

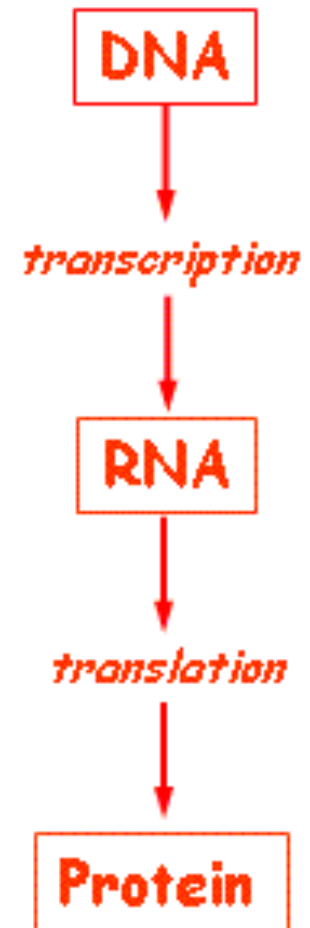
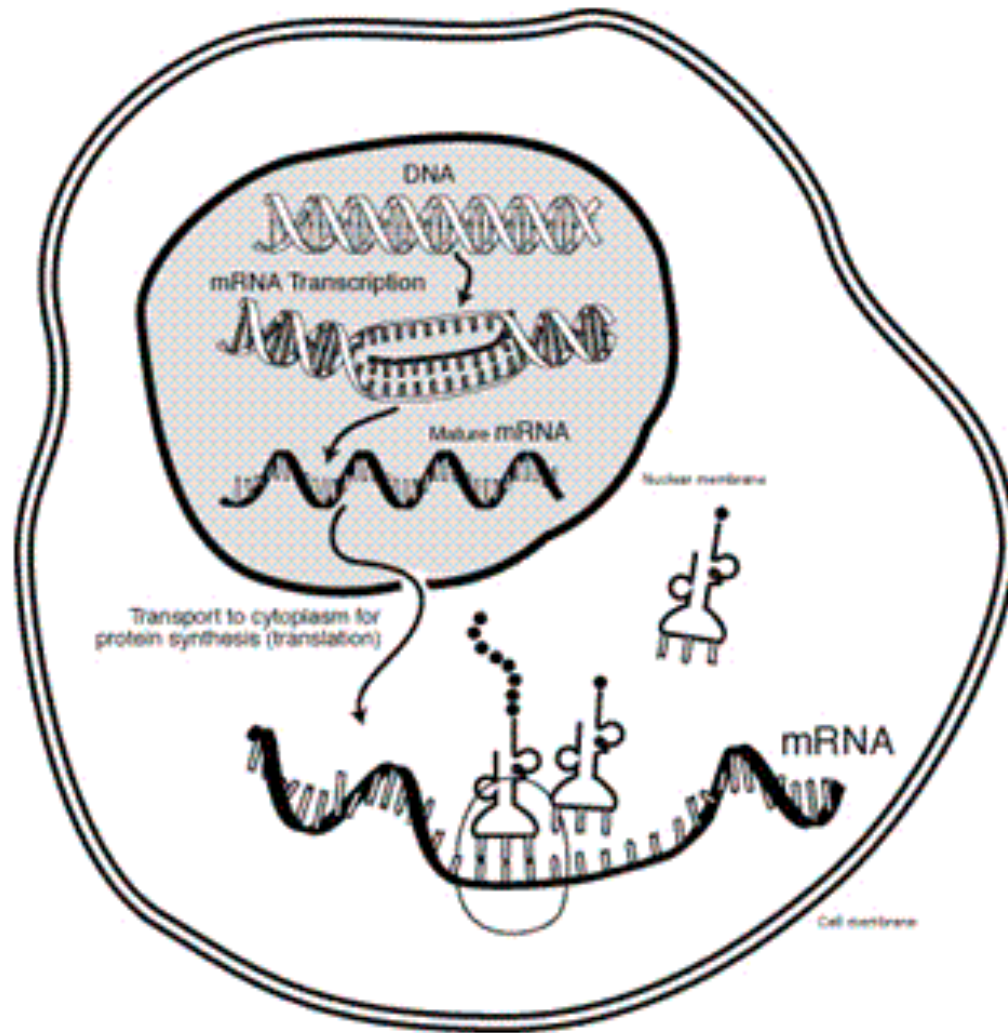
# RNA-Seq

Jim Knowles, MD-PhD

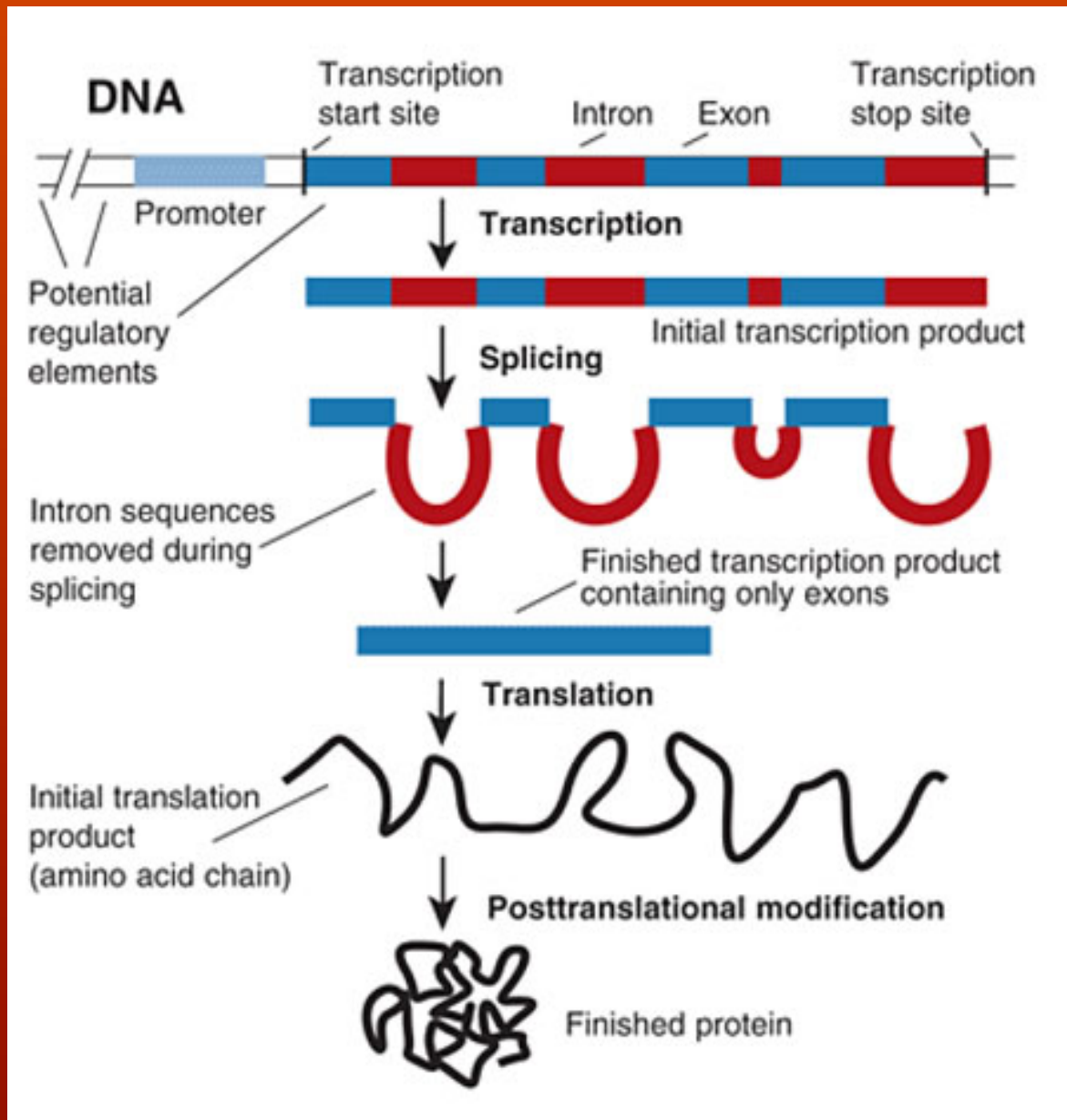
[knowles@med.usc.edu](mailto:knowles@med.usc.edu)

