

A photograph of a modern, multi-story building at night. The building has a dark facade with large glass windows that are illuminated from within, showing various rooms and offices. Two tall, cylindrical exhaust stacks are visible on the roof. The sky is a deep blue, suggesting twilight or early evening.

RNA-seq

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Canadian Center for Computational Genomics (C3G)



@guilbourque

Dec 6th 2017

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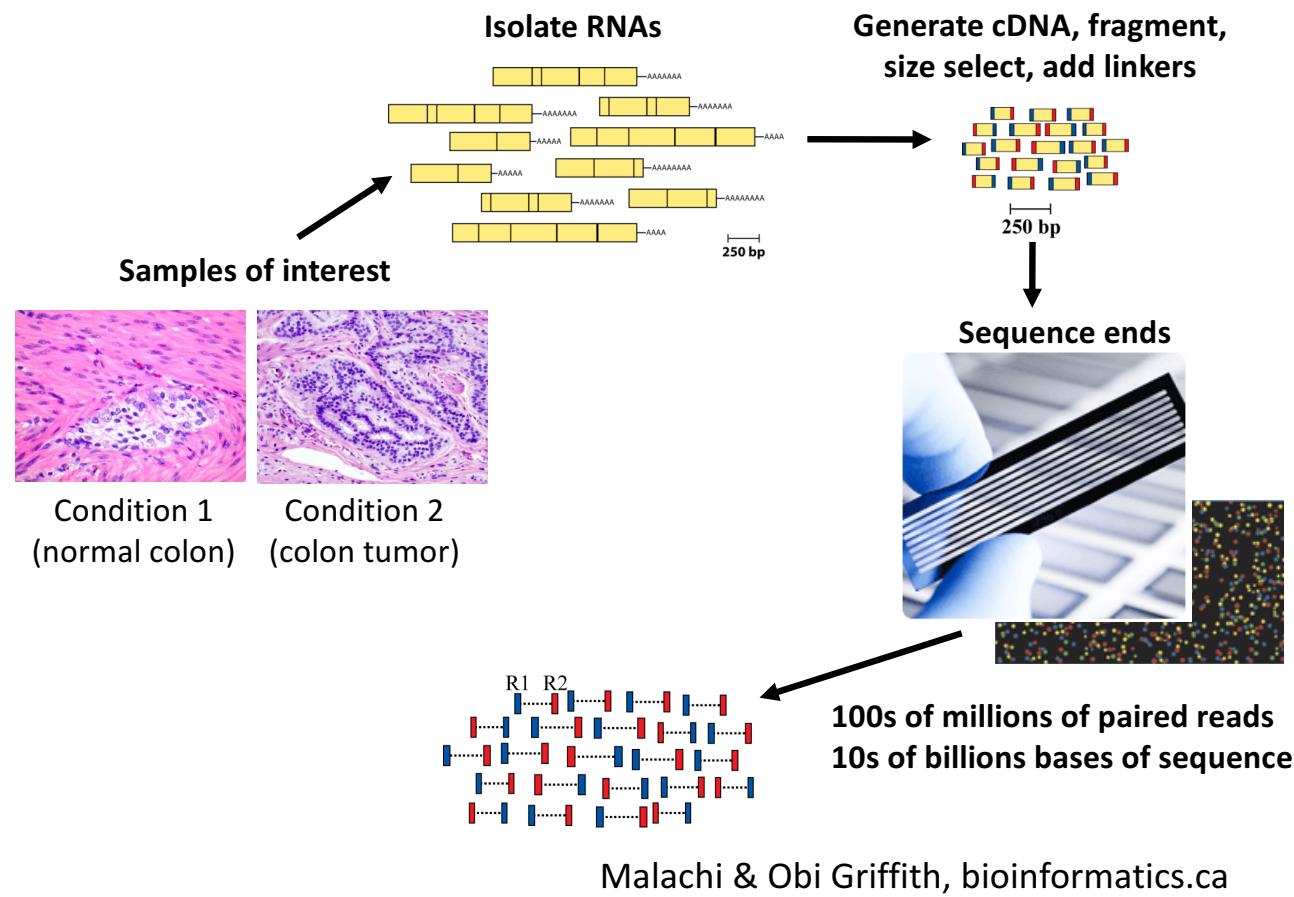
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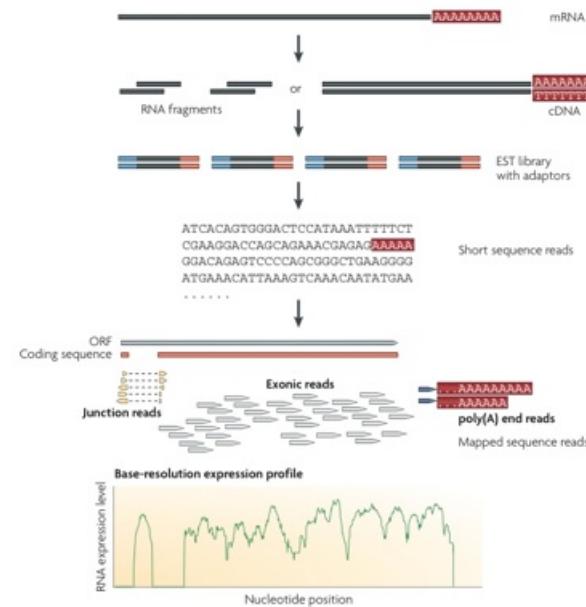
Outline

- Background on RNA-Seq
- Overview of RNA-Seq analysis pipeline
- Other RNA technologies
- Available web-based resources

RNA-seq



RNA-Seq: digital expression and much more



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

RNA-seq versus microarray

- Unbiased detection of novel transcripts
- Broader dynamic range
- Increased specificity and sensitivity
- Easier detection of rare and low-abundance transcripts

Source: illumina.com

Why sequence transcriptomes?

- Annotation & Discovery
 - New features of the transcriptome including genes, exons, splicing events, ncRNAs, lincRNAs
 - New transcript structures derived from chromosomal aberrations or structural polymorphisms
 - In cancer, novel events may reveal cancer drivers and/or may be “biomarkers”
- Measurement
 - Expression of individual genes/loci and the potential to quantitatively discriminate isoforms using junction reads and coverage of individual exons, introns etc.
 - Presence, genotype, and expression of alleles (may be relevant to disease, may reflect underlying epigenetic changes)

RNA-seq – Applications

- Gene expression and differential expression
- Transcript discovery
- SNV, RNA-editing events, variant validation
- Allele specific expression
- Gene fusion events detection
- Genome annotation and assembly
- etc ...

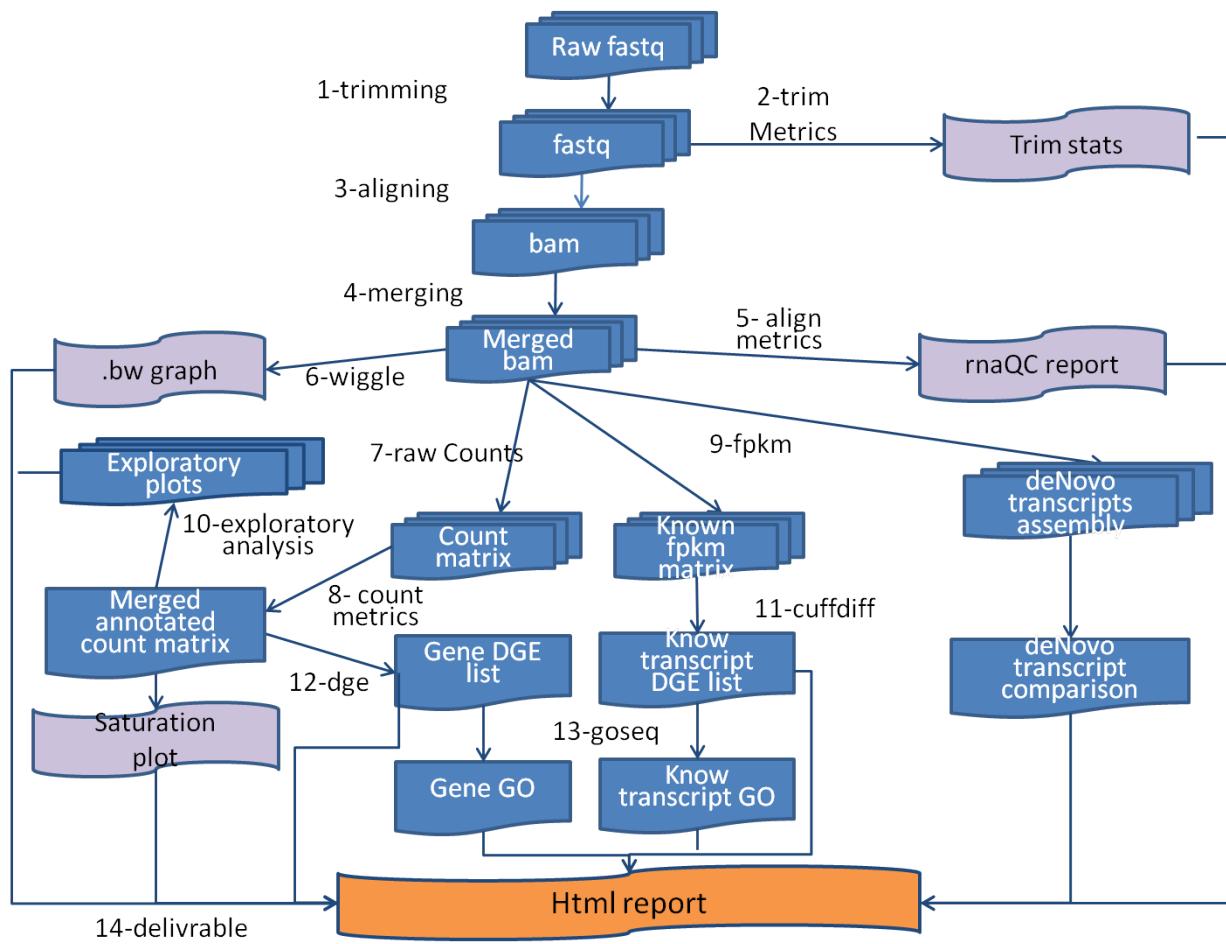
RNAseq Challenges

- RNAs consist of small exons that may be separated by large introns
 - Mapping splice-reads to the genome is challenging
 - Ribosomal and mitochondrial genes are misleading
- RNAs come in a wide range of sizes
 - Small RNAs must be captured separately
- RNA is fragile and easily degraded
 - Low quality material can bias the data

