

An Overview of Illumina's Expression and Regulation Portfolio

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Market Manager, Gene Expression & Regulation*

Agenda

- Expression/Transcriptomics
 - WG Arrays
 - WG-DASL (FFPE)
 - Transcriptome analysis
- Regulation/Epigenomics
 - Infinium Methylation Arrays
 - DNA Methylation Sequencing
 - ChIP-Seq

Comprehensive Set of Analysis Tools

TECHNOLOGY	Sequence-Based Discovery	BeadArray	VeraCode	
PLATFORM	 <p>Genome Analyzer Discovery & Profiling</p>	 <p>iScan System Profiling</p>	 <p>BeadXpress Reader Validation & Screening</p>	
				

PRODUCT	Sequence-Based Discovery		BeadArray		VeraCode
mRNA-Seq	ChIP-Seq	Human	Human FFPE WG- DASL	Expression: Custom DASL Assay	
SmallRNA	Bisulfite Sequencing	Mouse	miRNA	Methylation: Custom GG Assay	
Tag Profiling	MeDIP-Seq	Rat	Human Methylation27	Carboxyl Bead Sets	
				Universal Capture Bead Sets	

Gene Expression (Transcriptomics)

TECHNOLOGY

Sequence-Based Discovery

BeadArray

VeraCode

PLATFORM



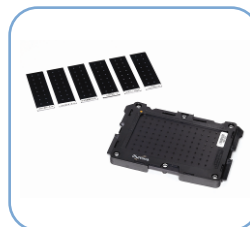
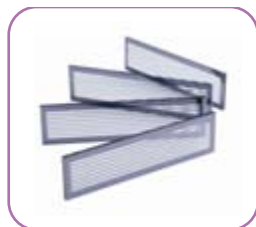
Genome Analyzer
Discovery & Profiling



iScan System
Profiling



BeadXpress Reader
Validation & Screening



PRODUCT

mRNA-Seq

ChIP-Seq

Human

Human FFPE
WG- DASL

Expression:
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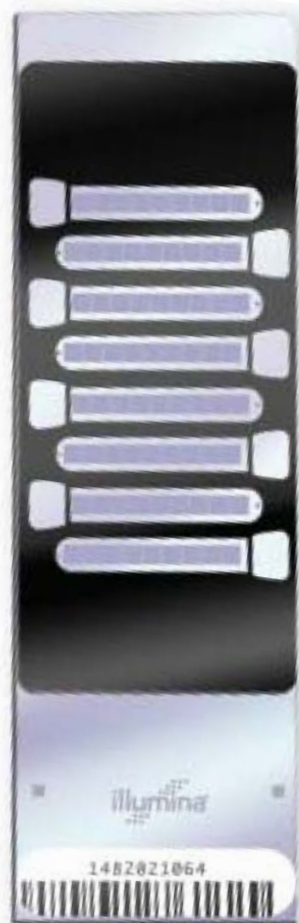
Whole Genome Expression Arrays



HumanHT-12 v3

- Ultra low cost
 - €63/array (<€100 per sample with labeling)
- High throughput
 - 12 samples per slide
 - >100 samples/day without specialized equipment
- Up to date content
 - RefSeq release 22
 - UniGene build 199

Whole Genome Expression with FFPE



Human WG-DASL

- Works with degraded samples
 - Based on the DASL assay
 - FFPE samples >20 years old
 - Brain samples
- Low sample input
 - As few as 100 cells
- Low cost
 - ~€126/sample (includes labeling)

mRNA-Seq: Sequencing Full Length mRNA

- **Quantitate levels of RNA expression**
- **Genome annotation**
 - Provide EST-like information to help annotate previously sequenced genomes
 - Discovery of novel transcripts
 - Characterize alternative splicing
- **Study diseases processes, such as cancer**
 - Find cSNPs and other point mutations
 - Discovery of disease-associated biomarkers
 - Quantitate aberrant expression in diseased tissue

