# What's New in Sequencing:

## A Sequencing System adapted to your Needs

Marco Cappelletti
Product Marketing Manager - Europe



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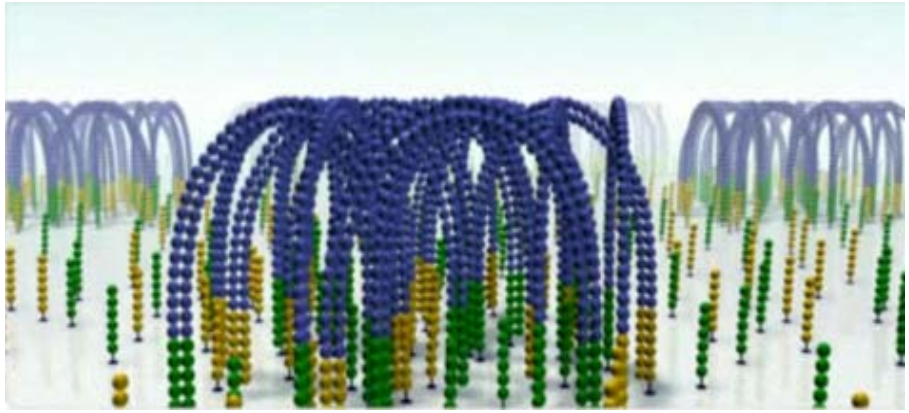
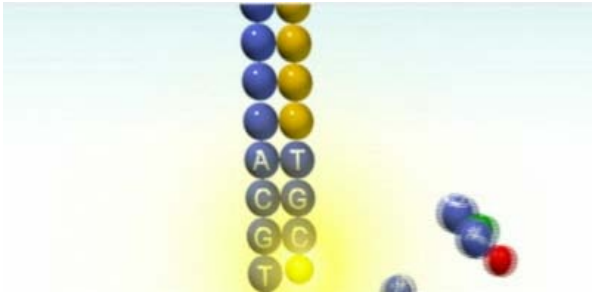
# Agenda

- Illumina Sequencing Technology
- Sequencing Workflow
- Applications



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# HiSeq 2000

*Redefining the trajectory of sequencing*

## HIGHEST OUTPUT

Initially capable of up to 200 Gb per run

## FASTEST DATA RATE

~25 Gb/day

7-8 days for 2 x 100 bp

## HIGHEST NUMBER OF READS

One billion single-end reads*

Two billion paired-end reads*


*Based on one billion clusters passing filter

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# An Illumina Sequencer for Every need

Next Generation Sequencing made accessible

**Genome Analyzer*IIe***

- 40 Gb/Run
- 260M Reads
- Read Length:150bp

Most widely adopted NGS platform

**Genome Analyzer*IIx***

- 50 Gb/Run
- 500M Reads
- Read Length:100bp

Unique combination of sequencing & arrays

**HiScanSQ**

- 50 Gb/Run
- 500M Reads
- Read Length:100bp

Redefining the trajectory of sequencing

**HiSeq 2000**

- 200 Gb/Run
- 2B Reads
- Read Length:100bp

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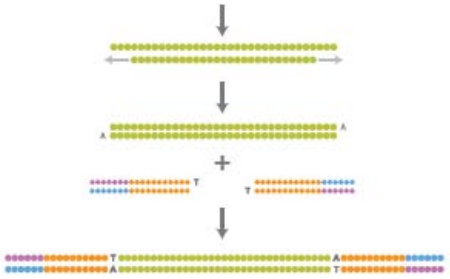
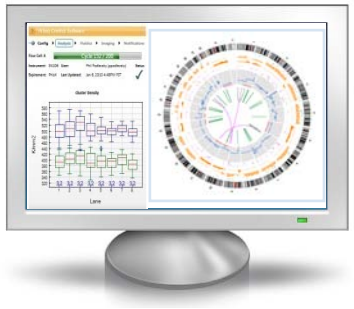
# Simplest Sequencing Workflow

| SIMPLIFIED SAMPLE PREP | cBot CLUSTER GENERATION | HiSeq 2000 SEQUENCING | DATA PROCESSING & ANALYSIS |
|---|---|---|---|
|  |  |  |  |
| Parallel sample processing | Automated cluster generation | Automated sequencing | Simple, efficient data analysis |

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# New Simplified Sample Prep Kits*

*Multiple DNA and mRNA samples for sequencing application*

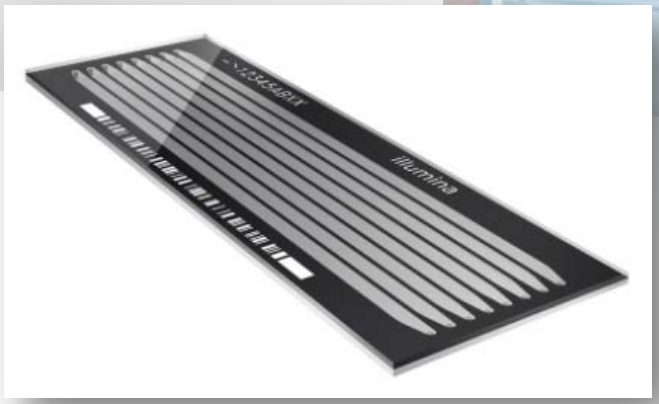
**HIGHLIGHTS**

**Streamlined Workflow:** Pre-mixed reagents reduce hands-on time when preparing multiple DNA or mRNA samples

**Faster Throughput:** Simultaneous preparation of 96 DNA or mRNA samples increases the number of samples processed/day

**Automation-Friendly Process:** Master-mixed reagents are compatible with liquid-handlers

**Cost-Effective Solution:** Significant reduction in sample preparation costs




*Expected availability Q2 2010

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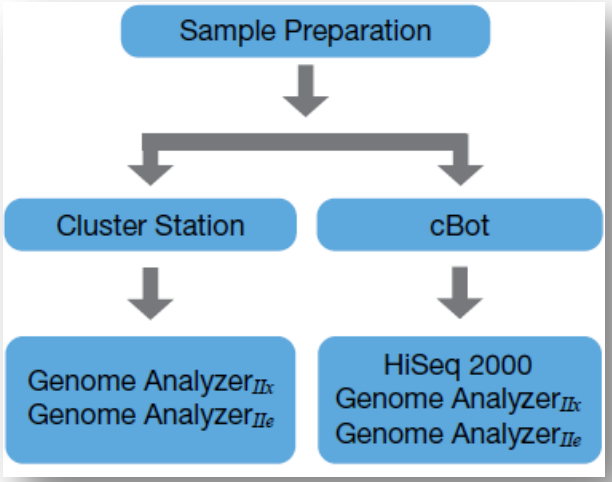
# Simplified Sample Preparation

Multiple DNA and mRNA samples for sequencing applications




## STREAMLINED WORKFLOW

Fast method for preparing gDNA or total RNA samples

Designed to process 12, 96, or more samples

Optimal for High-throughput studies

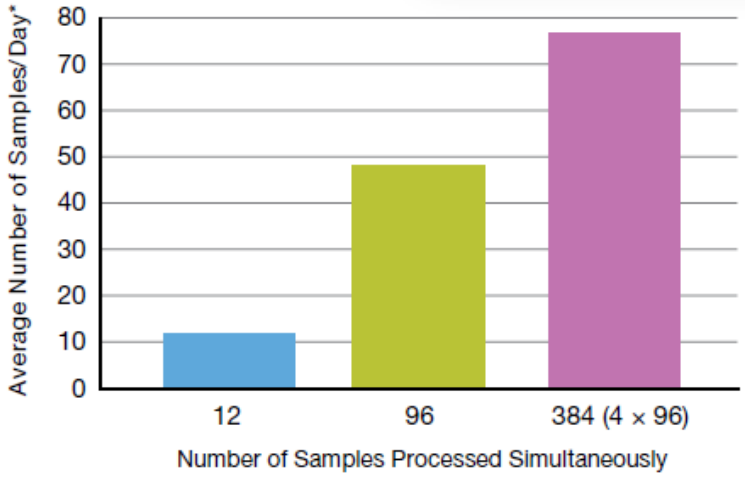
Universal Design, no need for multiple application kits

New Simplified Sample Multiplexing

Built-in Quality Control

Designed Around Automation

Part of the Illumina Solution

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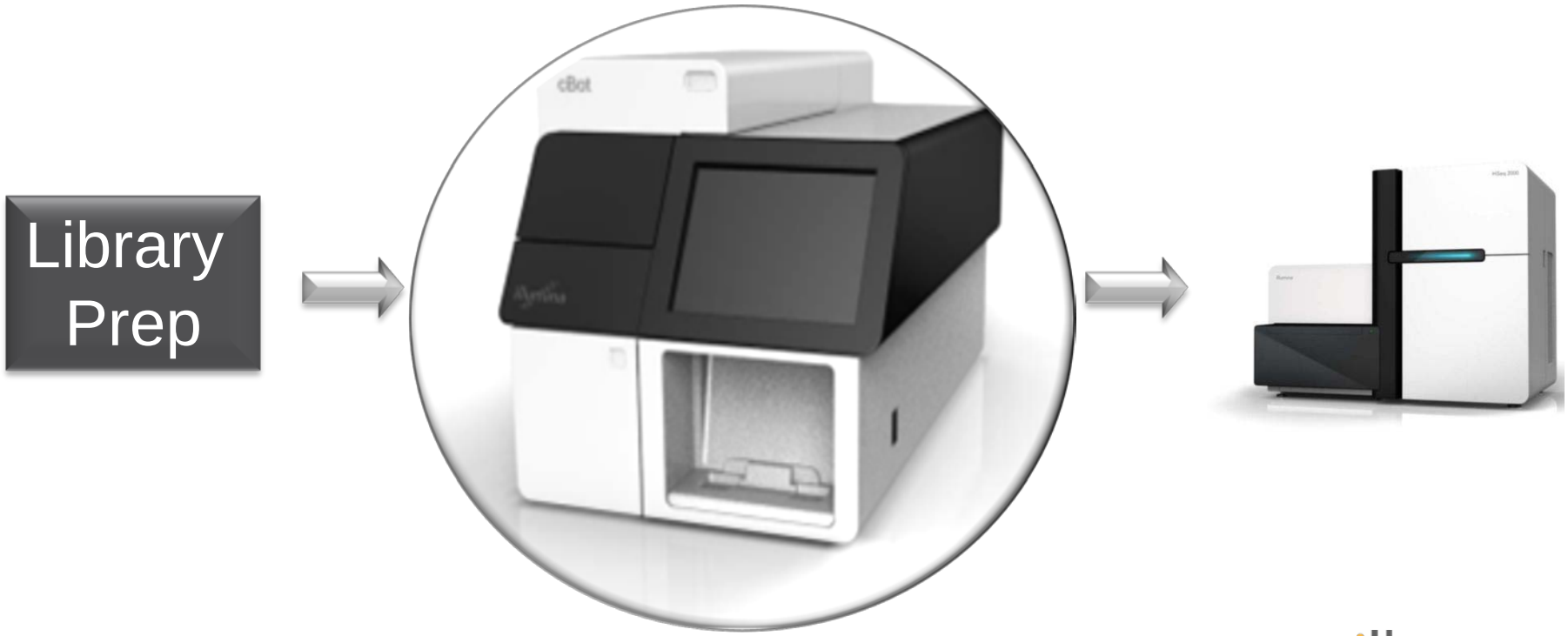
# cBot System – Cluster Generation Workflow

