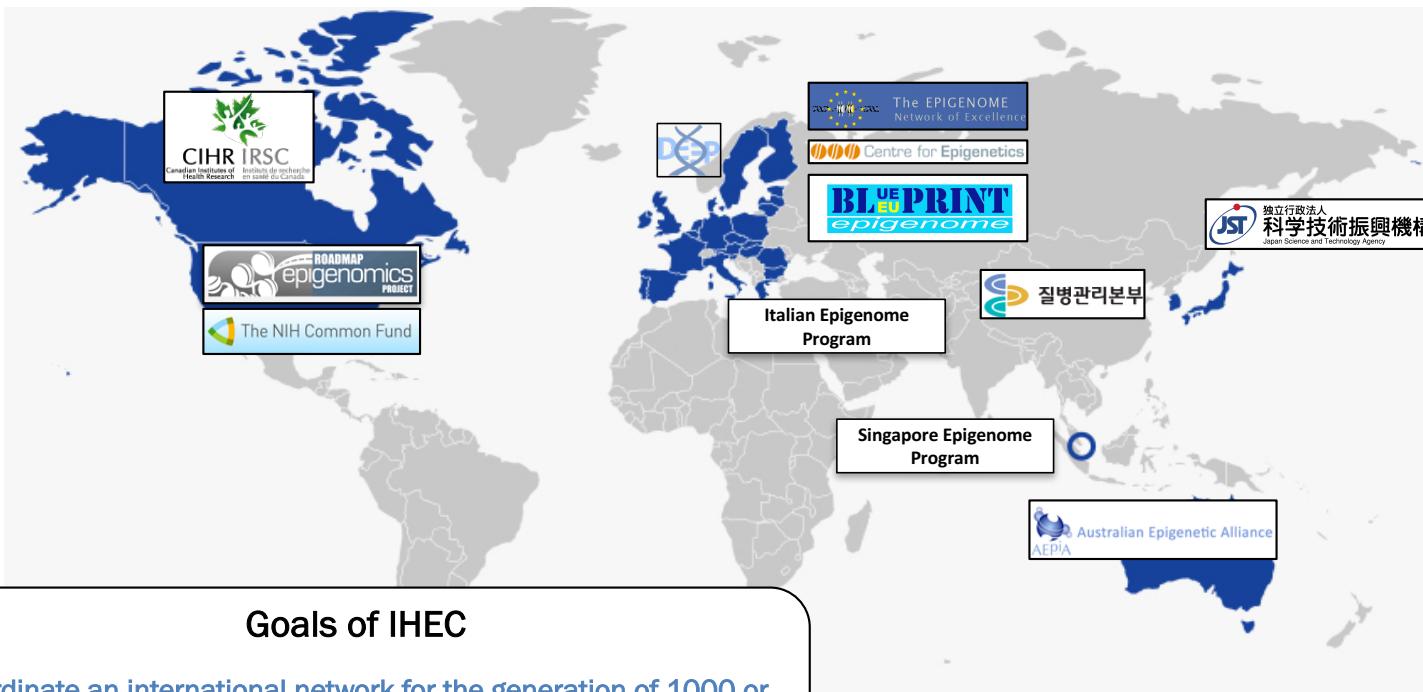
Technology development and  
characterization of functional elements  
mostly in cell lines



Reference epigenome mapping mostly in  
stem cells and primary ex vivo tissues



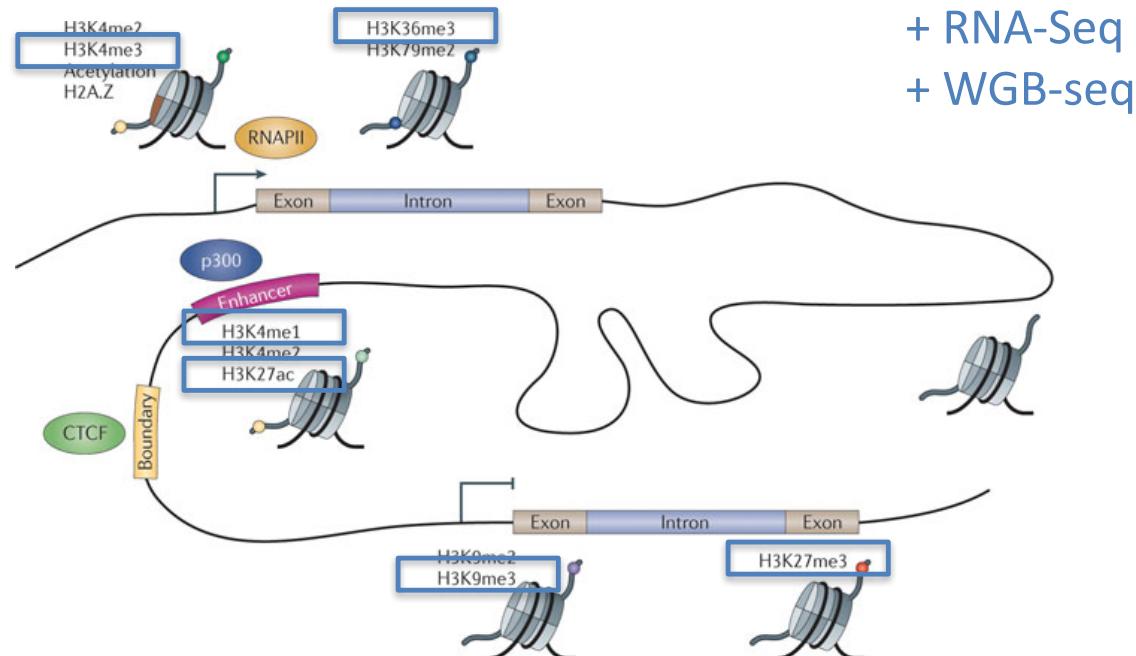
Most comprehensive reference  
epigenome mapping of **normal and  
disease tissues with replicates**



### Goals of IHEC

Coordinate an international network for the generation of 1000 or more reference epigenomes for a broad spectrum of human cell types and a wide range of developmental stages, laying the foundation to study the epigenetic mechanisms of human diseases.

# Reference epigenome



Nature Reviews | Genetics

Zhou et al., Nat Rev Genet, 2011

# CEEHRC Platform Centres (2012 - 2022): >200 Reference Epigenomes Planned



**CEEHRC PLATFORM**

Canadian Epigenetics, Environment and Health Research Consortium Platform

**Centre for Epigenomic Mapping Technologies (CEMT) - Martin Hirst, Marco Mara and Steven Jones**

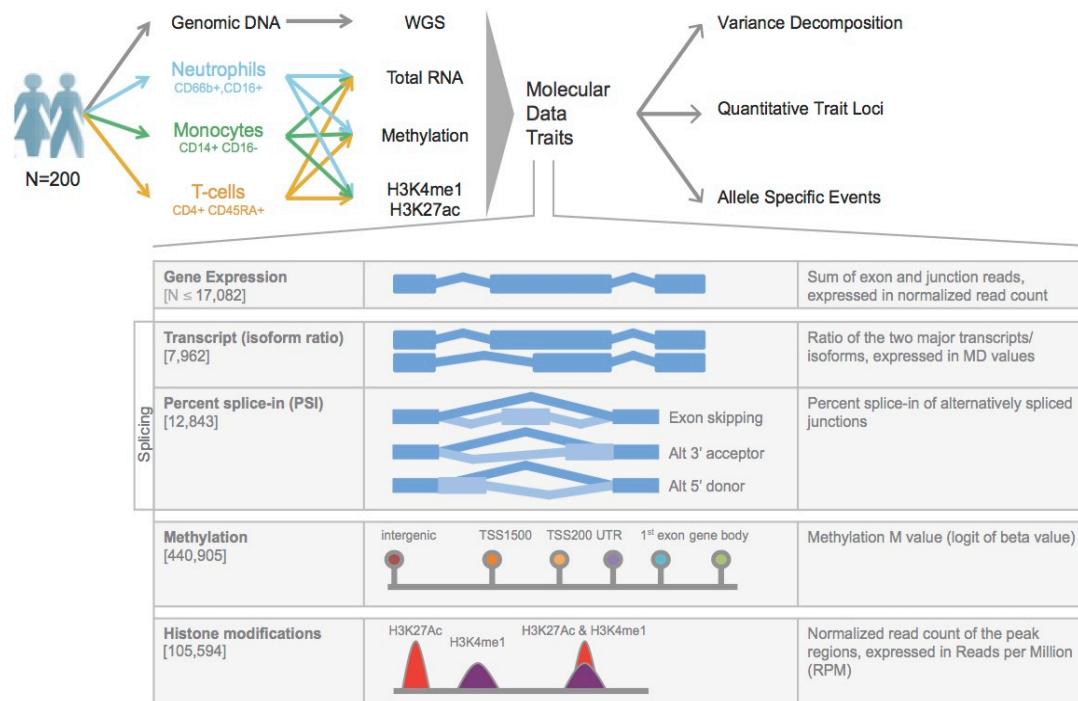
- Established Cell Groups, Biospecimen Core, Library Core, Sequencing Core and Informatics Core – with clear process flows
- Focusing on a number of cancer and stem cell types, including peripheral and lymphoid cells, hematopoietic cells, iPSCs, as well as thyroid cells and mammary, endometrial, fallopian and colonic epithelium.



**Guillaume Bourque, Tomi Pastinen, Luis Barreiro and Mark Lathrop**

- Focus on mapping population variation in human epigenomes
- Emphasis on longitudinal studies, population sampling in selected tissues, comparative epigenomic maps in other species, disease focus - especially autoimmune, inflammatory and metabolic

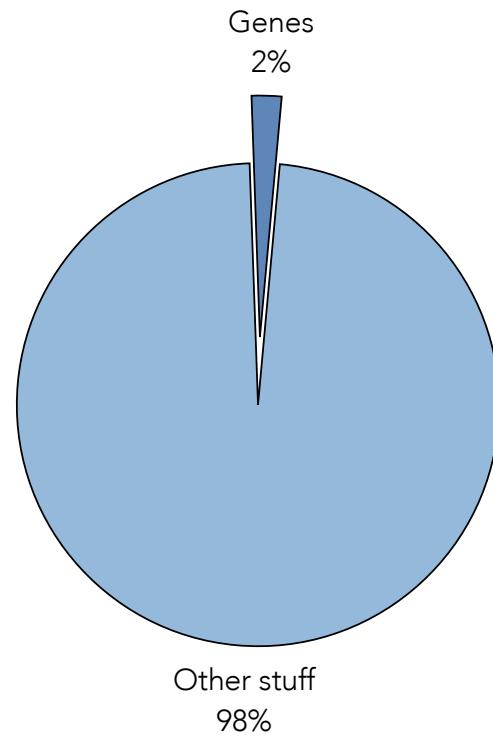
~40 IHEC papers (mid-Nov 2016)



Chen ... Pastinen, Sorenzo. *Cell*, 2016

Understanding non-coding DNA...

# Human Genome



# The Encyclopedia of DNA Elements (ENCODE)

Production and analysis of 1,640 data sets from 147 different cell types

## ARTICLE

[doi:10.1038/nature11247](https://doi.org/10.1038/nature11247)

# An integrated encyclopedia of DNA elements in the human genome

The ENCODE Project Consortium\*

The human genome encodes the blueprint of life, but the function of the vast majority of its nearly three billion bases is unknown. The Encyclopedia of DNA Elements (ENCODE) project has systematically mapped regions of transcription, transcription factor association, chromatin structure and histone modification. These data enabled us to assign biochemical functions for 80% of the genome, in particular outside of the well-studied protein-coding regions. Many discovered candidate regulatory elements are physically associated with one another and with expressed genes, providing new insights into the mechanisms of gene regulation. The newly identified elements also show a statistical correspondence to sequence variants linked to human disease, and can thereby guide interpretation of this variation. Overall, the project provides new insights into the organization and regulation of our genes and genome, and is an expansive resource of functional annotations for biomedical research.