Quantitative Measure of Gene Expression

FKBP8 Gene Expression



RPS3 Gene Expression



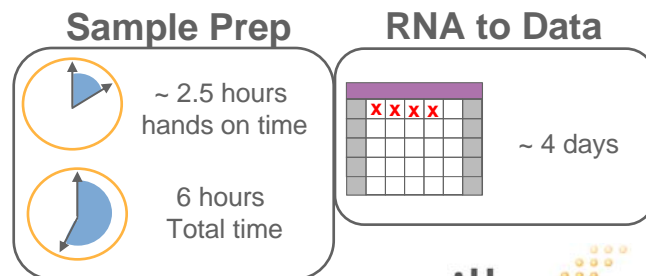
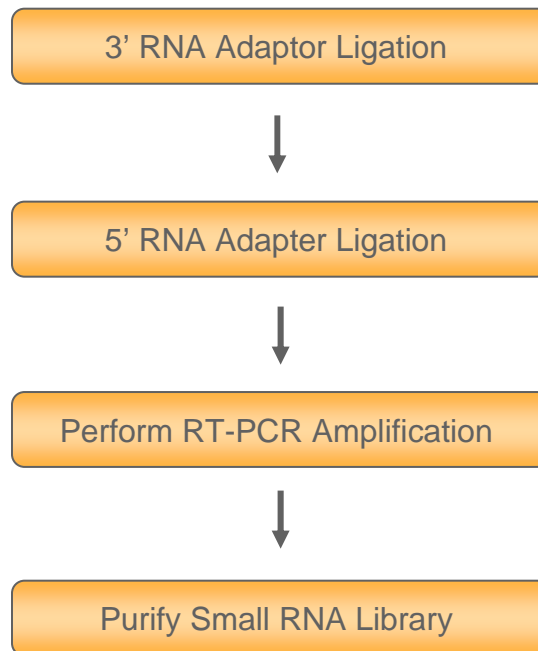
These screen shots were taken directly from the Illumina Genome Viewer (IGV), which is part of the GenomeStudio™ Software Suite

Illumina's Small RNA v1.5 library prep protocol

Just 6 hr library prep starting from total RNA

- Enhanced workflow
 - Shortest time from library to sequence
 - 2.5 hrs hands on time (6 hr total)
 - Single gel excision
- NO need for small RNA purification!
 - Starts directly from total RNA
- Reduced RNA input requirements
 - 1.0 μg total RNA input
 - Titrated to 100 ng total
- As always, strand specific information
 - Discover & profile microRNA
 - Sequence other non-coding RNAs

Streamlined Workflow



Comprehensive tools for transcriptome analysis

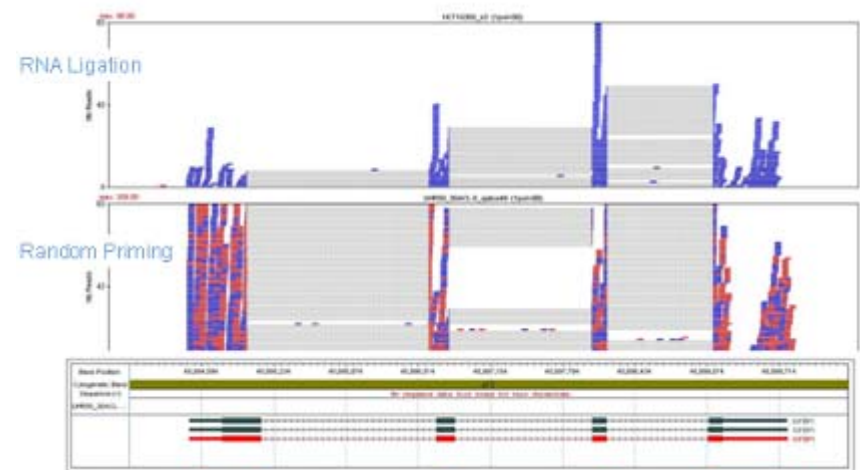
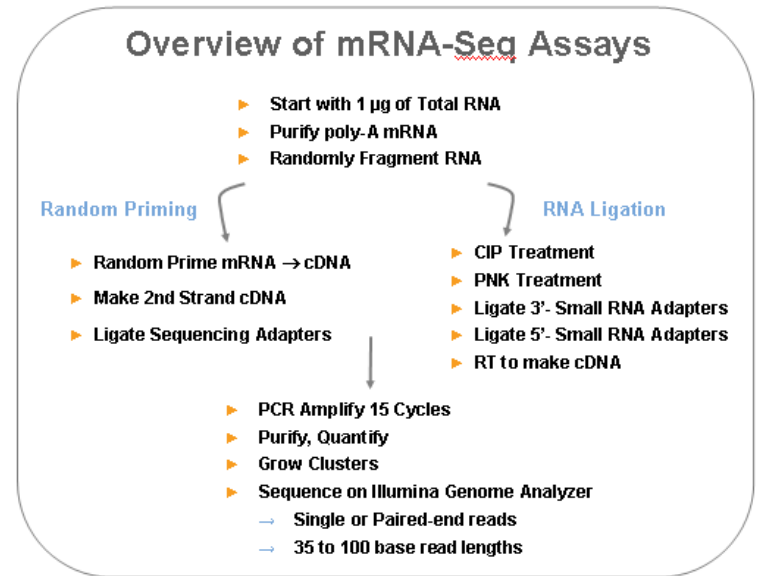
Two methods, complete answers