September 17, 2013

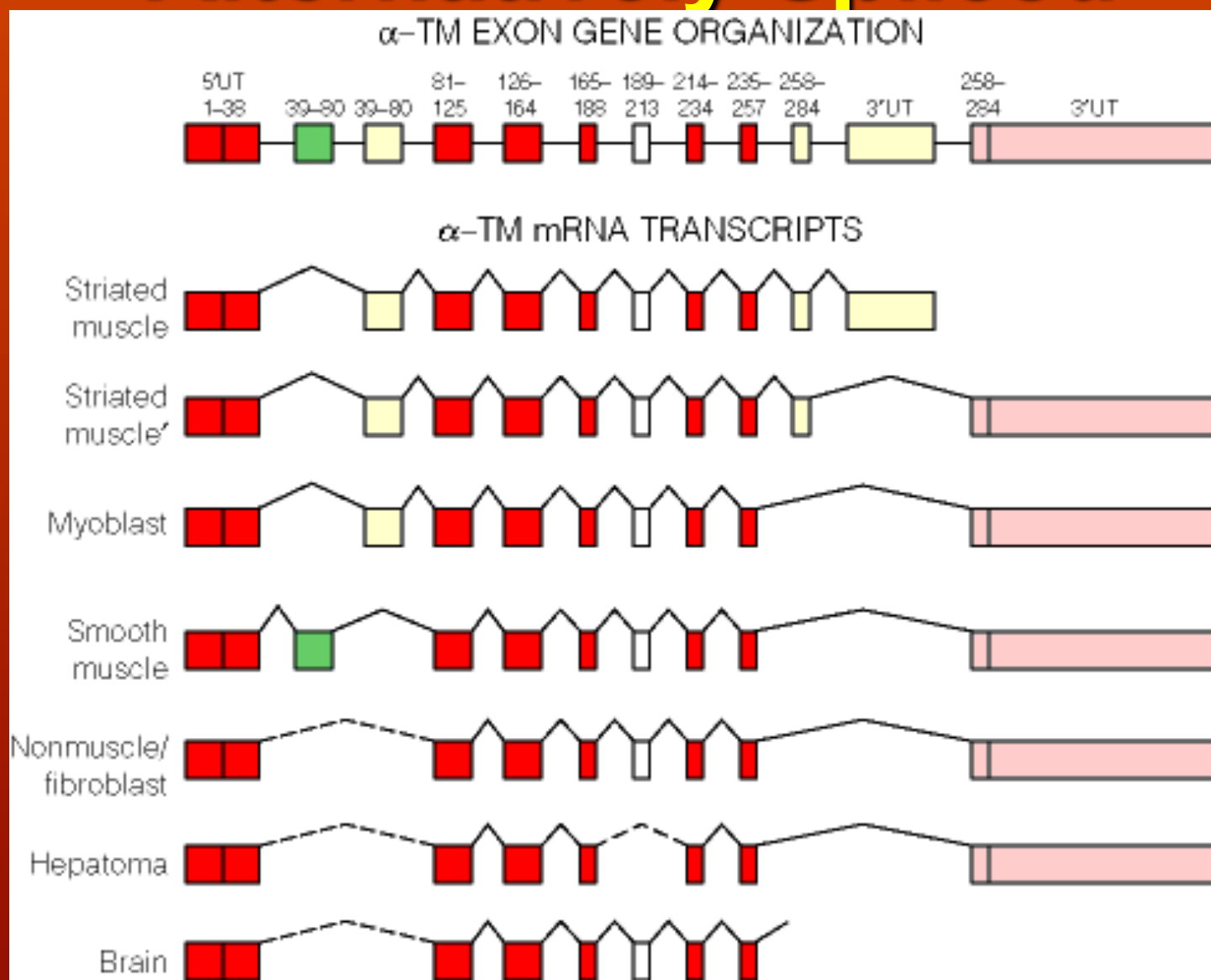
# Central Dogma of Molecular Biology



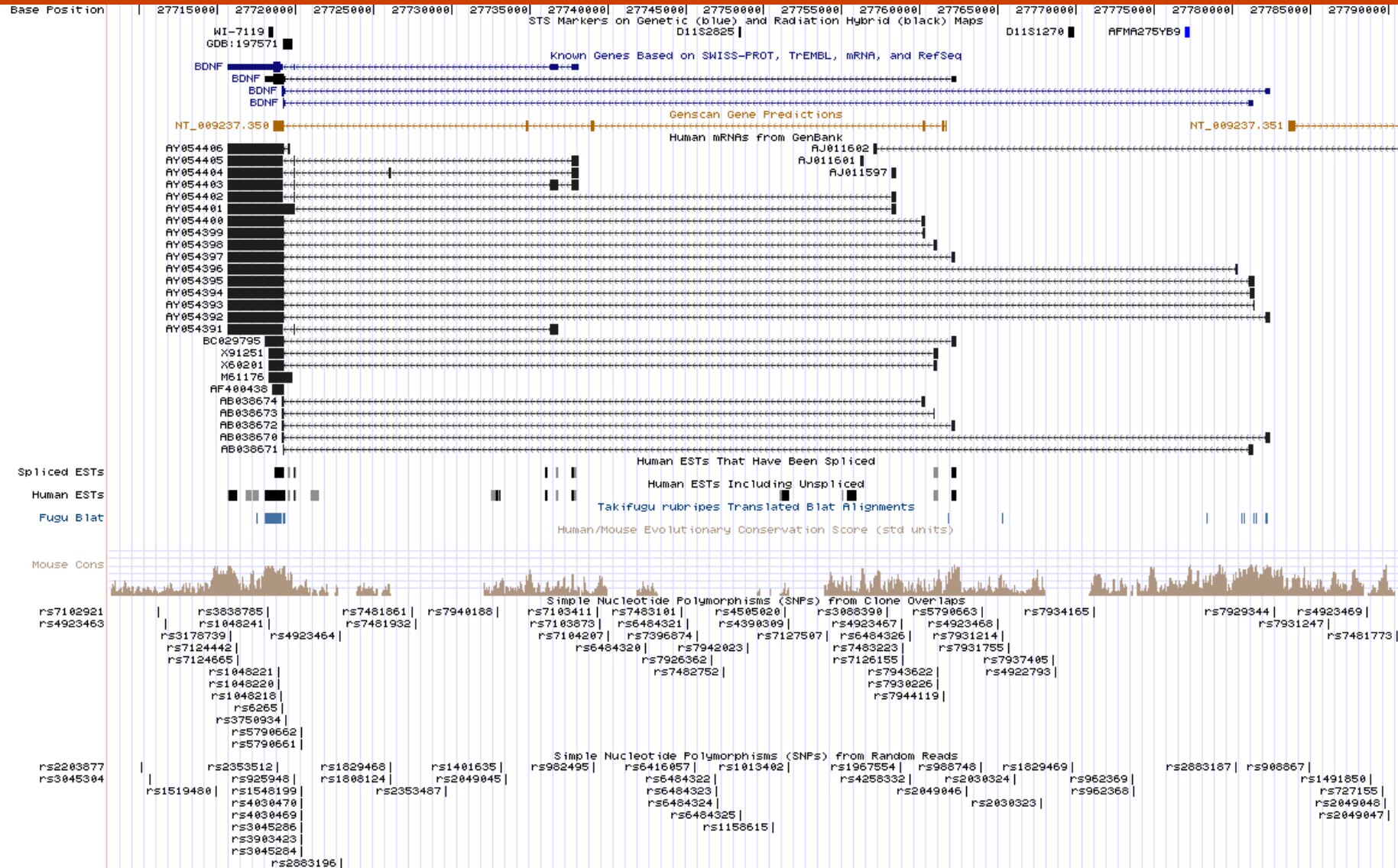
# Eukaryotic Genes are Spliced



# Eukaryotic Genes