September 2012

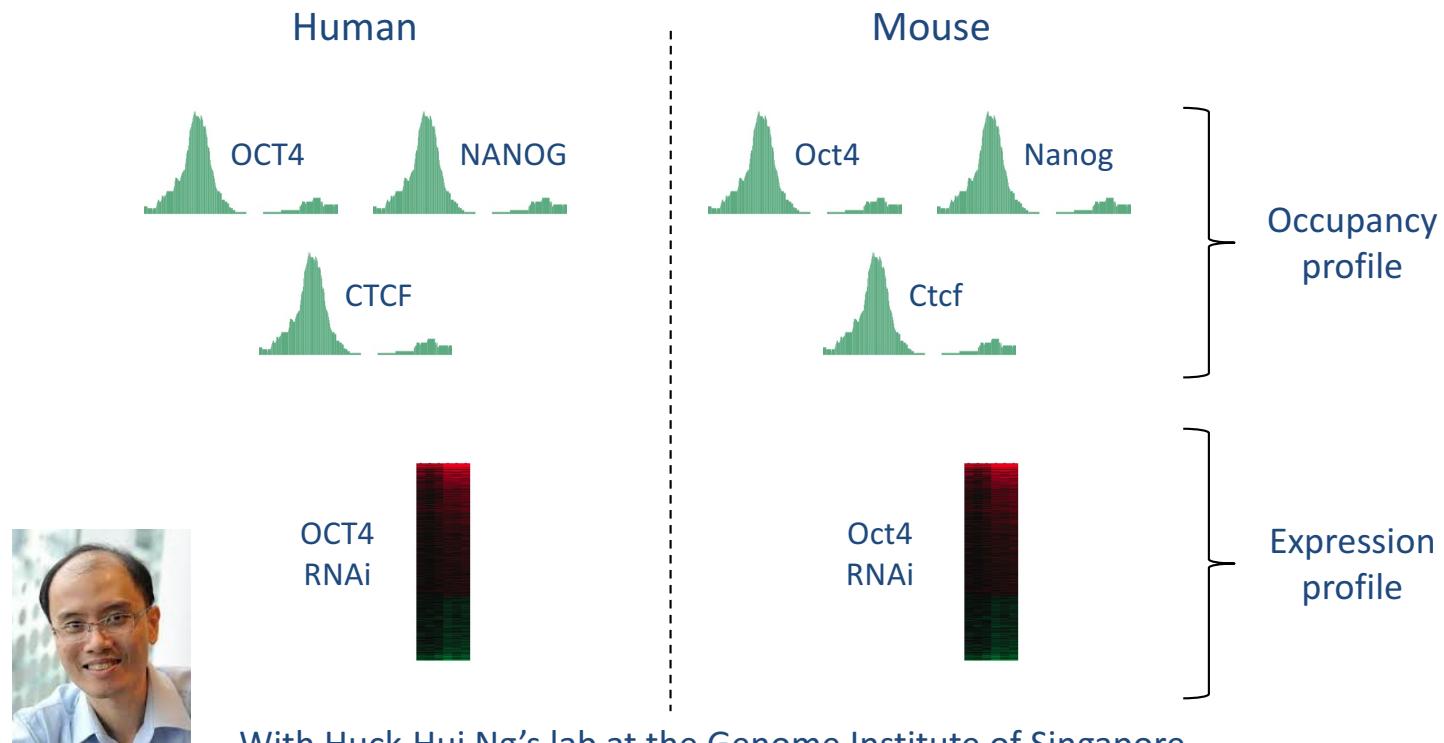
## Main ENCODE paper

- The vast majority (80.4%) of the human genome participates in at least one biochemical RNA- and/or chromatin-associated event:
  - 62% of the genome is expressed in at least one RNA assay,
  - 56% is observed in histone assays,
  - 15% corresponds to open chromatin and
  - 8% is bound by at least one TF.
- BUT... Only 3-8% of the genome show evidence of purifying selection in mammals.

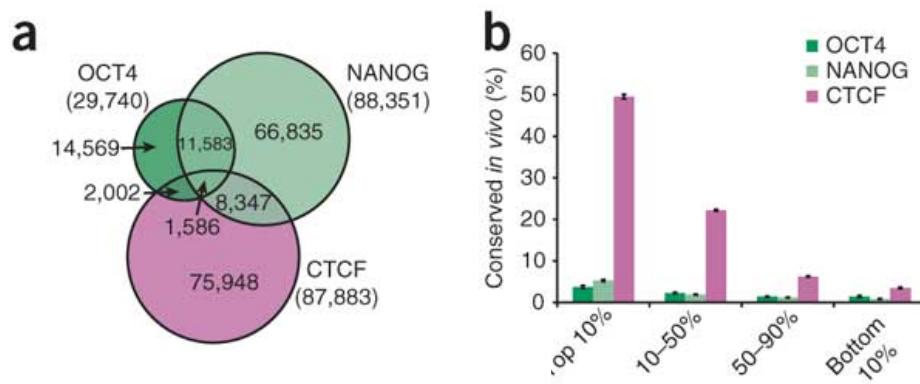
# Main ENCODE paper

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  - 56% is observed in histone assays,
  - 15% corresponds to open chromatin and
  - 8% is bound by at least one TF.
- BUT... Only 3-8% of the genome show evidence of purifying selection in mammals.
- What fraction is actually functional?
- What is the source of all of this biochemical activity?

## Human versus mouse ES cells



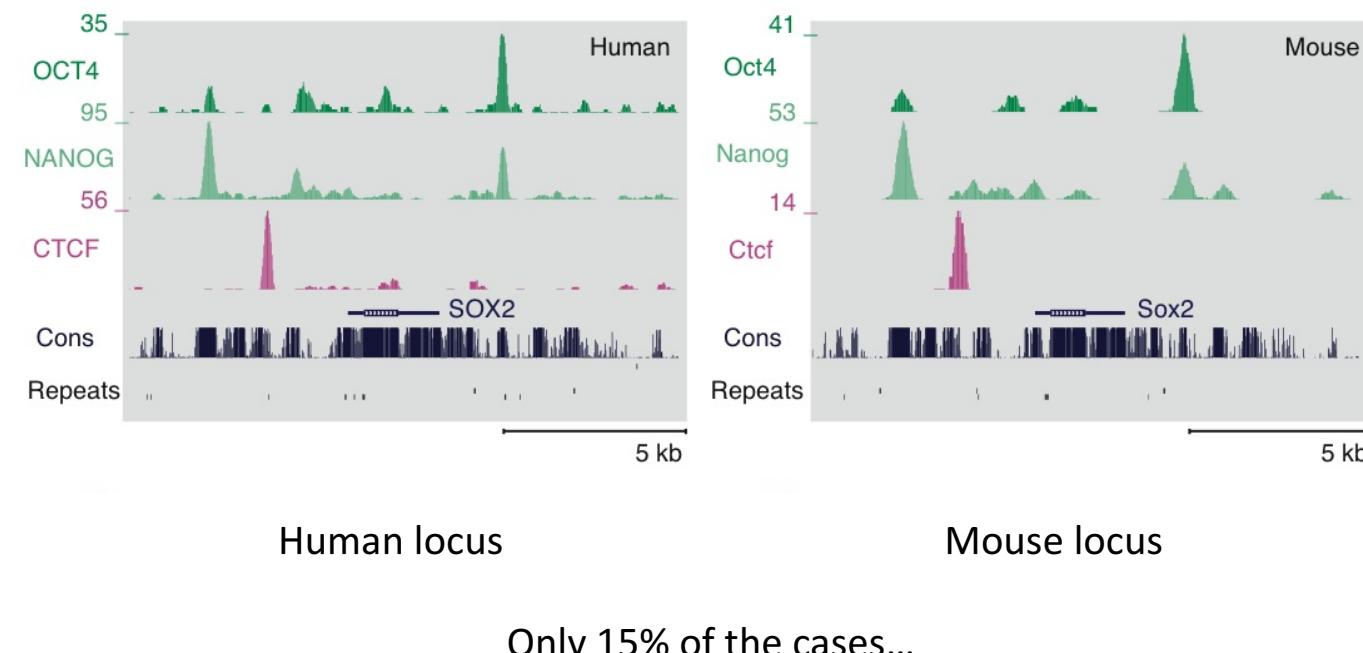
# Limited conservation of OCT4 and NANOG binding sites between human and mouse



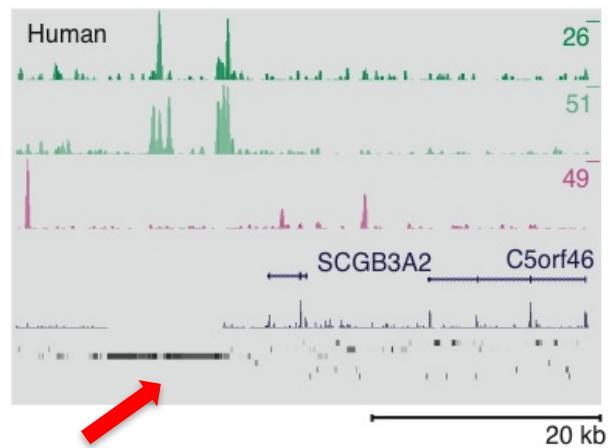
Kunarso et al. *Nat Genet*, 2010



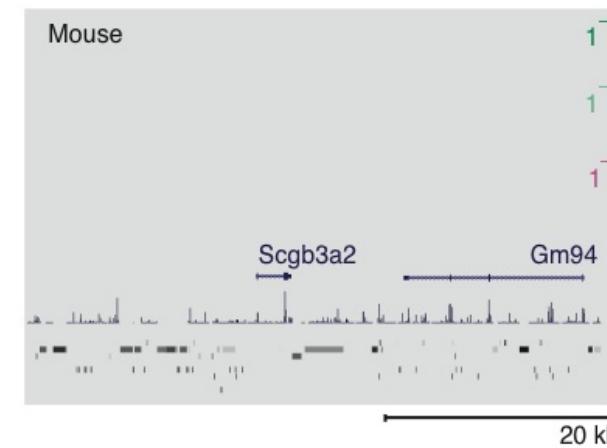
## SOX2 has a perfectly conserved occupancy profile



## Human-specific binding sites embedded in TEs

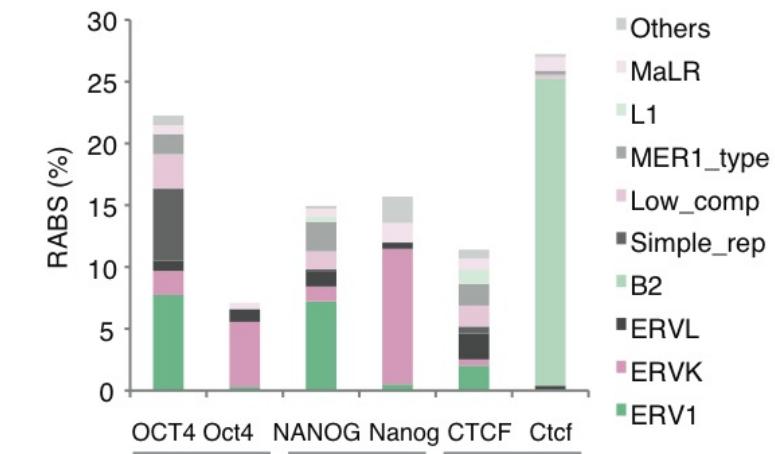
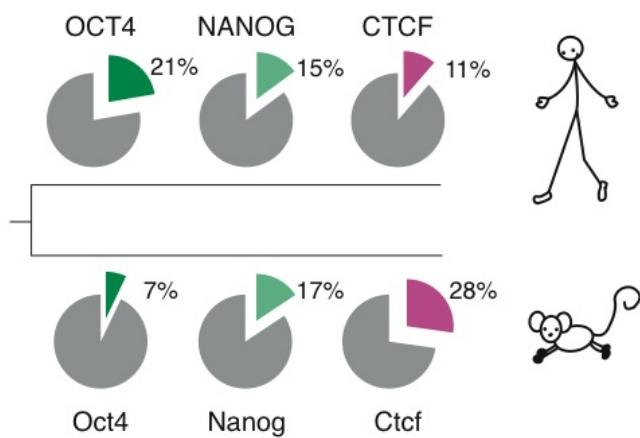