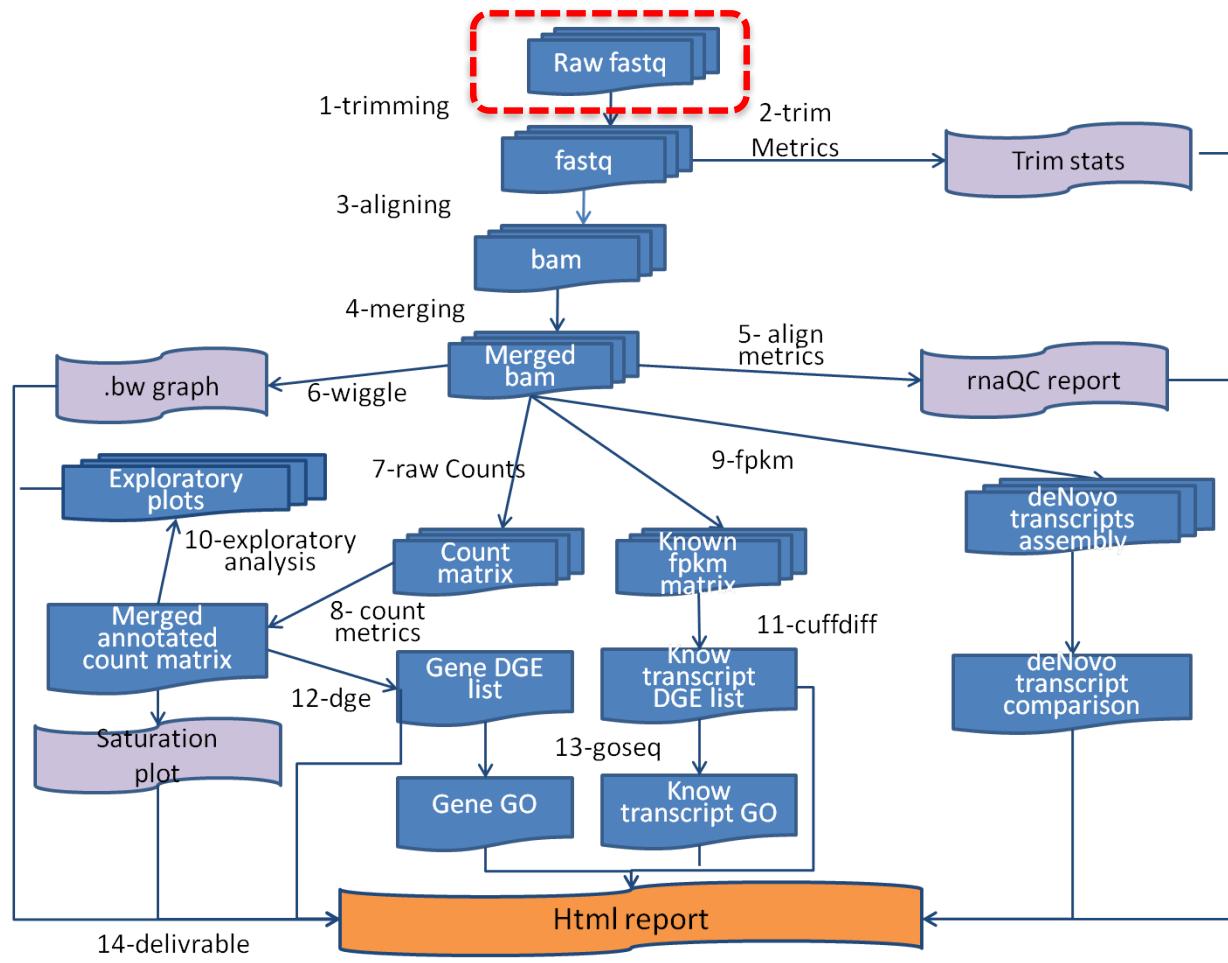
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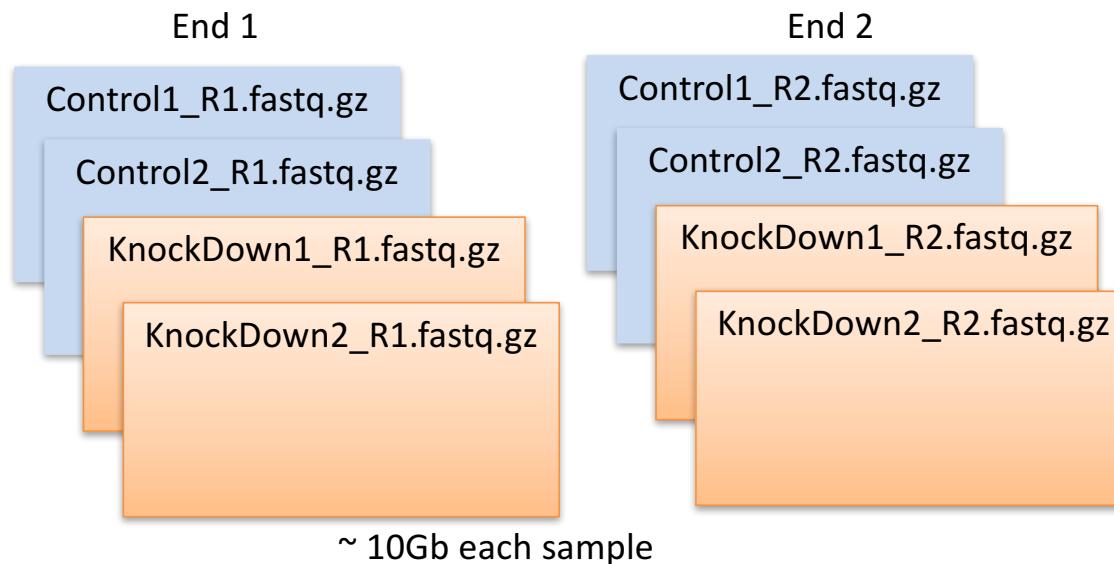
RNA-Seq: Pipeline Overview



RNA-Seq: Pipeline Overview



Input Data: FASTQ



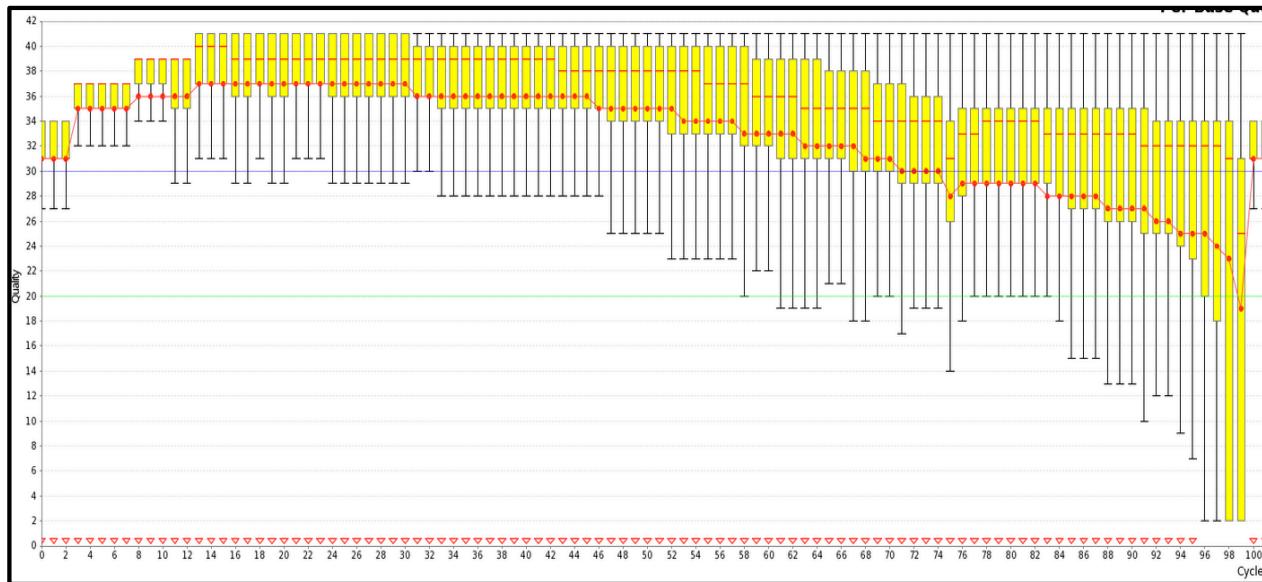
$$Q = -10 \log_{10} (p)$$

Where Q is the quality and p is the probability of the base being incorrect.

What is a base quality?