are Alternatively Spliced



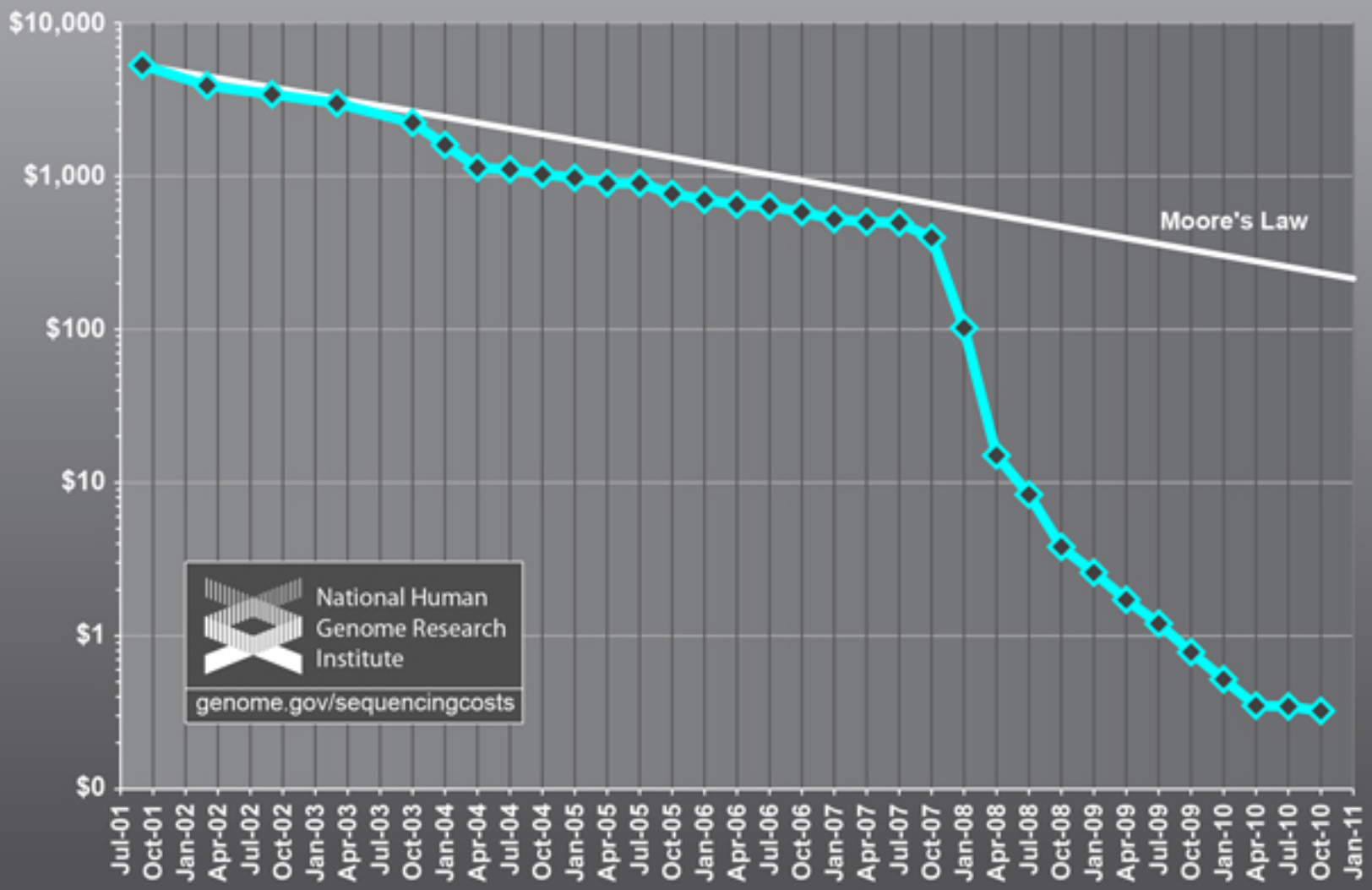
# UCSC Genome Browser genome.ucsc.edu




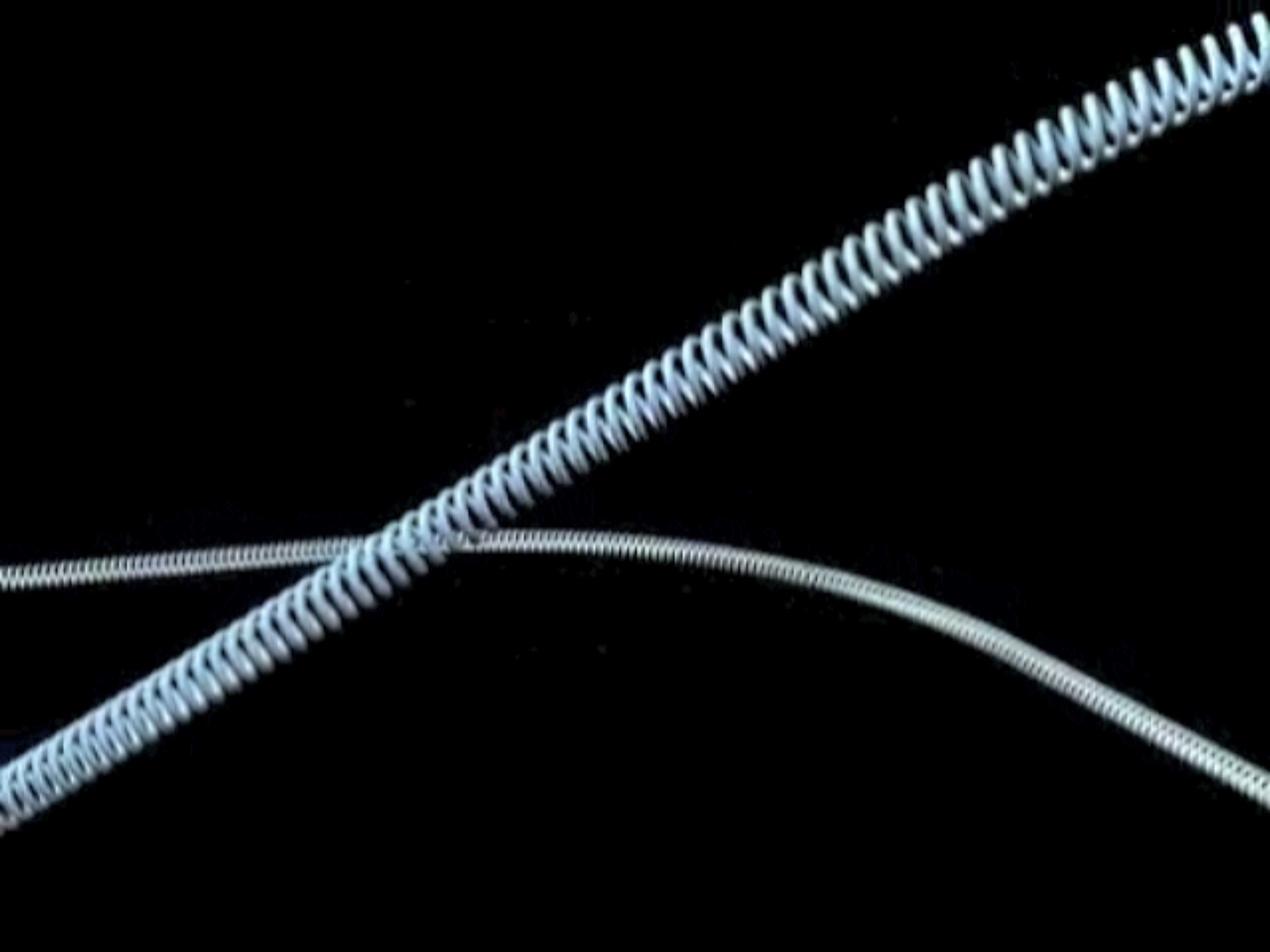
# Next Generation Sequencing Applications