Human locus



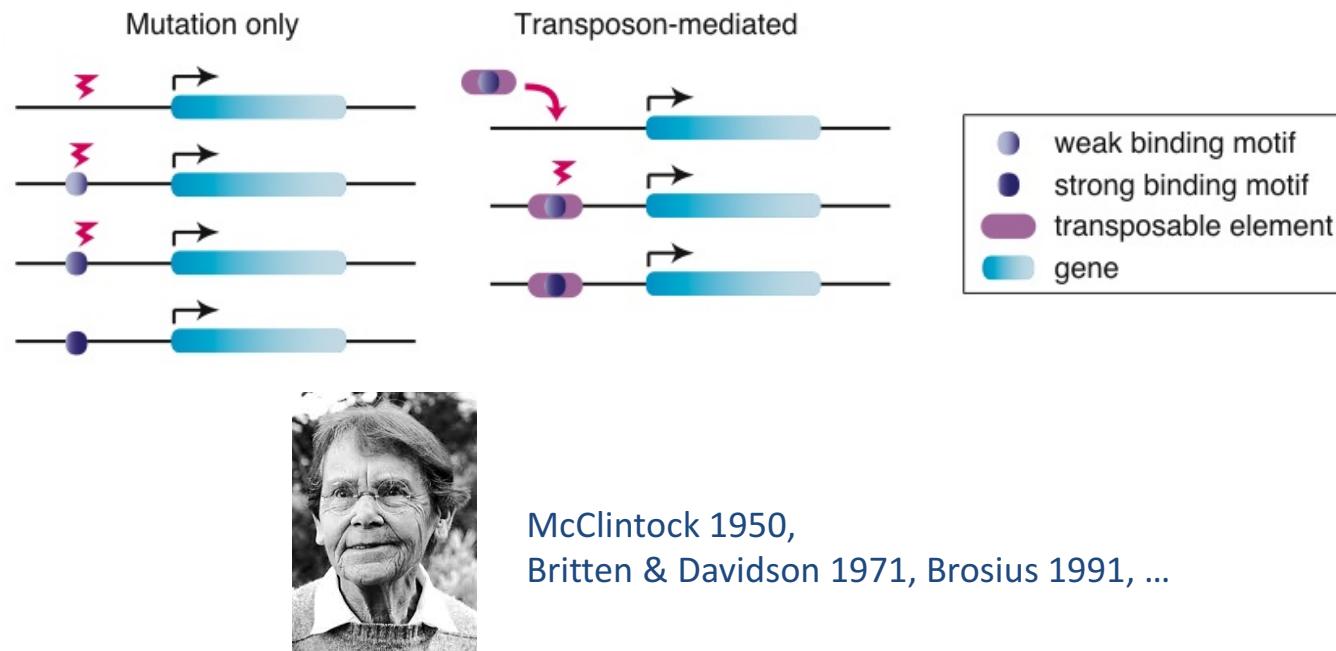
Mouse locus

## TE-associated binding sites in ES cells

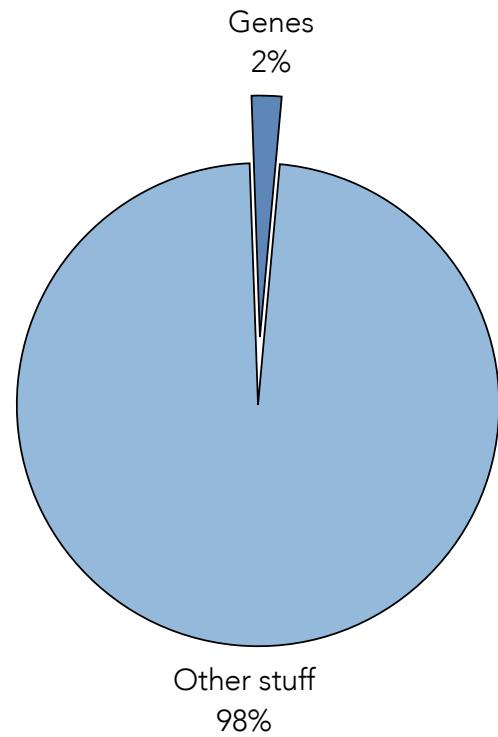


Different families of repeats have contributed a significant fraction of binding sites in both species.

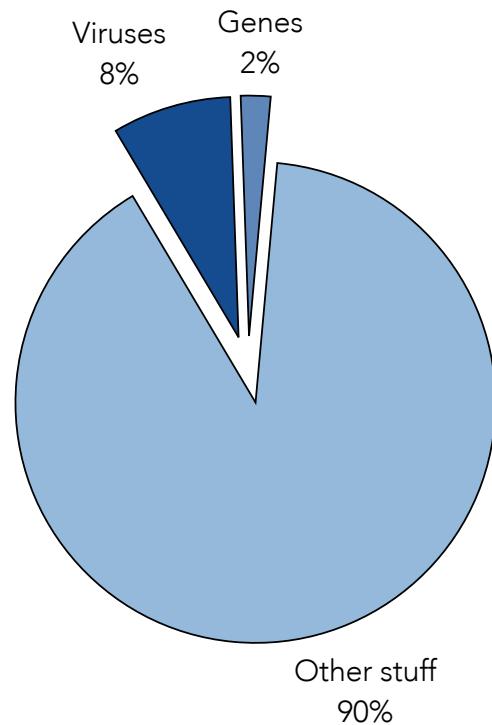
## Two models for regulatory site acquisition



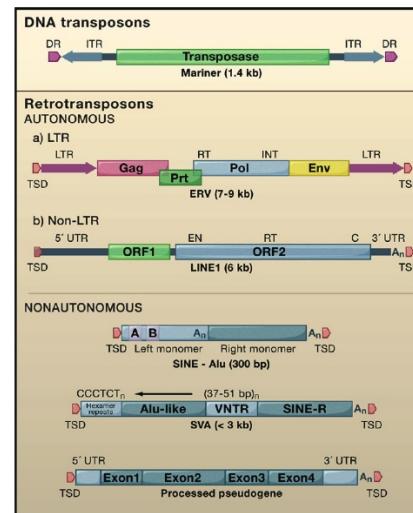
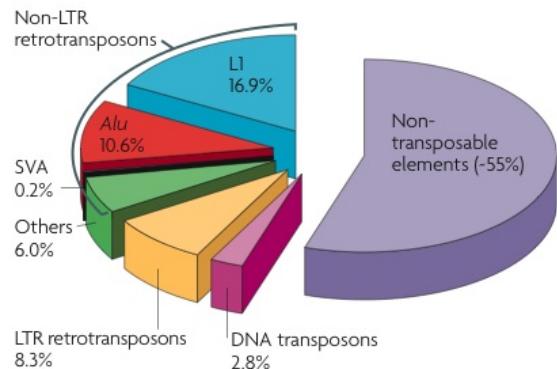
# Human Genome



# Human Genome



# TEs in the human genome



Cordeaux and Batzer,  
*Nat Rev Genet*, 2009

Goodier and Kazazian,  
*Cell* 2008

About 25% of the human genome consists of lineage-specific repeats.  
For the mouse genome it's 30%.

# Outline

- To what extent have transposable elements (TEs) contributed to regulatory regions in the human genome?
- Have TEs contributed to regions that are transcribed in the human genome?
- Is there evidence for functional TE regulatory innovation in the human genome?

# ENCODE Dnase I hypersensitive sites (DHS)

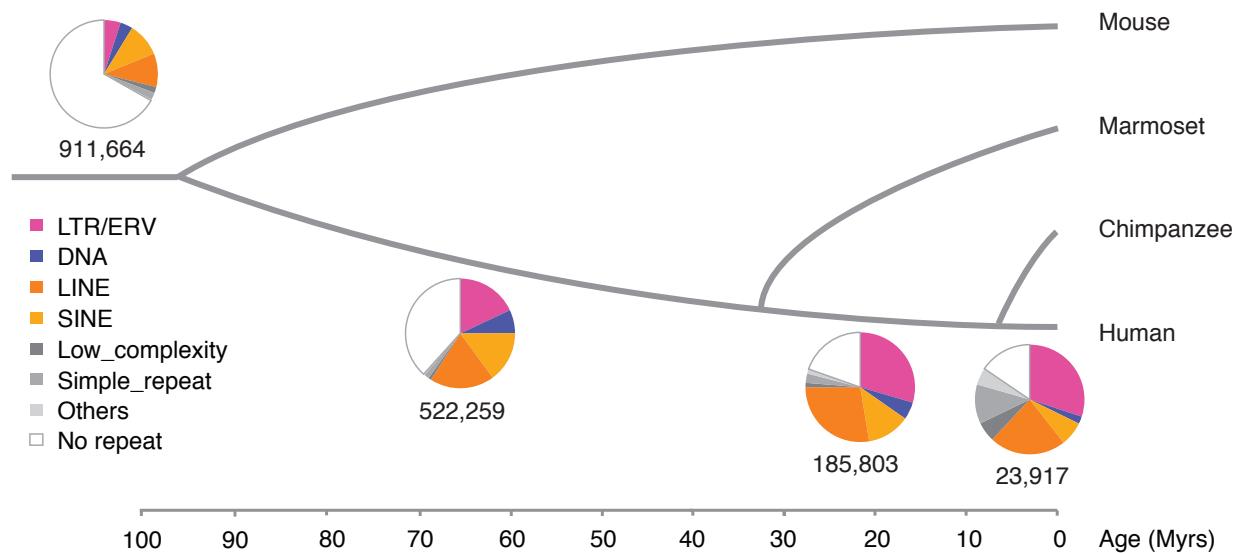
- 103 datasets from UW and Duke
- QC filtering (low quality, GC bias, duplicate rates, etc.)
- 75 datasets, 41 cell types, 9 “tissues”
- Total of 1,643,643 distinct regions of open chromatin

Tissue	Name
Fibroblast	Normal, Normal_Park., ProgFib, Neonatal, Fetal_lung (AG04450), Toe (AG09309), Gum (AG09319), Gingival (HGF), Abdominal (AG10803), Lung (NHLF), Skin (BJ-T), Cardiac (HCF)
Muscle	Myoblast (HSMM), Myotube (HSMMtube), Myocytes (HCM), Skeletal (SKMC), Aortic_smooth (AoSMC)
Epithelial	Small_air (SAEC), Esophageal (HEEpiC), Choroid_plex (HCPepiC), Retinal (HRPEpiC), Ciliary (HNPCepiC), Renal_cortical (HRCEpiC), Renal (HRE), Prostate (LHSR), Amniotic (HAEpiC)
B-Lymphocyte	GM12891, GM19238, GM19239, GM19240, GM12865
Lymphoblastoid	GM18507, GM12878
Others	Myometrial, PanIslets, Melanocytes, Epidermal (NHEK), Endothelial (HUVEC)
hESC	H1, H7, H9
Solid_tumor	HepG2, HeLa-S3, PANC-1, MCF-7, Medulloblastoma, Neuroblastoma
Leukemia	CMK, HL-60, NB4, K562, Jurkat



PE Jacques

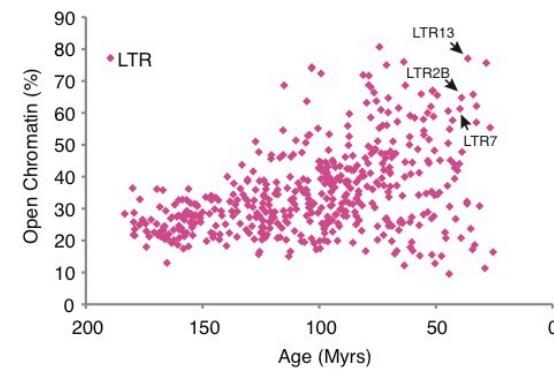
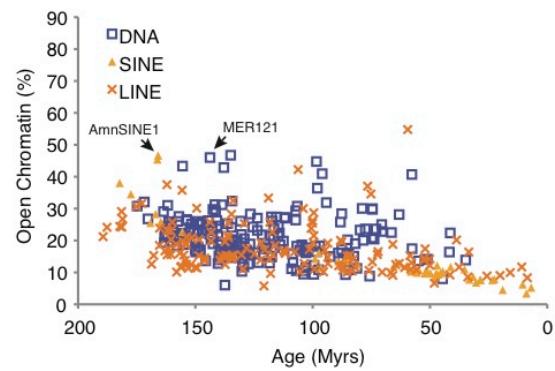
# A large fraction of human DHS are in TEs



Jacques et al. *PLoS Genetics* 2013

44% of all human DHS and 63% of primate-specific DHS

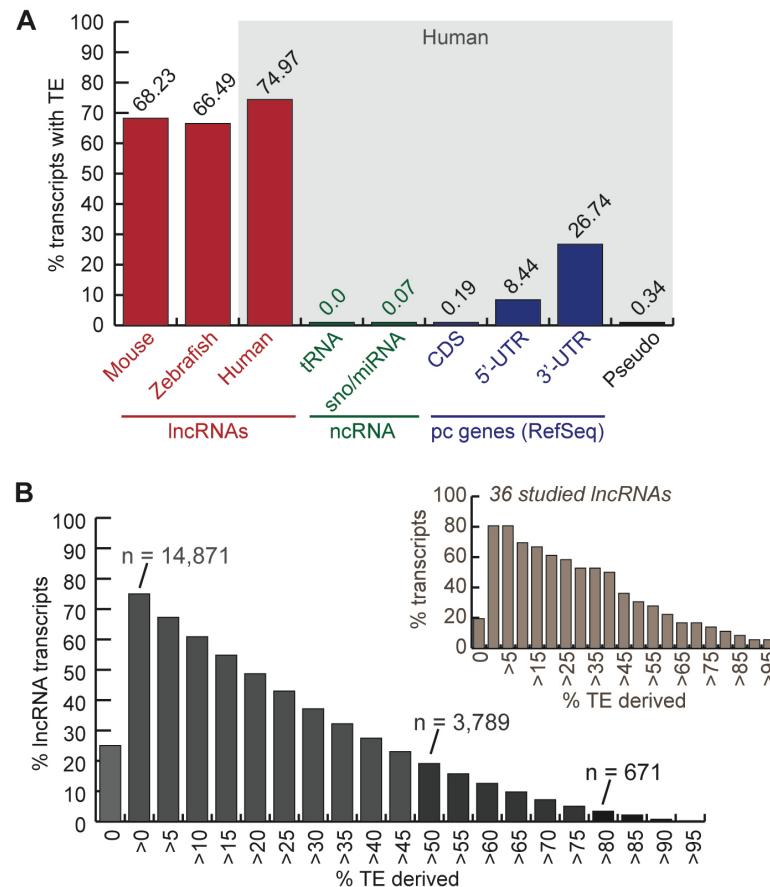
## Many LTR/ERV subfamilies are highly accessible



# Outline

- To what extent have transposable elements (TEs) contributed to regulatory regions in the human genome?  
*Significantly! 44% of all human DHS and 63% of primate-specific DHS are derived from TEs*
- Have TEs contributed to regions that are transcribed in the human genome?
- Is there evidence for functional TE regulatory innovation in the human genome?

