# RNA-Seq

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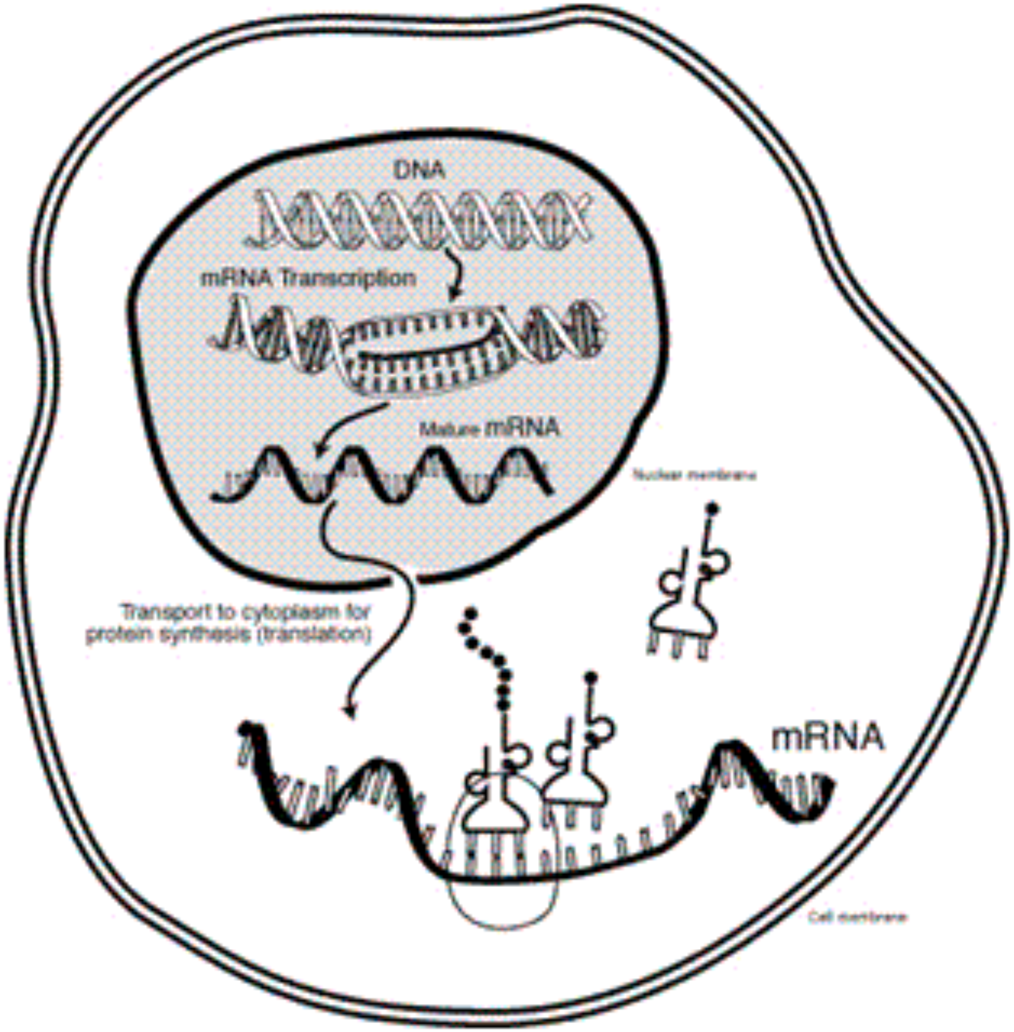
# Central Dogma of Molecular Biology



