More Than the Genes: Controlling the Genome

NHGRI Science Reporters Workshop

June 7, 2010

How can we “read” the human genome sequence?

- No instruction manual/punctuation marks
- Evolutionary conservation helps to identify functionally important regions

  ~5% conserved; ~1.5% protein coding
- Moderately good at identifying protein-coding regions, but fine structures difficult to predict from sequence
- Regulatory regions can be very far away from genes
- Need unbiased experimental investigation to identify all functional regions

ENCODE: Encyclopedia of DNA Elements

Goal: To compile a *comprehensive encyclopedia* of all of the sequence features in the human genome and in the genomes of selected model organisms

ENCODE

- Pilot Project Phase (9/03 – 9/07)
  Studied defined 1% of the human genome sequence using existing technologies
- Production Phase (9/07 – 9/11)
  New/continued pilot projects and expansion to whole genome studies in human

modENCODE (5/07 – 5/11)

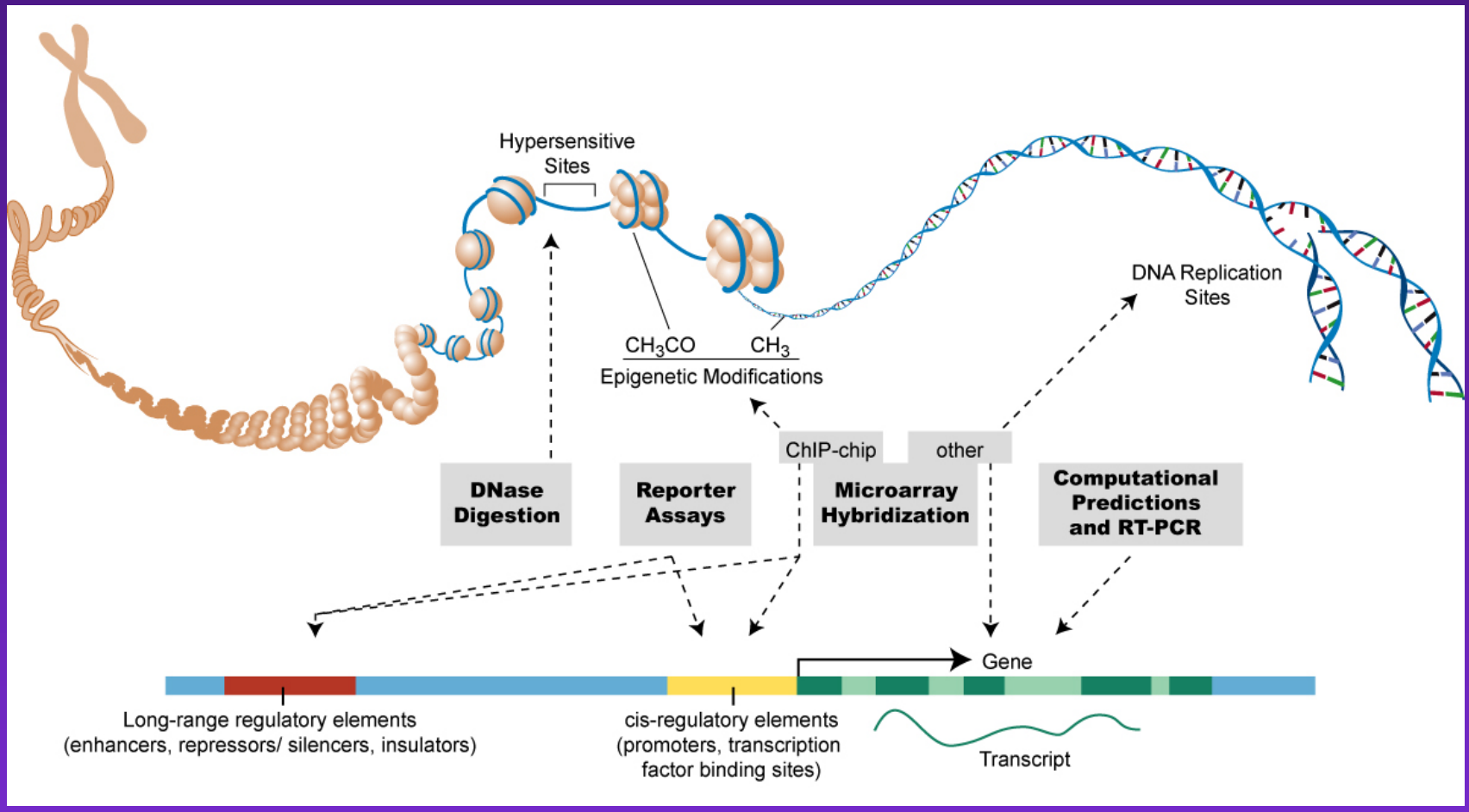
Production projects to comprehensively identify functional elements in the genomes of *C. elegans* and *D. melanogaster*