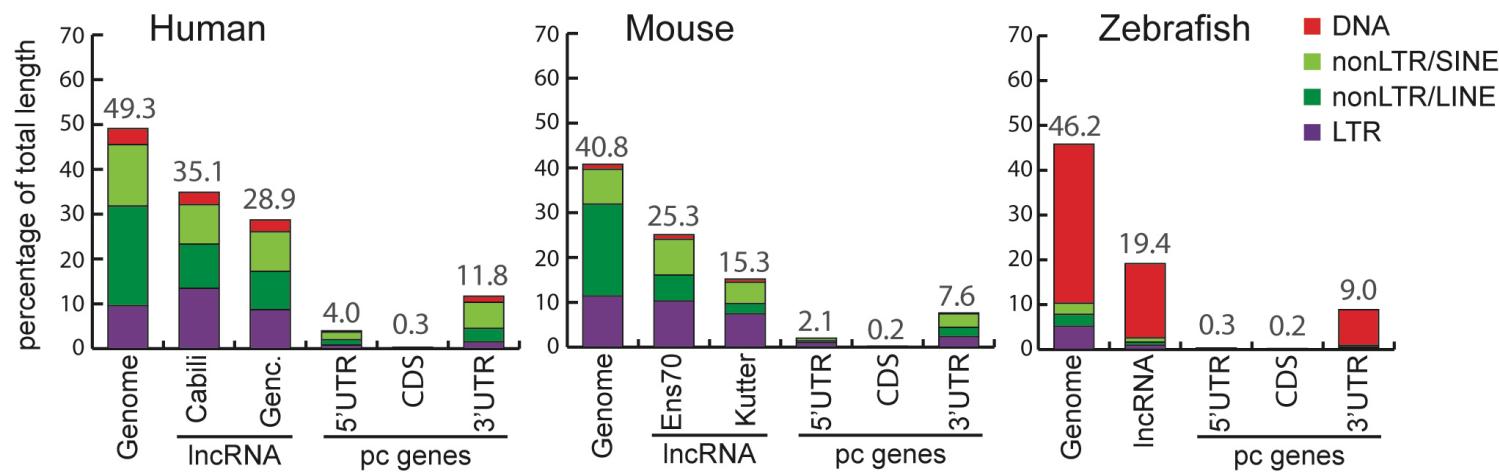
# TEs and the origin of lncRNAs

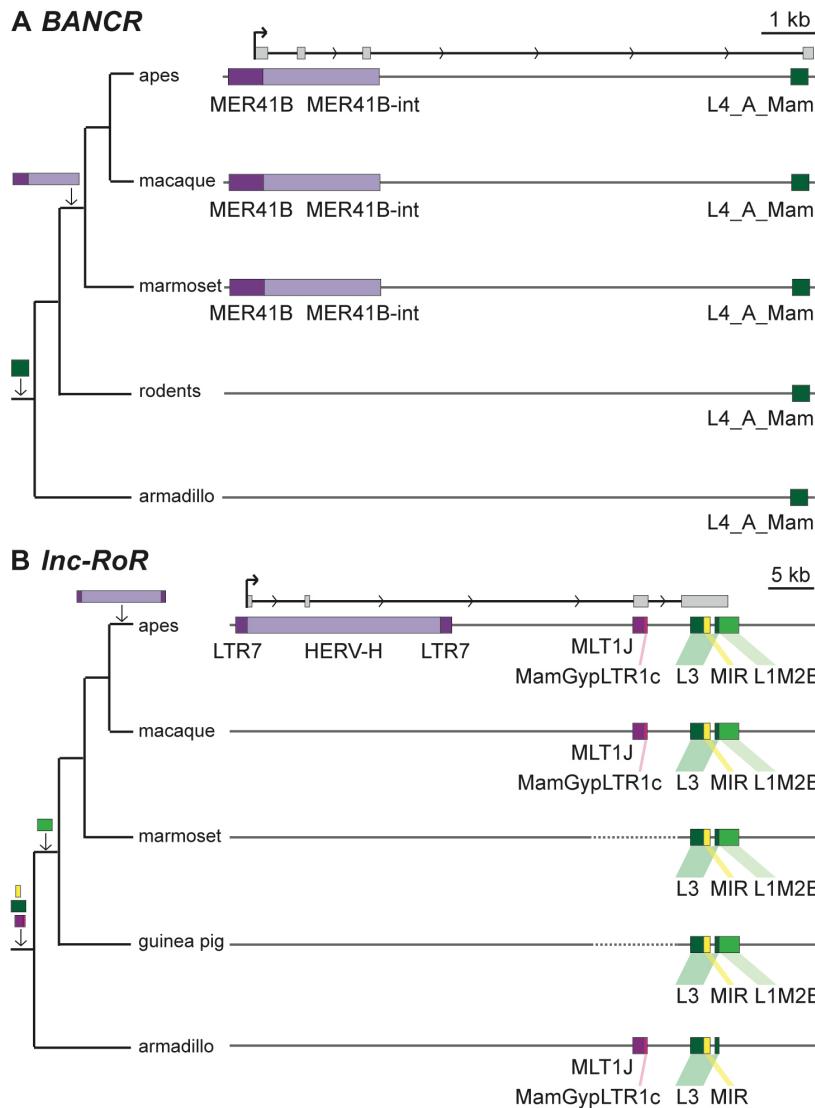


Kapusta et al. *PLoS Genet*, 2013

Collaboration with  
Feschotte's lab

# TEs and the origin of lncRNAs



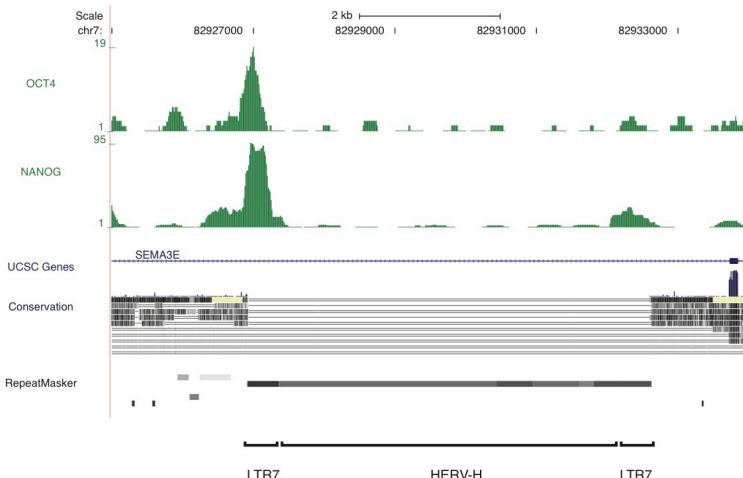


Actually,  
lncRNAs are  
frequently  
initiated in  
TEs

# Outline

- To what extent have transposable elements (TEs) contributed to regulatory regions in the human genome?  
*Significantly! 44% of all human DHS and 63% of primate-specific DHS are derived from TEs*
- Have TEs contributed to regions that are transcribed in the human genome?  
*YES! TEs occur in 2/3 of mature lncRNA transcripts and account for 30% of total lncRNA sequence*
- Is there evidence for functional TE regulatory innovation in the human genome?

# Expression of HERVH in hESCs



	Chromo	Start	End	Repeat family	hESCs reads	hESCs differ. reads	OCT4 binding
1	chr3	54645885	54648817	HERV-H	1606	2	yes
2	chr7	82927372	82930459	HERV-H	989	0	yes
3	chr23	113607832	113611015	HERV-H	640	1	yes
4	chr20	38331559	38334507	HERV-H	600	1	yes
5	chr13	55052694	55055771	HERV-H	486	0	yes
6	chr6	114852520	114855701	HERV-H	451	0	yes
7	chr4	180325471	180328265	HERV-H	446	0	yes
8	chr16	63823548	63826295	HERV-H	412	0	yes
9	chr3	113621411	113624448	HERV-H	330	10	no
10	chr20	38334639	38335103	HERV-H	320	1	yes
11	chr1	221266700	221269664	HERV-H	313	6	yes
12	chr13	50642453	50645421	HERV-H	304	0	yes
13	chr8	115363553	115366935	HERV-H	260	2	yes
14	chr20	38335105	38335769	HERV-H	257	0	yes
15	chr5	120063367	120066552	HERV-H	202	5	yes
16	chr23	91244228	91247089	HERV-H	189	10	no
17	chr1	221265402	221266059	HERV-H	174	0	yes
18	chr4	89062430	89065458	HERV-H	168	45	yes
19	chr24	5869603	5872627	HERV-H	162	0	no
20	chr19	59026922	59030071	HERV-H	152	7	no

# Unique profile of HERVH in stem cells

Kelley and Rinn *Genome Biology* 2012, **13**:R107  
<http://genomebiology.com/2012/13/11/R107>



RESEARCH

Open Access

Transposable elements reveal a stem cell-specific class of long noncoding RNAs

David Kelley<sup>1,2,3</sup> and John Rinn<sup>1,2,3\*</sup>

Kelley and Rinn *Genome Biology* 2012

Santoni et al. *Retrovirology* 2012, **9**:111  
<http://www.retrovirology.com/content/9/1/111>



RESEARCH

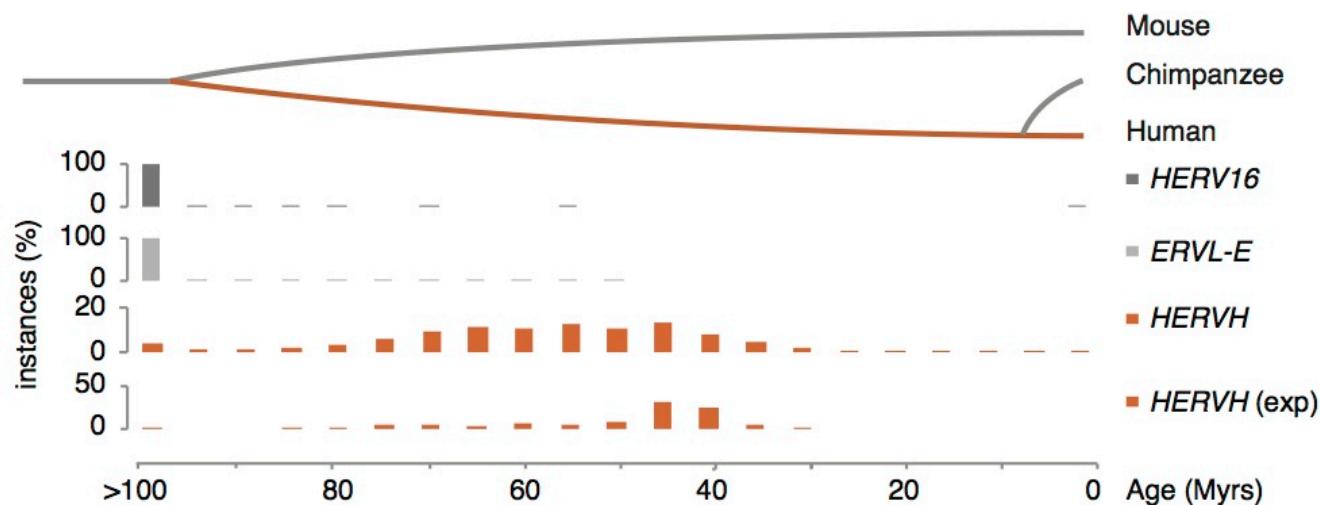
Open Access

HERV-H RNA is abundant in human embryonic stem cells and a precise marker for pluripotency

Federico A Santoni<sup>1,2\*</sup>, Jessica Guerra<sup>2</sup> and Jeremy Luban<sup>2,3</sup>