● mRNA-Seq

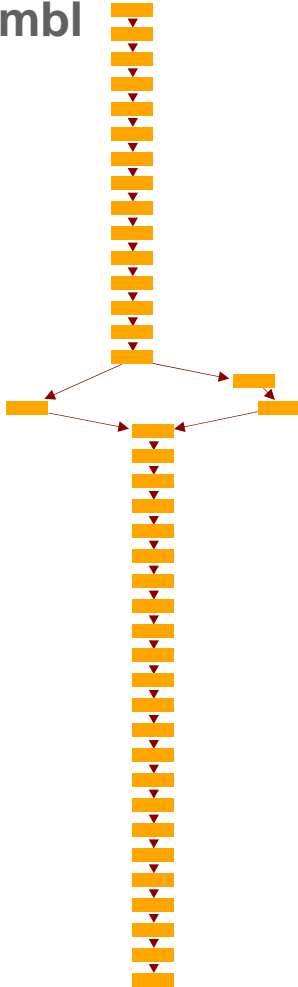
- Most uniform coverage across transcripts.
- Allows for more quantitative results.
- Offers better coverage for cSNP discovery and calling.
- Useful when strand specificity isn't needed

● Directional mRNA-Seq

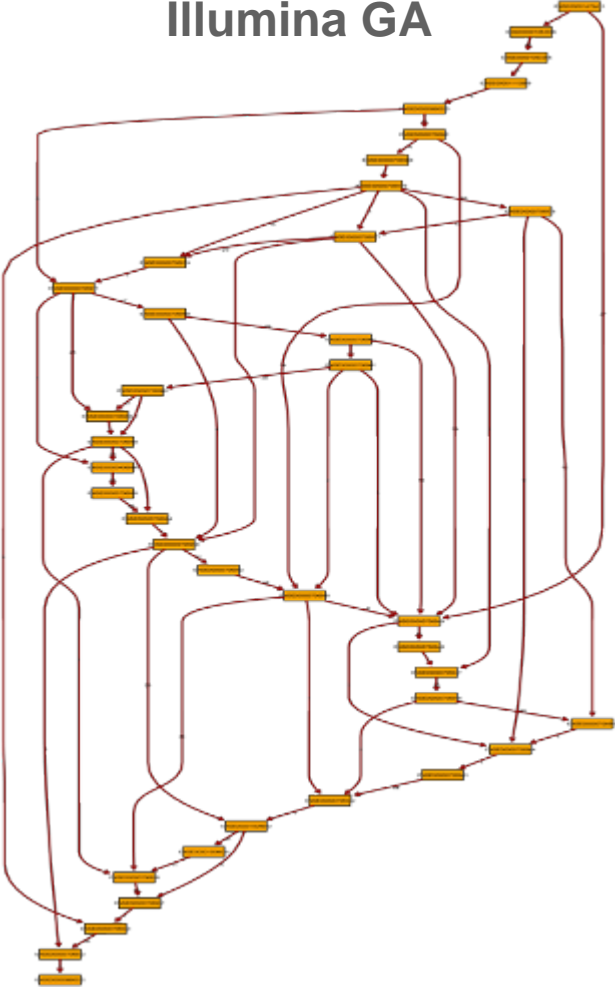
- Conserves strand specific information
- Key for discovery & annotation of novel transcripts and isoforms
- Uses Small RNA Kit & modified protocol



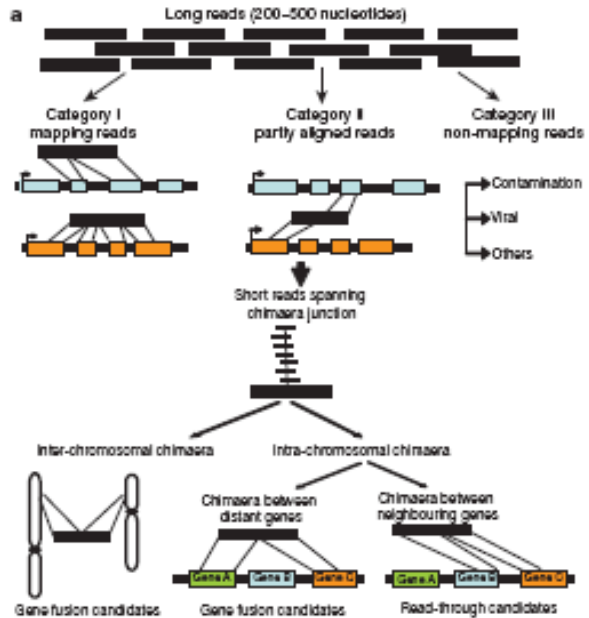
Ensembl



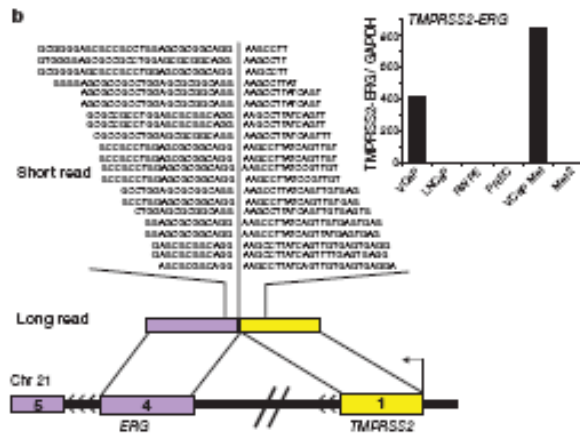
Illumina GA



Maher *et al*, Transcriptome sequencing to detect gene fusions in cancer. Nature Jan 09