Mouse ENCODE (9/09 – 9/11 with ARRA funds)

Limited production projects to identify functional elements in the mouse genome to inform annotation of human genome

Technology Development (9/03 -9/10)

Focused on less well-studied functional elements; funded solicitations in 2003, 2004, 2007


Hypersensitive Sites
DNA Replication Sites
CH_3CO CH_3
Epigenetic Modifications
ChIP-chip
other
DNase Digestion
Reporter Assays
Microarray Hybridization
Computational Predictions and RT-PCR
Gene
Long-range regulatory elements (enhancers, repressors/ silencers, insulators)
cis-regulatory elements (promoters, transcription factor binding sites)
Transcript

Lots of data and data types…

..... generated by:

RNA-seq

RNA-array

TF ChIP-seq

Histone modif ChIP-seq

DNaseHS-seq

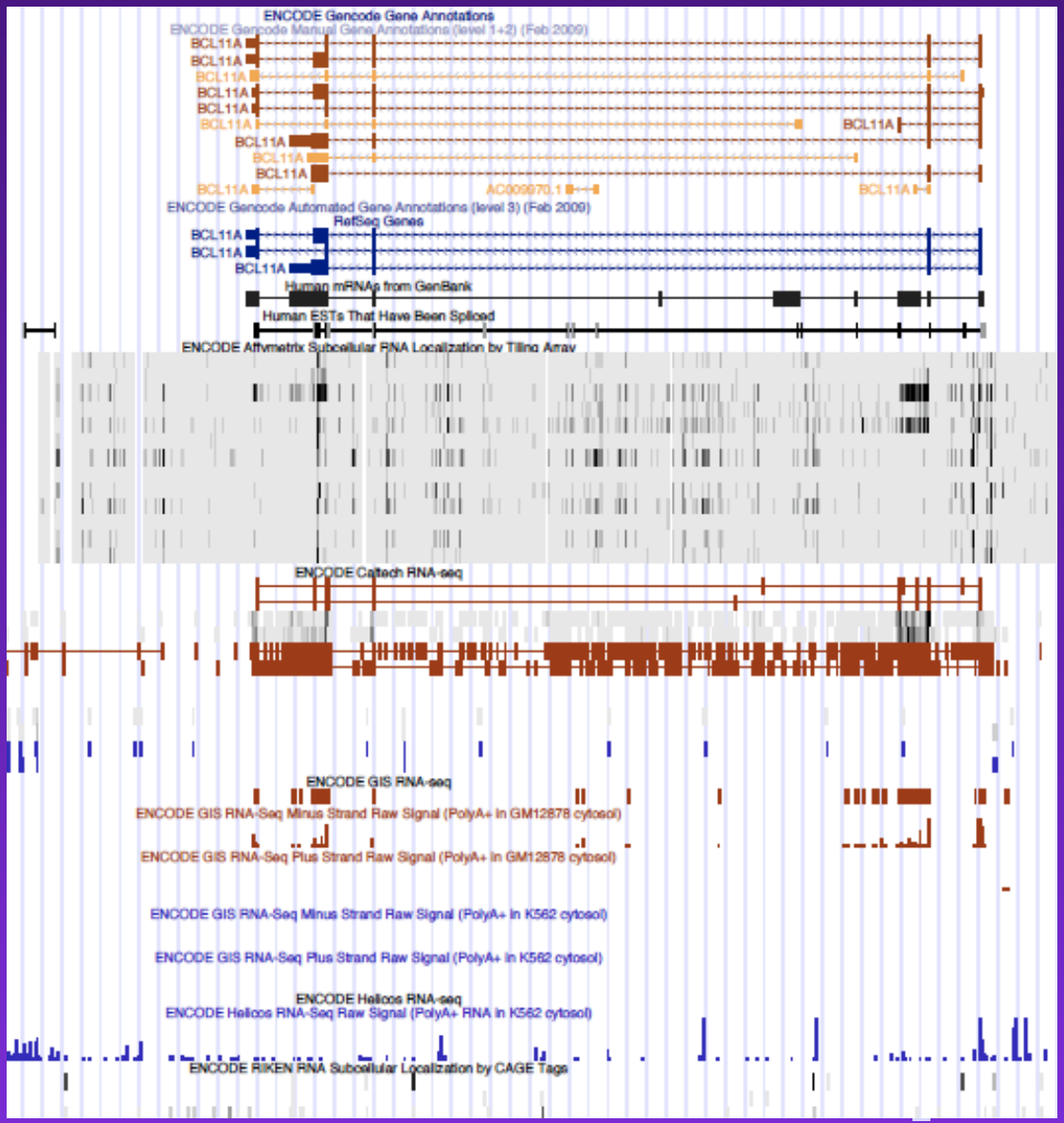
FAIRE-seq

Methyl-seq

Methyl27-bisulfite

1M SNP genotyping

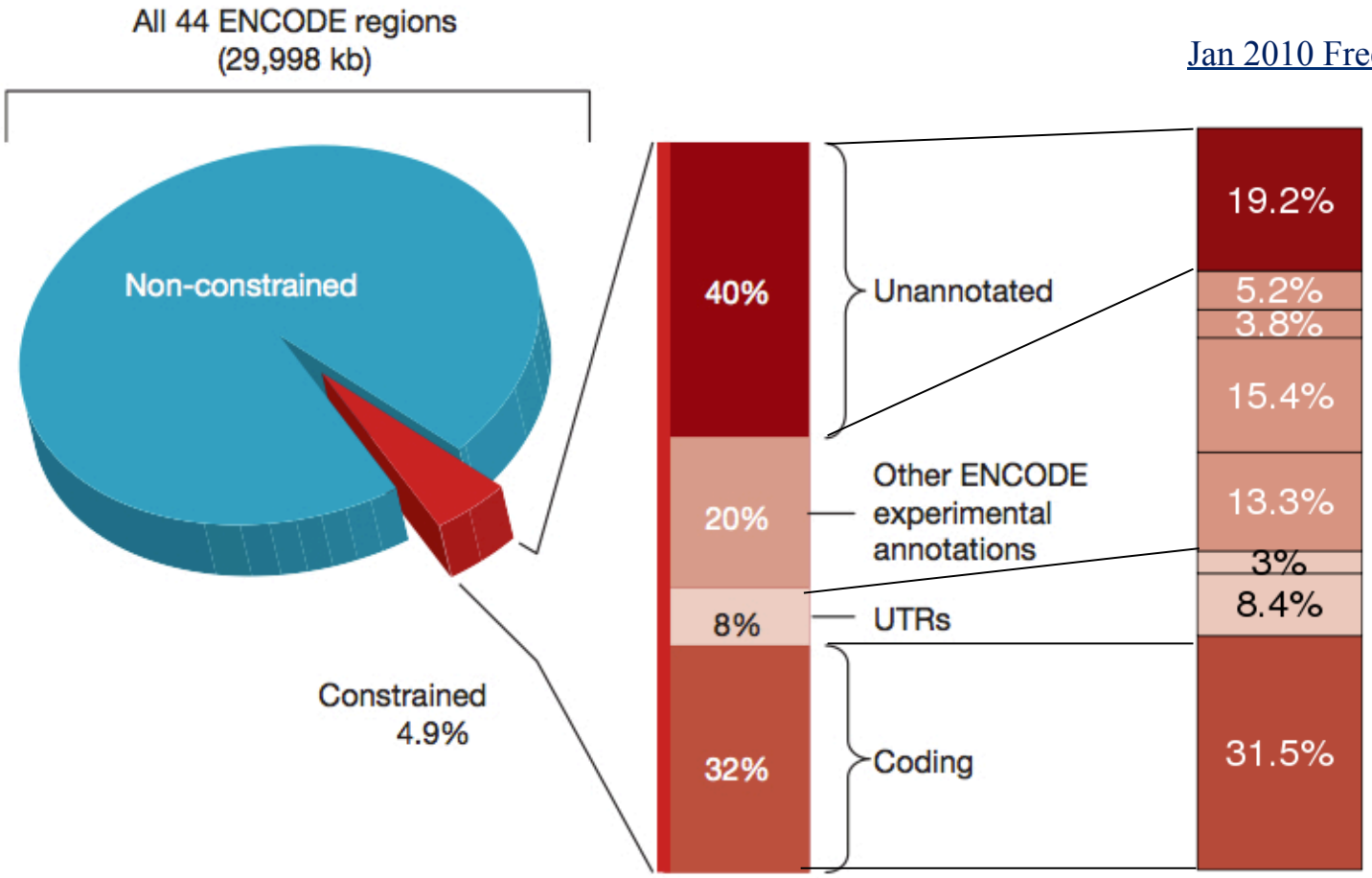
(+ WGS for GM12878)