- Breakthrough workflow automation system
  - Clonal amplification of DNA for Illumina sequencing

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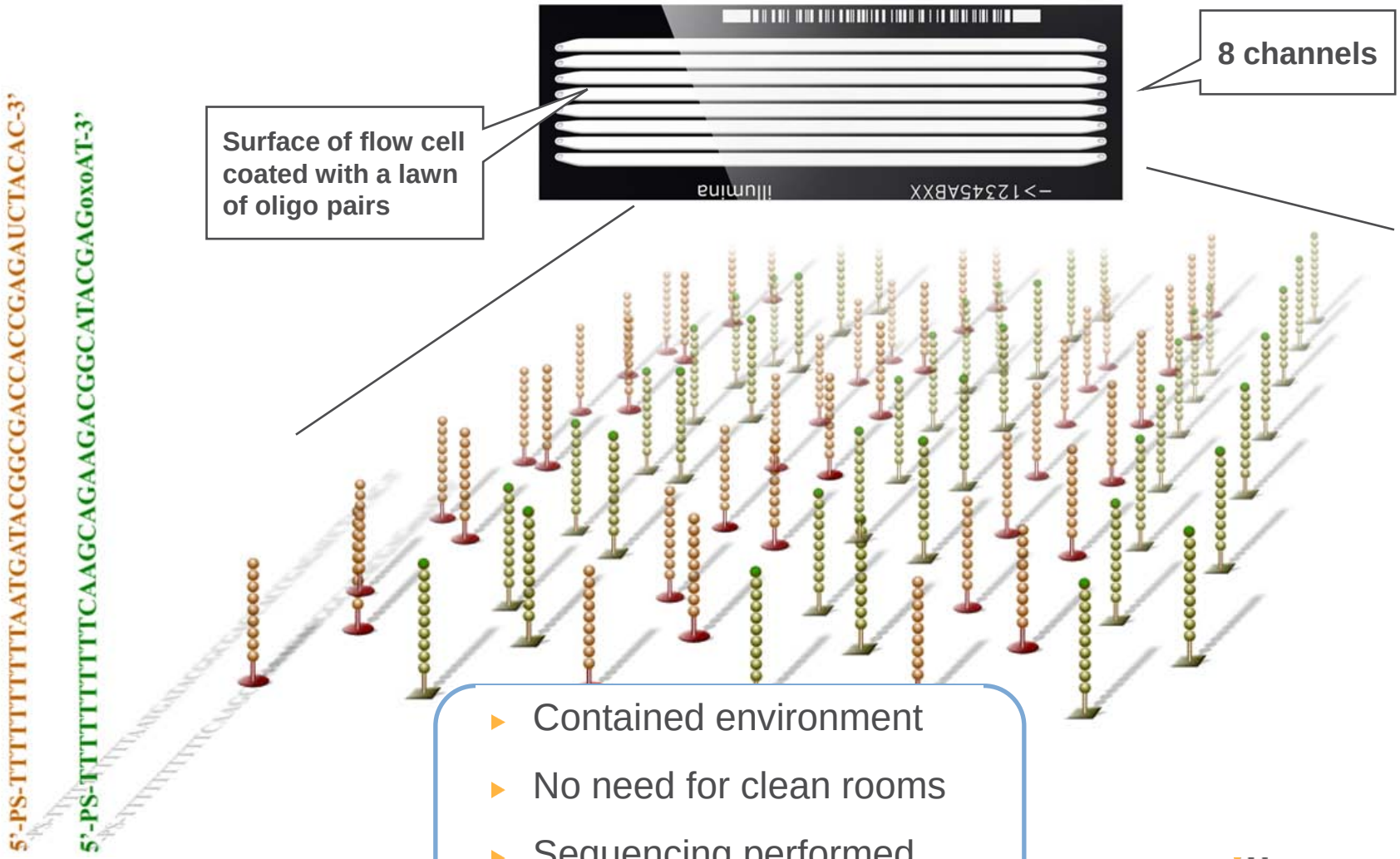
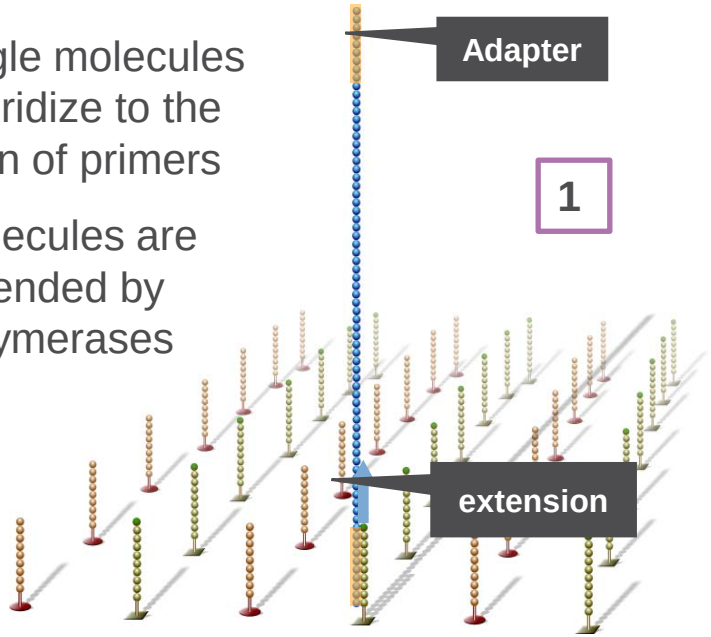
# The flow cell design



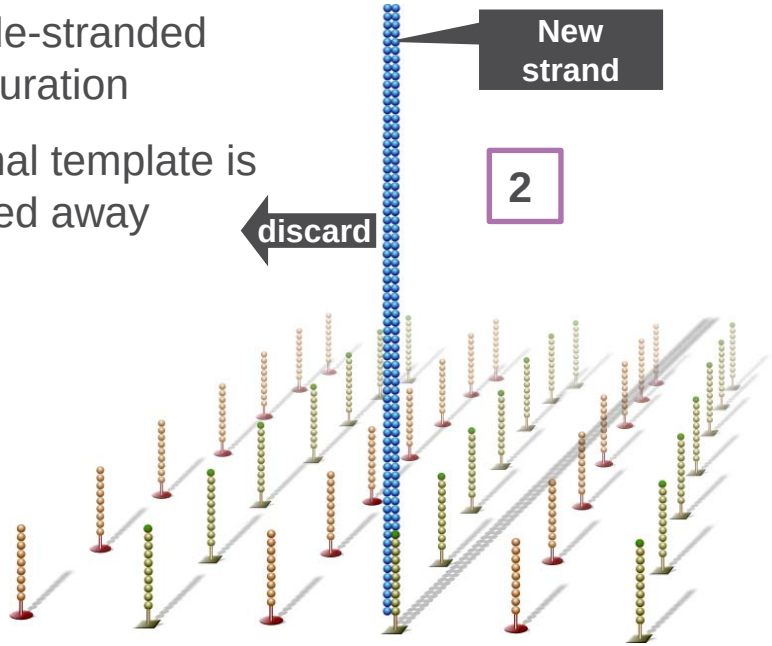

- Contained environment
- No need for clean rooms
- Sequencing performed inside the flow cell

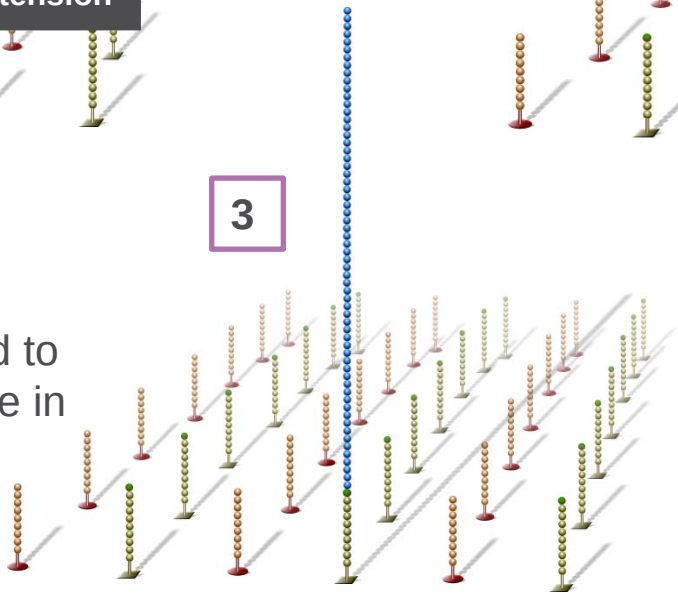
# Cluster Generation
## *Hybridize Fragment & Extend*

- Sngle molecules hybridize to the lawn of primers
- Molecules are extended by polymerases




- Double-stranded denaturation
- Original template is washed away




- Newly synthesized covalently attached to the flow cell surface in a random pattern