**DNA**

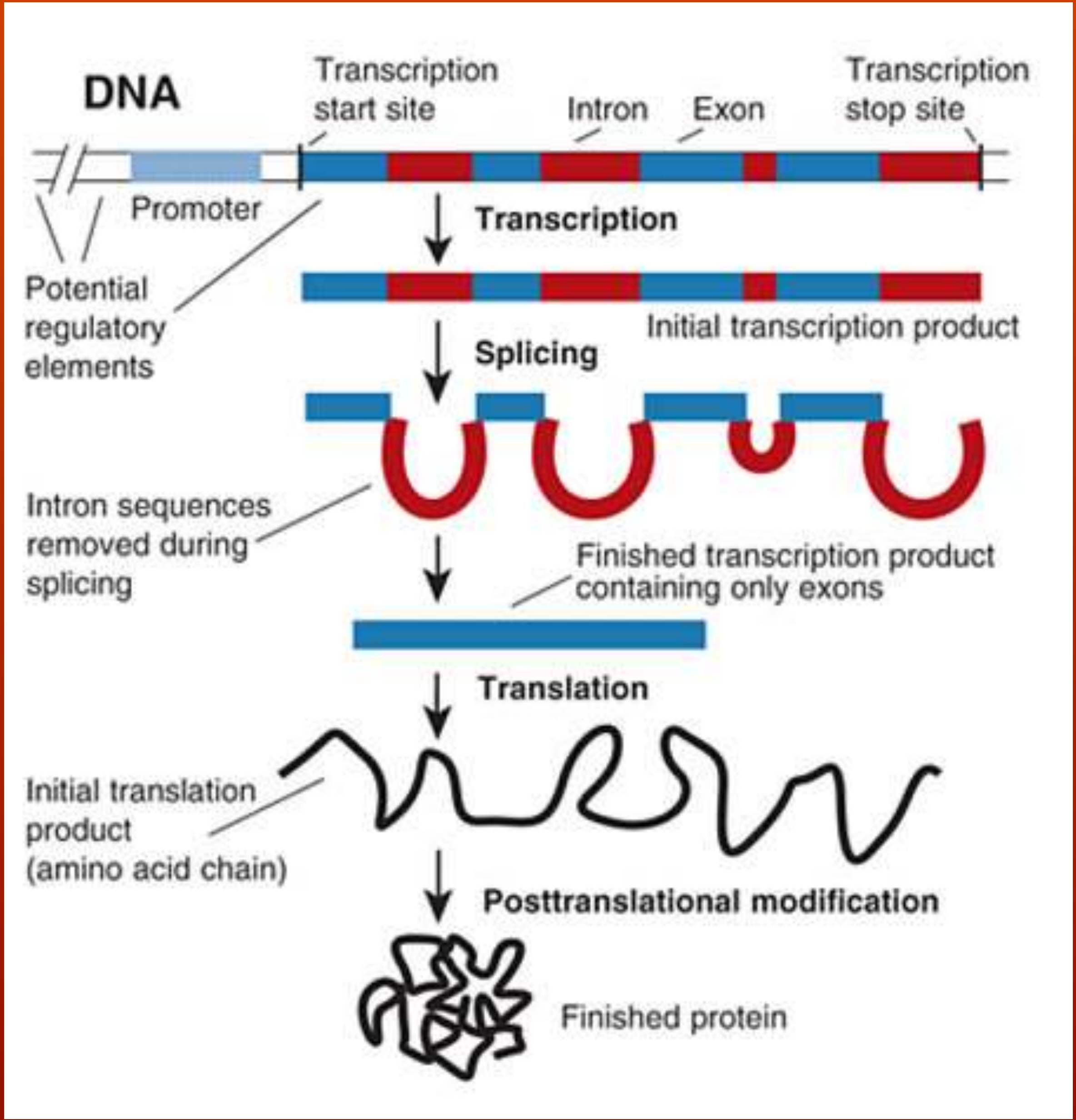
↓ *transcription* ↓

**RNA**

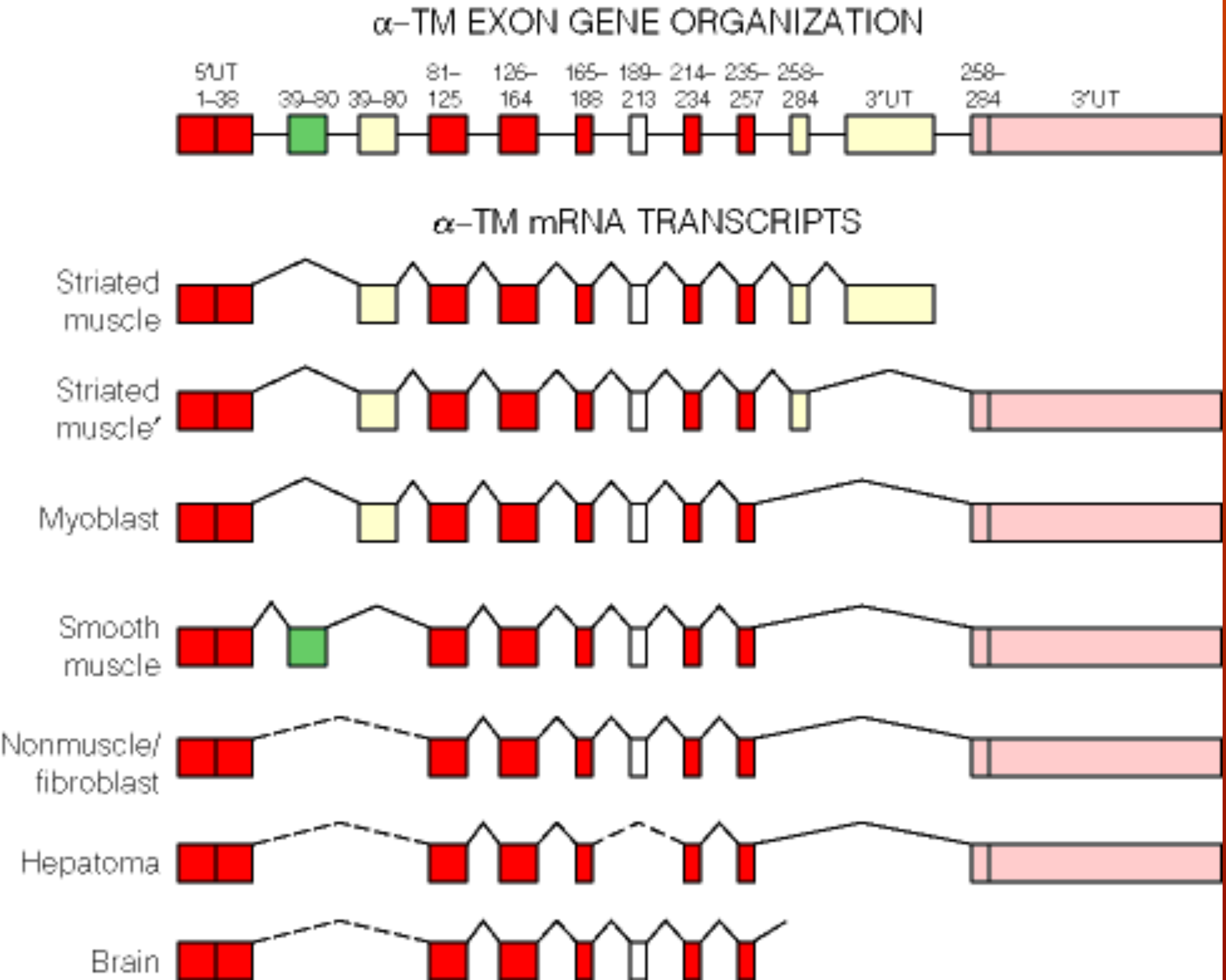
↓ *translation* ↓

**Protein**

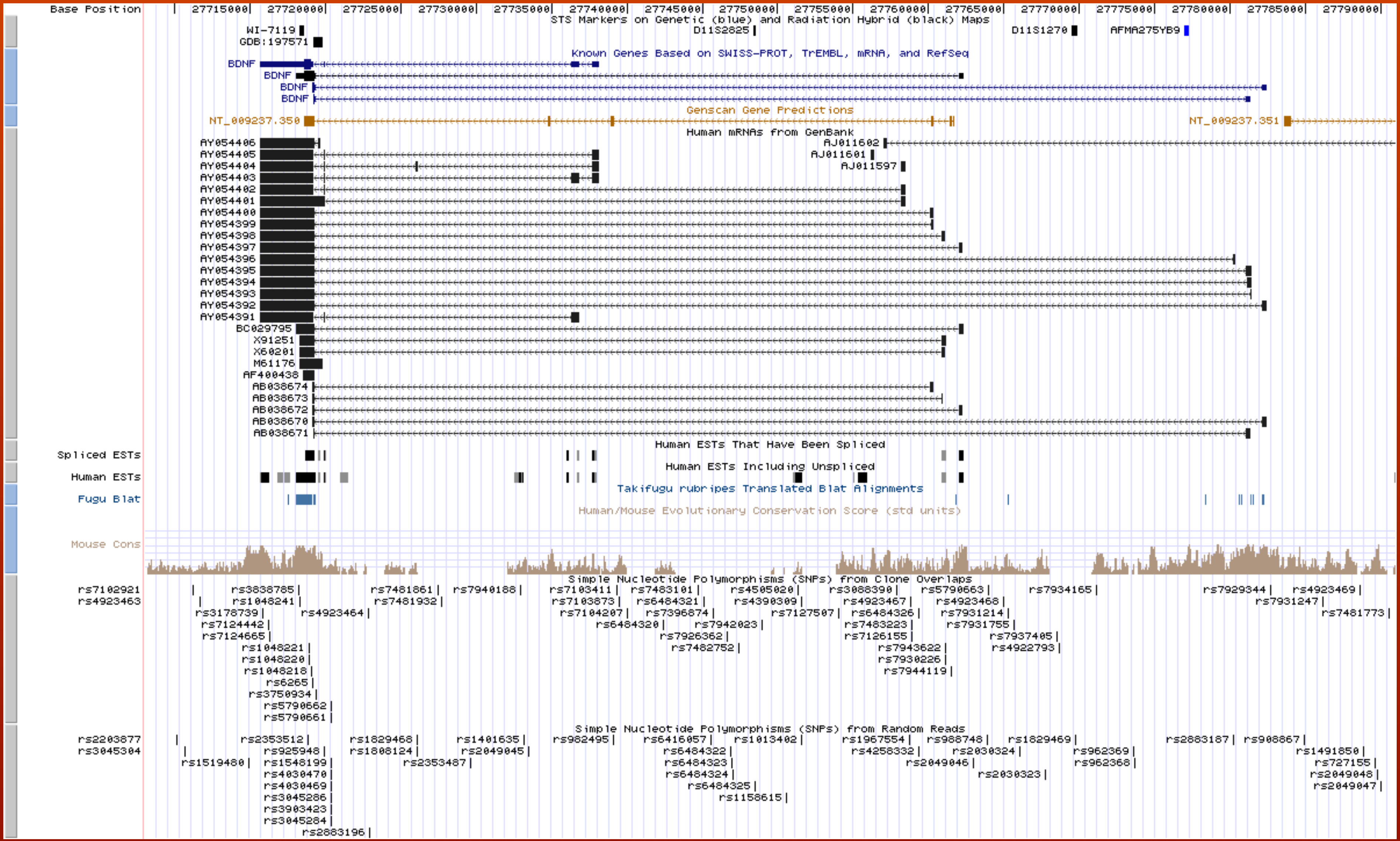
# Eukaryotic Genes are Spliced



# Eukaryotic Genes are Alternatively Spliced



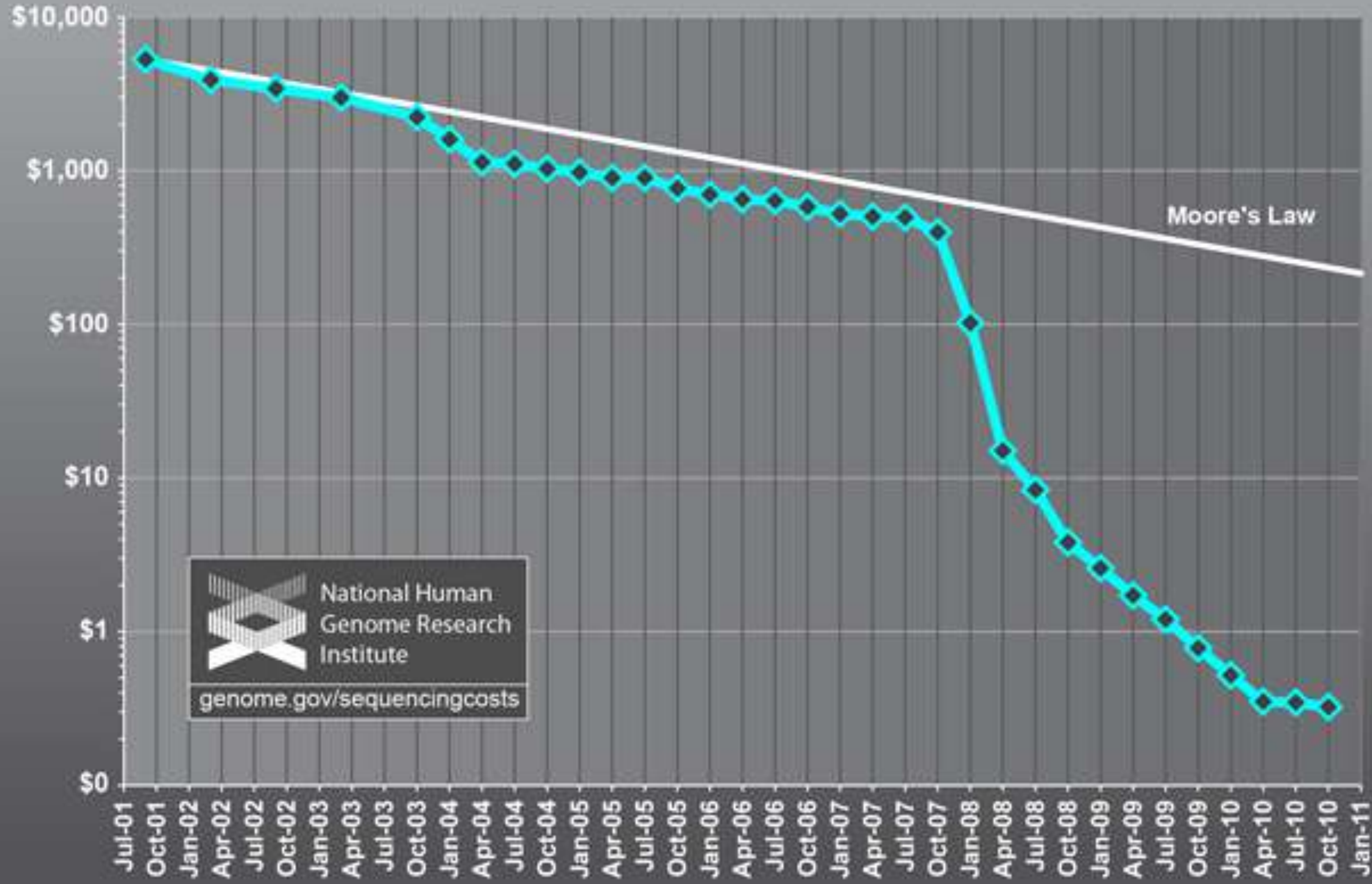
# UCSC Genome Browser
# genome.ucsc.edu

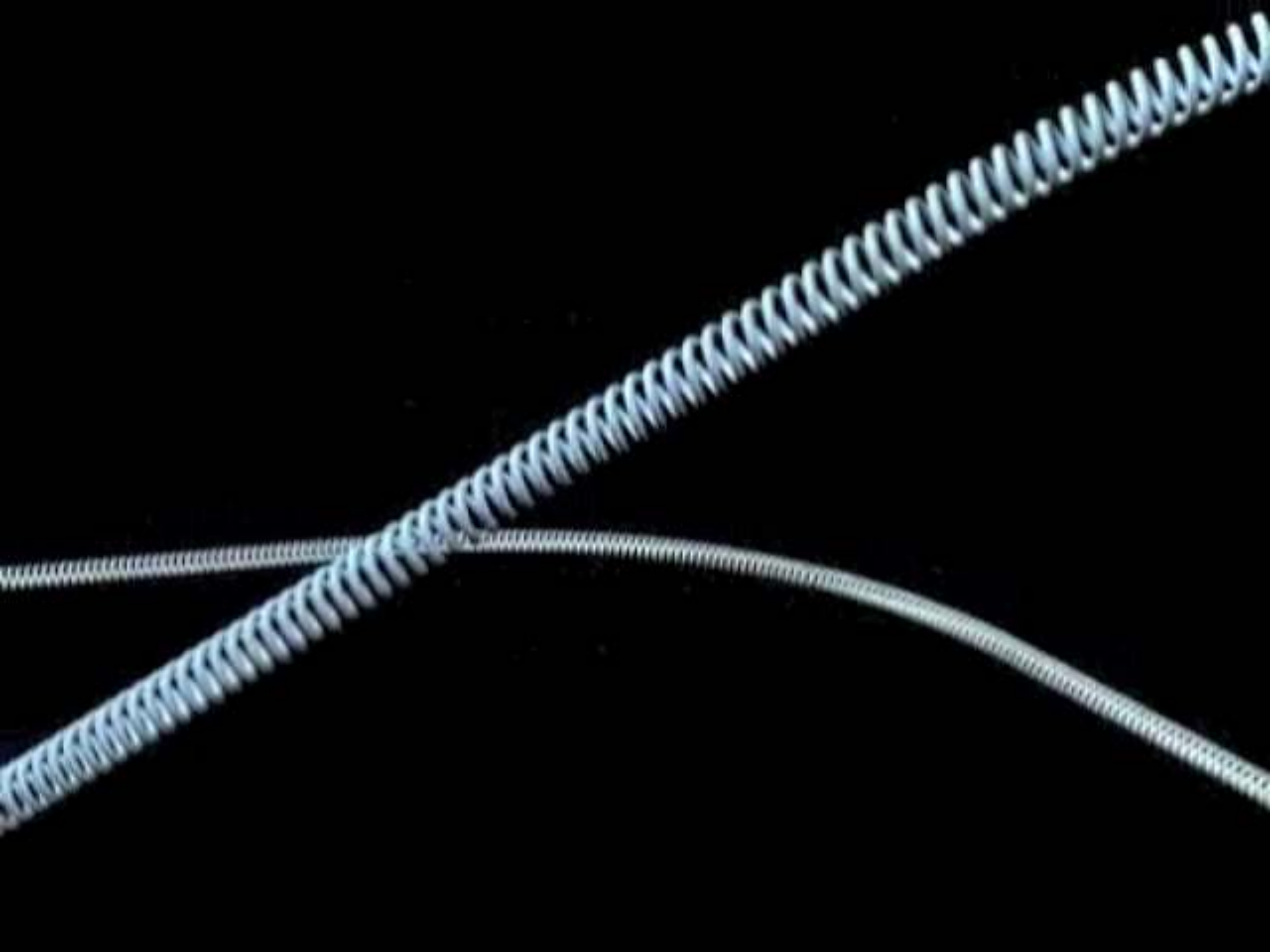