- Whole Genome Sequencing (WGS)
- Re-sequencing (Exons, other regions)
- **Transcriptome Sequencing (RNA-Seq)**
- Chromatin Immunoprecipitation Sequencing (ChIP-Seq)
- Meta-Genomics
- Other Methods
  - Footprinting
  - 3D mapping

# Cost per Megabase of DNA Sequence



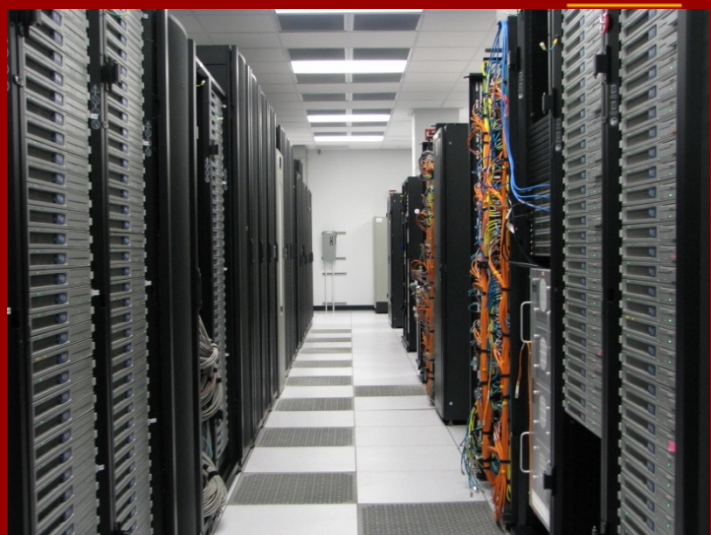
 National Human Genome Research Institute  
[genome.gov/sequencingcosts](http://genome.gov/sequencingcosts)