- Next generation sequencing technologies for identification of intra and inter-chromosomal gene fusions
- Long reads coupled with paired end technology could provide most complete view of transcriptome



Gene Regulation (Epigenomics)

TECHNOLOGY

Sequence-Based Discovery

BeadArray

VeraCode



Genome Analyzer
Discovery & Profiling

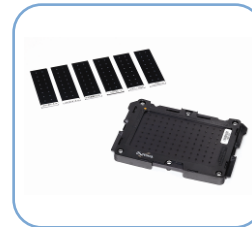


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PLATFORM



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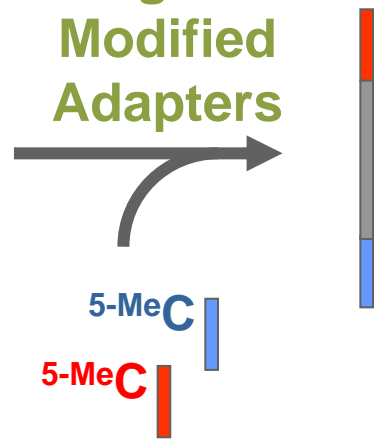
Carboxyl
Bead Sets

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Bisulfite Sequencing

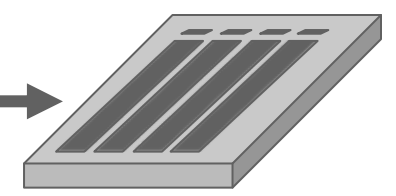
Fragment
gDNA

Ligate
Modified
Adapters

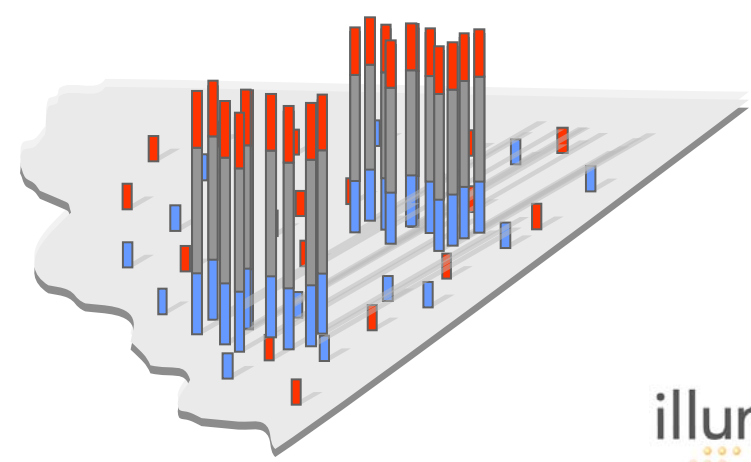


Bisulfite
Treatment

PCR & Size
Select



Generate Clusters



Sequence

Compare



Bisulfite Sequencing

- No change in protocol
- No homopolymer errors

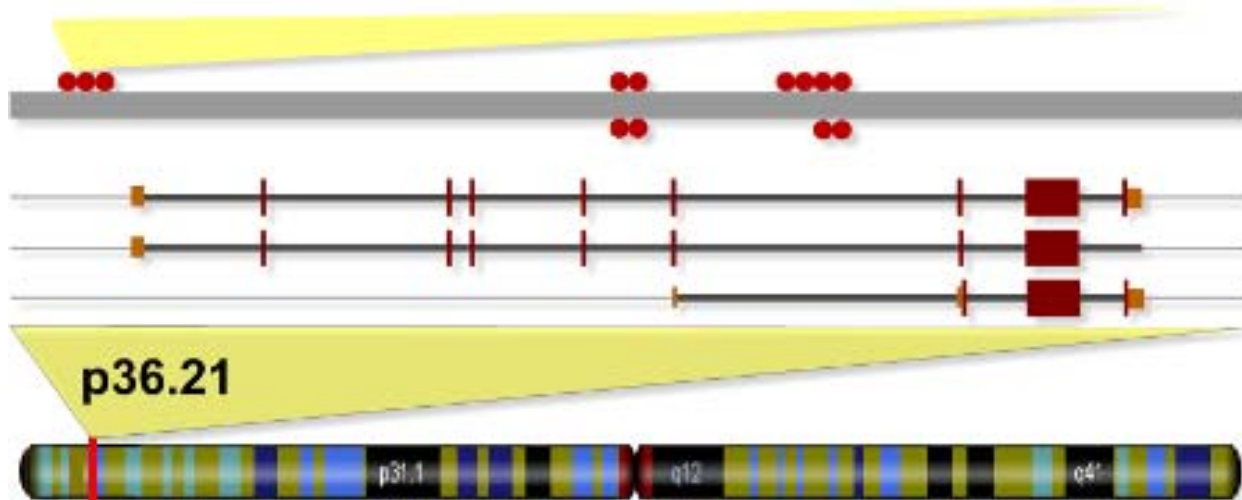
gctgaattacttcttggcacagcttgtctgaaatatgtaaagctaagtc