# Next Generation SequencingApplications

- 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





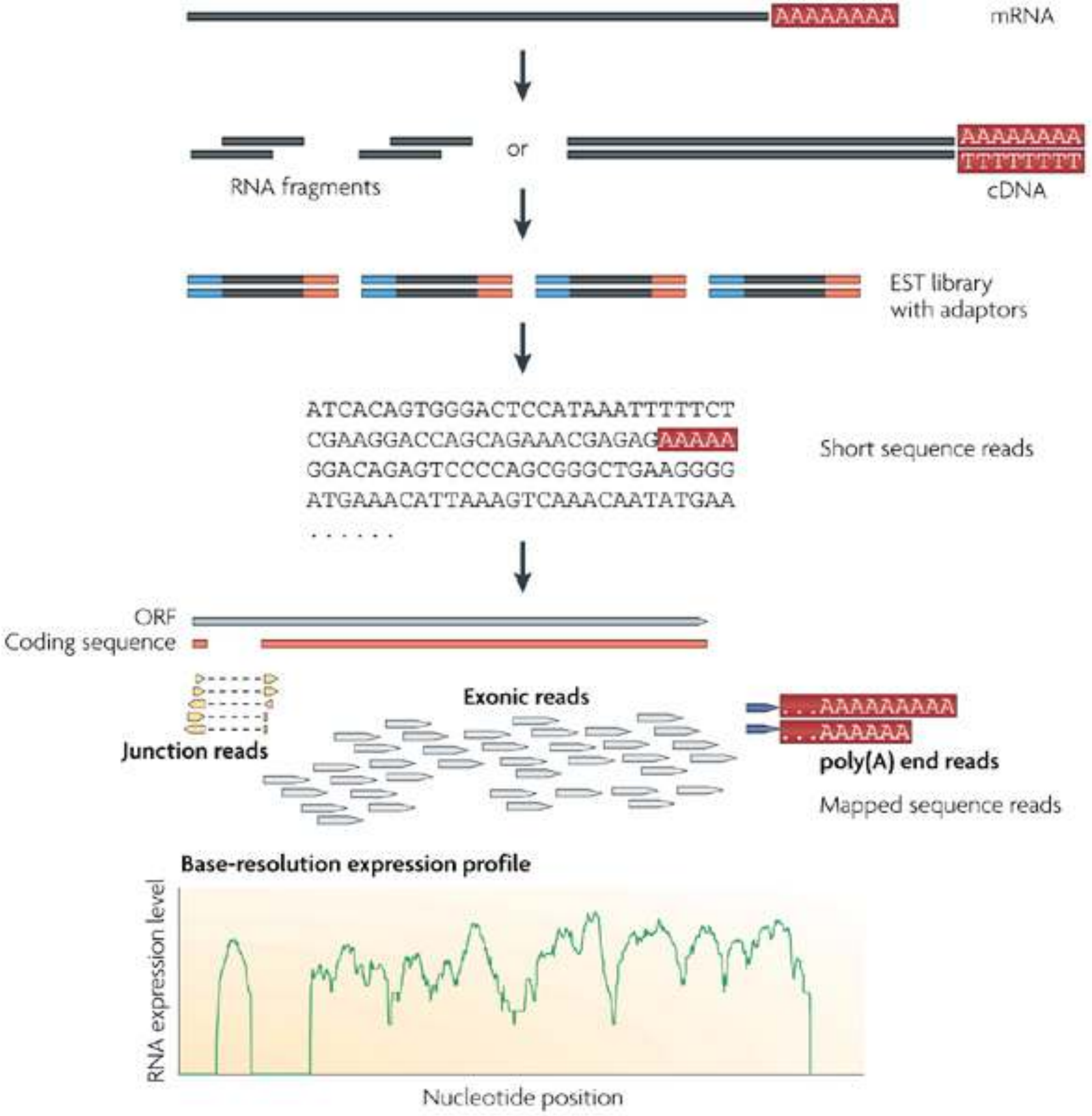
**ILMN HiSeq**
**@ ZNI**

**~1200 cores**
**@ HPCC**

**400 TB**
**@ ZNI**

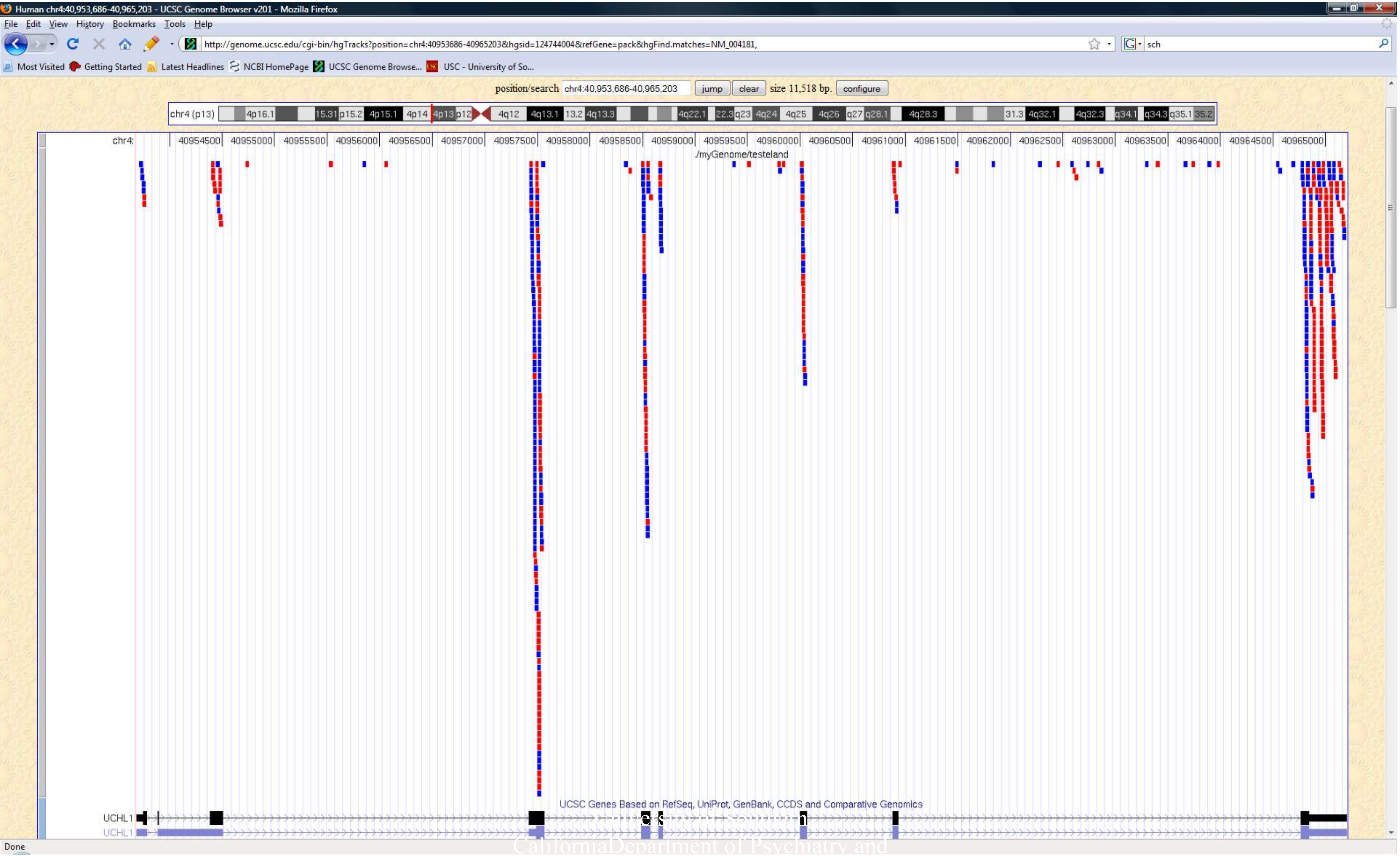
**1.2 PB**
**@ HPCC**

# RNA-Seq Method

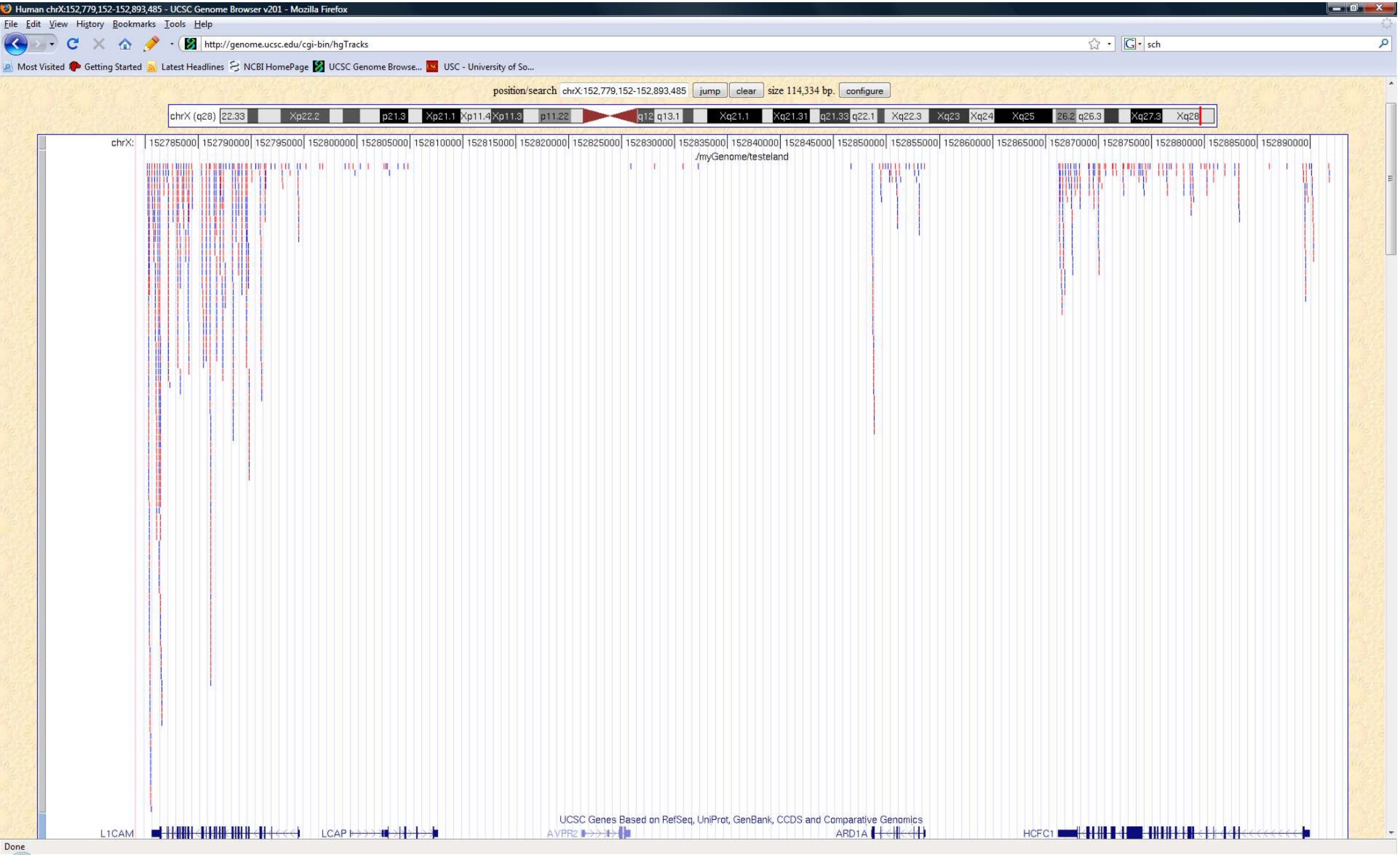


Nature Reviews | Genetics

# RNA-Seq: UCHL1 expression



# RNA-Seq: Differential Expression



# RseqFlow: Workflow