ILMN HiSeq @ ZNI



~1200 cores @ HPCC



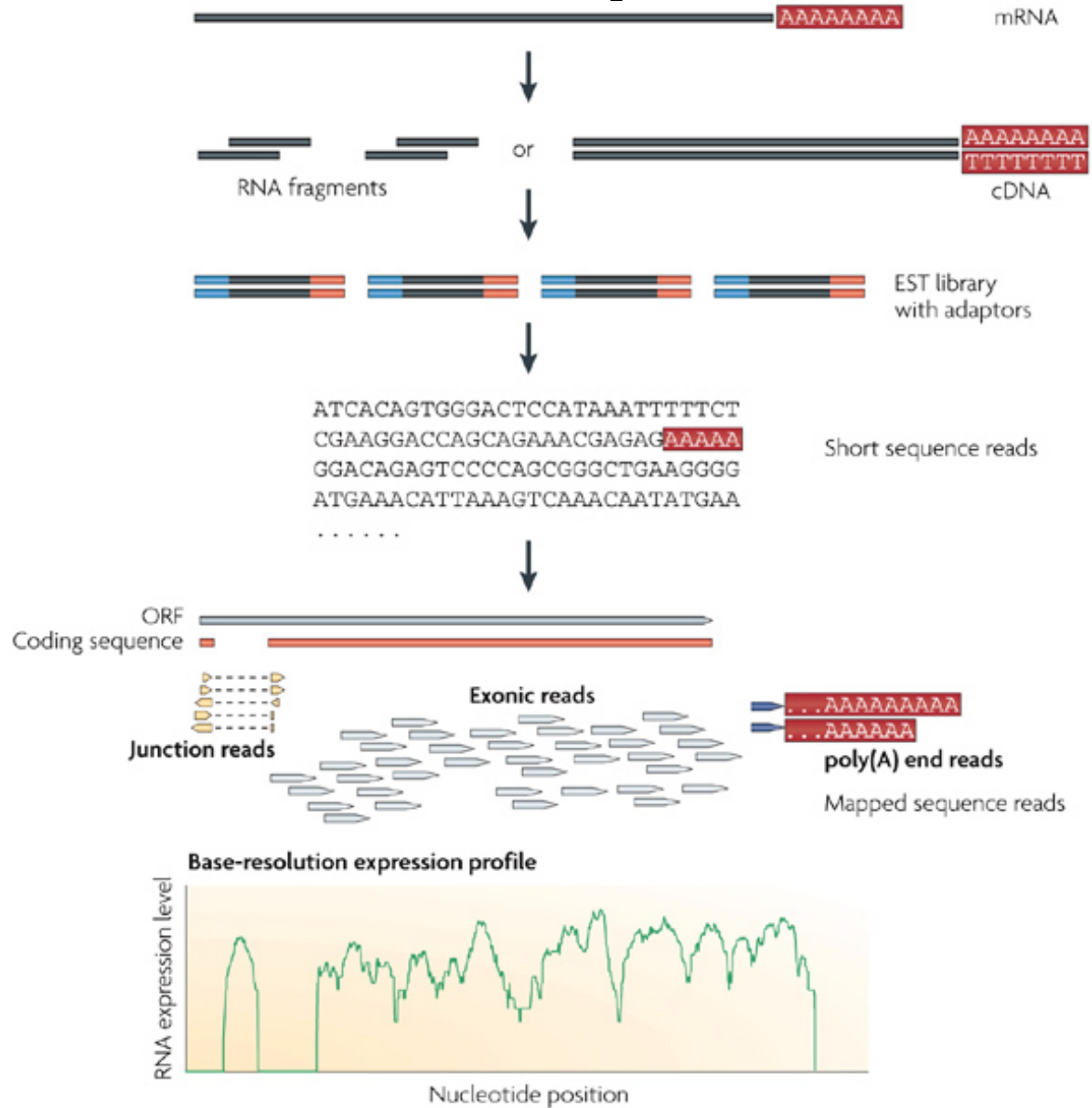
400 TB @ ZNI



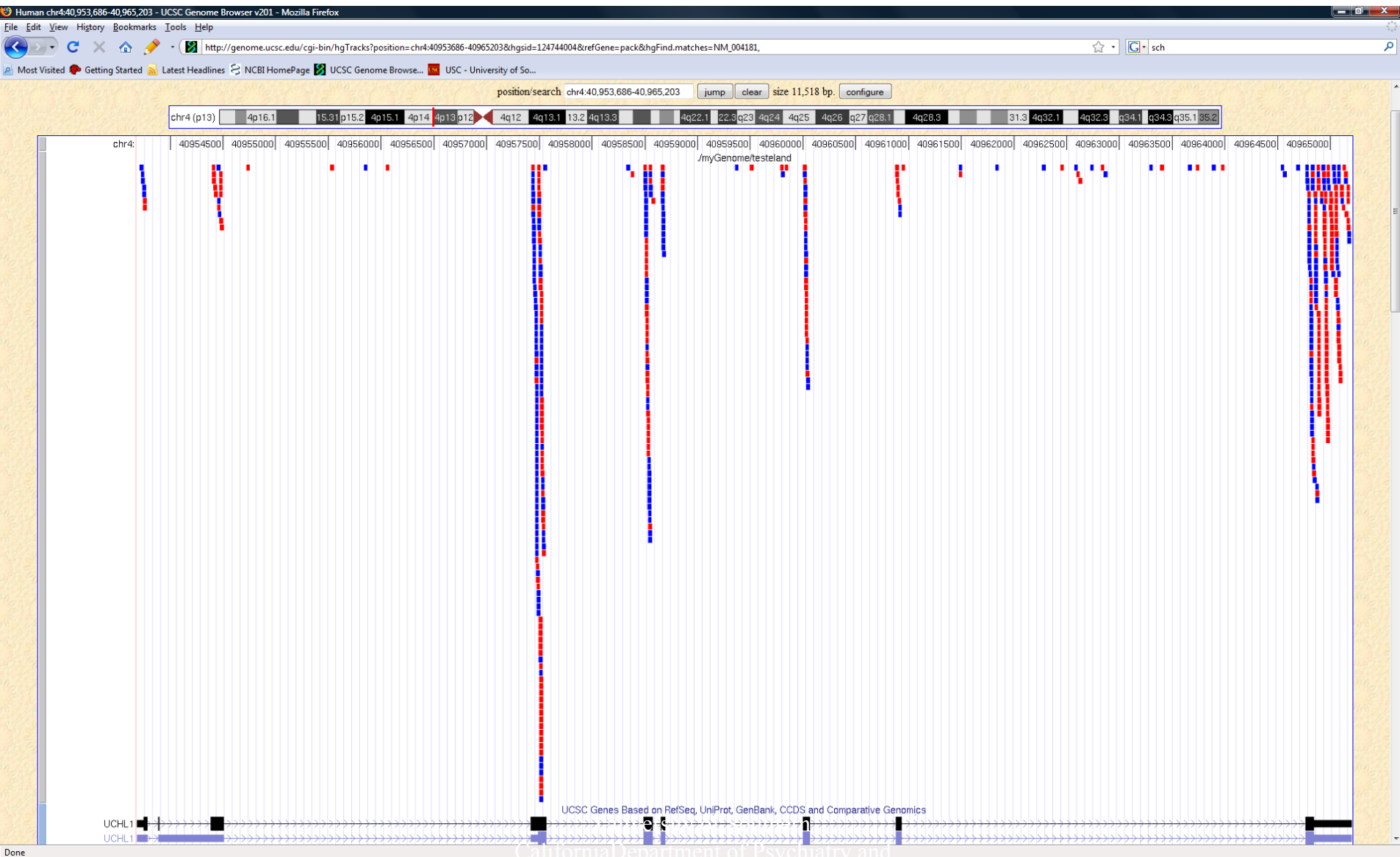
1.2 PB @ HPCC



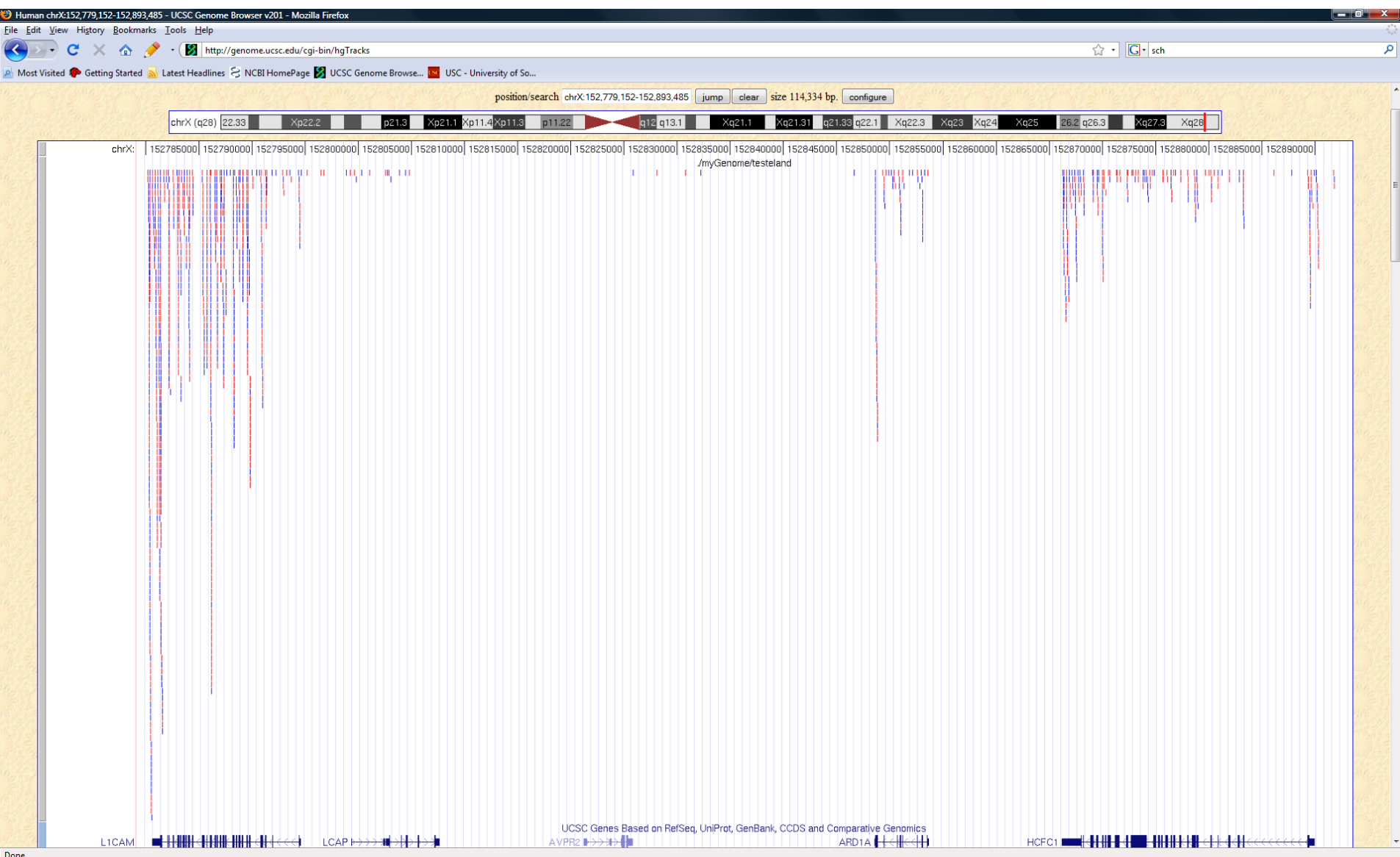
# RNA-Seq Method



# RNA-Seq: UCHL1 expression



# RNA-Seq: Differential Expression



# RseqFlow: Workflow for RNA-Seq Data Analysis

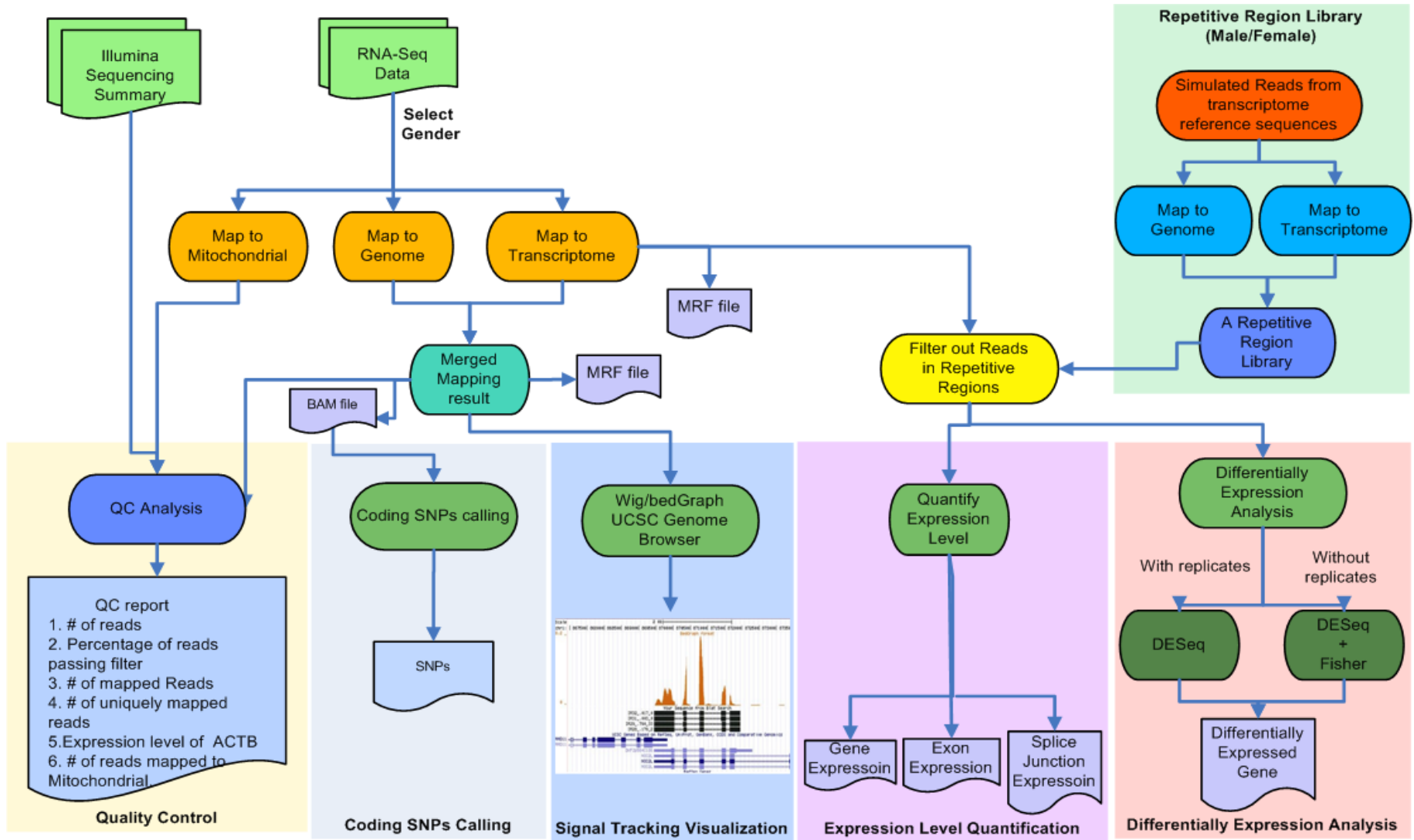