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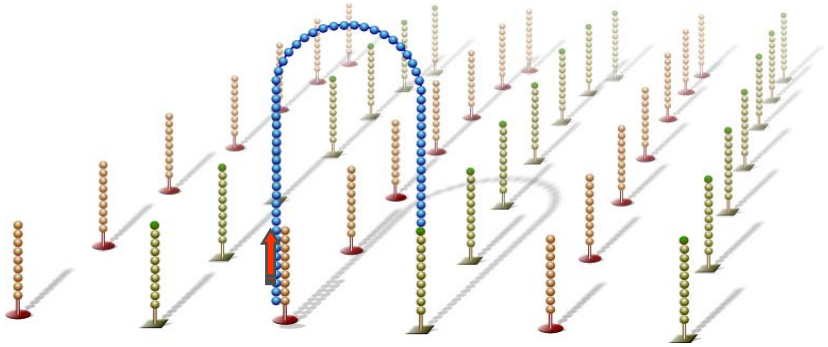
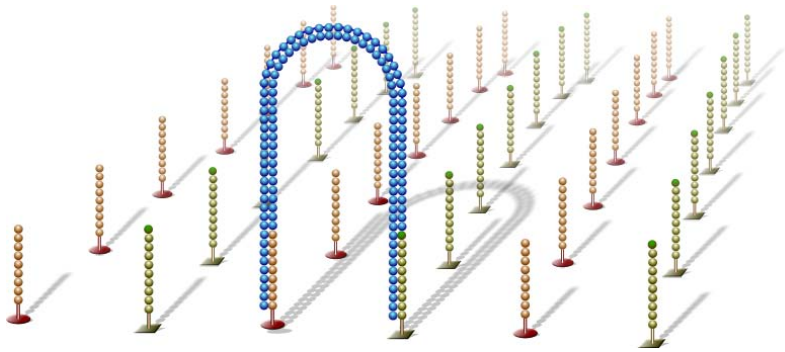
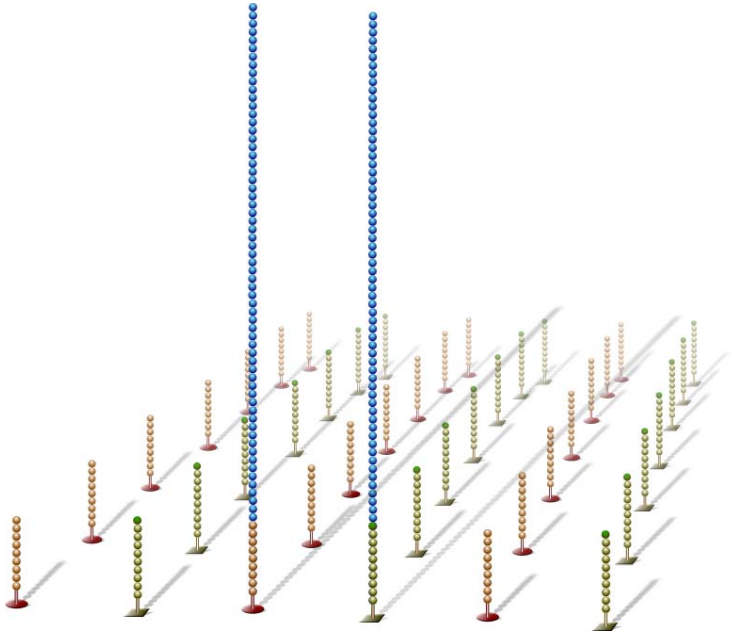
# Cluster Generation
## *Bridge Amplification*





- Single-strand flips over to hybridize to adjacent primers to form a bridge
- Hybridized primer is extended by polymerases
- Bridge is denatured



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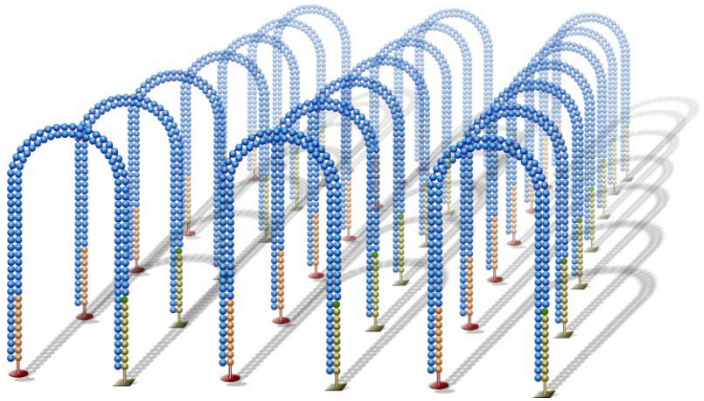
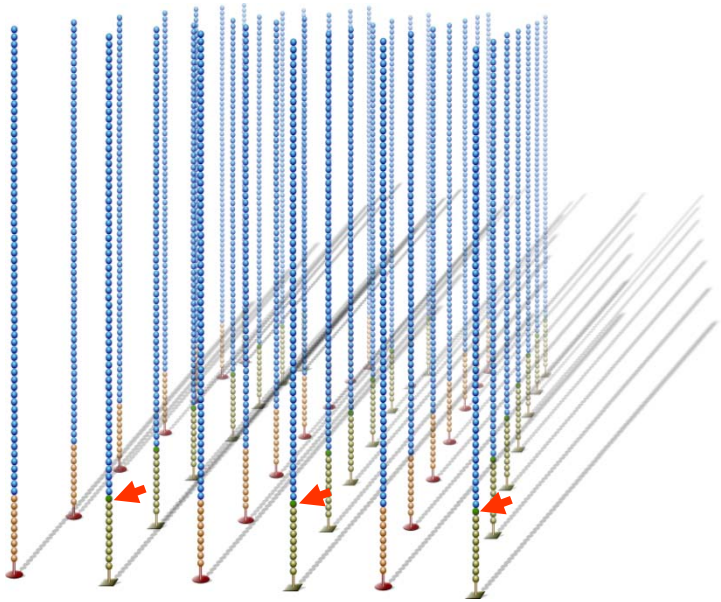
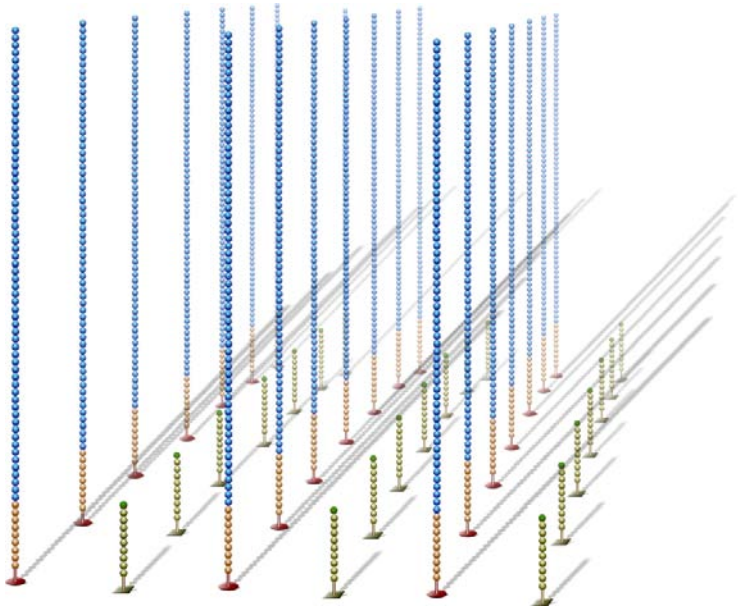
# Cluster Generation
## *Bridge Amplification*

- Bridge amplification cycle repeated until multiple bridges are formed
- Bridges denaturation
- Reverse strands cleaved and washed away







# cBot Performance Specifications

- Compatible with GA & iScan Seq Ext
- User installable
- Total hands-on time: <10 min
- Total run time: ~4 hrs
- Integrated barcode scanner
- Integrated touch-screen monitor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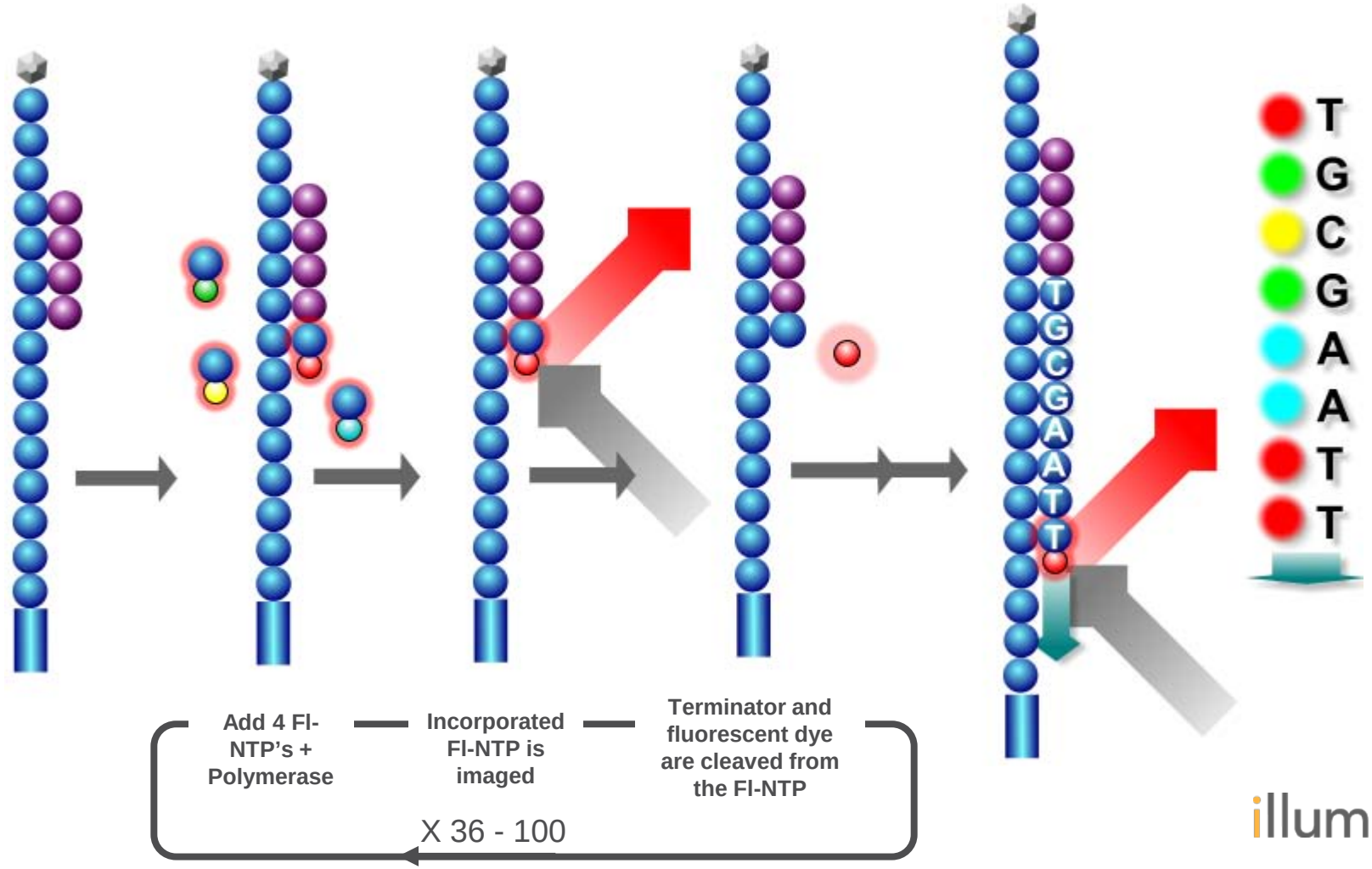
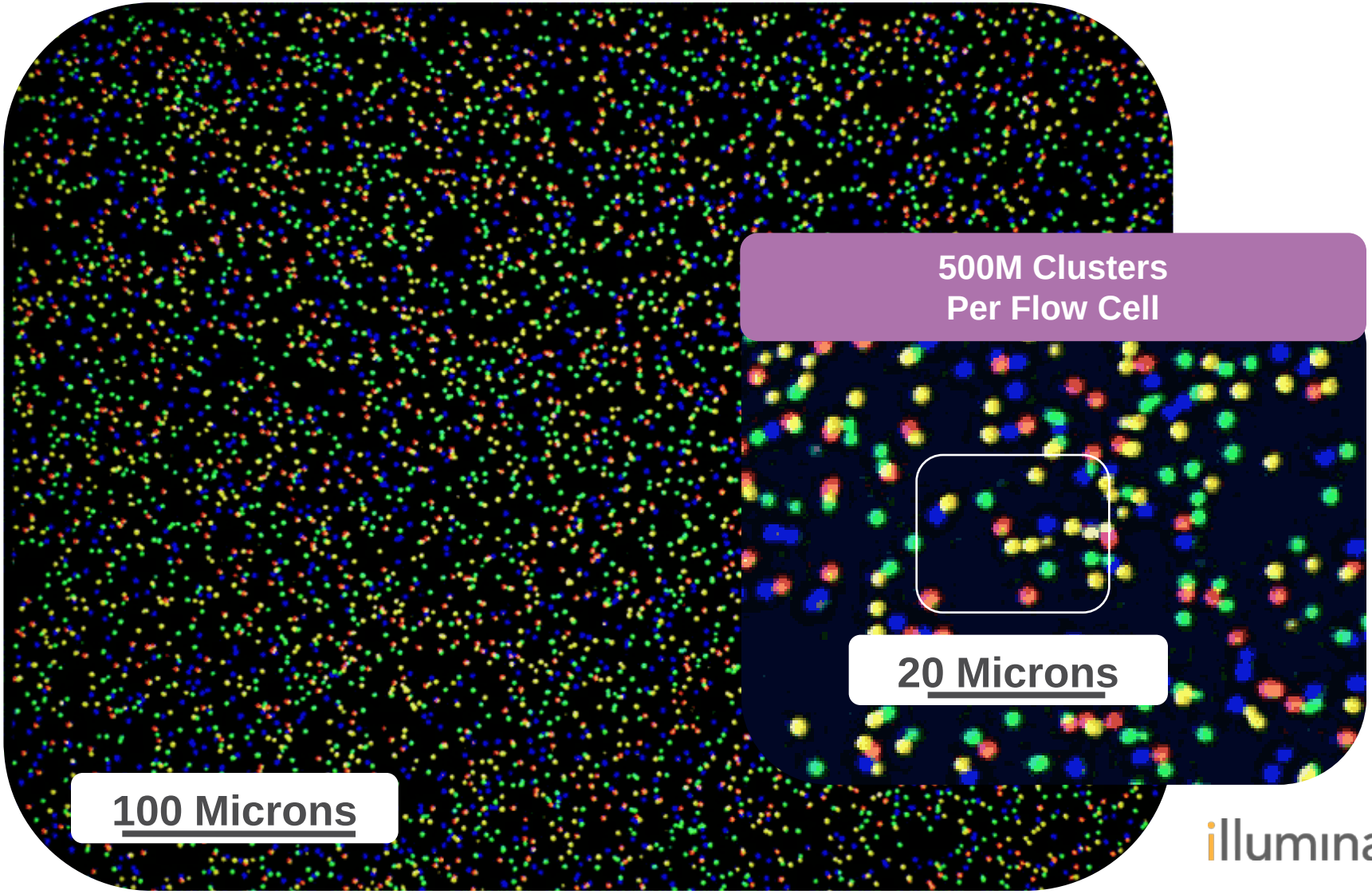
# Sequencing by Synthesis Chemistry