for RNA-Seq Data Analysis

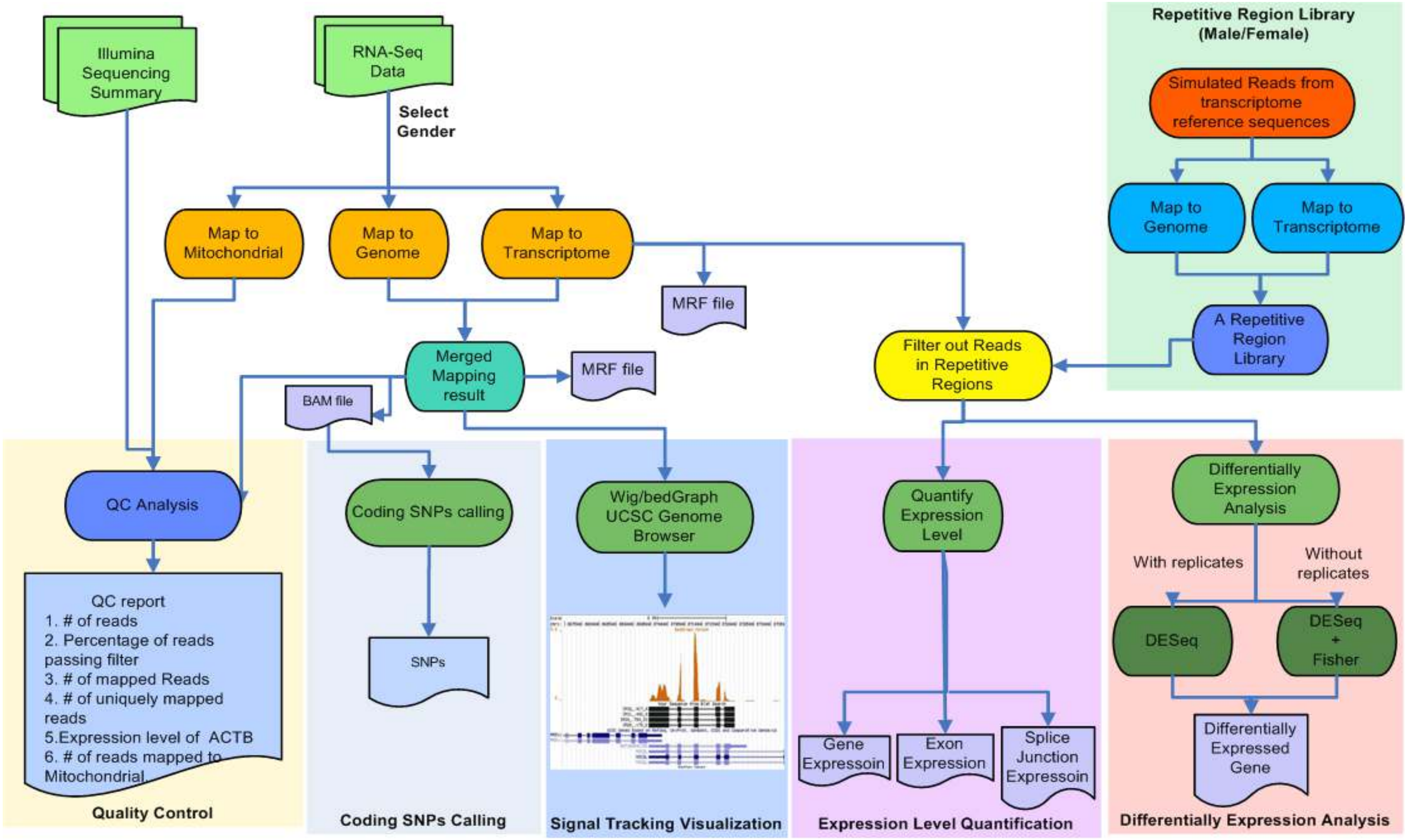
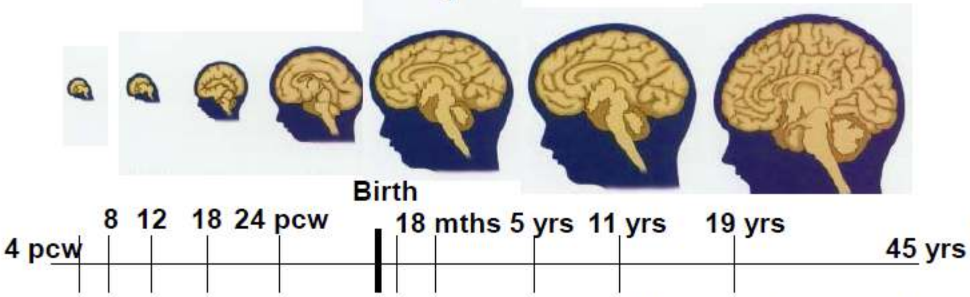


Figure 1. RNA-seq Workflow.

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



4 pcw 8 12 18 24 pcw Birth 18 mths 5 yrs 11 yrs 19 yrs 45 yrs

**Cortical neurogenesis**

**Synaptogenesis**

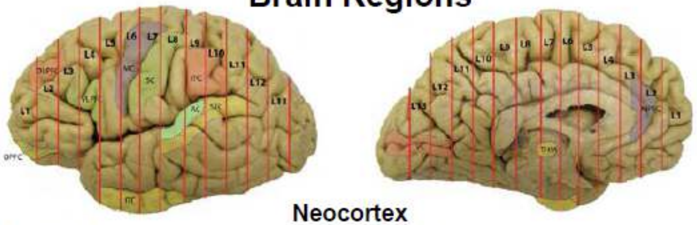
**Reorganization**

**Elimination**

**Peak Plasticity**

**Mature cortical