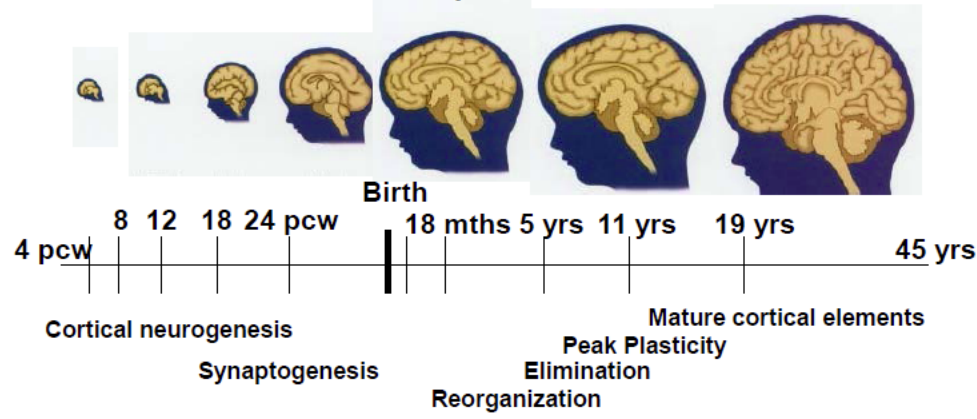


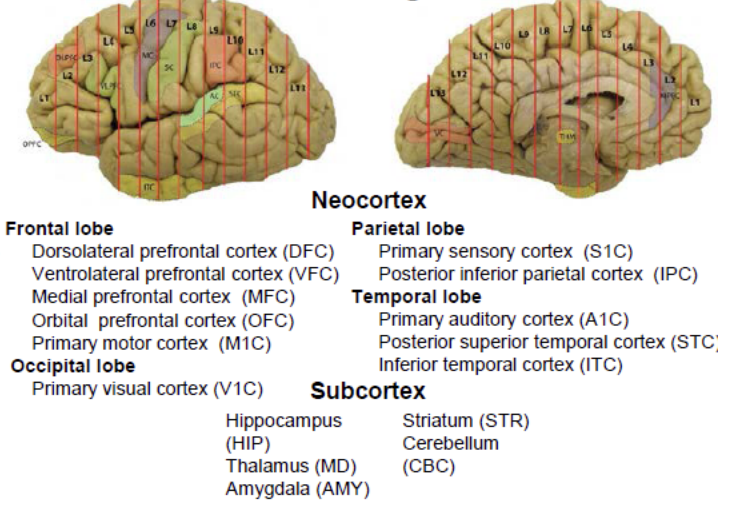
Figure 1. RNA-seq Workflow.

Wang Y, Mehta G, Mayani R, Lu J, Souaiaia T, Chen Y, Clark A, Yoon HJ, Wan L, Evgrafov OV, Knowles JA, Deelman E, Chen T. *Bioinformatics* (2011) 27 (18): 2598-2600. doi: 10.1093/bioinformatics/btr441