Cell line 1

g**C**tgaattaTttTttgg**C**aTag**C**ttgtTtgaaatatgtaaag**C**taagtg**C**

Cell line 2

g**T**tgaattaTttTttgg**T**aTag**T**ttgtTtgaaatatgtaaag**T**taagtg**T**



Whole Genome Bisulfite Sequencing

Cell

Lister *et al* '08

Highly Integrated Single-Base Resolution Maps of the Epigenome in *Arabidopsis*

nature

Cokus *et al* '08

Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning

nature

Meissner *et al* '09

Genome-scale DNA methylation maps of pluripotent and differentiated cells

CSH PRESS GENOME RESEARCH

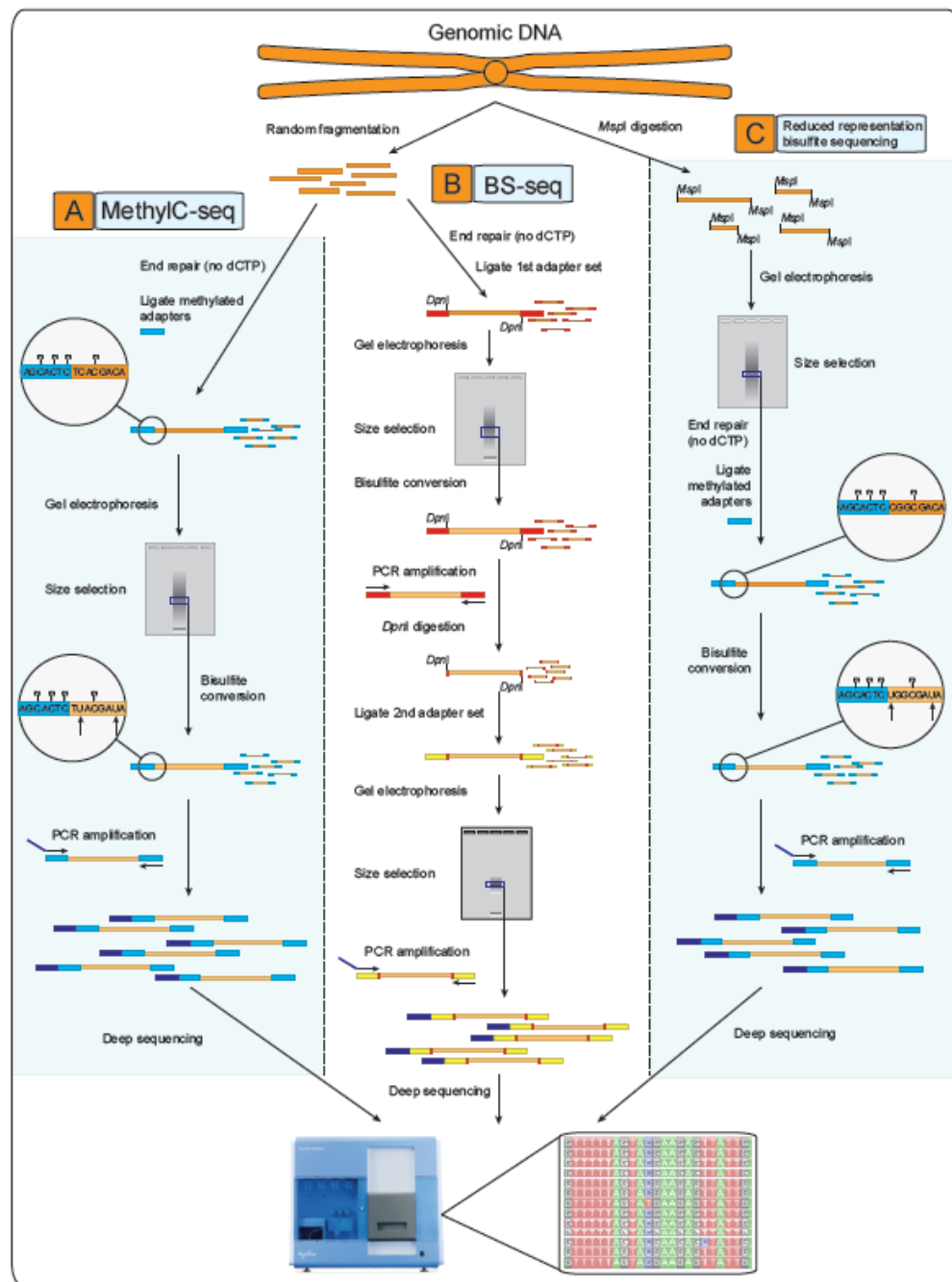
Brunner *et al* '09*

Distinct DNA methylation patterns characterize differentiated human embryonic stem cells and developing human fetal liver