elements**

## Brain Regions



**Neocortex**

**Frontal lobe**
- Dorsolateral prefrontal cortex (DFC)
- Ventrolateral prefrontal cortex (VFC)
- Medial prefrontal cortex (MFC)
- Orbital prefrontal cortex (OFC)
- Primary motor cortex (M1C)

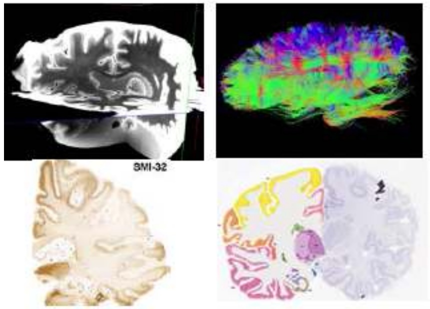
**Occipital lobe**
- Primary visual cortex (V1C)

**Parietal lobe**
- Primary sensory cortex (S1C)
- Posterior inferior parietal cortex (IPC)

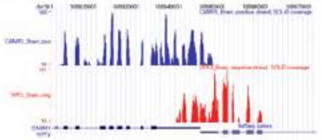
**Temporal lobe**
- Primary auditory cortex (A1C)
- Posterior superior temporal cortex (STC)
- Inferior temporal cortex (ITC)

**Subcortex**
- Hippocampus (HIP)
- Thalamus (MD)
- Amygdala (AMY)
- Striatum (STR)
- Cerebellum (CBC)

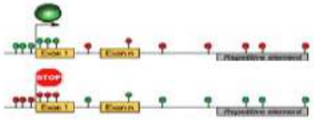
## Anatomy Reference Framework



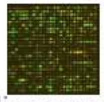
## Transcriptome, Epigenome, Genome Profiling