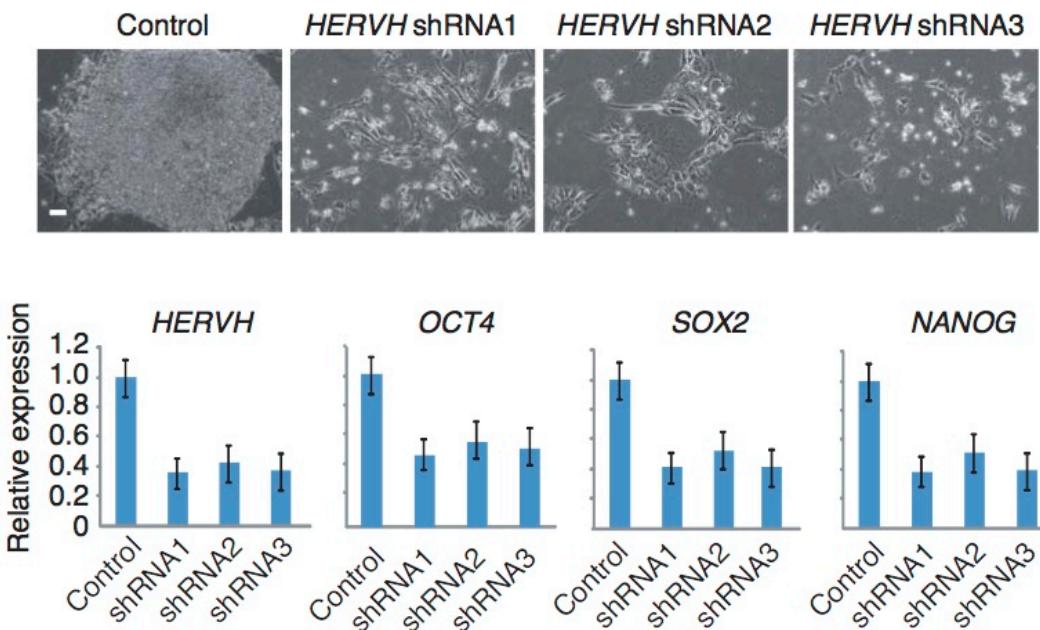
Santoni et al. *Retrovirology* 2012

## Expressed HERVH are primate specific



HERVH family was first discovered by Mager and Henthorn, PNAS 1984

# HERVH is required for pluripotency



With Huck-Hui Ng's lab

Lu et al. *NSMB* 2014

# More on HERVH...

## LETTER

doi:10.1038/nature13804

### Primate-specific endogenous retrovirus-driven transcription defines naïve-like stem cells

Jichang Wang<sup>1\*</sup>, Gangcai Xie<sup>1,2\*</sup>, Manvendra Singh<sup>1</sup>, Avazeh T. Ghanbarian<sup>3</sup>, Tamás Raskó<sup>1</sup>, Attila Szvetnik<sup>1</sup>, Huiqiang Cai<sup>1</sup>, Daniel Besser<sup>1</sup>, Alessandro Prigione<sup>1</sup>, Nina V. Fuchs<sup>1,4</sup>, Gerald G. Schumann<sup>4</sup>, Wei Chen<sup>1</sup>, Matthew C. Lorincz<sup>5</sup>, Zoltán Ivics<sup>4</sup>, Laurence D. Hurst<sup>3</sup> & Zsuzsanna Izsvák<sup>1</sup>

Wang et al. *Nature* 2014

Demonstrates that human naïve-like stem cells are associated with elevated transcription of HERVH

# Outline

- To what extent have transposable elements (TEs) contributed to regulatory regions in the human genome?  
*Significantly! 44% of all human DHS and 63% of primate-specific DHS are derived from TEs*
- Have TEs contributed to regions that are transcribed in the human genome?  
*YES! TEs occur in 2/3 of mature lncRNA transcripts and account for 30% of total lncRNA sequence*
- Is there evidence for functional TE regulatory innovation in the human genome?  
*YES! But...*

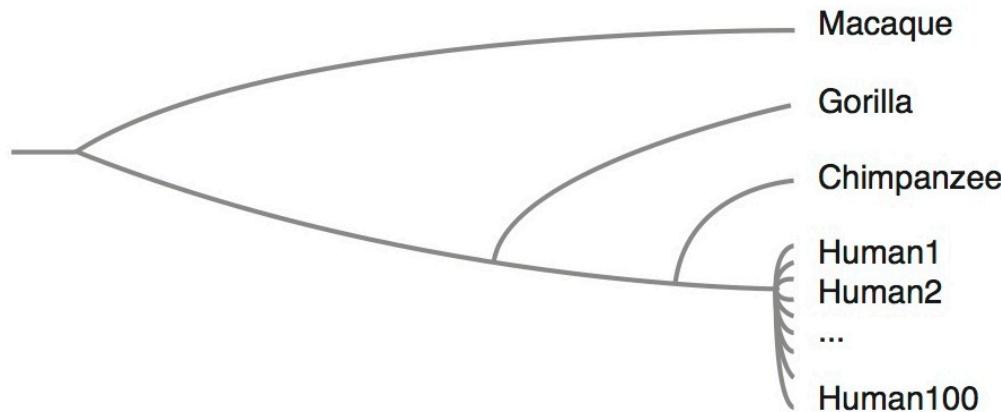
# Conclusions

- TEs have been a major source of new regulatory regions in the human genome (especially ERVs)
- Even if very few TE are “active” as retrotransposon in the human, TE-derived transcripts are frequently expressed (e.g. lncRNAs)
- There are examples of functional TE-derived regulatory regions and TE-derived transcripts (e.g. HERVH in hESCs)
- But... It's absolutely critical to take into account the source of all of this biochemical activity to distinguish the functional innovations from the noise (i.e. neutrally decaying transposons)

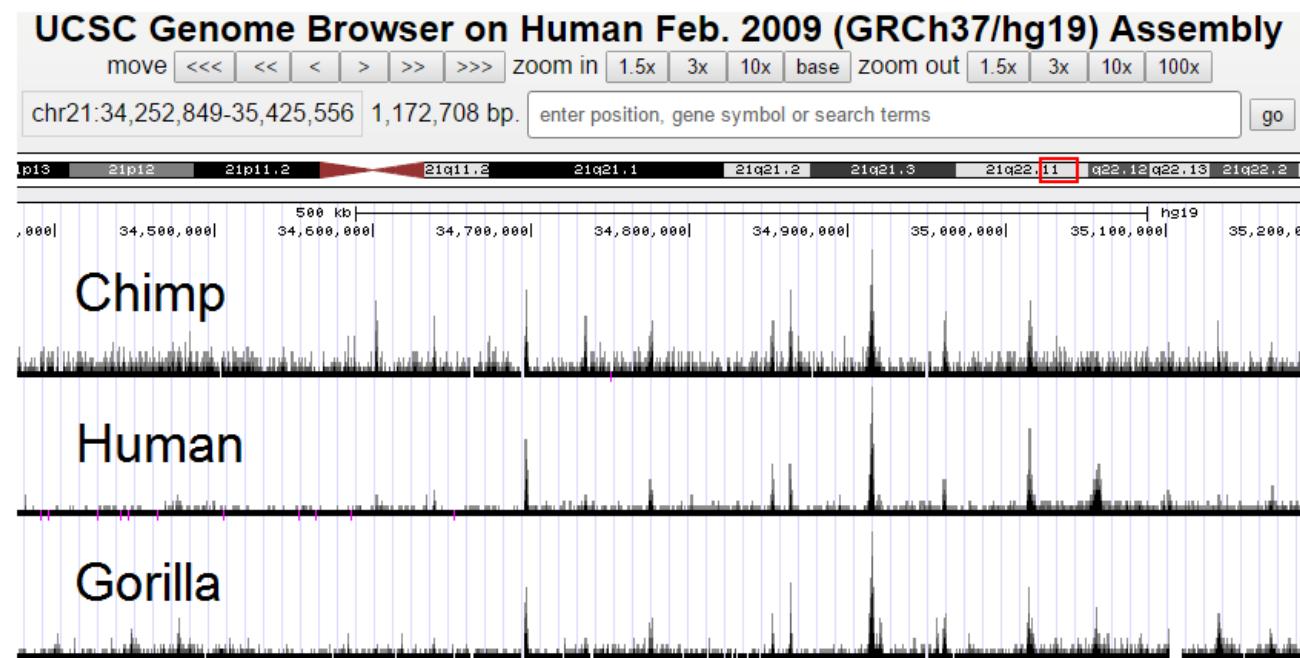
Next steps...

# Primate Comparative Epigenomics

- We are generating reference epigenomes for many human individuals and also non-human primates to look at both intra and inter-specie variability (iPSCs and also T-cells).
- We will also look at the impact of TEs on the evolution of transcription regulation.

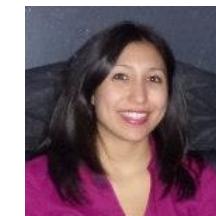
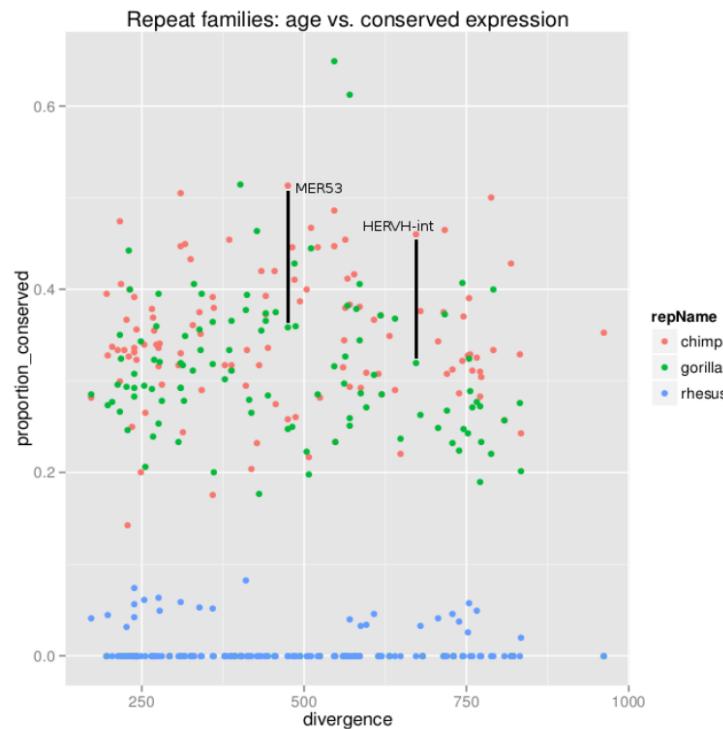


# Example of H3K27Ac Mark



Collaboration with Tomi Pastinen and Fred Gage (Salk)