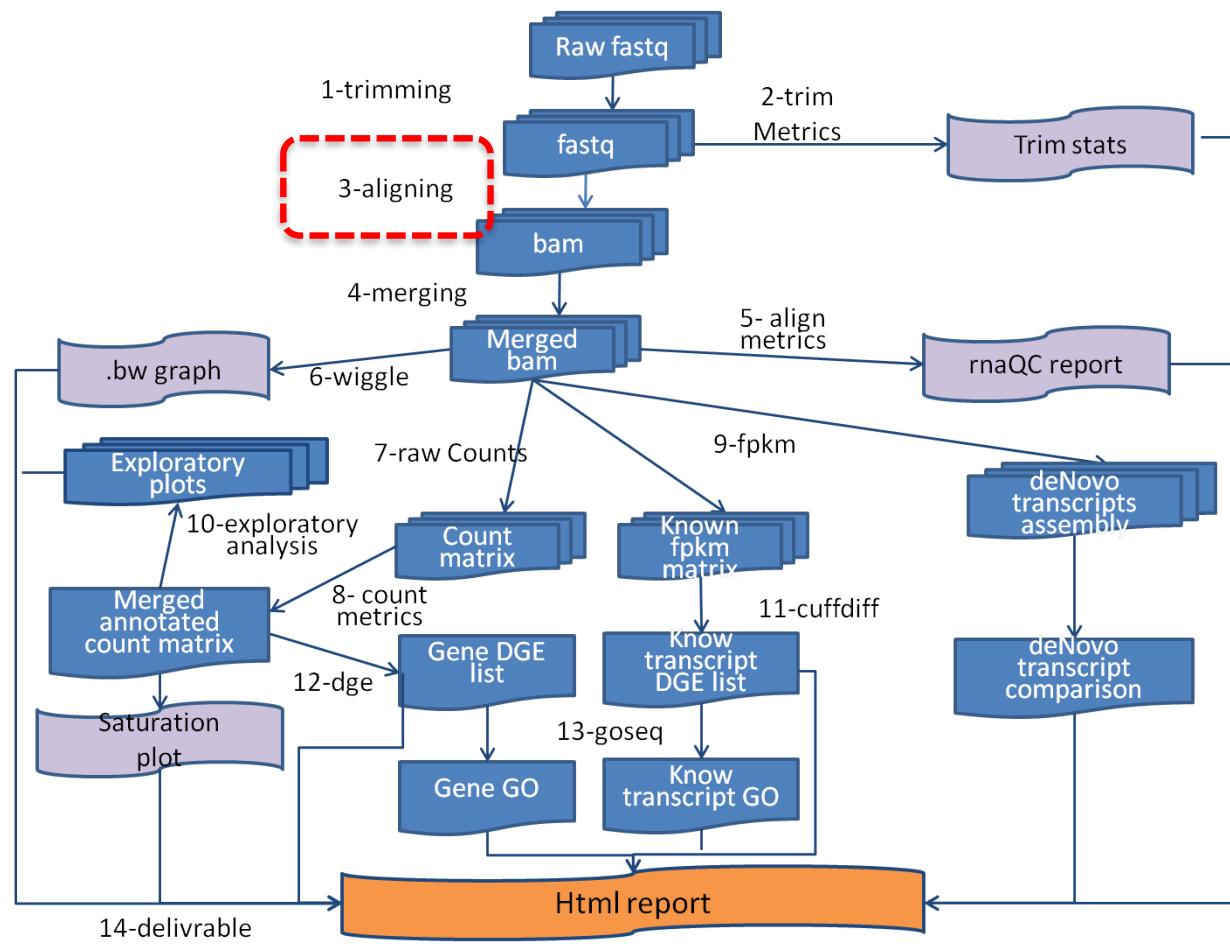
Base Quality	P _{error} (obs. base)
3	50 %
5	32 %
10	10 %
20	1 %
30	0.1 %
40	0.01 %

QC of raw sequences

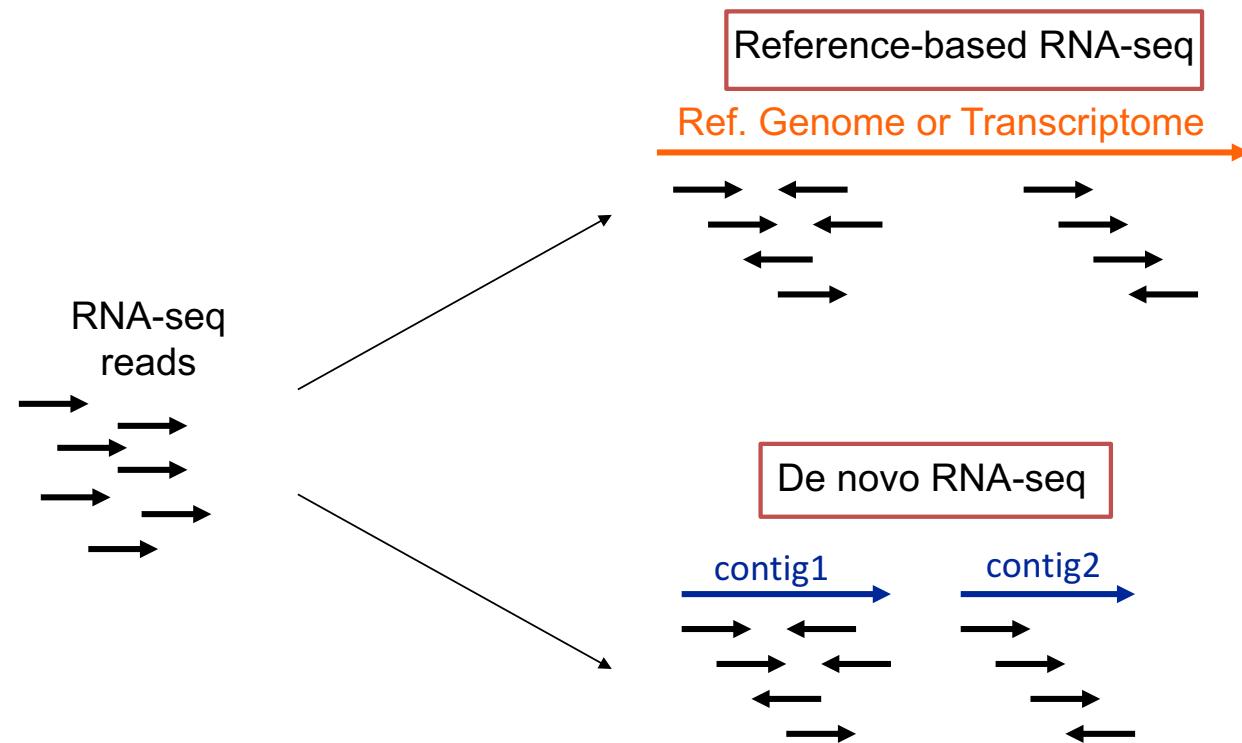


low quality bases can bias subsequent analysis
(i.e, SNP and SV calling, ...)

RNA-Seq: Overview

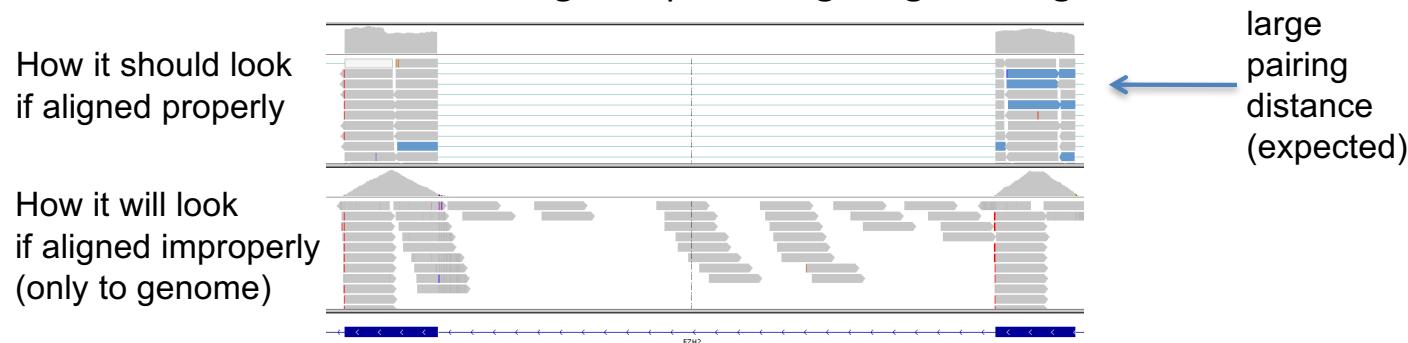


RNA-seq: Assembly vs Mapping



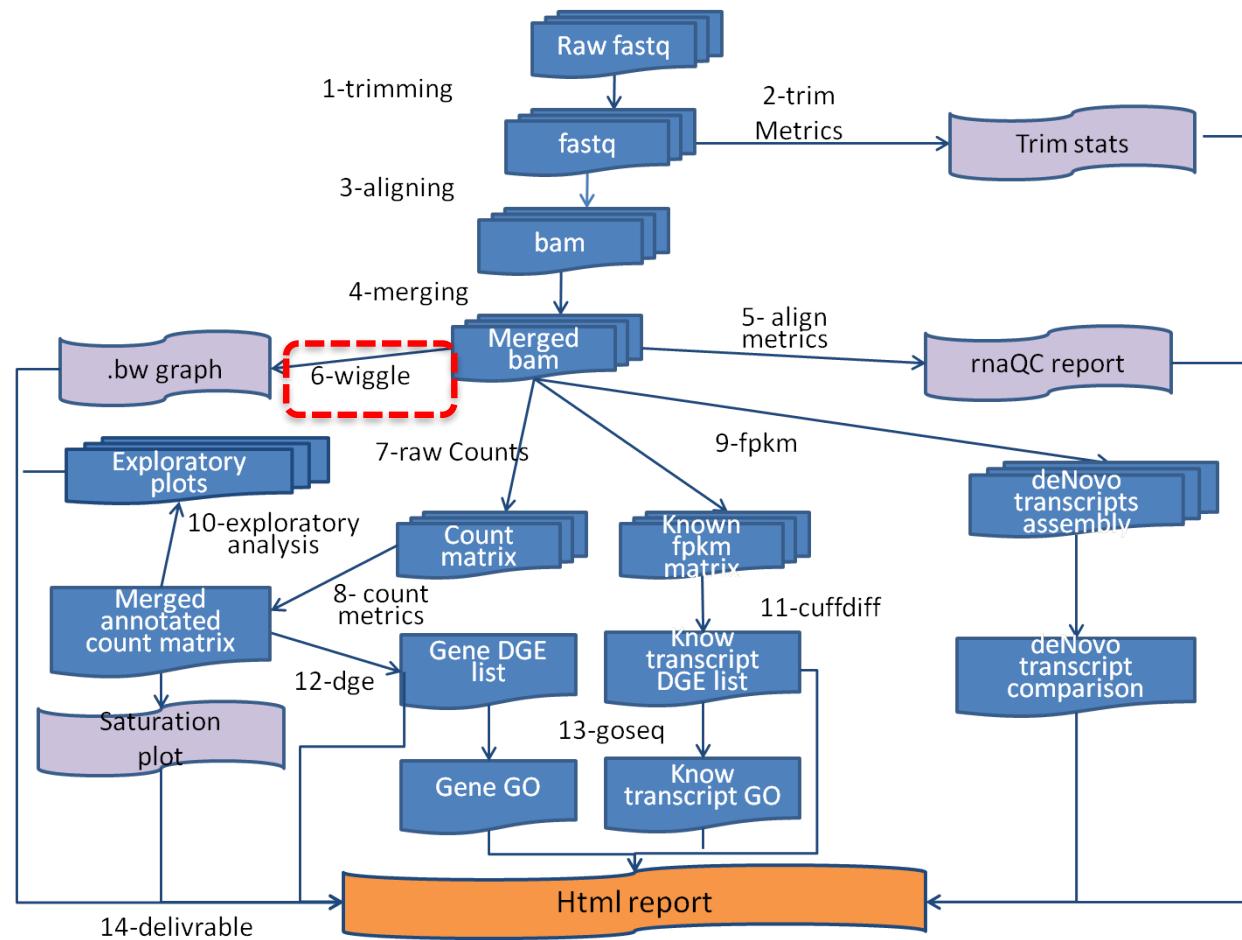
Why not treat RNA-seq reads like genomic DNA sequence?