Progress

- Large-scale data production ongoing
  - ENCODE: 959 datasets submitted
  - modENCODE: 1170 datasets submitted
- Analysis requires development of:
  - Common data reporting formats
  - Data standards
  - Analytical tools
- Integrative analyses for each species ongoing
  - Long-term plans for integration of fly/worm; fly/worm/human
- Follow up and expand on findings from pilot
  - Human genome is pervasively transcribed
  - Many functional elements are seemingly unconstrained across mammalian evolution

Annotation of Constrained Elements



Jan 2010 Freeze

How will the catalogs of functional elements be used?

1. Enhance understanding of regulation of gene expression on a spatial, temporal and quantitative level
   - Who are the players?
   - How do they interact?
   - How do variants affect gene expression?
   - Can we predict gene expression from sequence?
   - Can we manipulate gene expression?

How will the catalogs of functional elements be used?

2. Enhance understanding of genetic basis of disease
   - Many genome-wide association studies (GWAS) find SNPs in non-coding regions
   - How do SNPs/mutations in non-coding regions alter gene expression and contribute to disease?
3. Enhance understanding of epigenetic contributions to disease
   - Epigenomics (NIH Common Fund)

Functional Element Variation

- Genome-wide differences in transcription factor bindings sites between individual
  - RNA polymerase II: 25% difference
  - NF Kappa B: 7.5%
  - Binding differences frequently associated with SNPs and SVs, and differences in gene expression
  - Suggests functional consequences of binding variation
- Individual-specific and allele-specific chromatin signatures in humans
  - 10% active chromatin sites individual specific
  - 10% active chromatin sites allele-specific
  - Presence of individual-specific DHS site near TSS correlated with expression
  - Strong genetic component for individual and allele-specific differences

Kasowski et al. (2010) Science 328:232 & McDaniell et al. (2010) Science 328:235

Linking GWAS to Function & Disease

- Multiple regions in 8q24 have alleles predisposing to many cancers (e.g., prostate, breast and colon)
- Regions far from annotated genes; unknown biological function
- Profiled risk region (RNA expression, histone modifications, binding sites for Pol II & androgen receptor)
- Several enhancers identified
- SNP found in one enhancer within FoxA1 TF binding site
- Prostate cancer risk allele facilitating stronger FoxA1 binding and stronger androgen response

Jia et al. (2009) PLoS Genetics 5(8):e1000597

Pilot Project Findings

- The human genome is pervasively transcribed.
- Many novel non-protein-coding transcripts and transcription start sites identified.
- Regulatory sequences that surround transcription start sites are symmetrically distributed, with no bias towards upstream regions.
- Chromatin accessibility and histone-modification patterns are highly predictive of both the presence and activity of transcription start sites.
- Distal DNaseI hypersensitive sites have characteristic histone modification patterns that reliably distinguish them from promoters; some of these distal sites show marks consistent with insulator function.
- 5% of the bases in the genome can be confidently identified as being under evolutionary constraint in mammals.
  - For ~ 60% of these constrained bases, there is evidence of function based on the results of the experimental assays.
- Many functional elements are seemingly unconstrained across mammalian evolution.

ENCODE Funding

(4 years)

Project	ENCODE	modENCODE	Mouse ENCODE
Production /Pilot Grants	$81.5M $ 7.5M $89.0M	$57.2M $ 5.2M $62.4M	$4.2M
Data Coord. Center	$5.0M	$5.0M	
Data Analysis Center	$5.0M	$2.8M (2 yrs)	*2 year ARRA funds

RM Epigenomics Program and ENCODE

	RM Epigenomics Program	ENCODE (NHGRI)
Goal	Understanding epigenetic basis of disease	Generate comprehensive catalog of functional elements in the human genome
Cell types	>80 normal human cell types, selected based on relevance to disease	7 common cell types (cell lines, primary tissues and one hES cell line); Addnl ~9-80 cell sources depending on functional element
Epigenetic marks	Focus on Histone modifications, DNA methylation, and small ncRNAs	Histone modifications, small ncRNAs, DNase hypersensitive sites and pilot for DNA methylation
Disease Relevance	RFA on Epigenomics of Human Health and Disease	Data is resource to be mined by researchers, no disease focus

Epigenomics of Health and Disease
Investigates variety of human diseases