# Conserved expression of transposon-derived non-coding transcripts in primate iPSCs



LeeAnn Ramsay

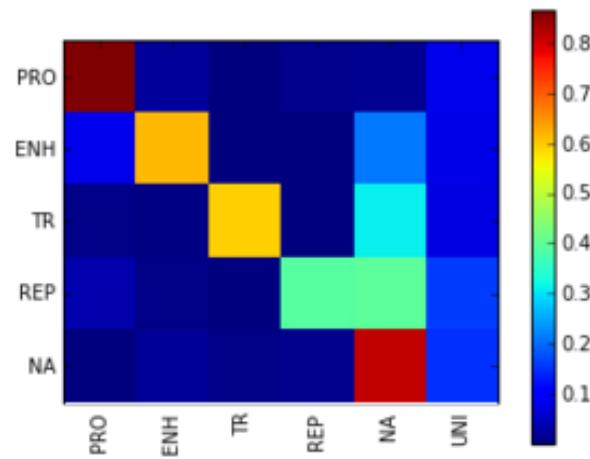
Ramsay et al. *BMC Genomics* 2017

# Comparing Human to Gorilla iPSCs

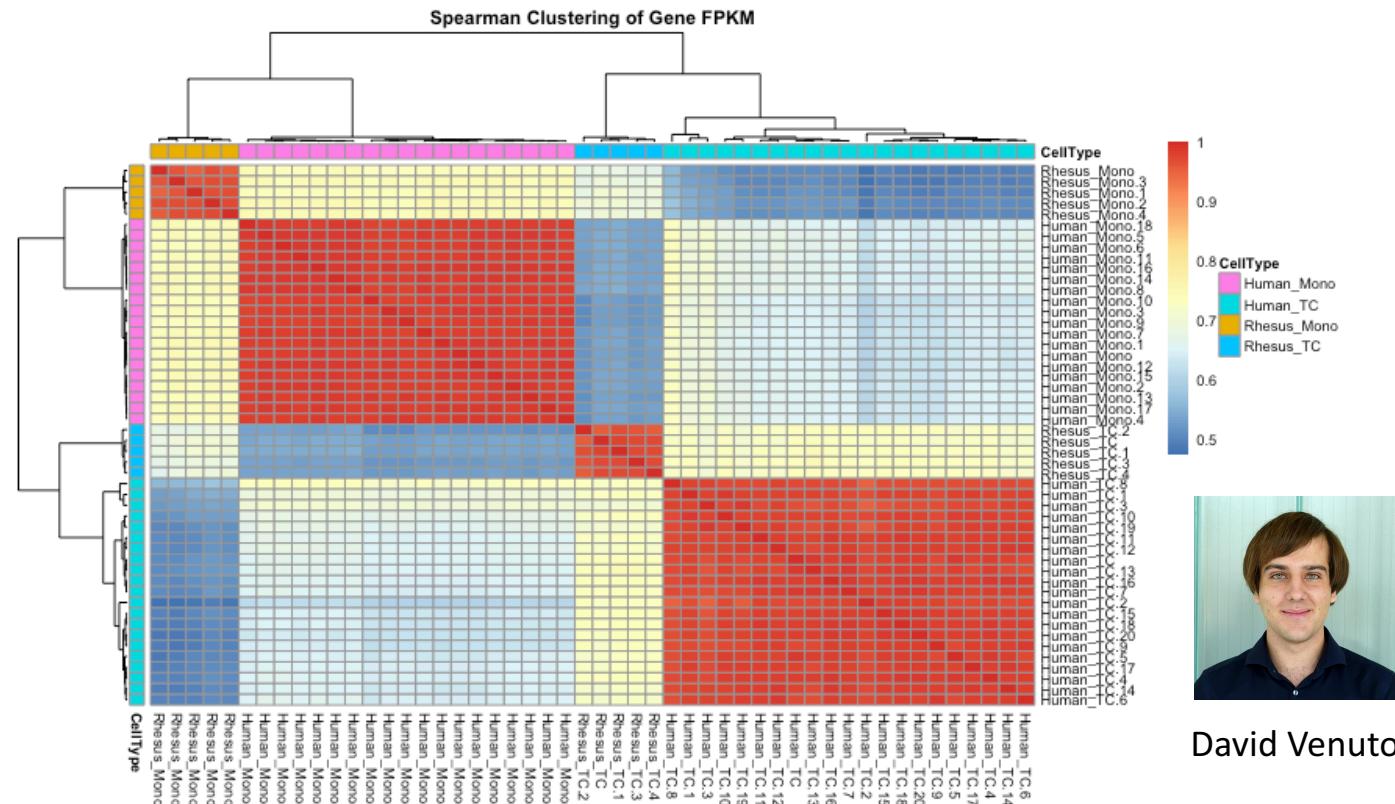
- ~90% of promoter
- ~60% of enhancer
- ~60% of transcribed
- ~40% of repressive



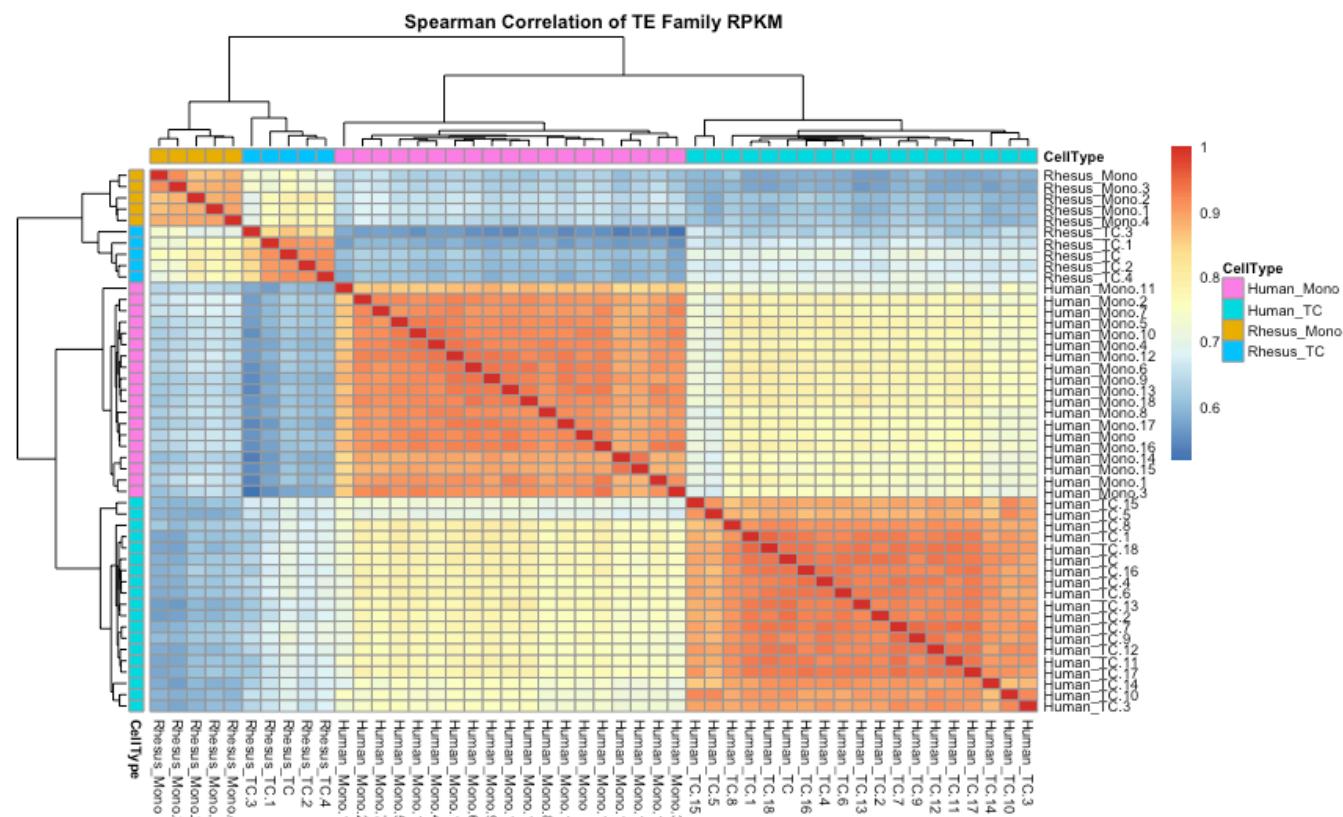
Joe Su



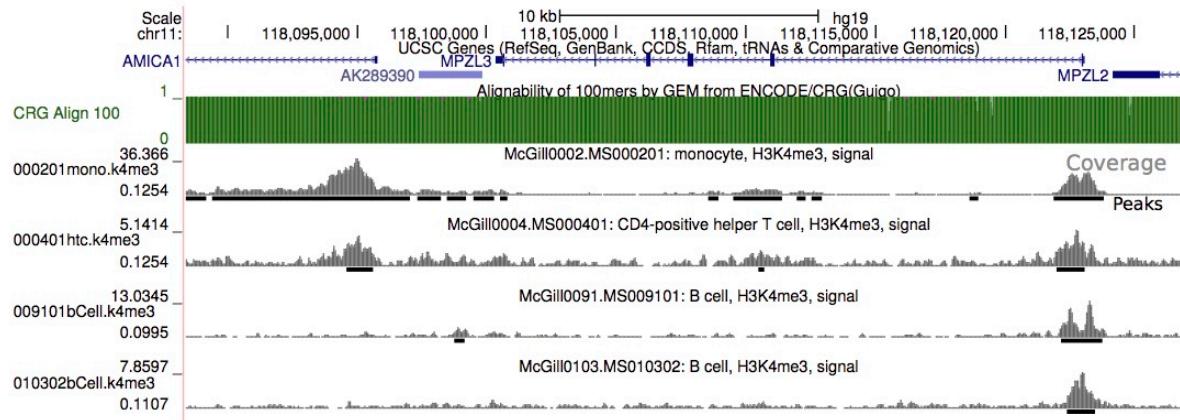
# Gene regulatory networks are mostly conserved across species



# TEs expression is highly species specific



# Limitations of current peak callers



Grey profiles are normalized aligned read count signals.

Black bars are “peaks” called by MACS2 (Zhang et al, 2008):

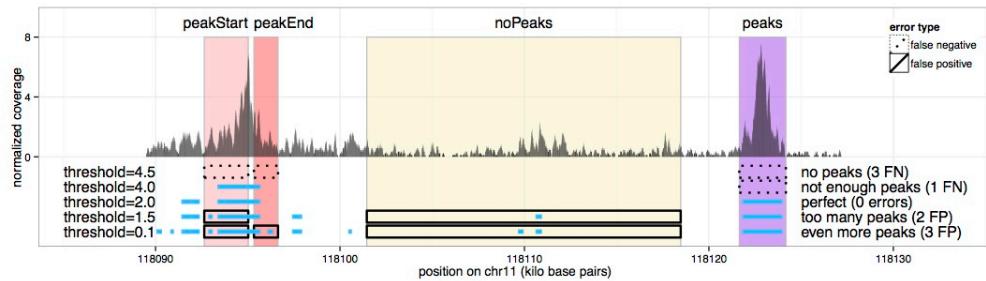
- ▶ many false positives.
- ▶ overlapping peaks have different start/end positions.



Toby Hocking

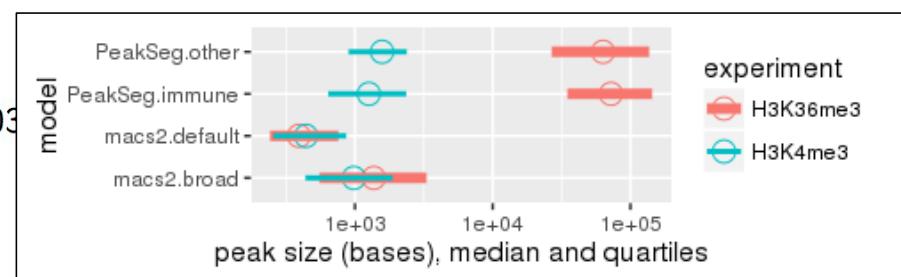
# PeakSeg: supervised machine-learning for peak calling

Input: data + labels, output: peaks.

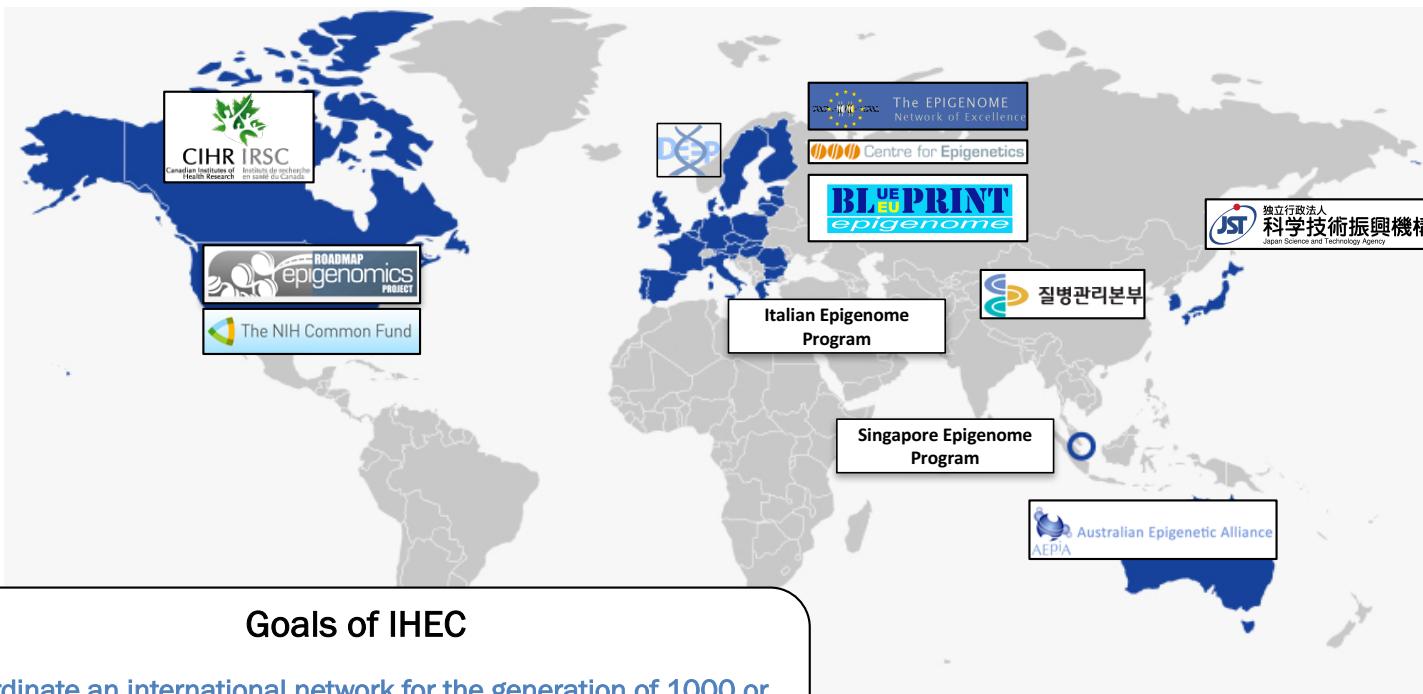


Advantage: automatically optimize peak detection parameters to maximize agreement with labels.