- Reads crossing one or more exon-exon junction will not align properly to the genome (longer reads, bigger issue)
- Reads deriving from a fragment that spans multiple exons will have a large pairing distance
 - skews any estimation of fragment length
 - Incompatible with any aligner requiring a sane pairing distance distribution or hard limit on pairing distance
 - More bias when correcting/compensating for gene length



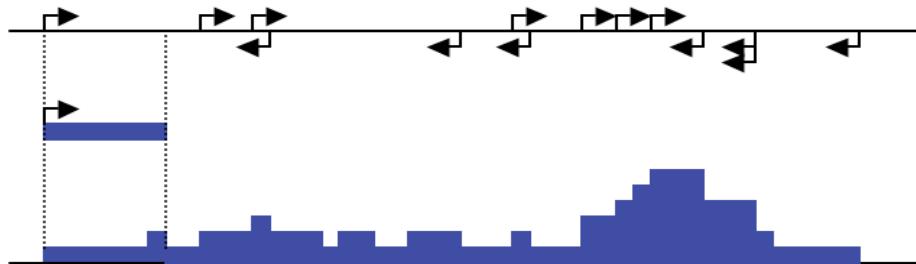
Ryan Morin, bioinformatics.ca

RNA-Seq:Wiggle



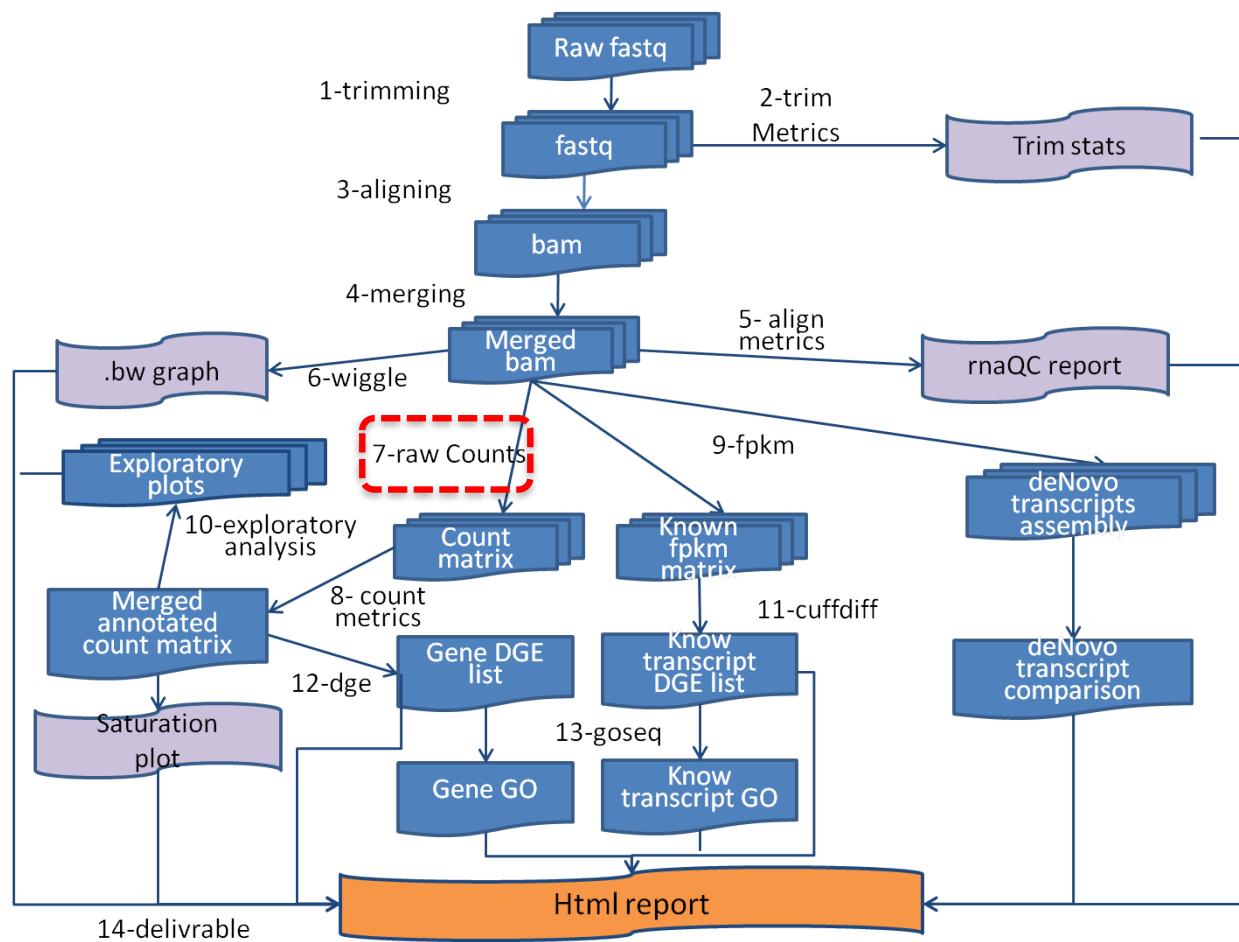
UCSC: bigWig Track Format

- The bigWig format is for display of dense, continuous data that will be displayed in the Genome Browser as a graph.
- Count the number of read (coverage at each genomic position):

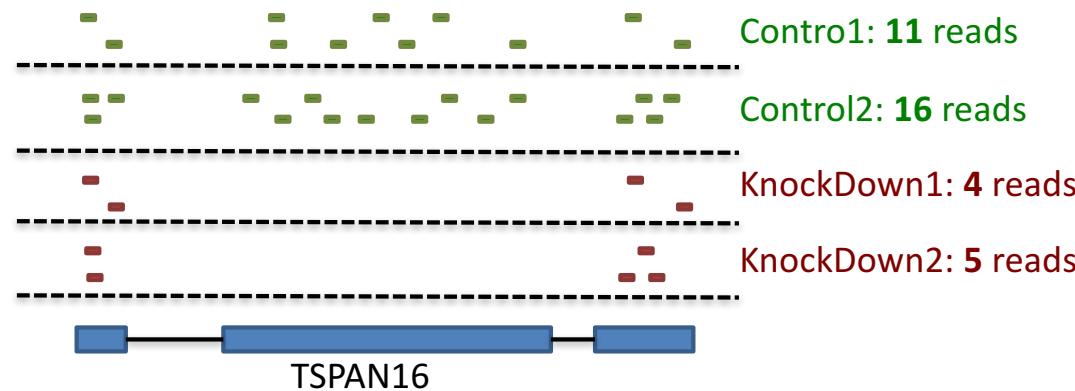


Modified from <http://biowhat.ucsd.edu/homer/chipseq/ucsc.html>

RNA-Seq: Gene-level counts



HTseq: Gene-level counts



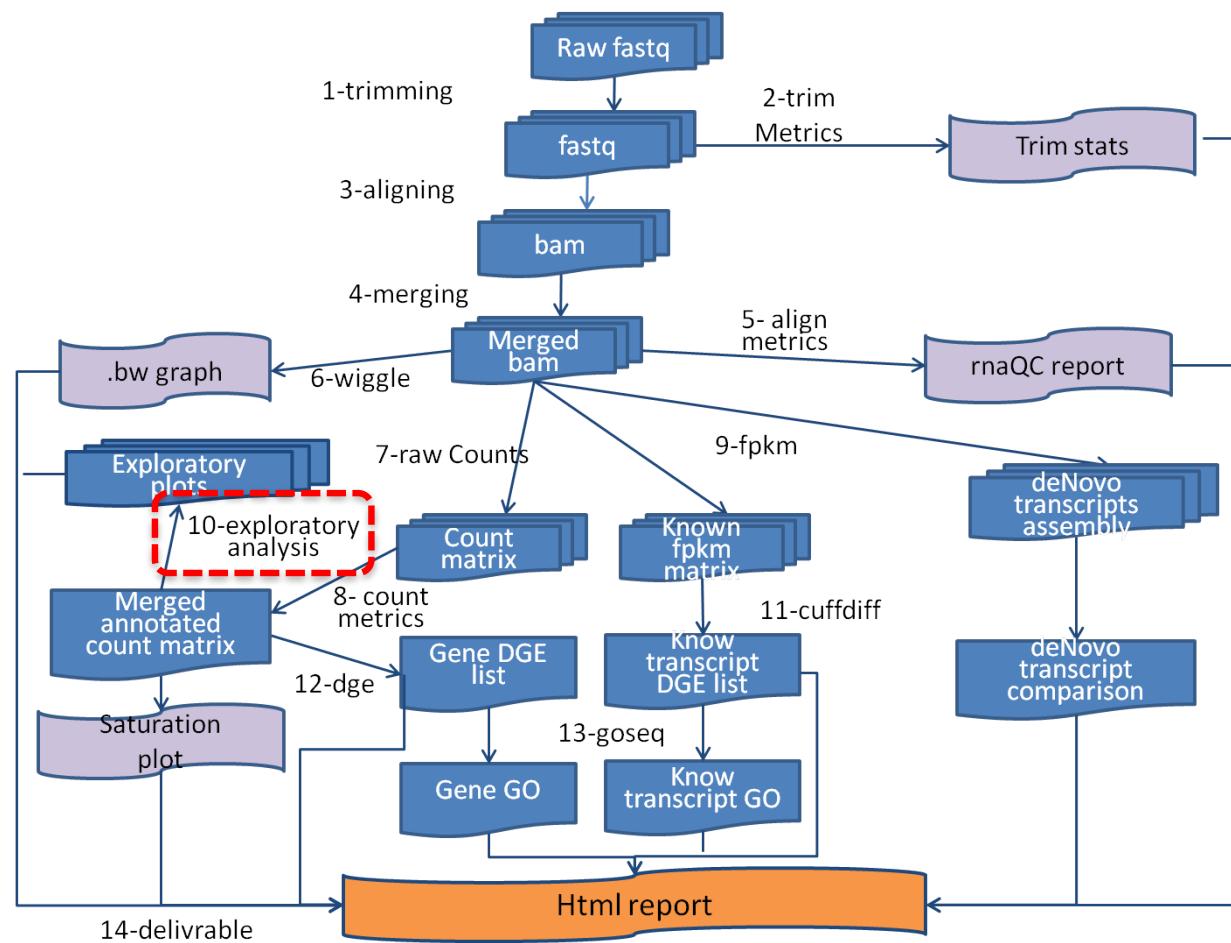
- Reads (BAM file) are counted for each gene model (gtf file) using *HTSeq-count*:

	Control1	Control2	KnockDown1	KnockDown2
TSPAN6	11	16	4	5
TNMD	1	0	0	0
DPM1	435	743	836	739
SCYL3	203	218	416	352
C1orf112	216	643	714	704
FGR	2365	5011	2828	2294
CFH	6	1	4	0
FUCA2	380	865	431	523
...
NFYA	888	827	1674	1580

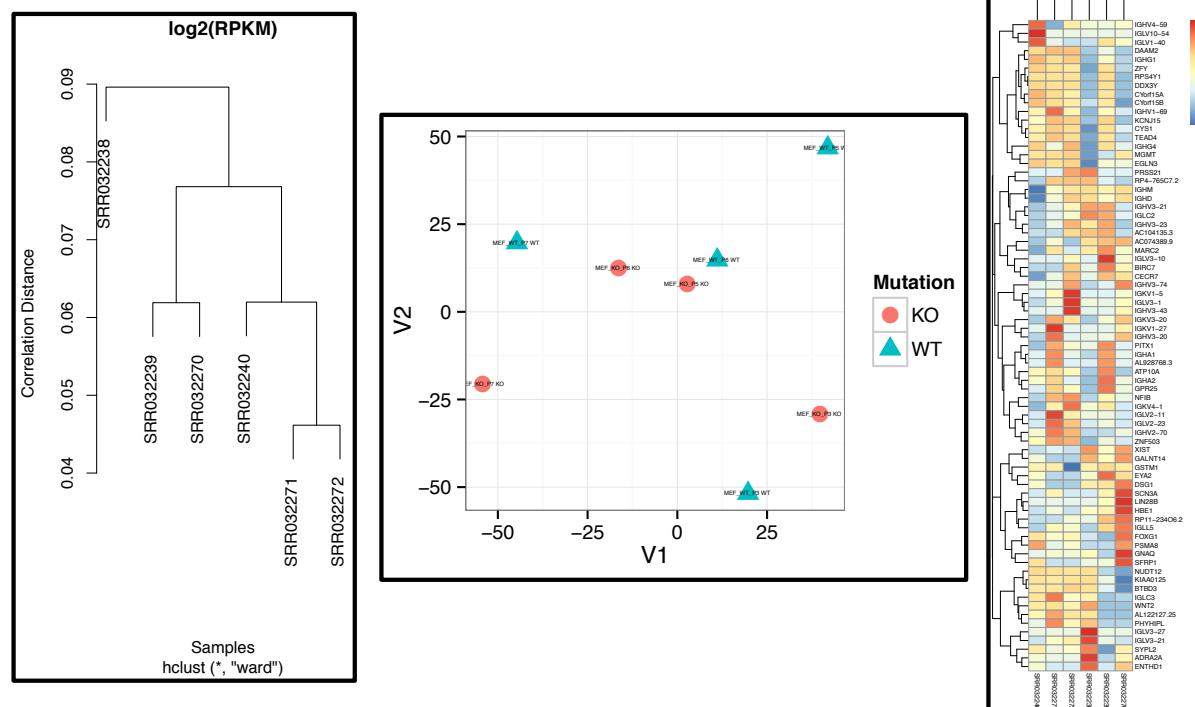
HTSeq

www-huber.embl.de/users/anders/HTSeq

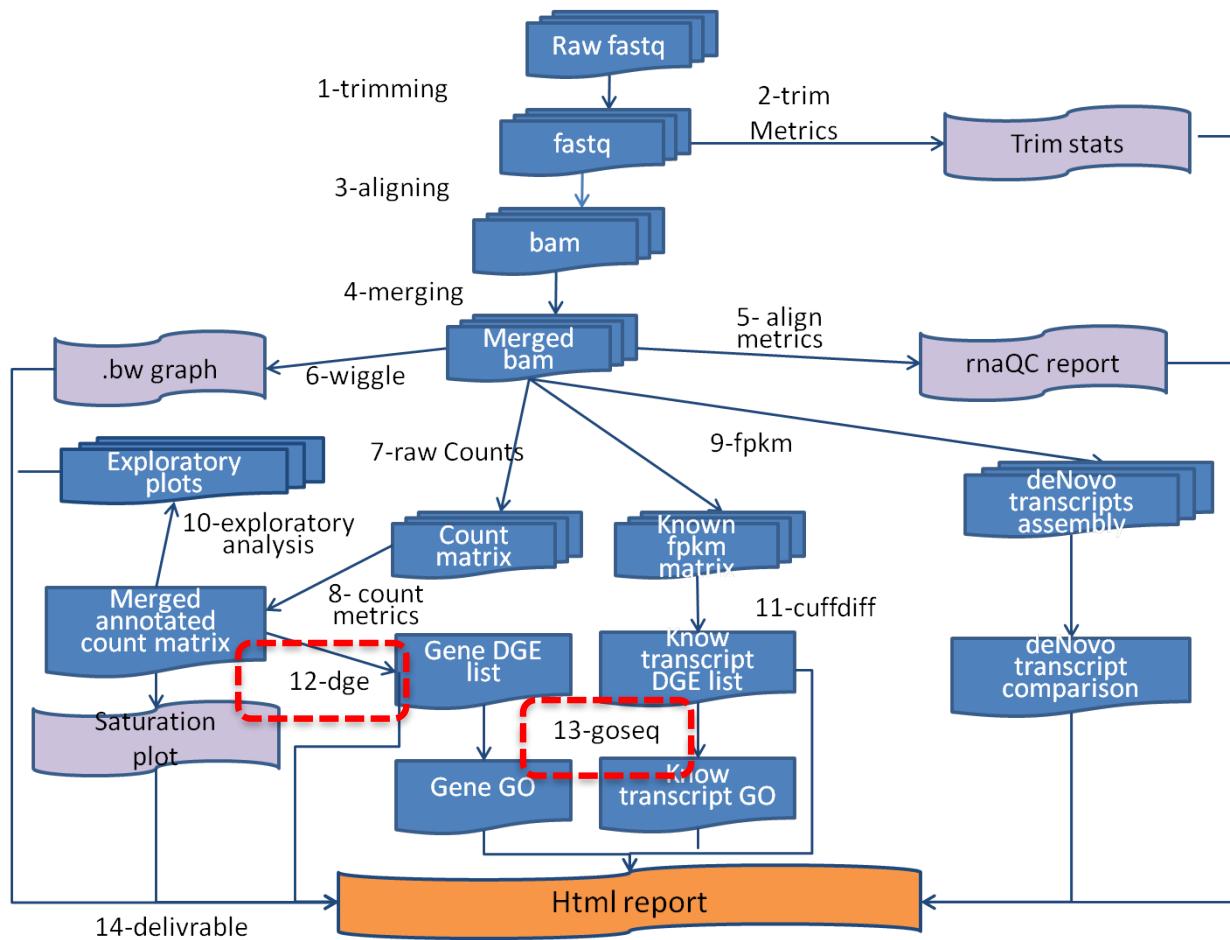
RNA-seq: EDA



gqSeqUtils R package: Exploratory Data Analysis



RNA-Seq: Gene-level DGE

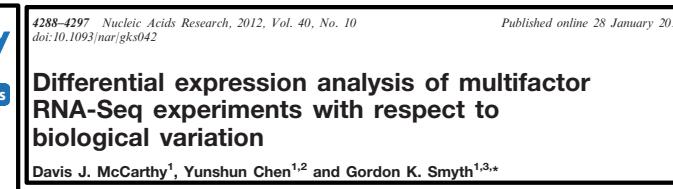


Home-made Rscript: Gene-level DGE

- *edgeR* and *DESeq* : Test the effect of exp. variables on gene-level read counts
- GLM with negative binomial distribution to account for biological variability (not Poisson!!)