CSH PRESS GENOME RESEARCH

Lister, Ecker '09

Finding the fifth base: Genome-wide sequencing of cytosine methylation



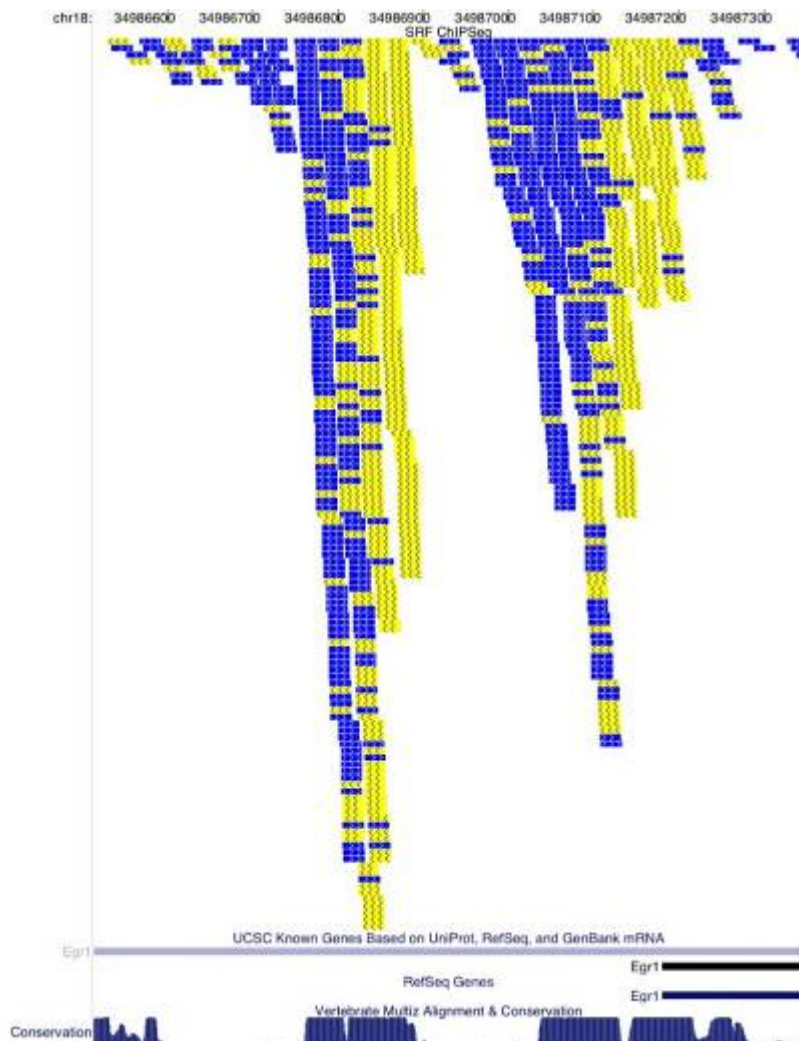
ChIP-Seq

(Genome Wide Characterization of Transcription Factor Binding Sites and Epigenetic Modifications)

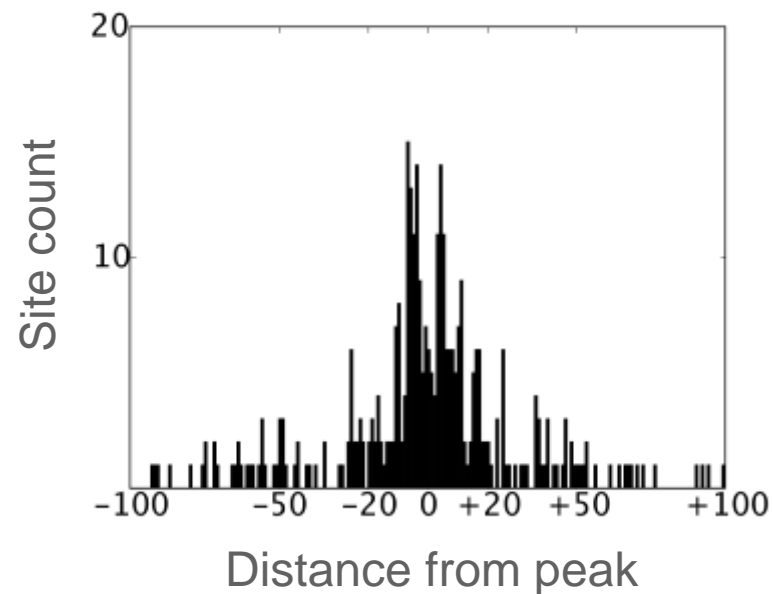
- Superior Performance
 - High sensitivity (millions of tags per sample)
 - Very low background and false positive rate
- Excellent Coverage
 - Genome-wide coverage
 - High specificity/fine resolution
- Low Sample Input
 - 1 to 10 nanograms of DNA

ChIP-Seq:

Serum Response Factor (SRF) in Mouse C2C12 Cells



CarG Boxes in SRF-enriched Regions



4.7 M reads

Renaissance in the analysis of regulatory elements

- High resolution mapping of regulatory elements including promoters, enhancers, control regions
- Nucleosome positioning
- Histone modification
- Transcription Factor binding
- Chromatin structure
- RNA-binding proteins



The ENCODE Project

The Original Plan

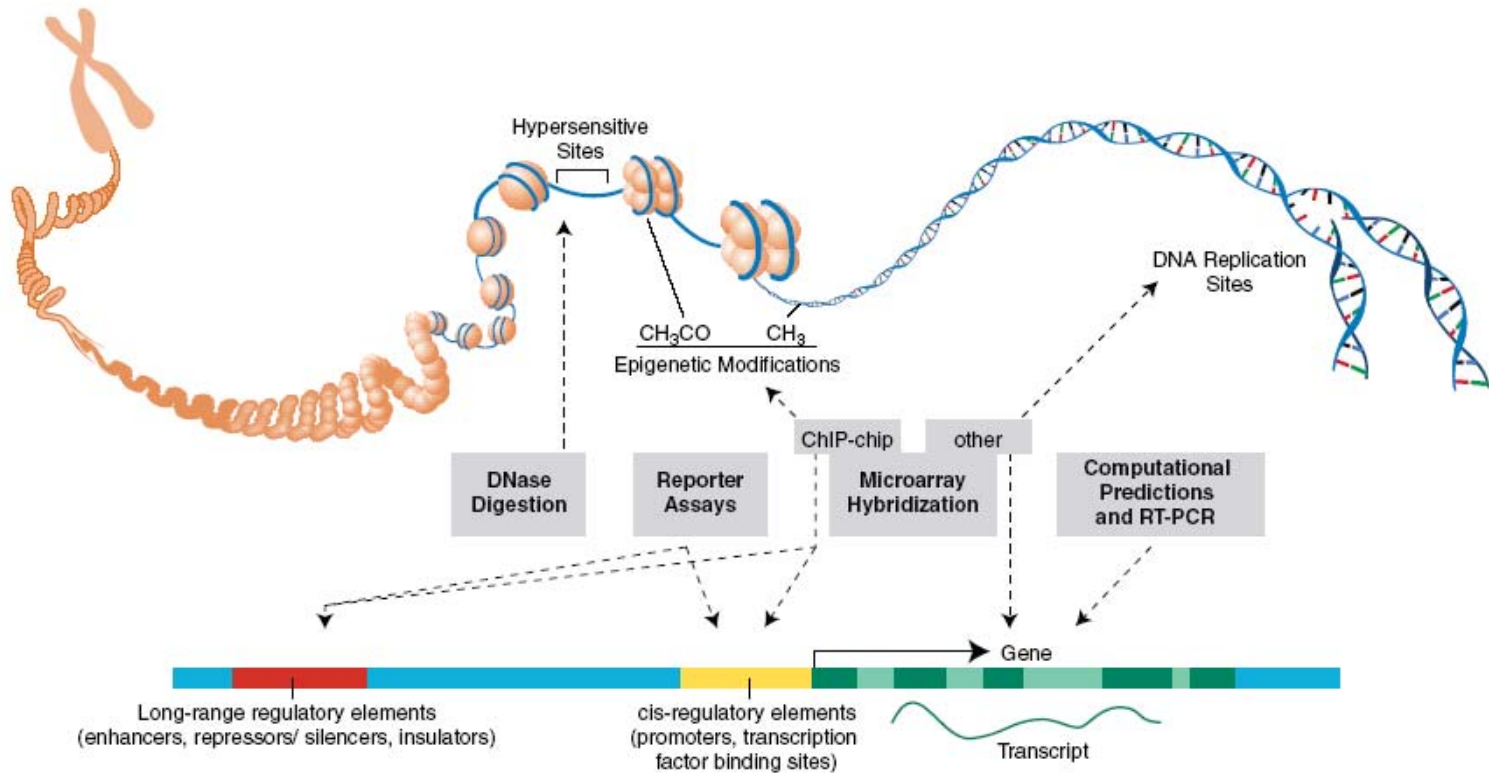


Fig. 1. Functional genomic elements being identified by the ENCODE pilot phase. The indicated methods are being used to identify different types of functional elements in the human genome.