- ▶ H et al., *Bioinformatics* 2017.
- ▶ Hocking and Bourque, arXiv:1506.01286.
- ▶ Hocking, Rigaill, Fearnhead, Bourque, arXiv:1703



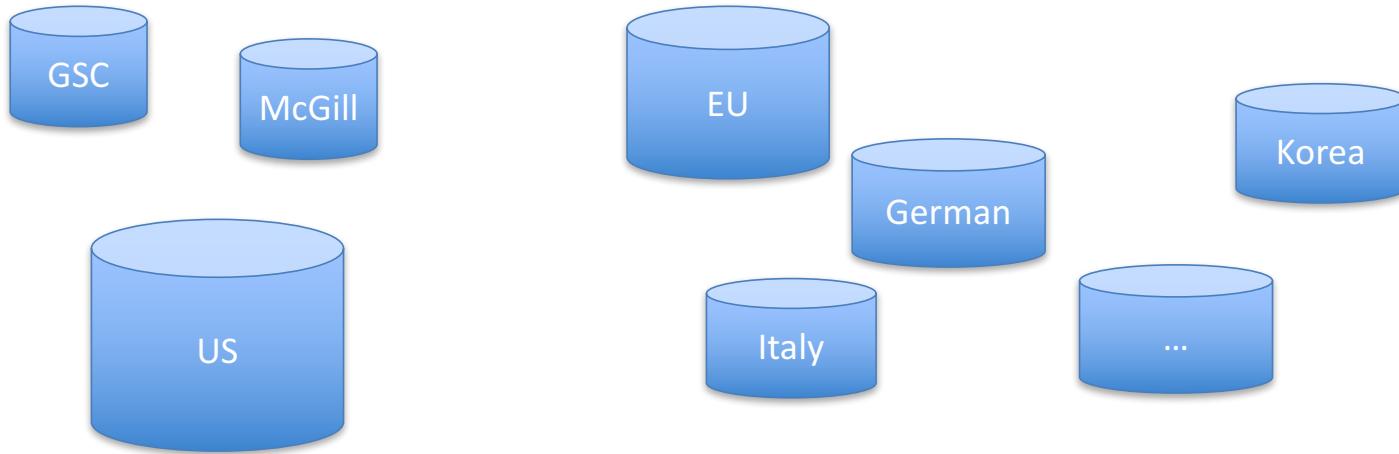
Data sharing...



### Goals of IHEC

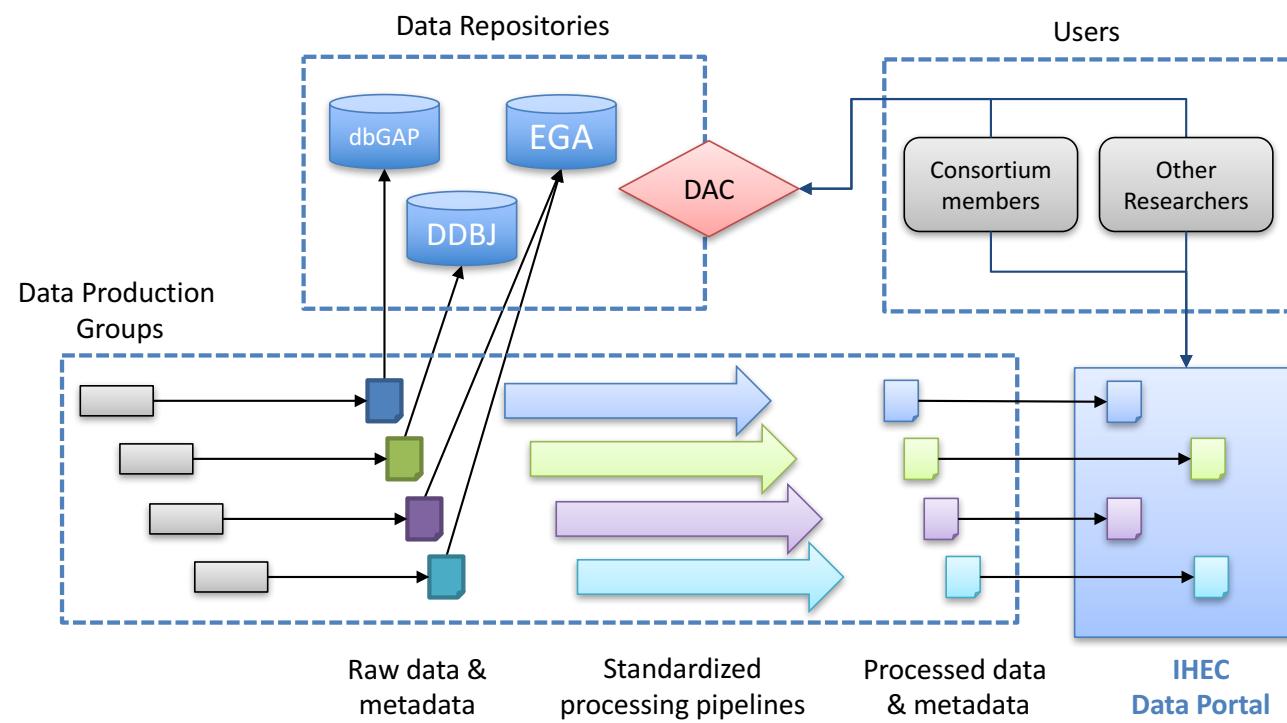
Coordinate an international network for the generation of 1000 or more reference epigenomes for a broad spectrum of human cell types and a wide range of developmental stages, laying the foundation to study the epigenetic mechanisms of human diseases.

# Need for data integration



Can we do better?

# IHEC data integration and sharing strategy

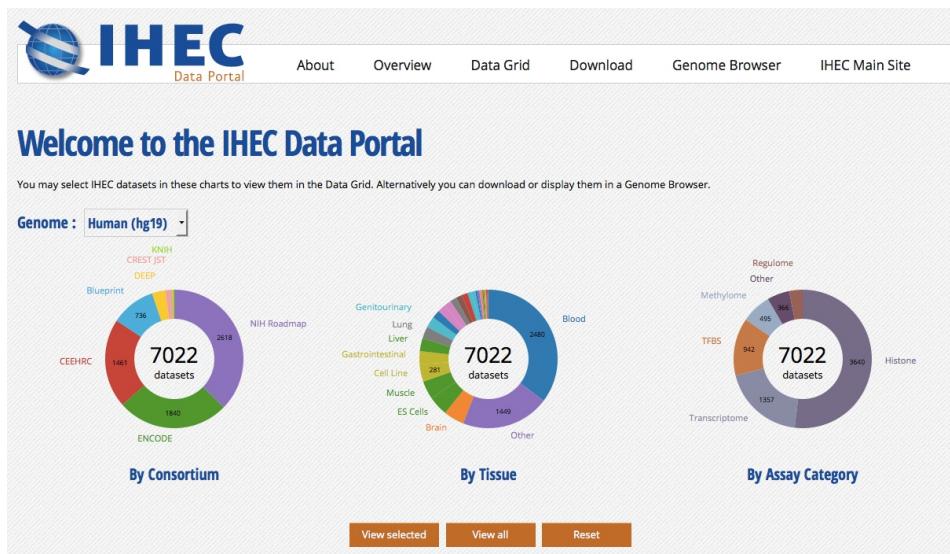


# IHEC Data Portal

- Launched in June 2014, <http://epigenomesportal.ca/ihec>
- As of **Nov 2017**, includes:
  - Over **10,800** human epigenomic datasets (hg19 and hg38)
  - Over **280** mouse and primate datasets
  - Over **>290** full reference epigenomes
  - Data from: Blueprint, CEEHRC, CREST, DEEP, ENCODE, KNIH, NIH Roadmap

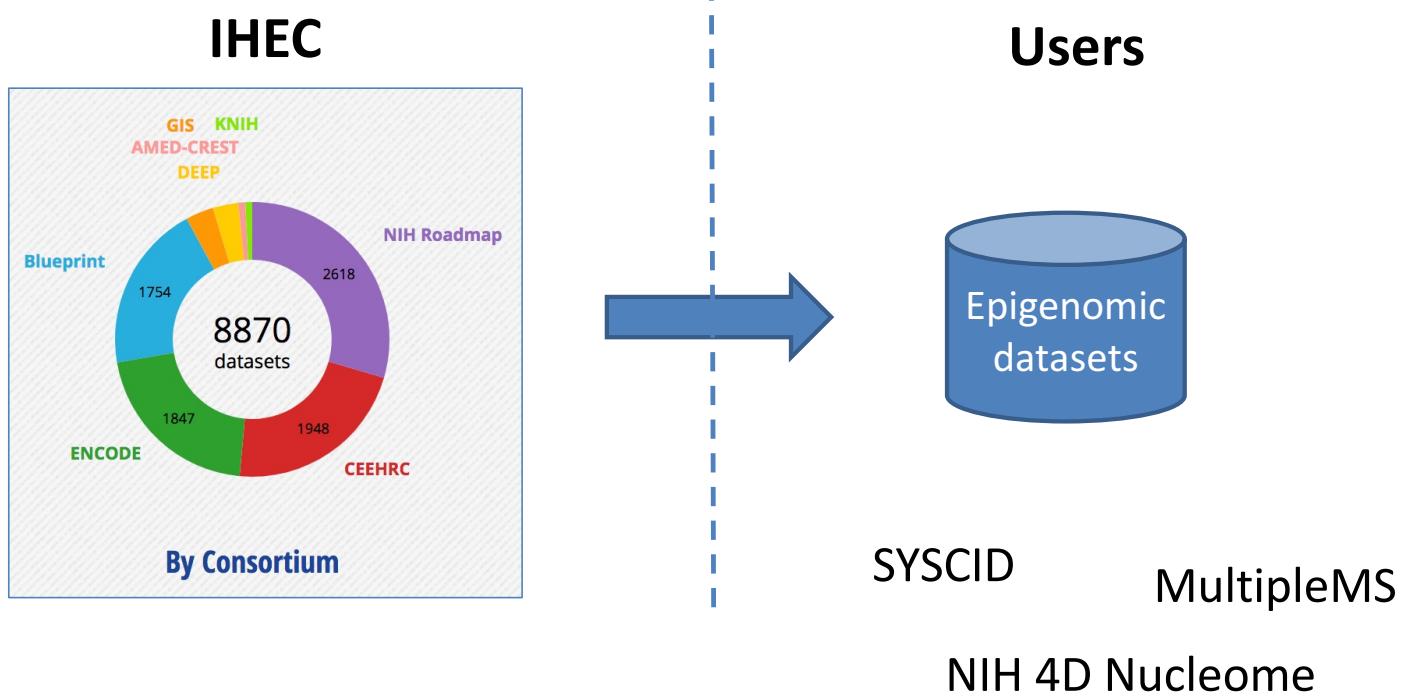


David Bujold

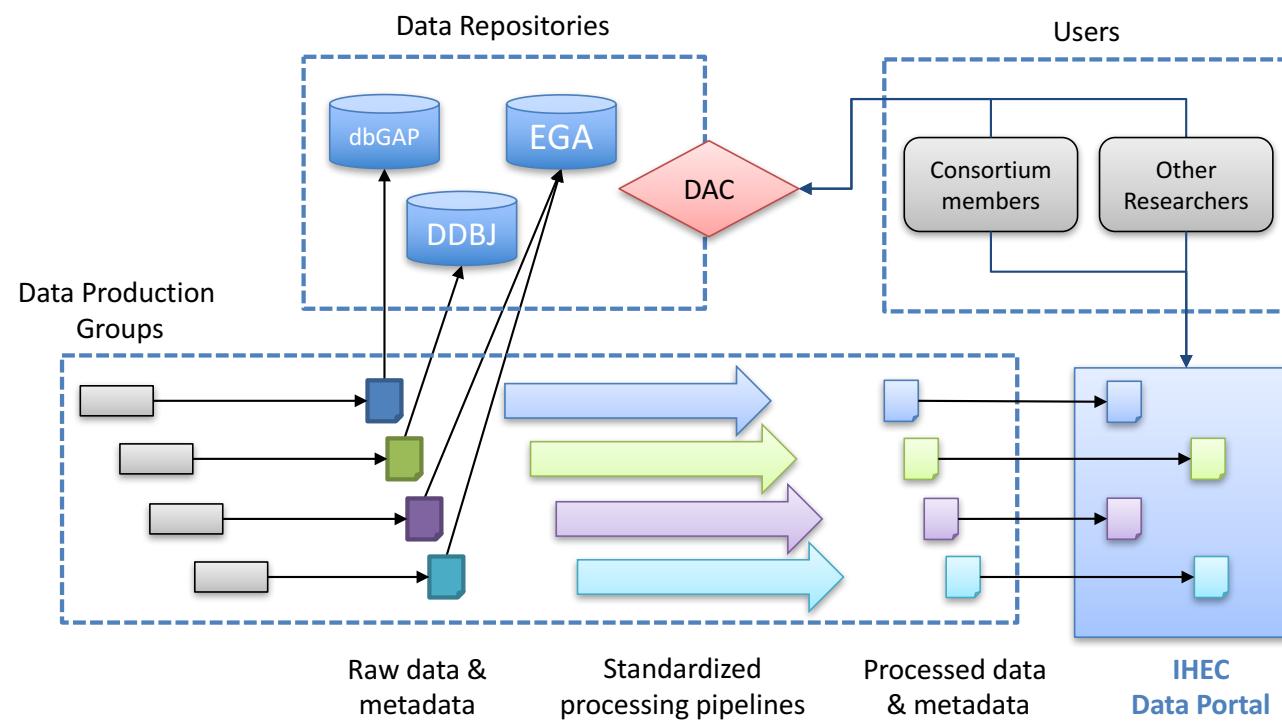


Bujold et al.  
*Cell Systems*  
2016

# 1000(s) human reference epigenomes



# IHEC data integration and sharing strategy



# IHEC Datasets

Consortium	Project	Data located at	Nb EpiRR (full & partial)	Nb EGA datasets	Nb Portal datasets	Access committee
Blueprint	Main	EGA	562	247	2309	DAC1
Blueprint	WP10	EGA	197	8	1018	
Blueprint	Progenitor	EGA	63	1	63	
CEEHRC	CEMT	EGA	53	16	558	DAC2
CEEHRC	McGill	EGA	278	12	614	DAC3
CREST	Kanai	DDBJ	8	?	72	DAC4
CREST	Sasaki	DDBJ	15	?	132	
DEEP	DEEP	EGA	50	1	193	DAC5
KNIH	KNIH	EGA	11	11	32	DAC6