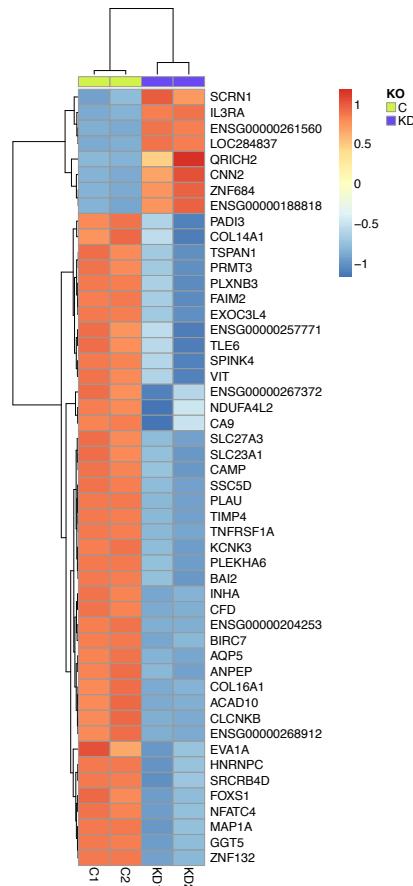
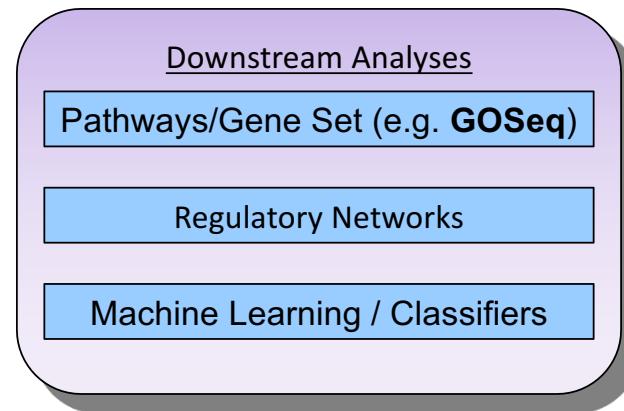
DESeq



edgeR

Differential Gene Expression

SYMBOL	logFC	PValue	FDR	counts.C1	counts.C2	counts.KD1	counts.KD2
HNRNPC	-5.26	9.19E-55	5.71E-50	12611	12404	244	443
FAIM2	-4.82	8.02E-29	2.49E-24	191	194	11	3
AC019178	-6.57	2.14E-28	4.42E-24	100	104	1	1
SSC5D	-2.95	2.39E-27	3.71E-23	2274	2123	318	276
GGT5	-3.03	1.03E-26	1.28E-22	838	803	93	117
EXOC3L4	-3.07	9.19E-21	9.51E-17	359	344	53	34
FOXS1	-4.02	1.69E-19	1.49E-15	113	92	5	8
AQP5	-3.73	2.82E-19	2.18E-15	106	113	9	8
SLC27A3	-2.39	6.97E-18	4.81E-14	736	637	144	129
TIMP4	-3.29	1.21E-17	7.52E-14	126	120	14	12



More info on RNA-seq data analysis

- https://bitbucket.org/mugqic/mugqic_pipelines
- Slides and code that explain step-by-step analysis of:
 - RNA-seq
 - ChIP-seq
 - DNA-seq
 - ...

Outline

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- How many genes are there in the genome?
- How many transcripts?
- How many types of RNA molecules?
- How many different RNA molecules?

Types of RNA

- RNAs involved in protein synthesis:
 - Messenger (mRNA), ribosomal (rRNA), signal recognition (7SL RNA), transfer (tRNA), transfer-messenger (tmRNA)
- RNAs involved in posttranscriptional modifications:
 - snRNA, snoRNA, SmY, ScaRNA, gRNA, RNase P, RNase MRP, TERC, SL RNA, ...
- Regulatory RNAs:
 - aRNA, cis-NAT, crRNA, lncRNA, miRNA, piRNA, siRNA, tasiRNA, rasiRNA, 7SK RNA, circRNA, ...

Source: wikipedia

How many RNAs are there?

- How many lncRNAs (> 200nt)?
- How many microRNAs?
- Why is it so hard to know how many there are?
 - How many experiments are needed to capture all types of RNAs?
 - How many tissues are there in the genome?

Different RNA sequencing technologies

- Just at Illumina:
 - mRNA-seq, total RNA, targeted RNA, small RNA, ribosome profiling, ultra-low or single cell RNA-seq
- CAGE-Seq
- GRO-Seq
- ...

CAGE-Seq

- Cap analysis gene expression (CAGE), was first published by Hayashizaki, Carninci and co-workers in 2003.
- Focuses on the 5' end of the messenger RNAs
- What are the advantages?
- What are the disadvantages?

GRO-Seq

- Global run-on sequencing (GRO-seq) was published by the Lis lab in 2008
- It maps the position, amount, and orientation of transcriptionally engaged RNA polymerases genome-wide
- What are the advantages?
- What are the disadvantages?

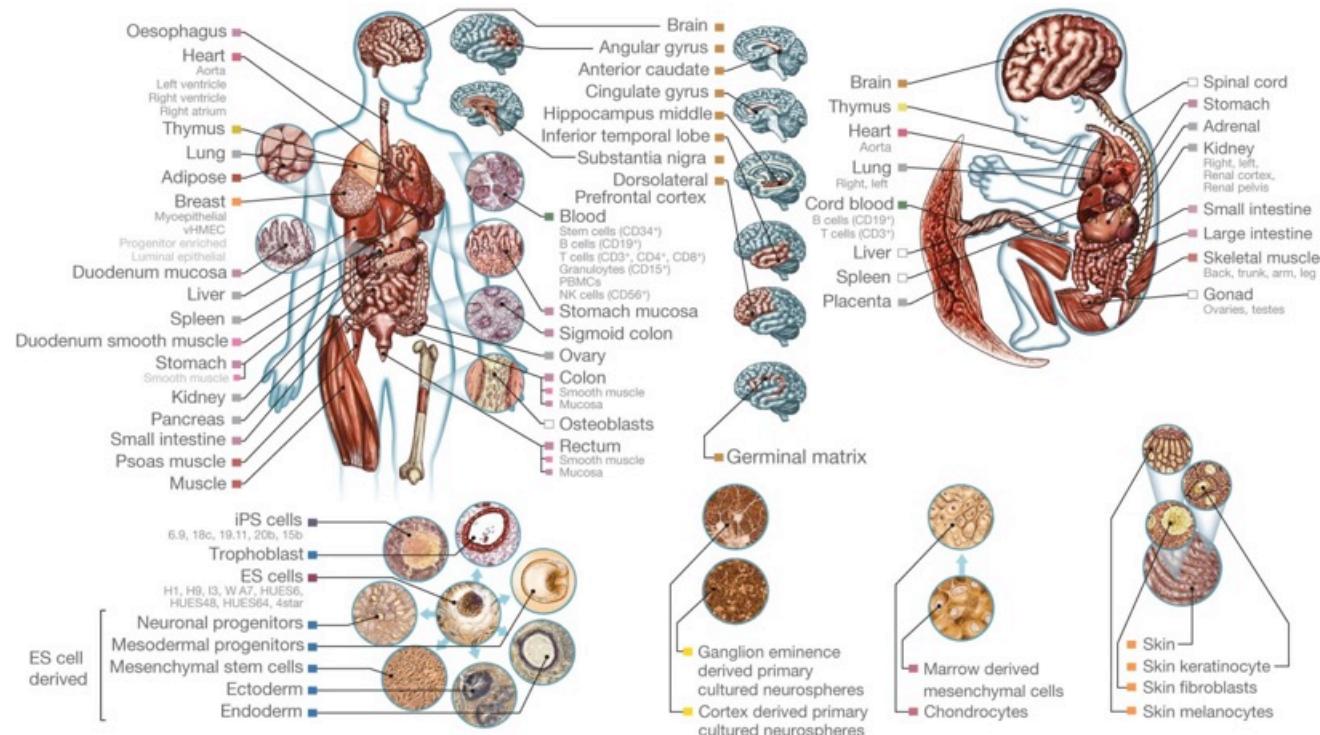
Some early results...

- FANTOM3 identified ~35,000 non-coding transcripts from ~10,000 distinct loci (Carninci 2005)
- More than 40% transcripts are non-polyadenylated (Cheng 2005)
- Only 20% of transcription is associated with protein-coding genes (Kapranov 2007)

How many RNAs are there?

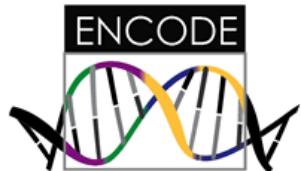
- How many lncRNAs (> 200nt)?
- How many microRNAs?
- Why is it so hard to know how many there are?
 - How many experiments are needed to capture all types of RNAs?
 - [How many tissues are there in the genome?](#)

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**

ENCODE RNA-seq

Genomes Genome Browser Tools Mirrors Downloads My Data Help About Us

CSHL Long RNA-seq Track Settings ENCODE Downloads

 **Long RNA-seq from ENCODE/Cold Spring Harbor Lab** ([ENC RNA-seq](#))

Maximum display mode:

Select views ([help](#)):

Contigs **Plus Signal** **Minus Signal** **Splice Junctions** **Alignments**

Select subtracks by localization and cell line:

All		Whole					
	Localization	Cell	Chromatin	Cytosol	Nucleolus	Nucleoplasm	Nucleus
Cell Line		[+/-]	[+/-]	[+/-]	[+/-]	[+/-]	[+/-]
GM12878 (Tier 1)		<input type="checkbox"/>		<input type="checkbox"/>	<input type="checkbox"/>		
H1-hESC (Tier 1)		<input type="checkbox"/>		<input type="checkbox"/>			
K562 (Tier 1)		<input checked="" type="checkbox"/>					
A549 (Tier 2)		<input type="checkbox"/>		<input type="checkbox"/>			
B cells CD20+ (Tier 2)		<input type="checkbox"/>					
HeLa-S3 (Tier 2)		<input type="checkbox"/>		<input type="checkbox"/>			
HepG2 (Tier 2)		<input type="checkbox"/>		<input type="checkbox"/>			
HUVEC (Tier 2)		<input type="checkbox"/>		<input type="checkbox"/>			
IMR90 (Tier 2)		<input type="checkbox"/>		<input type="checkbox"/>			
MCF-7 (Tier 2)		<input type="checkbox"/>		<input type="checkbox"/>			
Monocytes CD14+ (Tier 2)		<input type="checkbox"/>					
SK-N-SH (Tier 2)		<input type="checkbox"/>		<input type="checkbox"/>			
AG04450		<input type="checkbox"/>					
BJ		<input type="checkbox"/>					
CD34+ Mobilized		<input type="checkbox"/>					

ENCODE RNA-Seq

- >400 datasets
- Only in GM12878, 41 datasets:
 - InsertLength=200 base pairs ReadType=2x75
 - InsertLength=400 base pairs ReadType=2x75
 - InsertLength=None ReadType=1x75D
 - Localization=cell RnaExtract=Long PolyA+ RNA
 - Localization=cell RnaExtract=Long PolyA- RNA
 - Localization=cytosol RnaExtract=Long PolyA+ RNA
 - ...
 - Localization=cell Protocol=Tobacco Acid Pyrophosphatase Only RnaExtract=Short Total RNA
 - ...

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.