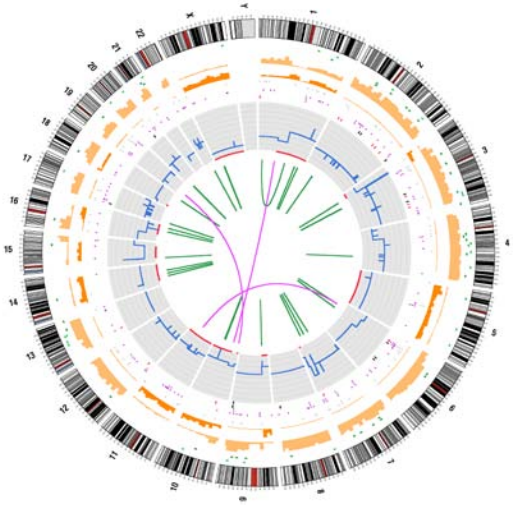


# Image acquisition



# Sequencing Data Analysis

## *A simple, familiar workflow*



**SEQUENCING CONTROL SOFTWARE**
**Read Generation**

**CASAVA**

**Alignments, variations, builds**

**VISUALIZATION**

**GenomeStudio, or favorite browser**

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# Broadest range of applications

***Optimized, streamlined and easy-to-use reagent solutions***

**Sample Prep**

**Genome**
- WG Reseq.
- De-novo
- Targeted

**Transcriptome**
- RNA-Seq
- Small RNA
- miRNA
- Directional Seq

**Regulation**
- Methylation
- ChIP-Seq

**Automated Cluster Generation**


**Sequencing**


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# Published Clinical Aapplications

- Copy Number Variation Analysis
- Pre-natal Diagnosis
- Metagenomics
- Expression Analysis from FFPET
- Epigenomics

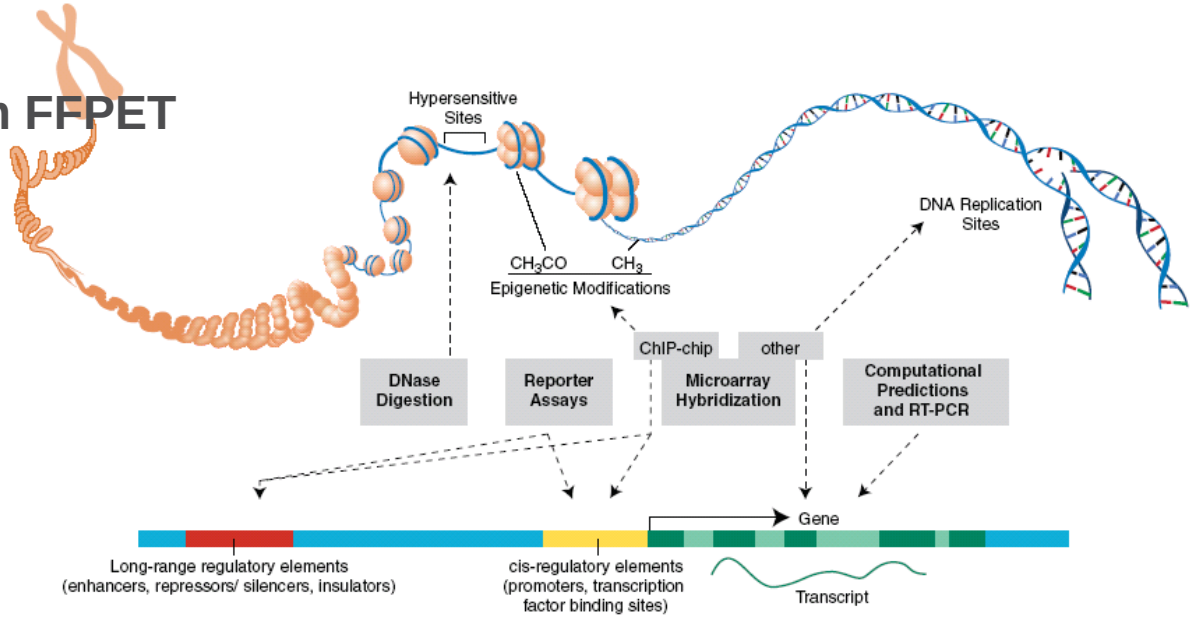


**Fig. 1.** Functional genomic elements being identified by the ENCODE pilot phase. The indicated methods are being used to identify different types of functional elements in the human genome.

www.sciencemag.org SCIENCE VOL 306 22 OCTOBER 2004

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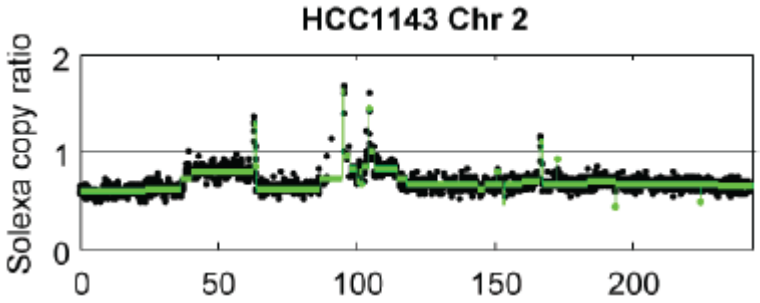
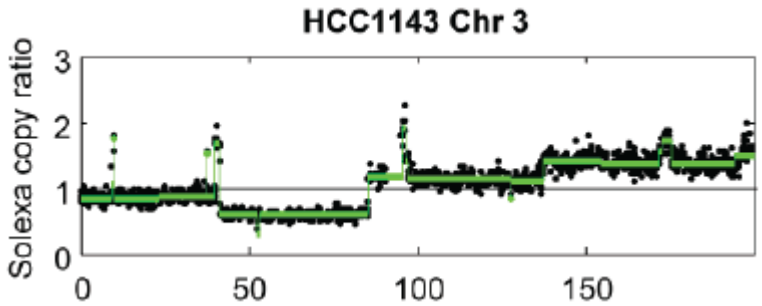
# Identify copy-number alterations

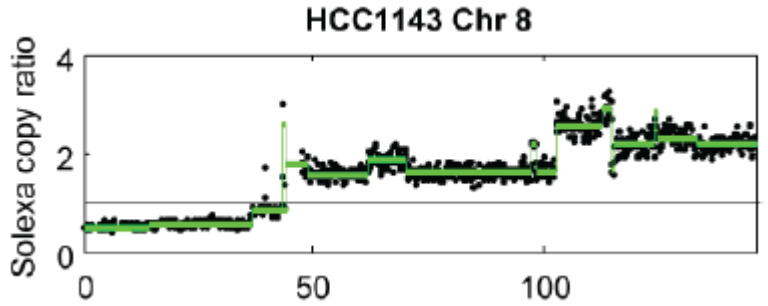
**Cancer High-resolution mapping of copy-number alterations with massively parallel sequencing** Nat Methods. 2009 Jan;6(1):99-103

- Cancer results from somatic alterations in key genes, including point mutations, copy-number alterations and structural rearrangements.