www.sciencemag.org SCIENCE VOL 306 22 OCTOBER 2004

The ENCODE Project

Sequencing as a Powerful Methodology

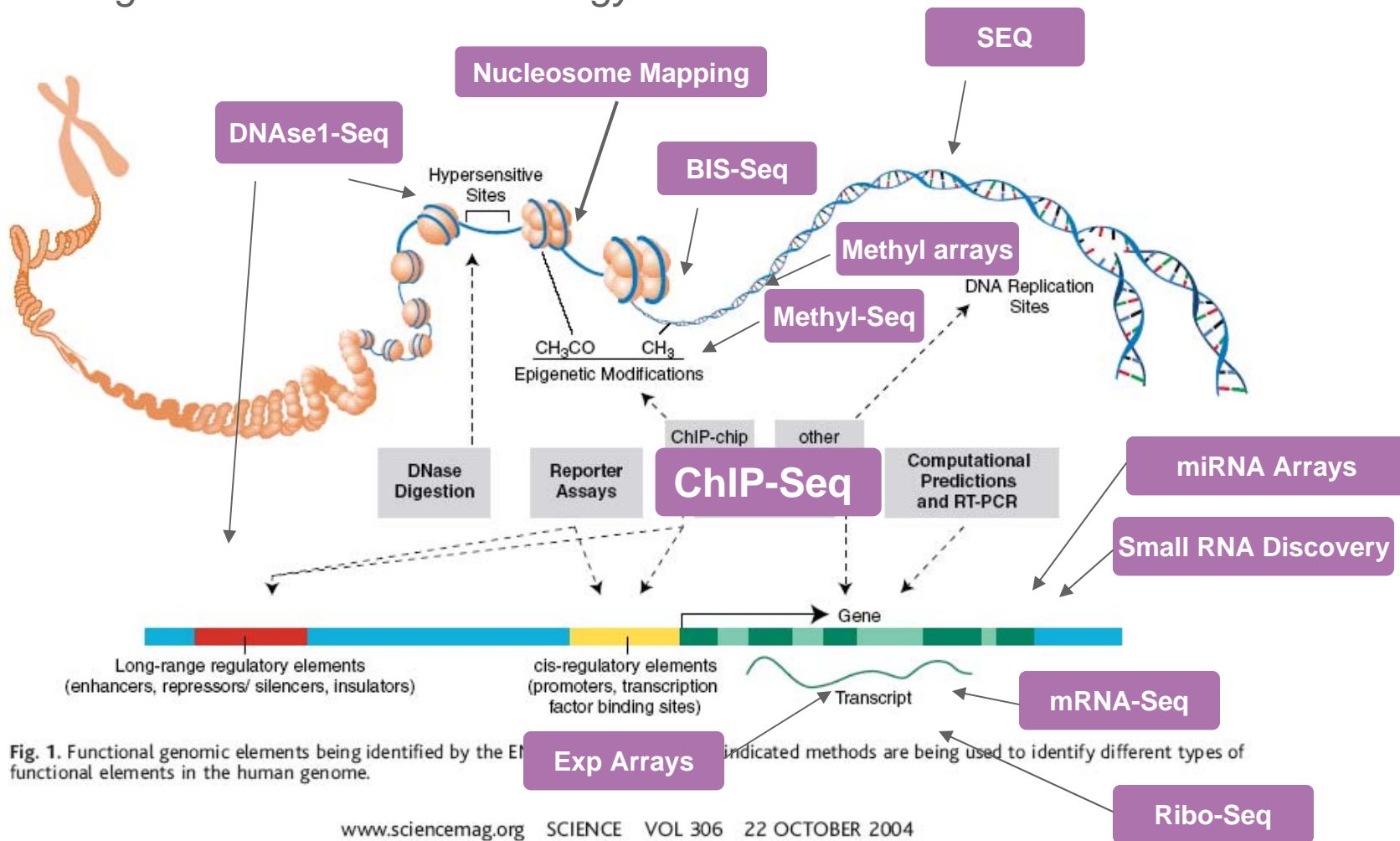
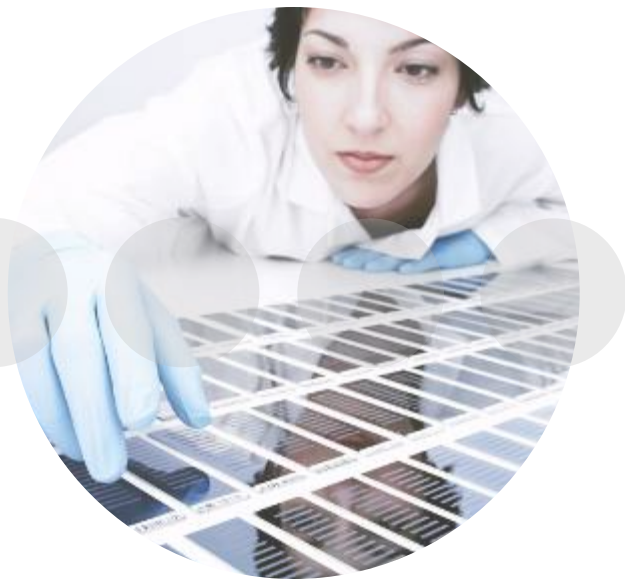
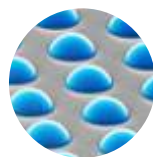
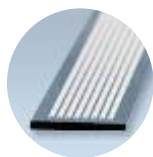


Fig. 1. Functional genomic elements being identified by the ENCODE Project. The indicated methods are being used to identify different types of functional elements in the human genome.



Thank You!