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Available resources

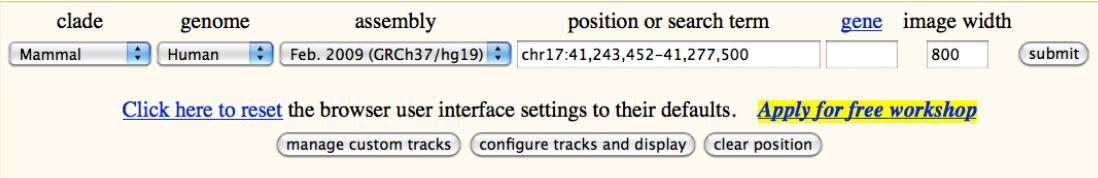
- UCSC Genome Browser
- FANTOM Portal
- ENCODE Data Portal
- IHEC Data Portal
- GTEx Portal
- ...

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	gene	image width
Mammal	Human	Feb. 2009 (GRCh37/hg19)	chr17:41,243,452-41,277,500		800

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

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



Francis Ouellette, bioinformatics.ca



Basic tracks

Mapping and Sequencing Tracks

Base Position	Chromosome Band	STS Markers	18 FISH Clones	Recomb Rate	Map Contigs
dense	hide	hide	hide	hide	hide
Assembly	GRC Map Contigs	Gap	BAC End Pairs	18 Fosmid End Pairs	GC Percent
hide	hide	hide	hide	hide	hide
GRC Patch Release	Hg18 Diff	NCBI Incident	Short Match	Restr Enzymes	Wiki Track
hide	hide	hide	hide	hide	hide
BU ORChID	Mapability				
hide	hide				

Phenotype and Disease Associations

18 GAD View	DECIPHER	OMIM AV SNPs	OMIM Genes	OMIM Pheno Loci	GWAS Catalog
hide	hide	hide	hide	hide	hide
18 RGD Human QTL	18 RGD Rat QTL	18 MGI Mouse QTL			
hide	hide	hide			

Genes and Gene Prediction Tracks

UCSC Genes	Alt Events	GENCODE Genes V4	GENCODE Genes V7	CCDS	RefSeq Genes
pack	hide	hide	hide	hide	hide
Other RefSeq	MGC Genes	ORFeome Clones	TransMap...	Vega Genes	Ensembl Genes
hide	hide	hide	hide	hide	hide
AccView Genes	N-SCAN	SGP Genes	Geneid Genes	Genscan Genes	Exoniphy
hide	hide	hide	hide	hide	hide
tRNA Genes	H-Inv 7.0	17 EvoFold	sno/miRNA	IKMC Genes	
hide	hide	hide	hide	Mapped	hide

mRNA and EST Tracks

Human mRNAs	Spliced ESTs	Human ESTs	Other mRNAs	Other ESTs	18 H-Inv
hide	hide	hide	hide	hide	hide
Gene Bounds	18 SIB Alt-Splicing	18 Poly(A)	18 CGAP SAGE	Human RNA Editing	
hide	hide	hide	hide	hide	

Conservation, variation and repeats

Comparative Genomics

Conservation	18 Cons Indels	GERP	18 Evo Cpg	Sheep Chain/Net	Primate Chain/Net
full	MmCf hide	hide	hide	hide	hide
Placental Chain/Net	hg19Patch2	Vertebrate Chain/Net	Sea hare Chain/Net		
hide	hide	hide	hide		

Neandertal Assembly and Analysis

18 H-C Coding Diffs	18 Sel Swp Scan (S)	18 5% Lowest S	18 S SNPs	18 Cand. Gene Flow	Neandertal Seq
hide	hide	hide	hide	hide	hide
18 Neandertal Mito [No data-chr17]					

Variation and Repeats

Common SNPs(132)	Flagged SNPs(132)	Mult. SNPs(132)	All SNPs(132)	SNPs (131)	Arrays
hide	hide	hide	hide	hide	hide
GIS DNA PET	HAIB Genotype	18 SNP Arrays	HGDP Allele Freq	18 HapMap SNPs	DGV Struct Var
hide	hide	hide	hide	hide	hide
Segmental Dups	RepeatMasker	Interrupted Rpts	Simple Repeats	Microsatellite	Self Chain
hide	full	hide	hide	hide	hide
18 Genome Variants	Numts Sequence				
hide	hide				

Expression and Regulation

Expression refresh

Affy Exon Array <input type="button" value="hide"/>	Affy GNF1H <input type="button" value="hide"/>	Affy RNA Loc <input type="button" value="hide"/>	Affy U133 <input type="button" value="hide"/>	Affy U133Plus2 <input type="button" value="hide"/>	Affy U95 <input type="button" value="hide"/>
Allen Brain <input type="button" value="hide"/>	Burge RNA-seq <input type="button" value="hide"/>	ENC Exon Array... <input type="button" value="hide"/>	ENC ProtGeno... <input type="button" value="hide"/>	ENC RNA-seq... <input type="button" value="hide"/>	GNF Atlas 2 <input type="button" value="hide"/>
18 Illumina WG-6 <input type="button" value="hide"/>	18 Sestan Brain <input type="button" value="hide"/>				

Regulation refresh

ENCODE Regulation... <input type="button" value="show"/>	18 CD34 DnaseI <input type="button" value="hide"/>	CpG Islands <input type="button" value="hide"/>	ENC DNA Methyl... <input type="button" value="hide"/>	ENC DNase/FAIRE... <input type="button" value="hide"/>	ENC Histone... <input type="button" value="hide"/>
ENC RNA Binding... <input type="button" value="hide"/>	ENC TF Binding... <input type="button" value="hide"/>	18 ORegAnno <input type="button" value="hide"/>	Stanf Nucleosome <input type="button" value="hide"/>	SUNY SwitchGear <input type="button" value="hide"/>	17 SwitchGear TSS <input type="button" value="hide"/>
TFBS Conserved <input type="button" value="hide"/>	TS miRNA sites <input type="button" value="hide"/>	UMMS Brain Hist <input type="button" value="hide"/>	Vista Enhancers <input type="button" value="hide"/>	18 NKI Nuc Lamina... <input type="button" value="hide"/>	18 UCSF Brain Methyl <input type="button" value="hide"/>

Integrated ENCODE tracks

Home Genomes Genome Browser Blat Tables Gene Sorter PCR Session FAQ Help

ENCODE Regulation Super-track Settings

 **Integrated Regulation from ENCODE Tracks** ([▲ All Regulation tracks](#))

Display mode:

Transcription Transcription Levels Assayed by RNA-seq on 7 Cell Lines from ENCODE

Layered H3K4Me1 H3K4Me1 Mark (Often Found Near Regulatory Elements) on 7 cell lines from ENCODE

Layered H3K4Me3 H3K4Me3 Mark (Often Found Near Promoters) on 7 cell lines from ENCODE

Layered H3K27Ac H3K27Ac Mark (Often Found Near Active Regulatory Elements) on 7 cell lines from ENCODE

DNase Clusters Digital DNaseI Hypersensitivity Clusters from ENCODE

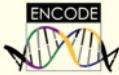
Txn Factor ChIP Transcription Factor ChIP-seq from ENCODE

NOTE: Early access to additional track data may be available on the [Preview Browser](#).

RNA-Seq tracks

Home Genomes Genome Browser Blat Tables Gene Sorter PCR Session FAQ Help

Transcription Track Settings

 **Transcription Levels Assayed by RNA-seq on 7 Cell Lines**

Display mode: full [Reset to defaults](#)

Overlay method: transparent

Type of graph: bar

Track height: 30 pixels (range: 11 to 100)

Vertical viewing range: min: 0 max: 8 (range: 0 to 65500)

Data view scaling: use vertical viewing range setting Always include zero: OFF

Transform function: Transform data points by: LOG (ln(1+x))

Windowing function: mean+whiskers Smoothing window: OFF pixels

Draw y indicator lines: at y = 0.0: OFF at y = 0 OFF

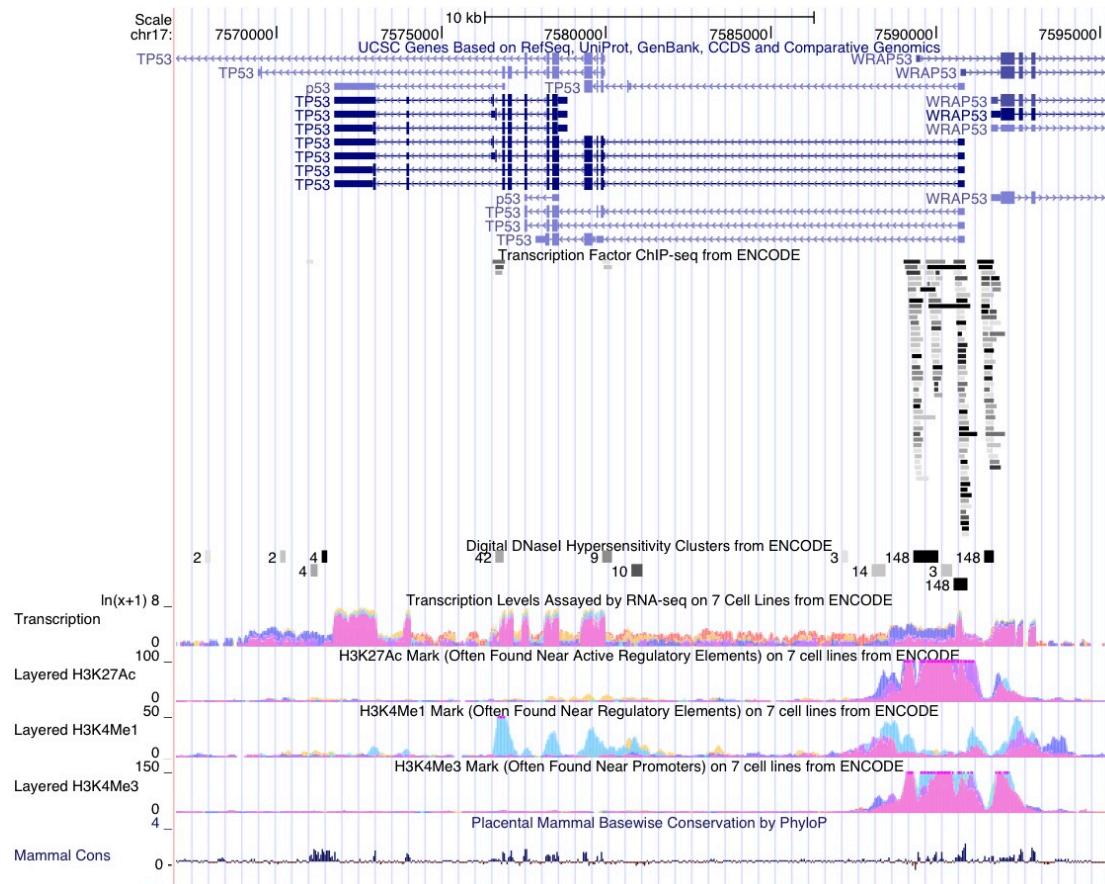
[Graph configuration help](#)