- The collection of million aligned sequence reads has comparable power to detect events as the current generation of DNA microarrays and has over twofold better precision for localizing breakpoints

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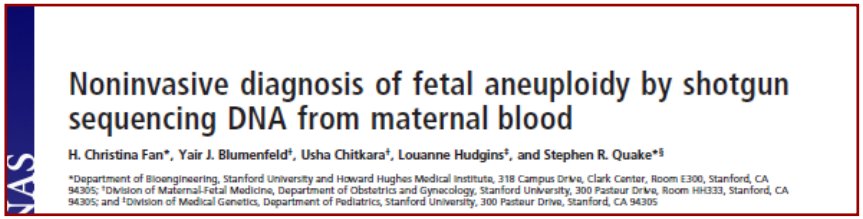
# Prenatal Diagnostics by Deep Sequencing

**(PNAS December 23, 2008 vol. 105 no. 51)**

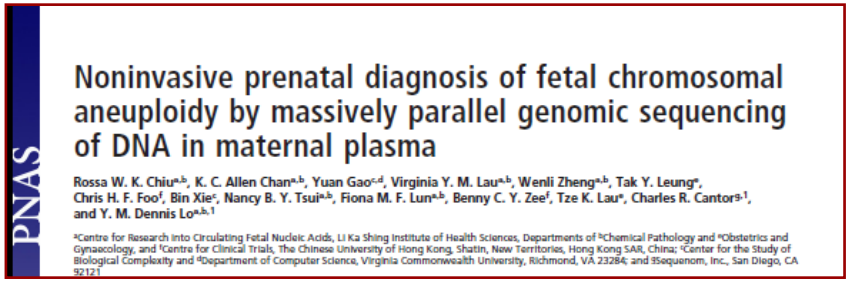
- "Fetal DNA has been found in maternal plasma but exists as a minor fraction among a high background of maternal DNA"…quantitative perturbations caused by an aneuploid chromosome in the fetal genome would be small"
- "The use of a locus-independent method would greatly increase the number of target molecules from the aneuploid chromosome that could be analyzed within the same fixed volume of plasma
- Sequencing is the clear way to do non-invasive prenatal testing. … existing noninvasive Down syndrome tests are not very informative and provide variable results depending on the ethnicity of those taking the test."



Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood

H. Christina Fan*, Yair J. Blumenfeld†, Usha Chitkara†, Louanne Hudgins†, and Stephen R. Quake*§

*Department of Bioengineering, Stanford University and Howard Hughes Medical Institute, 318 Campus Drive, Clark Center, Room E300, Stanford, CA 94305; †Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Stanford University, 300 Pasteur Drive, Room HH333, Stanford, CA 94305; and ‡Division of Medical Genetics, Department of Pediatrics, Stanford University, 300 Pasteur Drive, Stanford, CA 94305



PNAS

Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma

Rossa W. K. Chiu[a,b], K. C. Allen Chan[a,b], Yuan Gao[c,d], Virginia Y. M. Lau[a,b], Wenli Zheng[a,b], Tak Y. Leung[e], Chris H. F. Foo[f], Bin Xie[c], Nancy B. Y. Tsui[a,b], Fiona M. F. Lun[a,b], Benny C. Y. Zee[f], Tze K. Lau[e], Charles R. Cantor[g,1], and Y. M. Dennis Lo[a,b,1]

[a]Centre for Research into Circulating Fetal Nucleic Acids, Li Ka Shing Institute of Health Sciences, Departments of [e]Chemical Pathology and [e]Obstetrics and Gynaecology, and [f]Centre for Clinical Trials, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China; [c]Center for the Study of Biological Complexity and [d]Department of Computer Science, Virginia Commonwealth University, Richmond, VA 23284; and [g]Sequenom, Inc., San Diego, CA 92121

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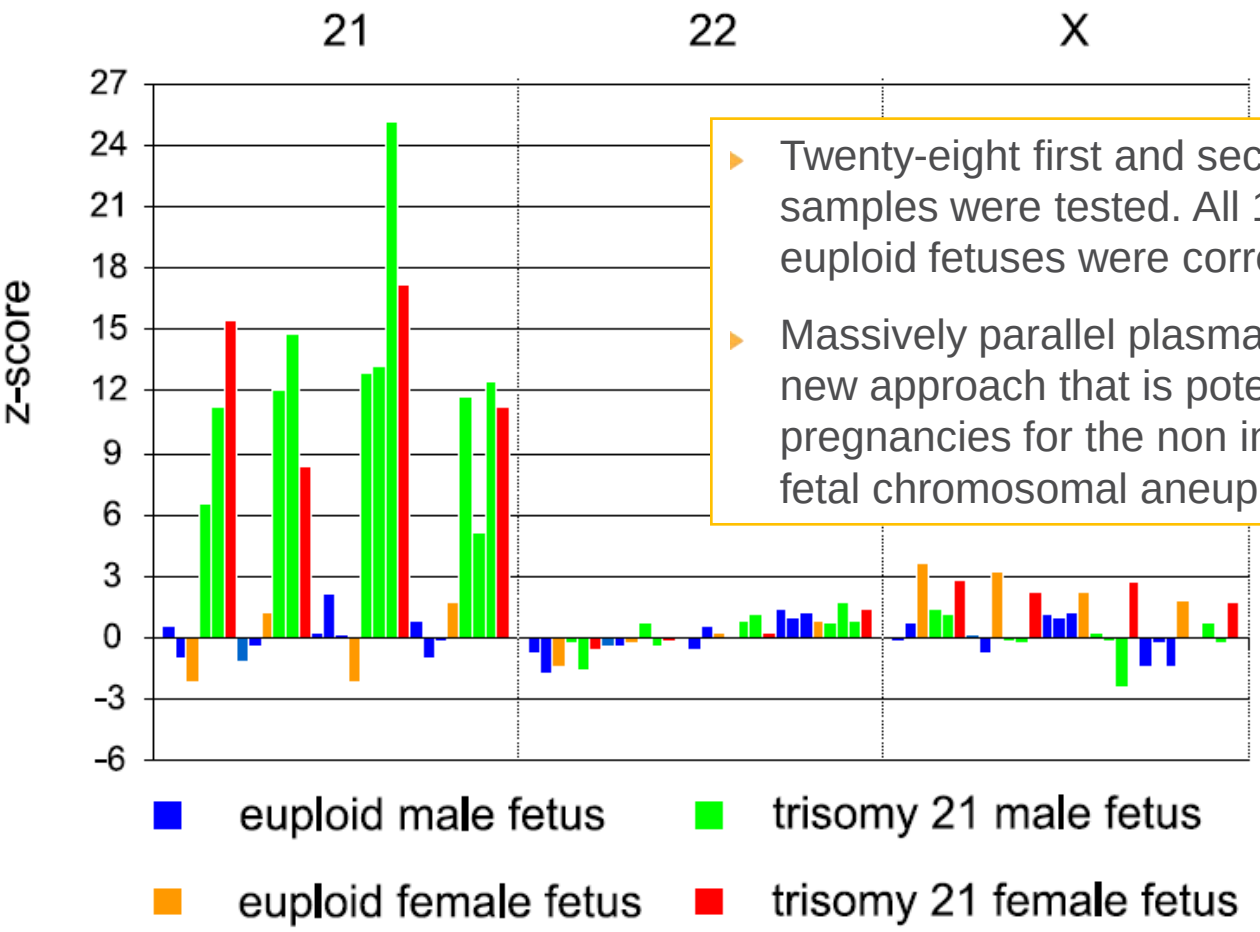
**(PNAS December 23, 2008 vol. 105 no. 51)**




- Twenty-eight first and second trimester maternal plasma samples were tested. All 14 trisomy 21 fetuses and 14 euploid fetuses were correctly identified.
- Massively parallel plasma DNA sequencing represents a new approach that is potentially applicable to all pregnancies for the non invasive prenatal diagnosis of fetal chromosomal aneuploidies.

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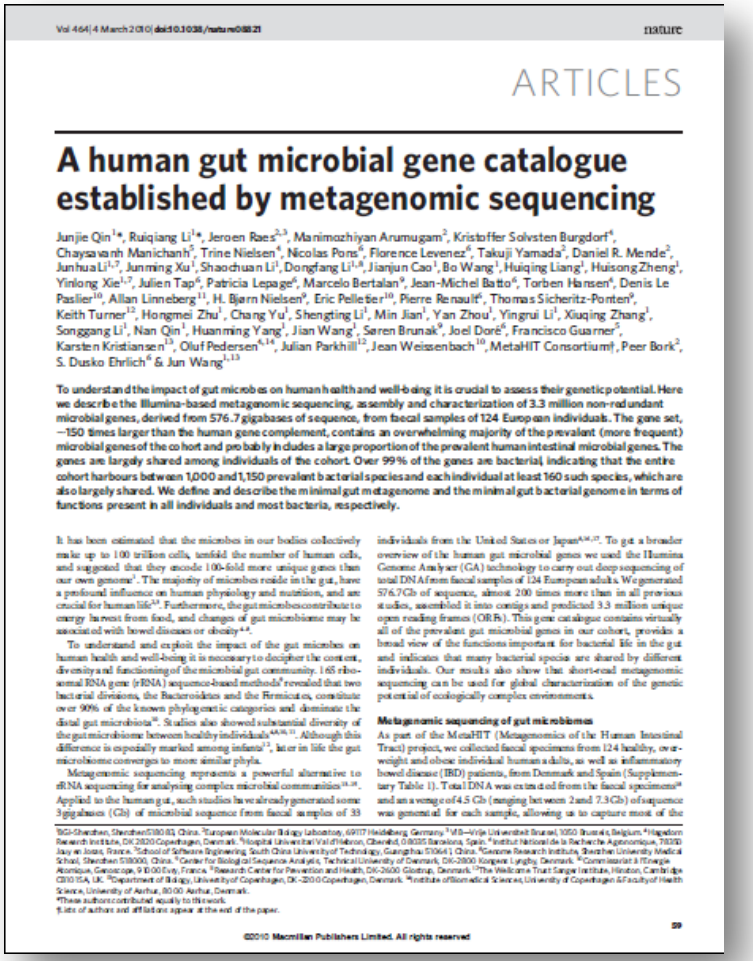
# A human gut microbial gene catalogue established by metagenomic sequencing

*Nature* (2010), Vol 464|4 March 2010| doi:10.1038/nature08821

- To understand the impact of **gut microbes on human health** and well-being it is crucial decipher the content, diversity and functioning of the microbial gut community
- Metagenomic sequencing, assembly and characterization of **3.3 million non-redundant microbial genes** from human faecal samples (576.7 Gb of sequence, 124 European individuals)
- The gene set contains an overwhelming majority of the prevalent microbial genes..