RNA sequencing



DNA methylation



Microarray

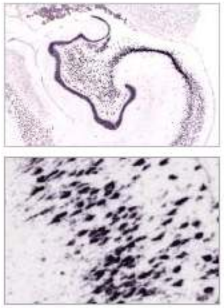
Small RNAs

**....ACGGCTT.....**

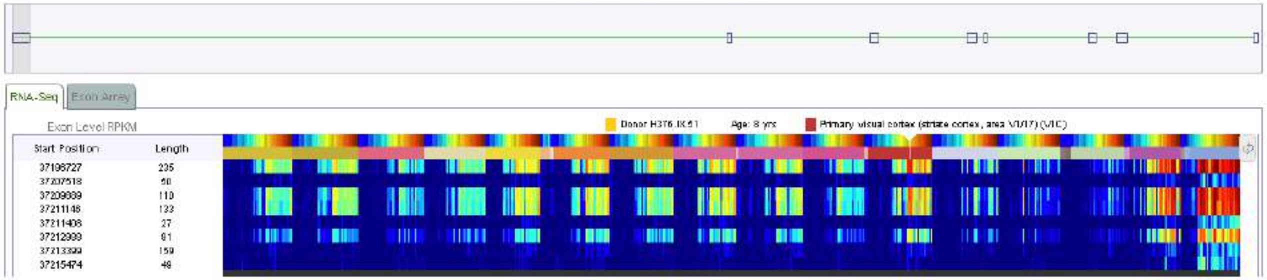
**....ACGACTT......**

SNPS

## Cellular Resolution Gene Expression

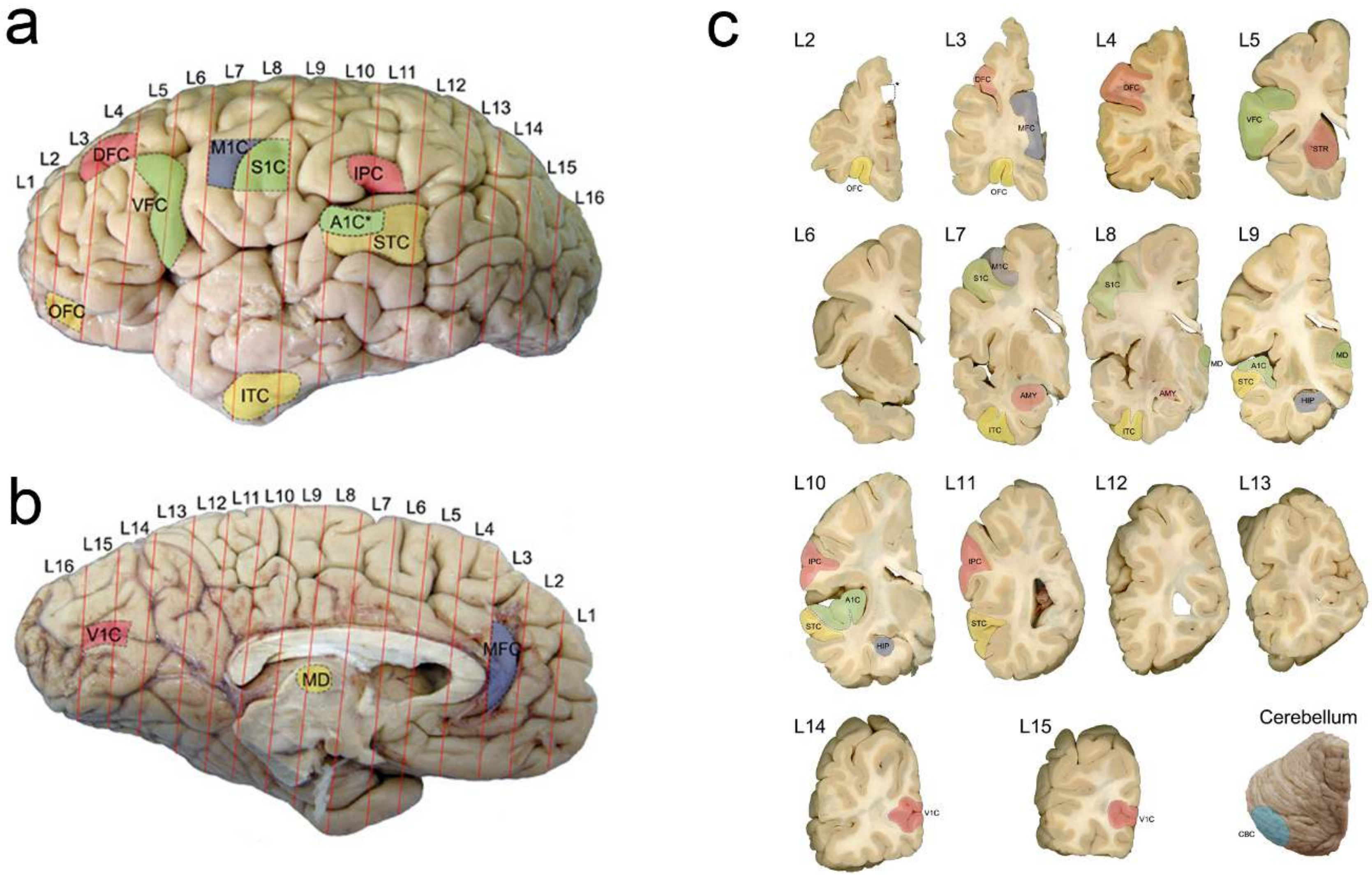


## Public Resource

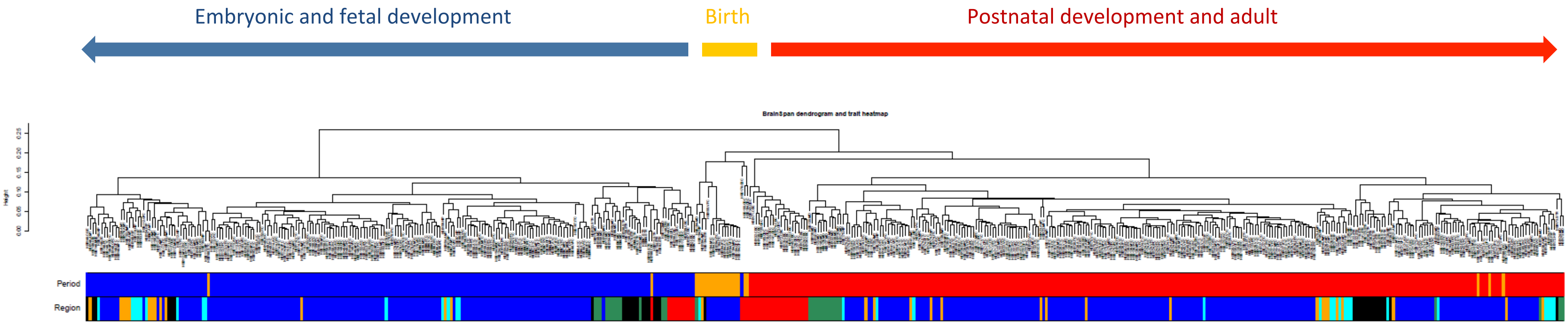


| Start Position | Length |
|---|---|
| 37196727 | 235 |
| 37207518 | 50 |
| 37209889 | 110 |
| 37211148 | 133 |
| 37211408 | 27 |
| 37212999 | 81 |
| 37213399 | 159 |
| 37215474 | 48 |