List subtracks: only selected/visible all (7 of 7 selected) **Restricted Until**

<input checked="" type="checkbox"/> Gm12878	Transcription of Gm12878 cells from ENCODE	<input type="button"/> schema	2011-10-21
<input checked="" type="checkbox"/> H1-hESC	Transcription of H1-hESC cells from ENCODE	<input type="button"/> schema	2011-10-21
<input checked="" type="checkbox"/> HSMM	Transcription of HSMM cells from ENCODE	<input type="button"/> schema	2011-10-21
<input checked="" type="checkbox"/> HUVEC	Transcription of HUVEC cells from ENCODE	<input type="button"/> schema	2011-10-21
<input checked="" type="checkbox"/> K562	Transcription of K562 cells from ENCODE	<input type="button"/> schema	2011-10-21
<input checked="" type="checkbox"/> NHEK	Transcription of NHEK cells from ENCODE	<input type="button"/> schema	2011-10-21
<input checked="" type="checkbox"/> NHLF	Transcription of NHLF cells from ENCODE	<input type="button"/> schema	2011-10-21

7 of 7 selected

P53 + ENCODE



FANTOM Portal

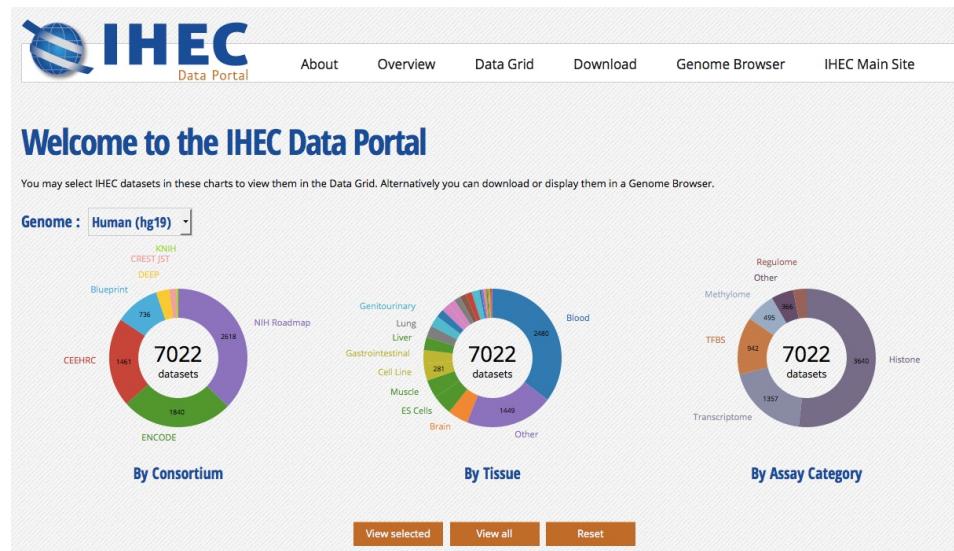
- **FANTOM1-3** focused on identifying the transcribed components of mammalian cells
- **FANTOM4** focused on the dynamics of transcription start site (TSS) usage via deepCAGE monitoring during monocytic differentiation
- **FANTOM5** focused on systematically investigating transcriptional expression in virtually all cell types across the human body and mice
- <http://fantom.gsc.riken.jp/>

ENCODE Data Portal

- Over 8000 experiments from ENCODE and NIH Roadmap
 - 4934 ChIP-Seq
 - 1079 RNA-Seq
 - 729 DNA-Seq
 - ...
- Can navigate through all meta-data fields to find most relevant datasets
- Can download datasets
- <https://www.encodeproject.org/>

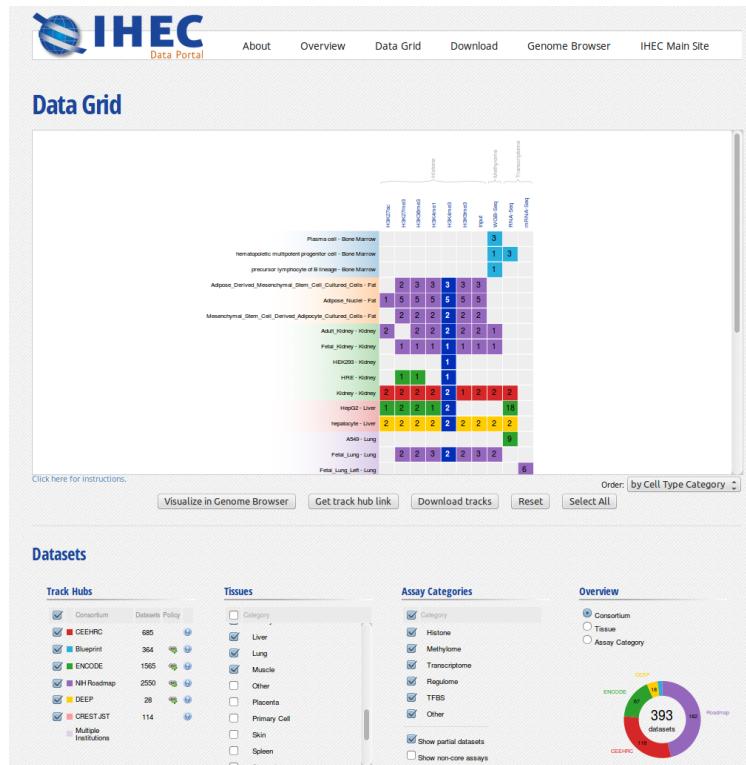
IHEC Data Portal

- Launched in June 2014: <http://epigenomesportal.ca/ihec>
- As of now, includes:
 - Over 7300 human datasets (hg19 and hg38)
 - Over 5000 core set of assays
 - Over >200 full reference epigenomes
 - Data from: Blueprint, CEEHRC, CREST, DEEP, ENCODE, KNIH, NIH Roadmap



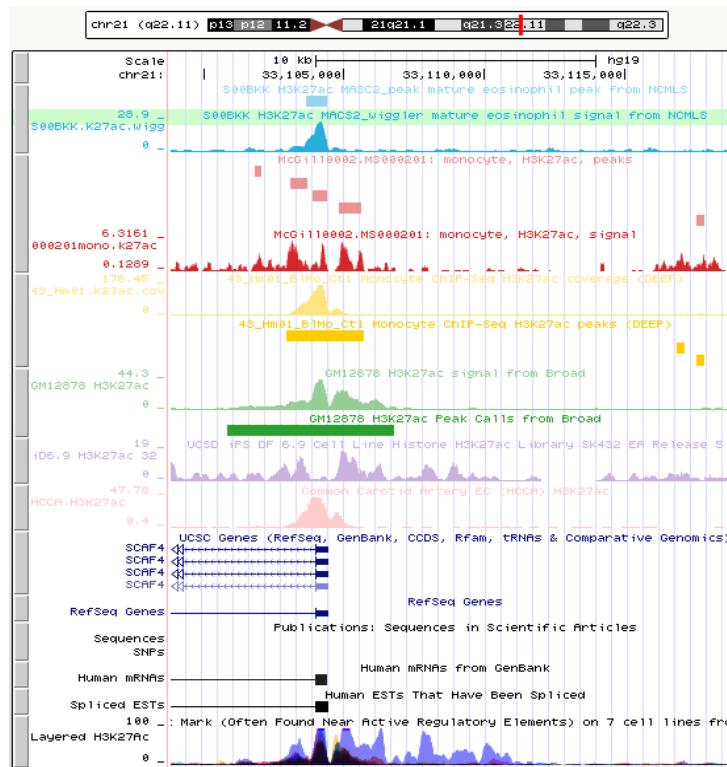
Datasets Grid

- X axis: Assay
- Y axis: Cell type
- Grid cell displays number of available datasets
- Cell color: Consortium, or light pink for multiple consortiums
- Sortable on:
 - Cell type
 - Tissue
 - Consortium



Dynamic track hubs and Genome Browser

- Usable in UCSC Genome Browser, and other tools that support hubs, such as Ensembl
- Hub includes all metadata recommended by IHEC Ecosystem Workgroup, when available



GTEx

- Genotype-Tissue Expression (GTEx) project is a resource to study human gene expression and regulation and its relationship to genetic variation
- Also includes a large collection of transcriptome datasets (>8000) from many tissues
- <http://www.gtexportal.org/home/>

Questions?

Acknowledgements

Lab

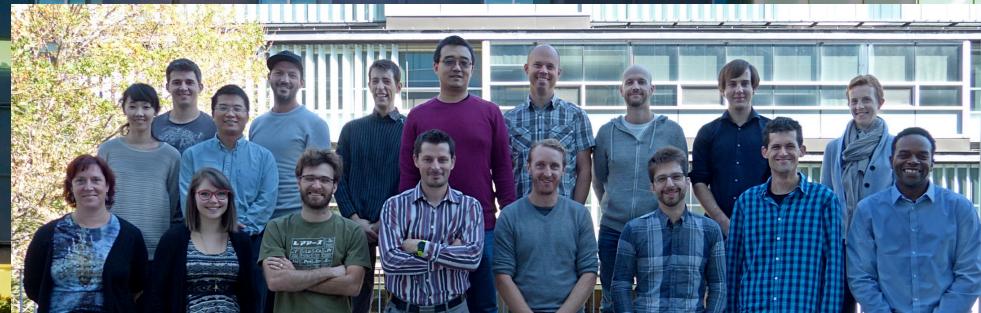
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