Vol 464|4 March 2010|doi:10.1038/nature08821 nature

ARTICLES

## A human gut microbial gene catalogue established by metagenomic sequencing

Junjie Qin[1]*, Ruiqiang Li[1]*, Jeroen Raes[2,3], Manimozhiyan Arumugam[2], Kristoffer Solvsten Burgdorf[4], Chaysavanh Manichanh[5], Trine Nielsen[4], Nicolas Pons[6], Florence Levenez[6], Takuji Yamada[2], Daniel R. Mende[2], Junhua Li[1,7], Junming Xu[1], Shaochuan Li[1], Dongfang Li[1,8], Jianjun Cao[1], Bo Wang[1], Huiqing Liang[1], Huisong Zheng[1], Yinlong Xie[1,7], Julien Tap[6], Patricia Lepage[6], Marcelo Bertalan[9], Jean-Michel Batto[6], Torben Hansen[4], Denis Le Paslier[10], Allan Linneberg[11], H. Bjørn Nielsen[9], Eric Pelletier[10], Pierre Renault[6], Thomas Sicheritz-Ponten[9], Keith Turner[12], Hongmei Zhu[1], Chang Yu[1], Shengting Li[1], Min Jian[1], Yan Zhou[1], Yingrui Li[1], Xiuqing Zhang[1], Songgang Li[1], Nan Qin[1], Huanming Yang[1], Jian Wang[1], Søren Brunak[9], Joel Doré[6], Francisco Guarner[5], Karsten Kristiansen[13], Oluf Pedersen[4,14], Julian Parkhill[12], Jean Weissenbach[10], MetaHIT Consortium†, Peer Bork[2], S. Dusko Ehrlich[6] & Jun Wang[1,13]

**To understand the impact of gut microbes on human health and well-being it is crucial to assess their genetic potential. Here we describe the Illumina-based metagenomic sequencing, assembly and characterization of 3.3 million non-redundant microbial genes, derived from 576.7 gigabases of sequence, from faecal samples of 124 European individuals. The gene set, ~150 times larger than the human gene complement, contains an overwhelming majority of the prevalent (more frequent) microbial genes of the cohort and probably includes a large proportion of the prevalent human intestinal microbial genes. The genes are largely shared among individuals of the cohort. Over 99% of the genes are bacterial, indicating that the entire cohort harbours between 1,000 and 1,150 prevalent bacterial species and each individual at least 160 such species, which are also largely shared. We define and describe the minimal gut metagenome and the minimal gut bacterial genome in terms of functions present in all individuals and most bacteria, respectively.**

It has been estimated that the microbes in our bodies collectively make up to 100 trillion cells, tenfold the number of human cells, and suggested that they encode 100-fold more unique genes than our own genome[1]. The majority of microbes reside in the gut, have a profound influence on human physiology and nutrition, and are crucial for human life[2,3]. Furthermore, the gut microbes contribute to energy harvest from food, and changes of gut microbiome may be associated with bowel diseases or obesity[4–6].

To understand and exploit the impact of the gut microbes on human health and well-being it is necessary to decipher the content, diversity and functioning of the microbial gut community. 16S ribosomal RNA gene (rRNA) sequence-based methods[7] revealed that two bacterial divisions, the Bacteroidetes and the Firmicutes, constitute over 90% of the known phylogenetic categories and dominate the distal gut microbiota[8]. Studies also showed substantial diversity of the gut microbiome between healthy individuals[4,6,9–11]. Although this difference is especially marked among infants[12], later in life the gut microbiome converges to more similar phyla.

Metagenomic sequencing represents a powerful alternative to rRNA sequencing for analysing complex microbial communities[13–15]. Applied to the human gut, such studies have already generated some 3 gigabases (Gb) of microbial sequence from faecal samples of 33 individuals from the United States or Japan[8,16,17]. To get a broader overview of the human gut microbial genes we used the Illumina Genome Analyser (GA) technology to carry out deep sequencing of total DNA from faecal samples of 124 European adults. We generated 576.7 Gb of sequence, almost 200 times more than in all previous studies, assembled it into contigs and predicted 3.3 million unique open reading frames (ORFs). This gene catalogue contains virtually all of the prevalent gut microbial genes in our cohort, provides a broad view of the functions important for bacterial life in the gut and indicates that many bacterial species are shared by different individuals. Our results also show that short-read metagenomic sequencing can be used for global characterization of the genetic potential of ecologically complex environments.

### Metagenomic sequencing of gut microbiomes

As part of the MetaHIT (Metagenomics of the Human Intestinal Tract) project, we collected faecal specimens from 124 healthy, overweight and obese individual human adults, as well as inflammatory bowel disease (IBD) patients, from Denmark and Spain (Supplementary Table 1). Total DNA was extracted from the faecal specimens[18] and on average of 4.5 Gb (ranging between 2 and 7.3 Gb) of sequence was generated for each sample, allowing us to capture most of the

BGI-Shenzhen, Shenzhen 518083, China. [2]European Molecular Biology Laboratory, 69117 Heidelberg, Germany. [3]VIB—Vrije Universiteit Brussel, 1050 Brussels, Belgium. [4]Hagedorn Research Institute, DK-2820 Copenhagen, Denmark. [5]Hospital Universitari Vall d'Hebron, Ciberehd, 08035 Barcelona, Spain. [6]Institut National de la Recherche Agronomique, 78350 Jouy en Josas, France. [7]School of Software Engineering, South China University of Technology, Guangzhou 510641, China. [8]Genome Research Institute, Shenzhen University Medical School, Shenzhen 518000, China. [9]Center for Biological Sequence Analysis, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark. [10]Commissariat à l'Energie Atomique, Genoscope, 91000 Evry, France. [11]Research Centre for Prevention and Health, DK-2600 Glostrup, Denmark. [12]The Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK. [13]Department of Biology, University of Copenhagen, DK-2200 Copenhagen, Denmark. [14]Institute of Biomedical Sciences, University of Copenhagen & Faculty of Health Sciences, University of Aarhus, 8000 Aarhus, Denmark.
*These authors contributed equally to this work.
†Lists of authors and affiliations appear at the end of the paper.





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# A human gut microbial gene catalogue established by metagenomic sequencing

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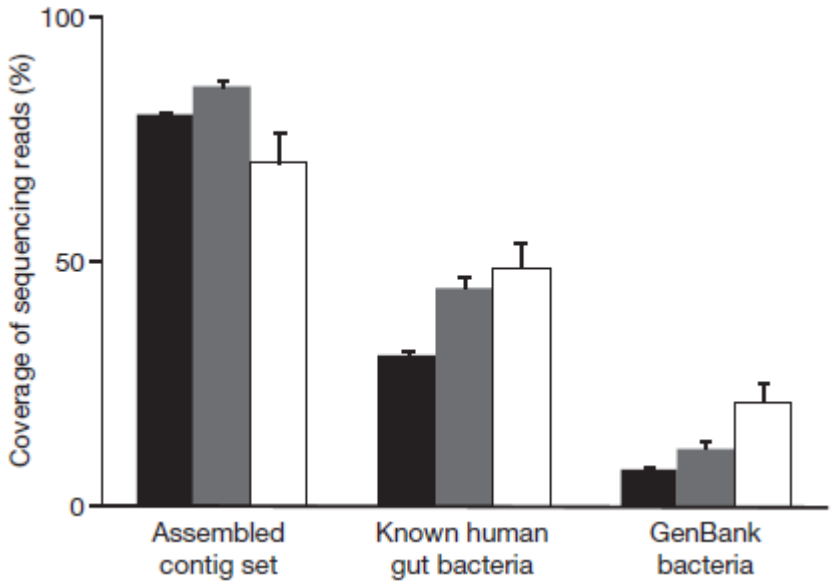


- **85.7% and 69.5% of our contigs were not covered by the reads from the two previous studies highlighting the novelty we captured**

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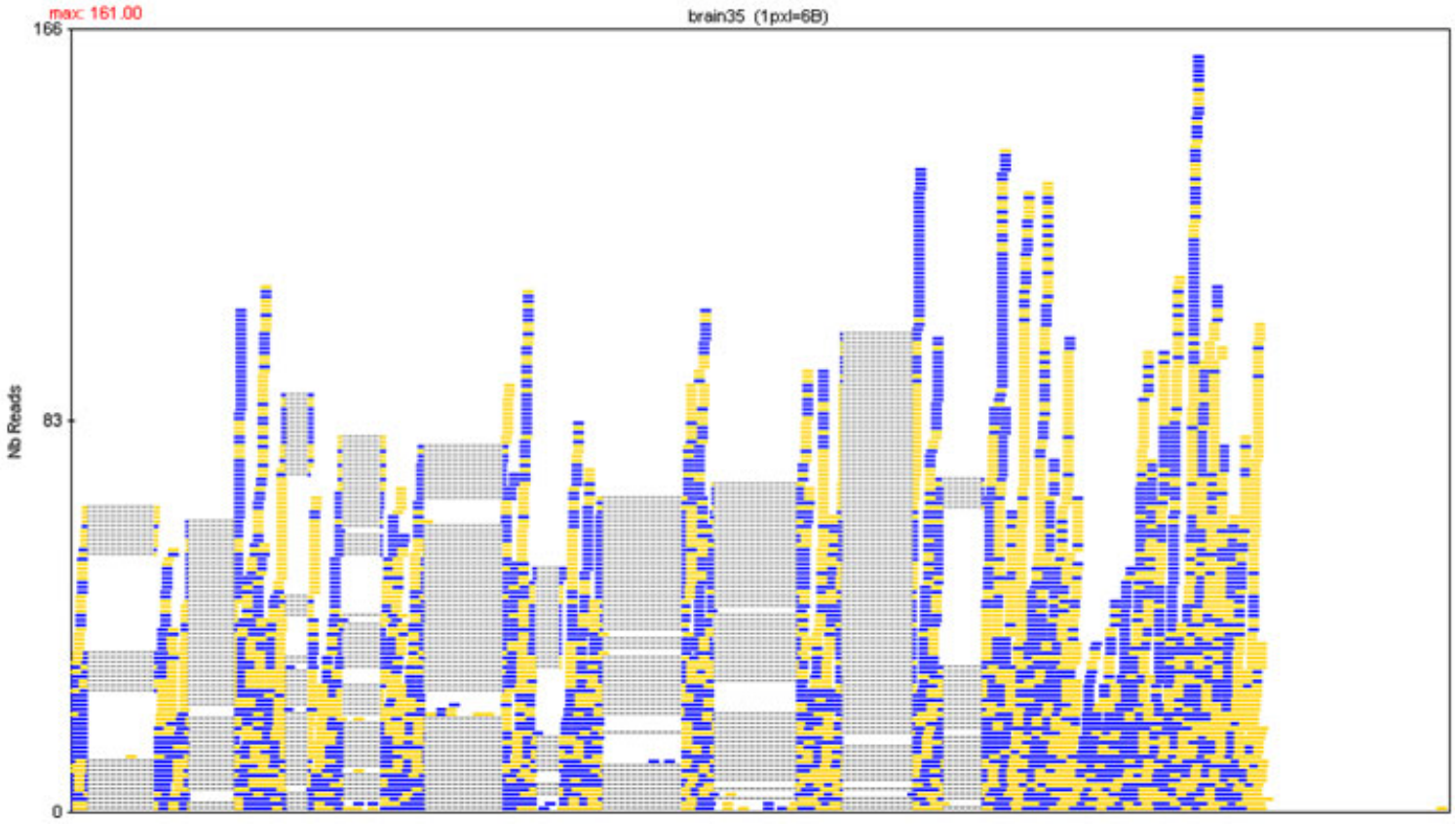
# Transcriptomics