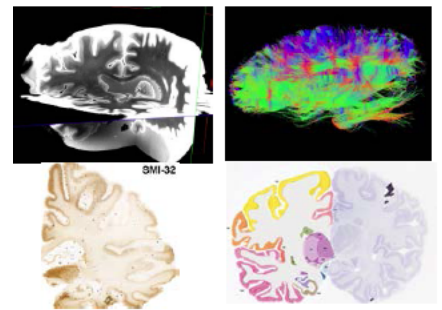
# Brain Development



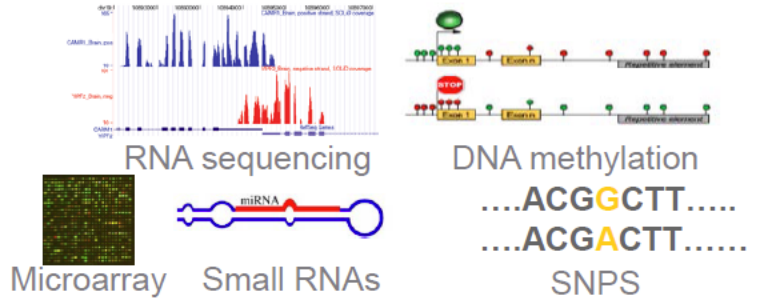
# Brain Regions



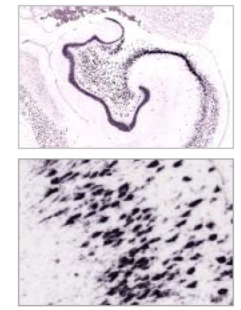
## Anatomy Reference Framework



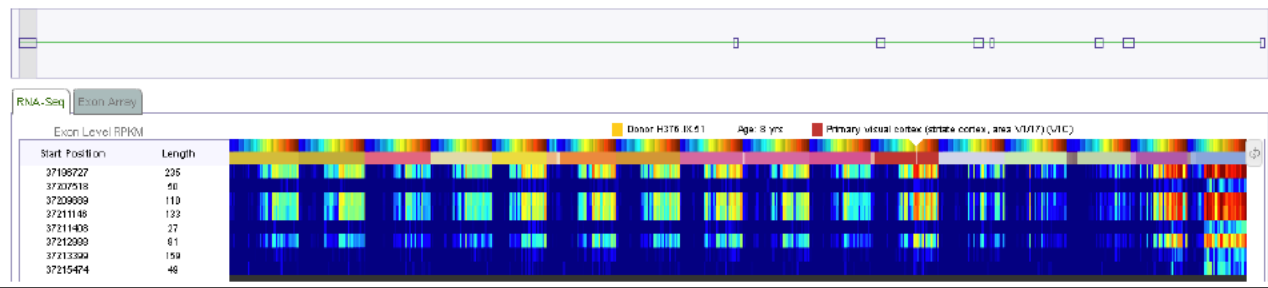
## Transcriptome, Epigenome, Genome Profiling



## Cellular Resolution Gene Expression

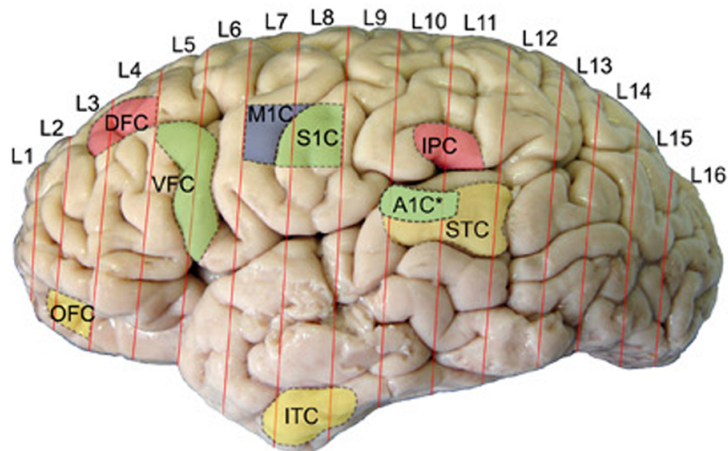


## Public Resource

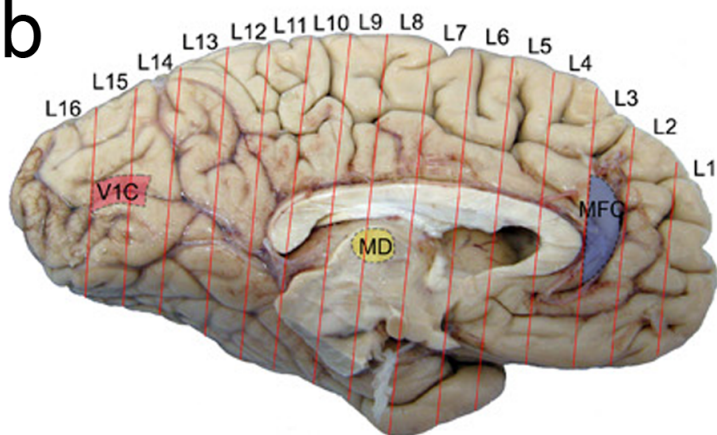


# Adult Tissue Sampling

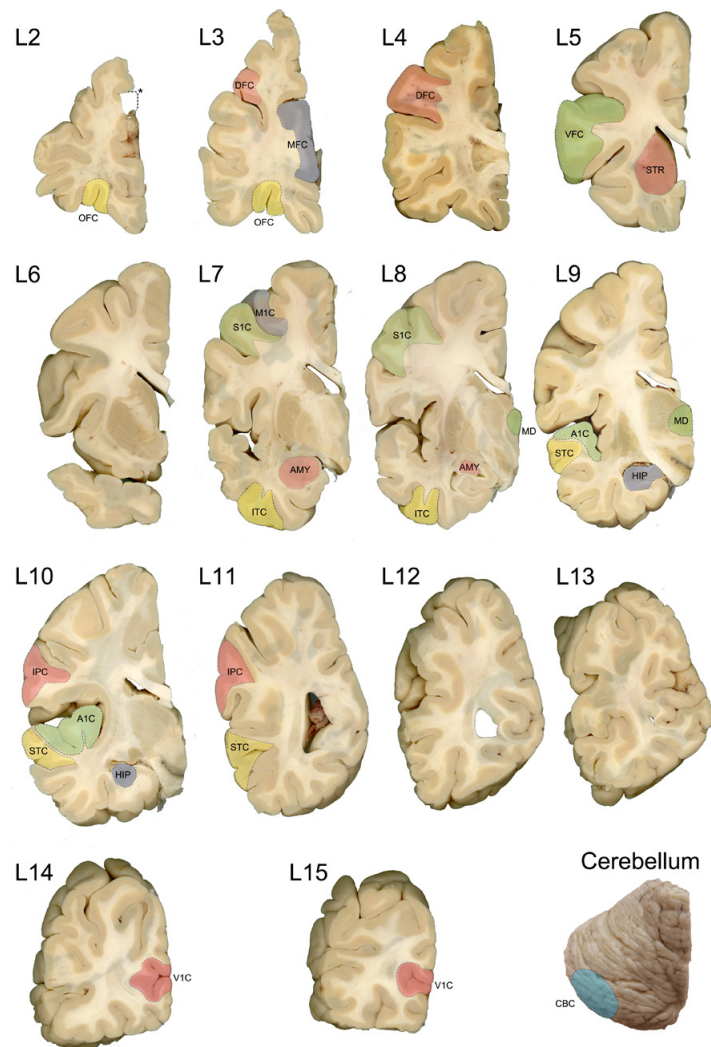
a



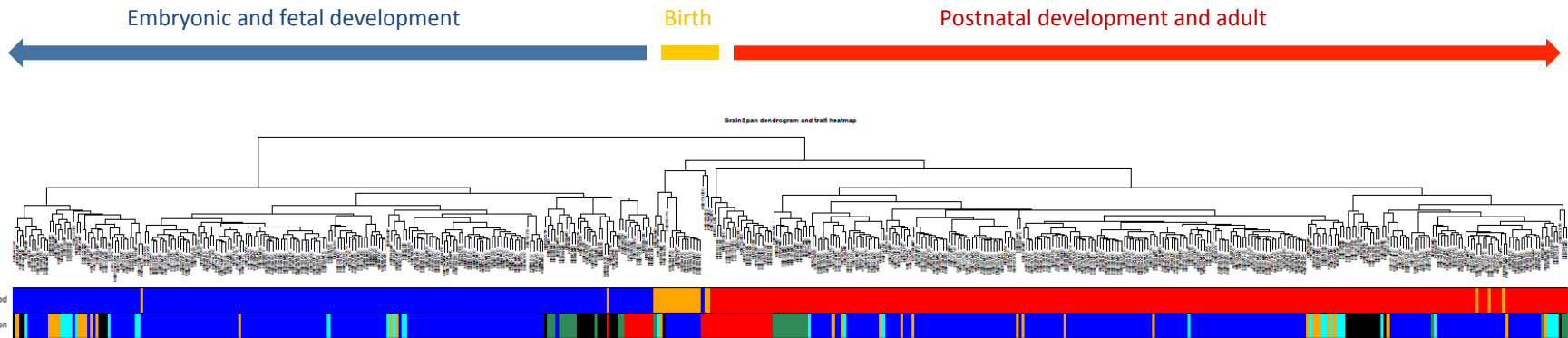
b



c



# Hierarchical clustering and analyses of all RNA-seq samples



## Top row color:

Blue is prenatal development  
Orange is period 7 (late fetal to birth)  
Red is postnatal development and adulthood