# Adult Tissue Sampling



# Hierarchical clustering and analyses of all RNA-seq samples

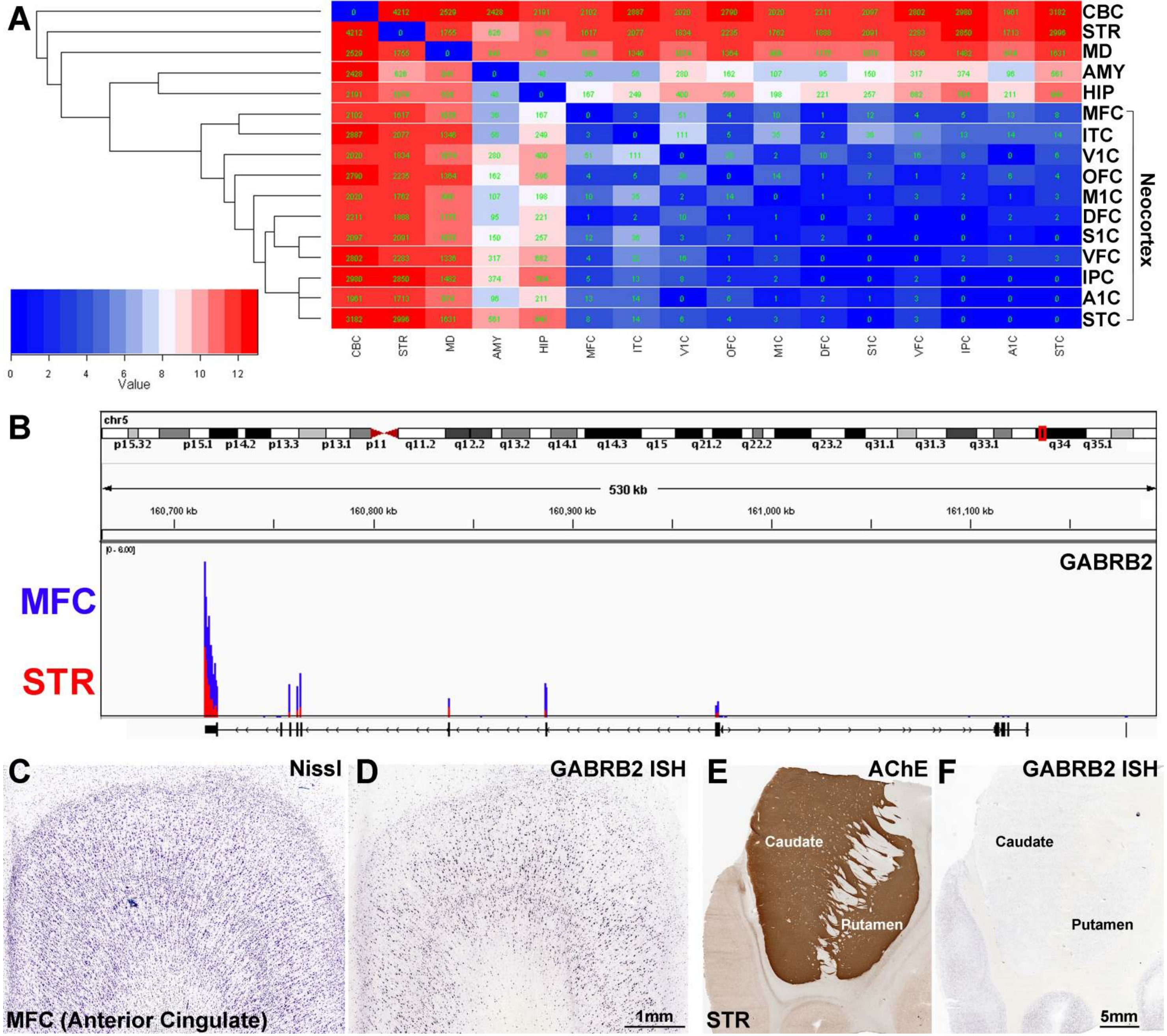


**Top row color:**
Blue is prenatal development
Orange is period 7 (late fetal to birth)
Red is postnatal development and adulthood

**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



| | CBC | STR | MD | AMY | HIP | MFC | ITC | V1C | OFC | M1C | DFC | S1C | VFC | IPC | A1C | STC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CBC | 0 | 4212 | 2529 | 2428 | 2191 | 2102 | 2887 | 2020 | 2790 | 2020 | 2211 | 2097 | 2802 | 2980 | 1961 | 3182 |
| STR | 4212 | 0 | 1755 | 626 | [illegible] | 1617 | 2077 | 1834 | 2235 | 1762 | 1888 | 2091 | 2283 | 2850 | 1713 | 2996 |
| MD | 2529 | 1755 | 0 | 841 | [illegible] | [illegible] | 1346 | [illegible] | 1364 | [illegible] | [illegible] | [illegible] | 1336 | 1482 | 974 | 1631 |
| AMY | 2428 | 626 | 841 | 0 | 40 | 36 | 58 | 280 | 162 | 107 | 95 | 150 | 317 | 374 | 96 | 561 |
| HIP | 2191 | [illegible] | [illegible] | 40 | 0 | 167 | 249 | 400 | 596 | 198 | 221 | 257 | 682 | 704 | 211 | [illegible] |
| MFC | 2102 | 1617 | [illegible] | 36 | 167 | 0 | 3 | 51 | 4 | 10 | 1 | 12 | 4 | 5 | 13 | 8 |
| ITC | 2887 | 2077 | 1346 | 58 | 249 | 3 | 0 | 111 | 5 | 35 | 2 | 36 | [illegible] | 13 | 14 | 14 |
| V1C | 2020 | 1834 | [illegible] | 280 | 400 | 51 | 111 | 0 | [illegible] | 2 | 10 | 3 | 16 | 8 | 0 | 6 |
| OFC | 2790 | 2235 | 1364 | 162 | 596 | 4 | 5 | [illegible] | 0 | 14 | 1 | 7 | 1 | 2 | 6 | 4 |
| M1C | 2020 | 1762 | [illegible] | 107 | 198 | 10 | 35 | 2 | 14 | 0 | 1 | 1 | 3 | 2 | 1 | 3 |
| DFC | 2211 | 1888 | [illegible] | 95 | 221 | 1 | 2 | 10 | 1 | 1 | 0 | 2 | 0 | 0 | 2 | 2 |
| S1C | 2097 | 2091 | [illegible] | 150 | 257 | 12 | 36 | 3 | 7 | 1 | 2 | 0 | 0 | 0 | 1 | 0 |
| VFC | 2802 | 2283 | 1336 | 317 | 682 | 4 | [illegible] | 16 | 1 | 3 | 0 | 0 | 0 | 2 | 3 | 3 |
| IPC | 2980 | 2850 | 1482 | 374 | 704 | 5 | 13 | 8 | 2 | 2 | 0 | 0 | 2 | 0 | 0 | 0 |
| A1C | 1961 | 1713 | 974 | 96 | 211 | 13 | 14 | 0 | 6 | 1 | 2 | 1 | 3 | 0 | 0 | 0 |
| STC | 3182 | 2996 | 1631 | 561 | [illegible] | 8 | 14 | 6 | 4 | 3 | 2 | 0 | 3 | 0 | 0 | 0 |

# RNA-Seq of *DHRS11* and Correlated Genes Across Human Brain Development