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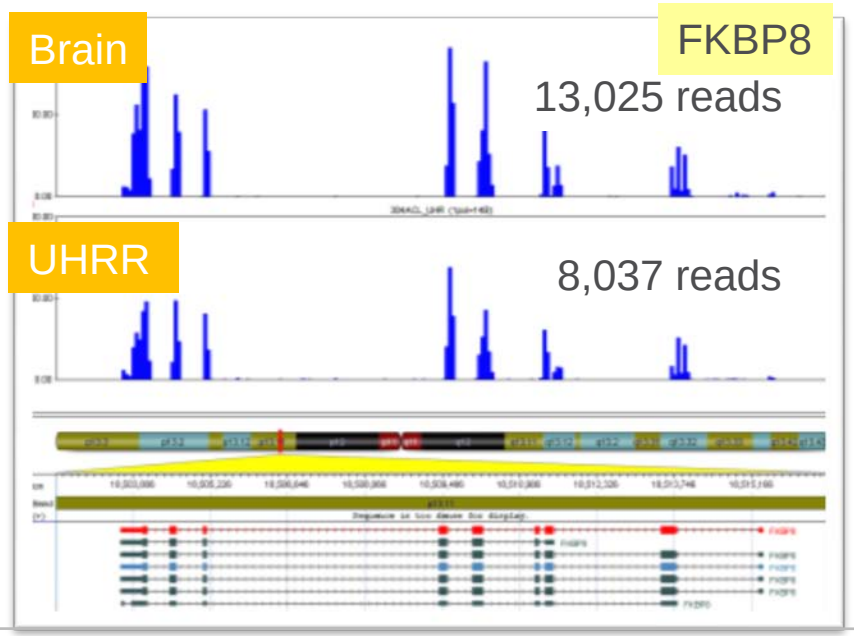
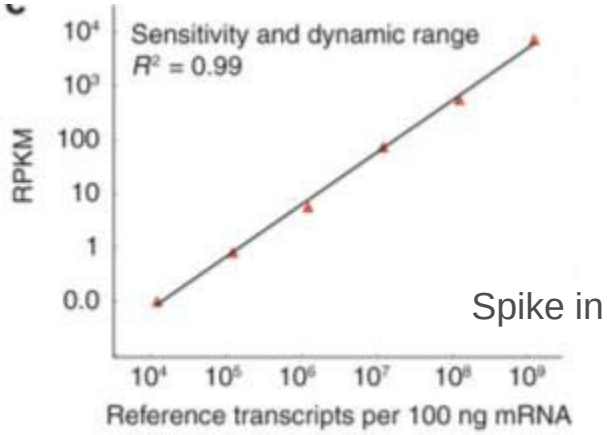
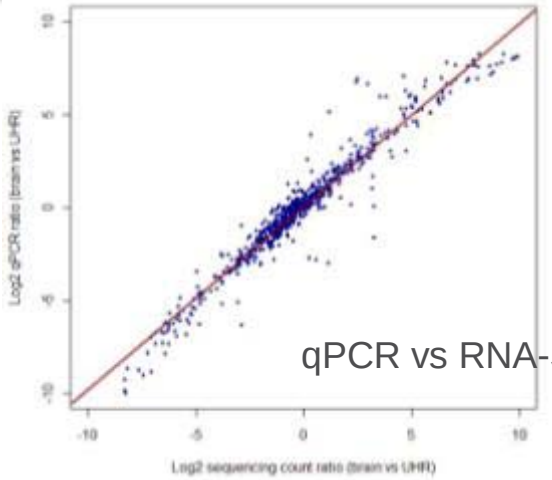
# Quantitative Measure of Gene Expression








Spike in




qPCR vs RNA-Seq

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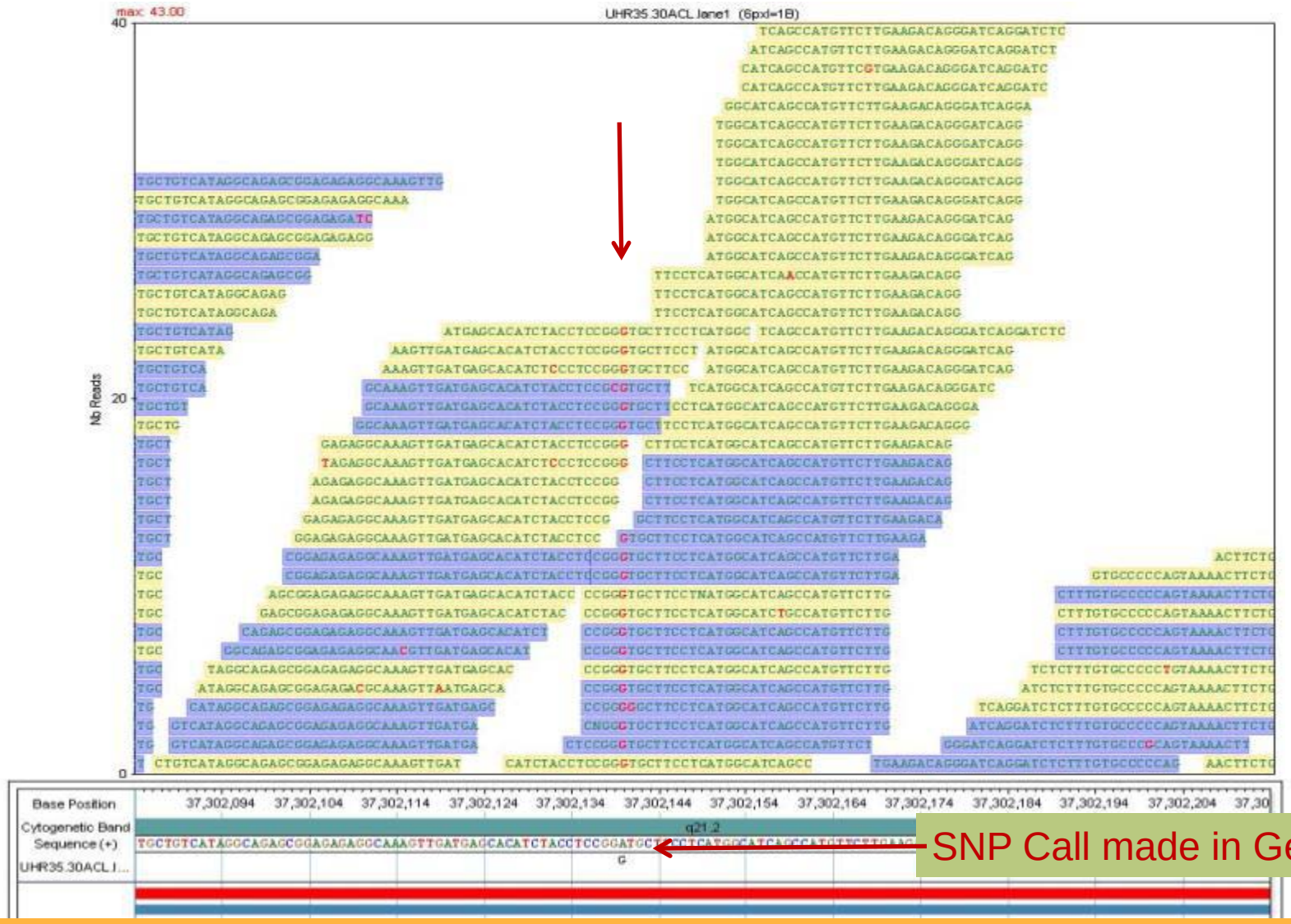
# Visualization of Polymorphisms in the Transcriptome



# 3’-End Sequencing for Expression Quantification (3SEQ) from Archival Tumor Samples

January 2010 | Volume 5 | Issue 1 | e8768

- A novel procedure (3SEQ) for gene expression profiling from FFPET using NGS.
- GEX profiling by 3SEQ and microarray on both frozen tissue and FFPET from two soft tissue tumors (desmoid type fibromatosis (DTF) and solitary fibrous tumor (SFT))
- Analysis of 3SEQ data revealed many genes differentially expressed between the tumor types on both the frozen tissue (~9.6K genes) and FFPET (~8.1K genes)
- Found Biological pathways known to be important in DTF and SFT pathogenesis

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PLoS one

3′-End Sequencing for Expression Quantification (3SEQ) from Archival Tumor Samples

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**Abstract**

Gene expression microarrays are the most widely used technique for genome-wide expression profiling. However, microarrays do not perform well on formalin fixed paraffin embedded tissue (FFPET). Consequently, microarrays cannot be effectively utilized to perform gene expression profiling on the vast majority of archival tumor samples. To address this limitation of gene expression microarrays, we designed a novel procedure (3′-end sequencing for expression quantification (3SEQ)) for gene expression profiling from FFPET using next-generation sequencing. We performed gene expression profiling by 3SEQ and microarray on both frozen tissue and FFPET from two soft tissue tumors (desmoid type fibromatosis (DTF) and solitary fibrous tumor (SFT)) (total $n = 23$ samples), which were each profiled by at least one of the four platform-tissue preparation combinations). Analysis of 3SEQ data revealed many genes differentially expressed between the tumor types (FDR<0.01) on both the frozen tissue (~9.6K genes) and FFPET (~8.1K genes). Analysis of microarray data from frozen tissue revealed fewer differentially expressed genes (~4.6K), and analysis of microarray data on FFPET revealed very few (69) differentially expressed genes. Functional gene set analysis of 3SEQ data from both frozen tissue and FFPET identified biological pathways known to be important in DTF and SFT pathogenesis and suggested several additional candidate oncogenic pathways in these tumors. These findings demonstrate that 3SEQ is an effective technique for gene expression profiling from archival tumor samples and may facilitate significant advances in translational cancer research.

**Citation:** Beck AH, Weng Z, Witten DM, Zhu S, Foley JW, et al. (2010) 3′-End Sequencing for Expression Quantification (3SEQ) from Archival Tumor Samples. PLoS ONE 5(1): e8768. doi:10.1371/journal.pone.0008768

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**Competing Interests:** The authors have declared that no competing interests exist.

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☯ These authors contributed equally to this work.

**Introduction**

The development of gene expression microarrays in the mid-1990s represented a significant technical achievement that, for the first time, permitted the systematic genome-wide evaluation of gene expression [1,2]. Since their introduction, these technologies have been widely used for gene expression profiling of cancer samples, leading to the identification of gene expression patterns that predict the biological and clinical features of a wide range of human malignancies [3–16].

Despite the large numbers of gene expression profiling experiments performed on human cancers, the full potential of these technologies for impacting the clinical management of cancer patients has not yet been realized [17–21]. A major limitation of gene expression microarrays for translational cancer research is that they rely on the availability of fresh frozen tissue and show inconsistent performance on formalin fixed paraffin embedded tissue (FFPET) [22–27]. Consequently, gene microarrays cannot be used effectively on the vast majority of tumor specimens, since few samples are stored frozen. In contrast, essentially all tumor samples are stored as FFPET in pathology laboratories around the world [28]. In an attempt to utilize this rich source of human tumor samples, investigators have resorted to measuring the expression of relatively small numbers of known transcripts from FFPET through the use of a variety of targeted approaches, including reverse transcriptase-polymerase chain reaction (RT-PCR) [29,30] and cDNA-mediated annealing, selection, extension and ligation (DASL) [31,32]. No technique currently exists for accurate quantitative genome-wide expression profiling from FFPET.