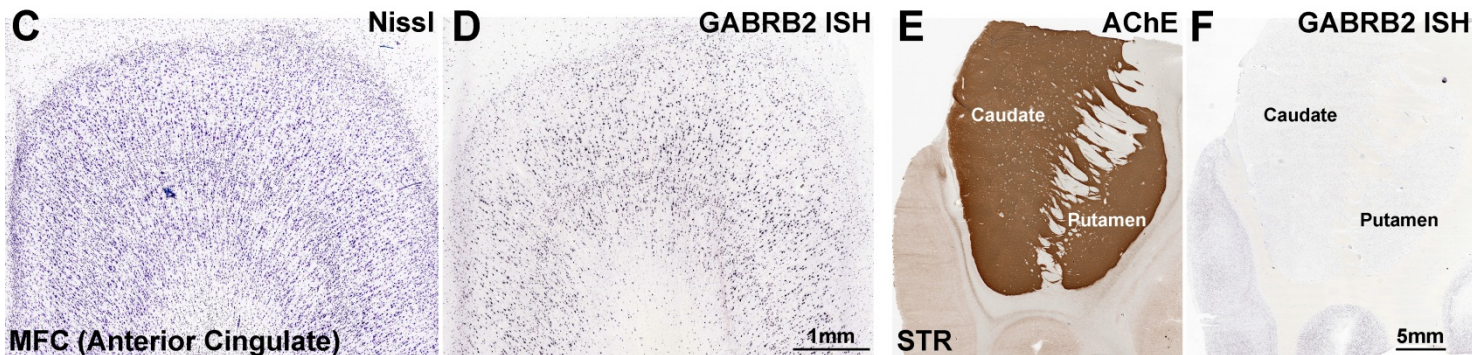
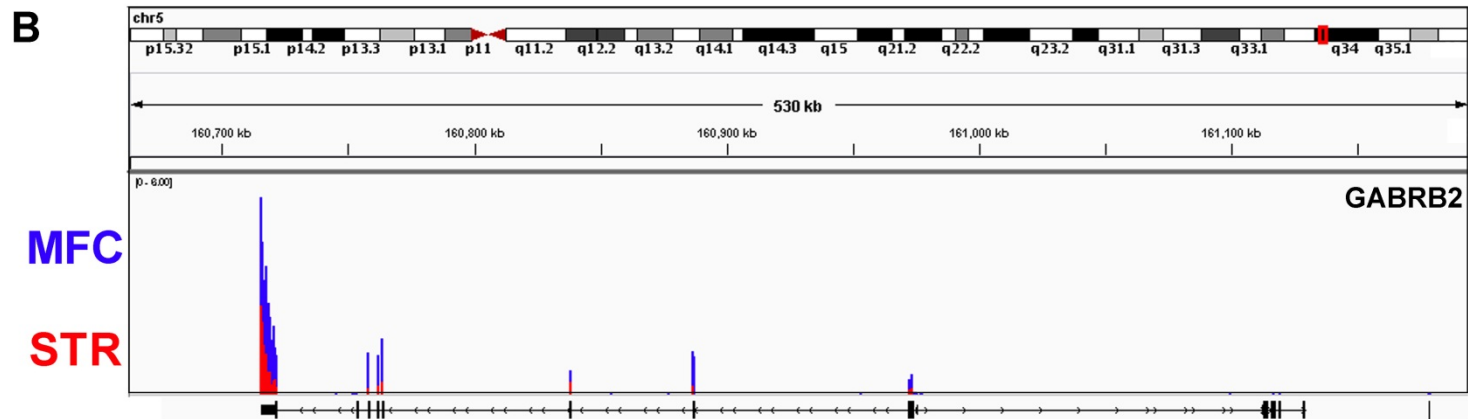
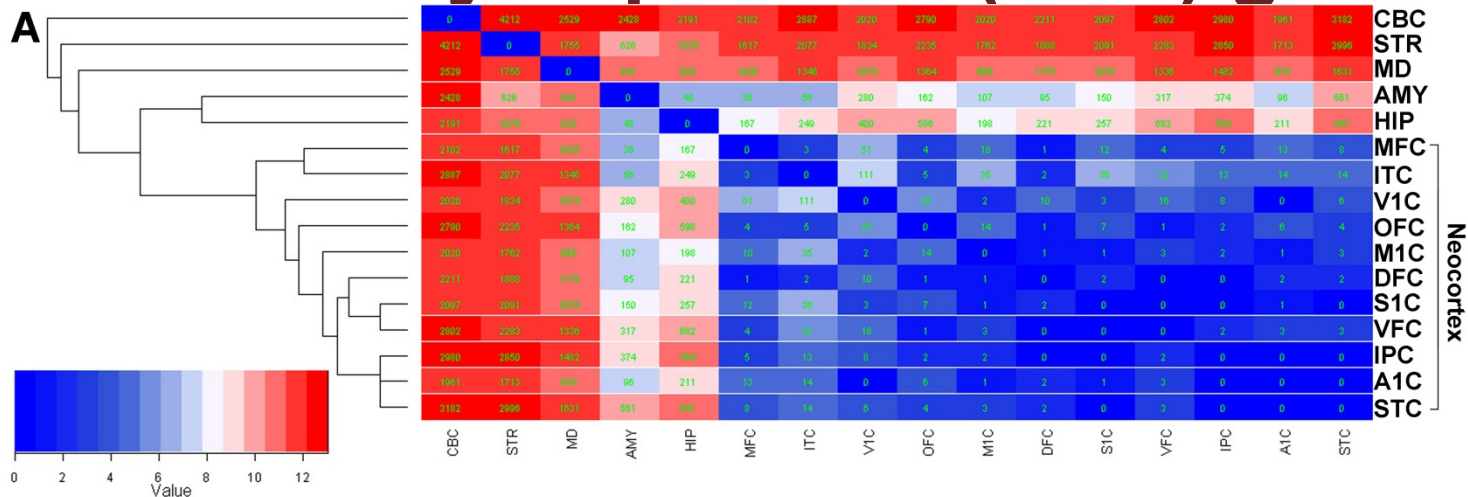
**Bottom row color :** Blue is NCX, Cyan is HIP, Orange is AMY,  
Black is STR, Sea green is MD, Red is CBC

## Bioinformatics Analyses:

- Comprehensive QC analyses
- mRNA transcript detection
- Small non-coding RNA detection (microRNAs...)
- Spatio-temporal differential expression (DEX)
- Spatio-temporal differential splicing and exon usage (DEU)
- Allele-specific expression
- RNA-editing
- Weighted correlation network analysis
- eQTL



# Differentially Expressed (DEX) genes



# RNA-Seq of *DHR511* and Correlated Genes Across Human Brain Development



**BRAINSPAN**

ATLAS OF THE DEVELOPING HUMAN BRAIN