# Data access challenges

**Table 1: Clauses Identified across IHEC Agreements**

	#1	#2	#3	#4	#5	#6	#7
<b>Constraints on Use</b>							
<b>Application Renewal</b>							
<b>Evidence of Competence</b>							
<b>Student Access</b>							
<b>Specific External Laws</b>							
<b>Specific Policies</b>							
<b>Jurisdiction</b>							
<b>External Access</b>							
<b>Acknowledgements</b>							
<b>Liability</b>							
<b>Report to Project</b>							
<b>Publication Delays</b>							
<b>Destruction of Data</b>							
<b>Ethics Review</b>							
<b>IT Practices</b>							
<b>Intellectual Property</b>							
<b>Unique Provisions</b>							

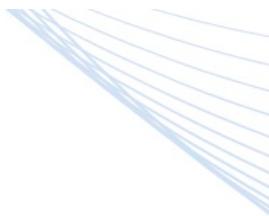
Yann Joly, McGill University

# PROFYLE project



The Terry Fox Research Institute  
L'Institut de recherche Terry Fox

**Terry Fox PROFYLE,** a research project for children and young adults who have been told they are out of treatment options, giving them another chance.



## Project Leader

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The Hospital for Sick Children

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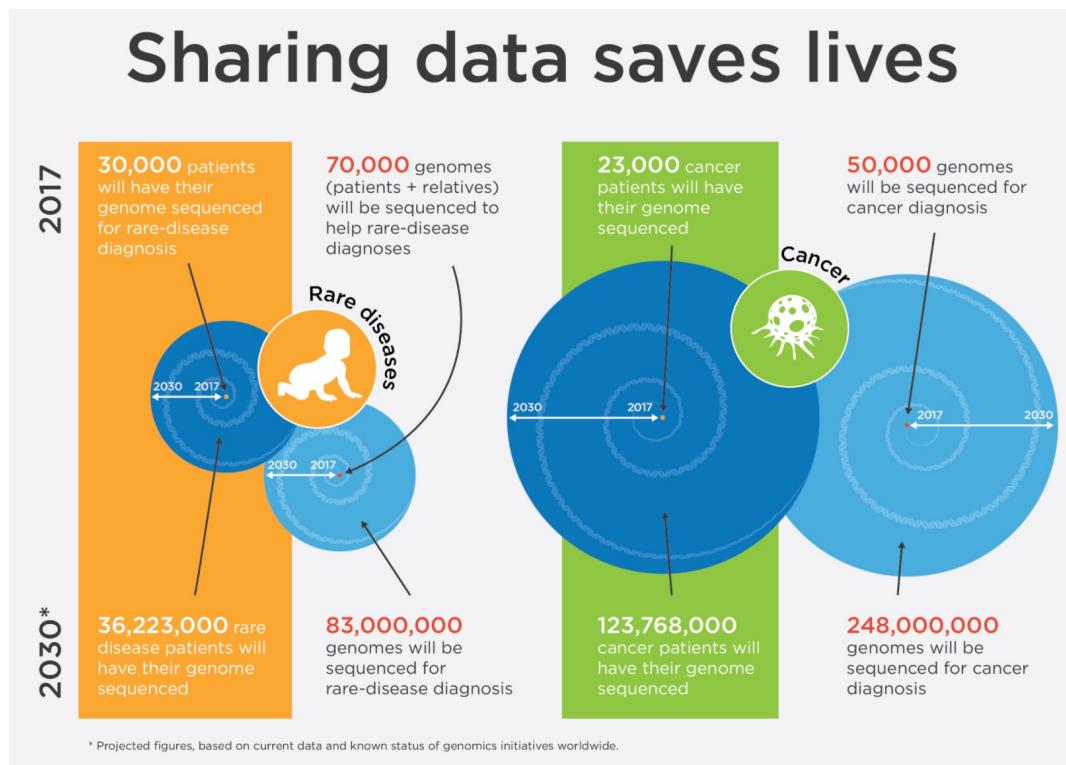
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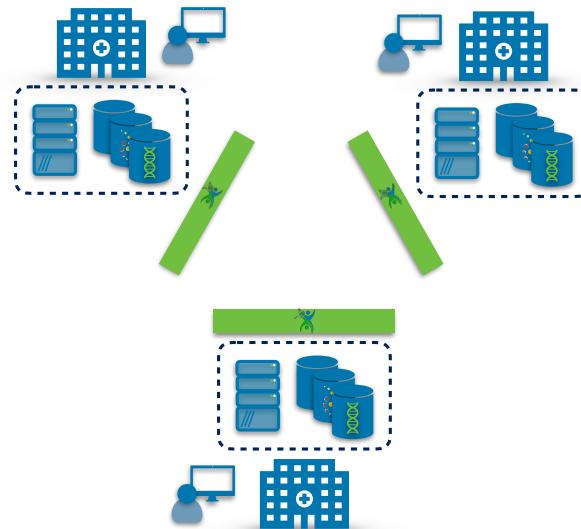
**Dr. Jim Whitlock,**  
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# Global Alliance for Genomics and Health (GA4GH)



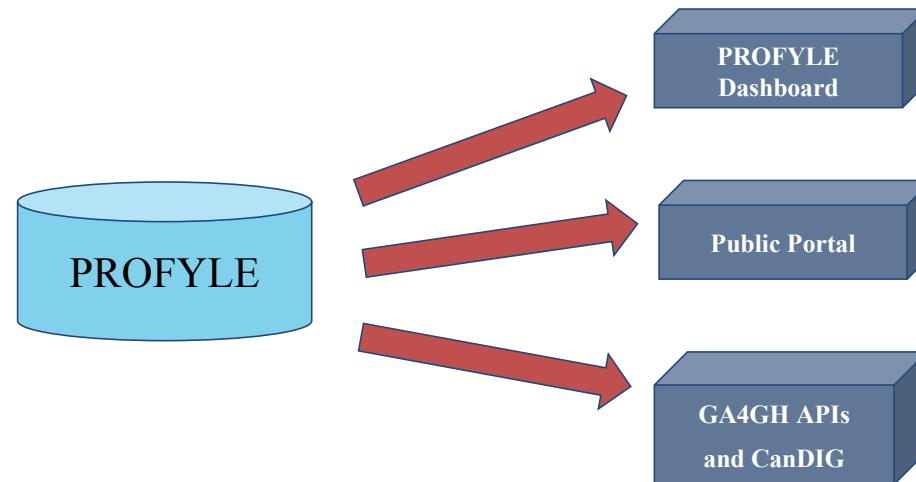
# CanDIG: CFI-cyberinfrastructure and GA4GH driver project

- Fully distributed
- Participating sites: data providers, source of user requests
- Distributed synchronization of metadata, apps available, etc
- Access to data through API requests, either for data as it stands or for processing through some pipelines
- Local sites control access to their data
- Sites authenticate their users

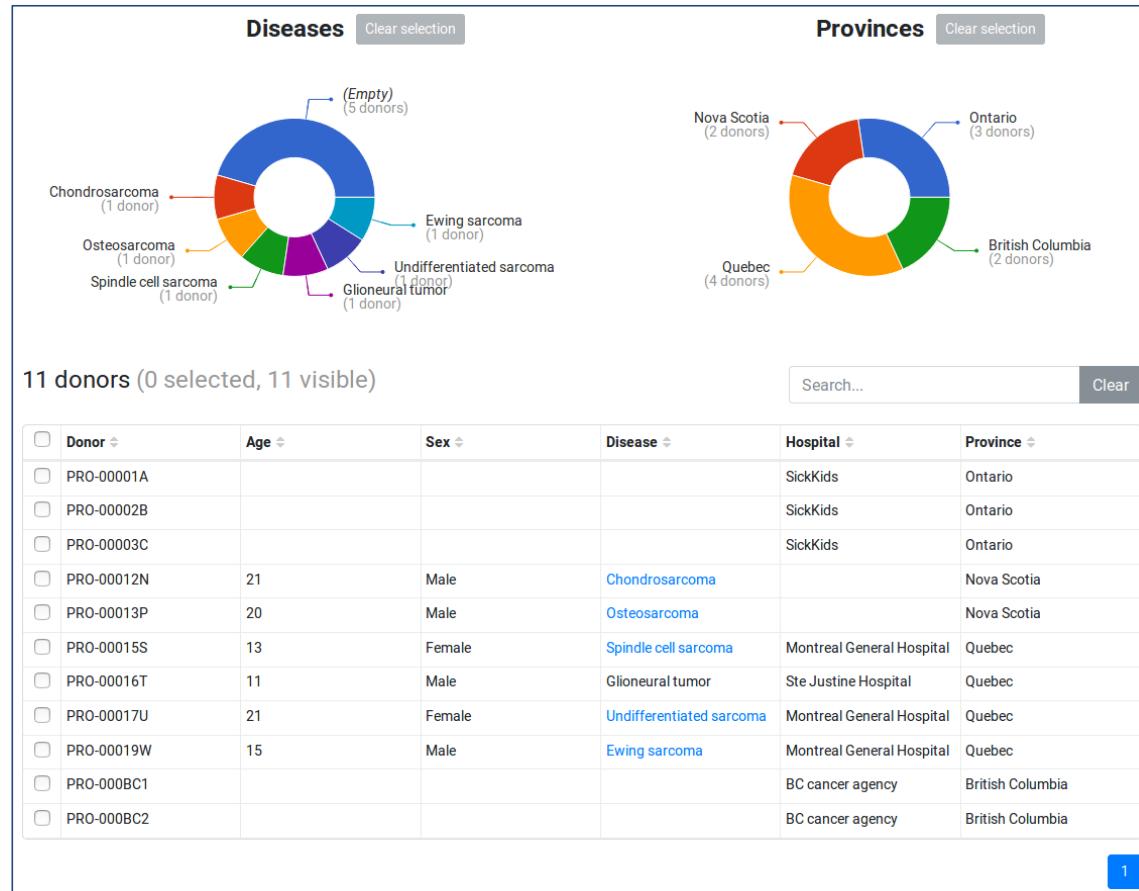


# Why should we improve metadata organization?

- Well organized metadata is necessary to make data useful and discoverable



# PROFYLE dashboard



# Acknowledgements

## Lab

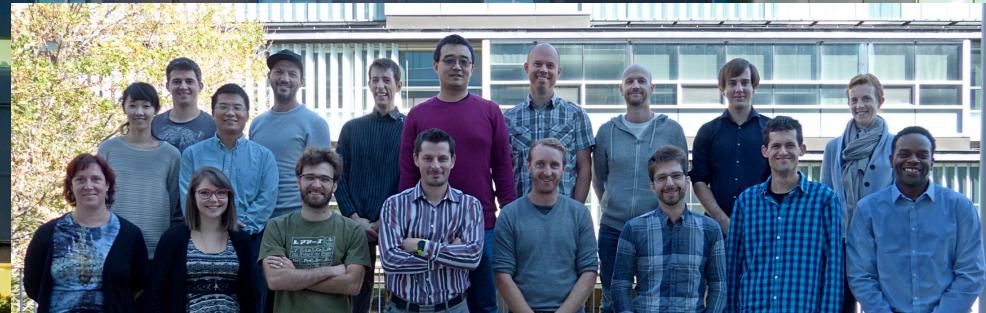
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