In the past several years, there have been major advances in sequencing technologies, resulting in the development of ultra high-throughput sequencing (UHTS) platforms that have allowed significant increases in sequencing throughput and decreases in sequencing cost [33,34]. There is considerable hope in the scientific community that UHTS will overcome the major limitations of microarray technology and revolutionize the field of functional genomics [35,36]. UHTS has been developed in several platforms, including Roche 454, Illumina Genome Analyzer, and ABI SOLID. These technologies have been used



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# 3’-End Sequencing for Expression Quantification (3SEQ) from Archival Tumor Samples

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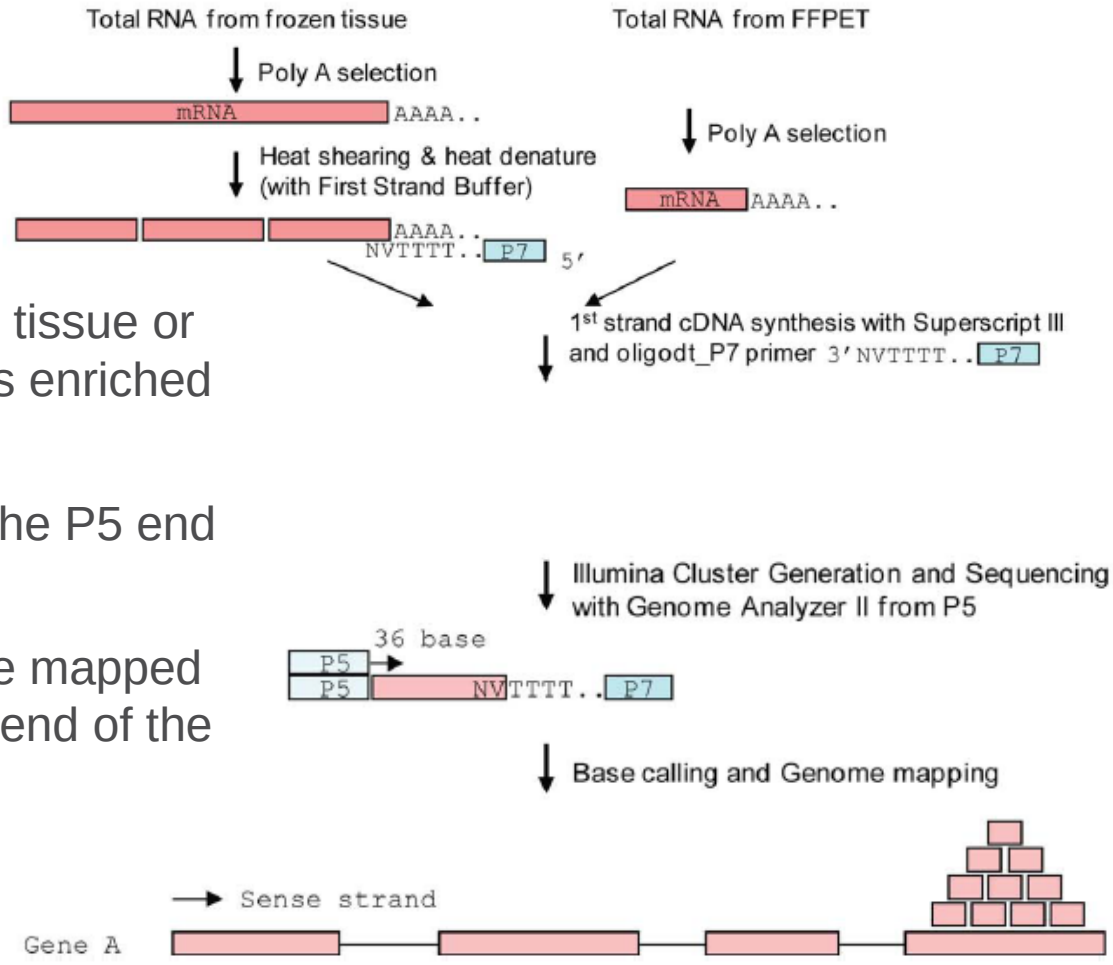


- Either intact mRNA from frozen tissue or degraded mRNA from FFPET is enriched by poly-A selection
- The library is sequenced from the P5 end to generate 36 bp reads
- These reads are expected to be mapped towards to the 3’ UTR or the 3’ end of the expressed genes

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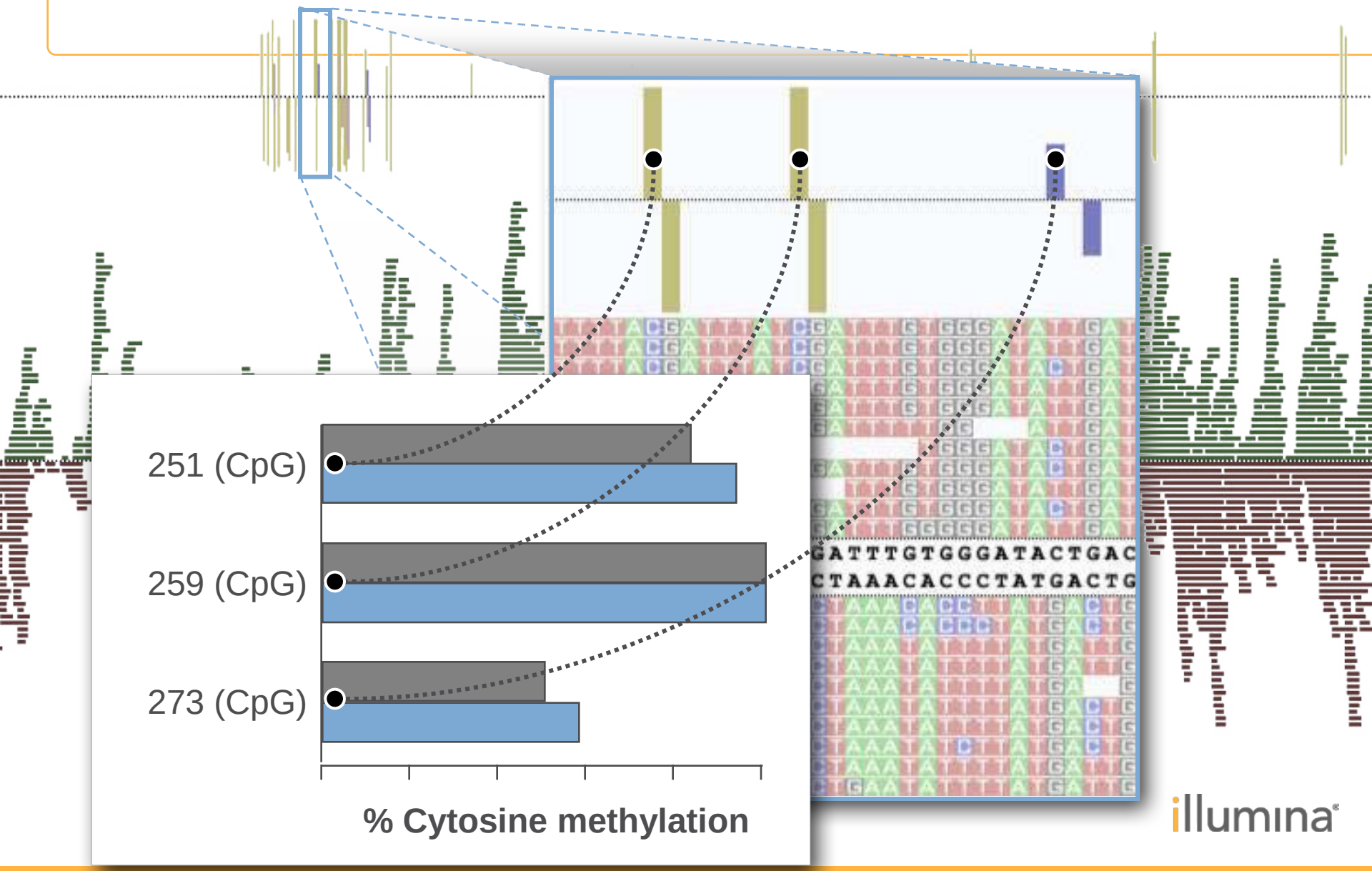
# Methylation Analysis

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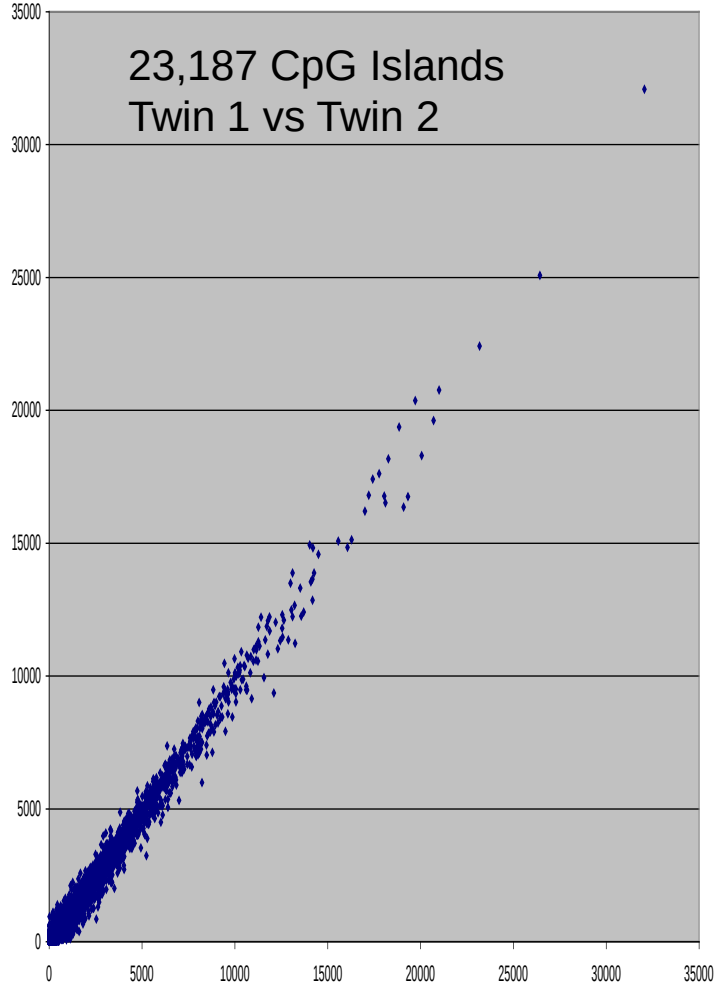
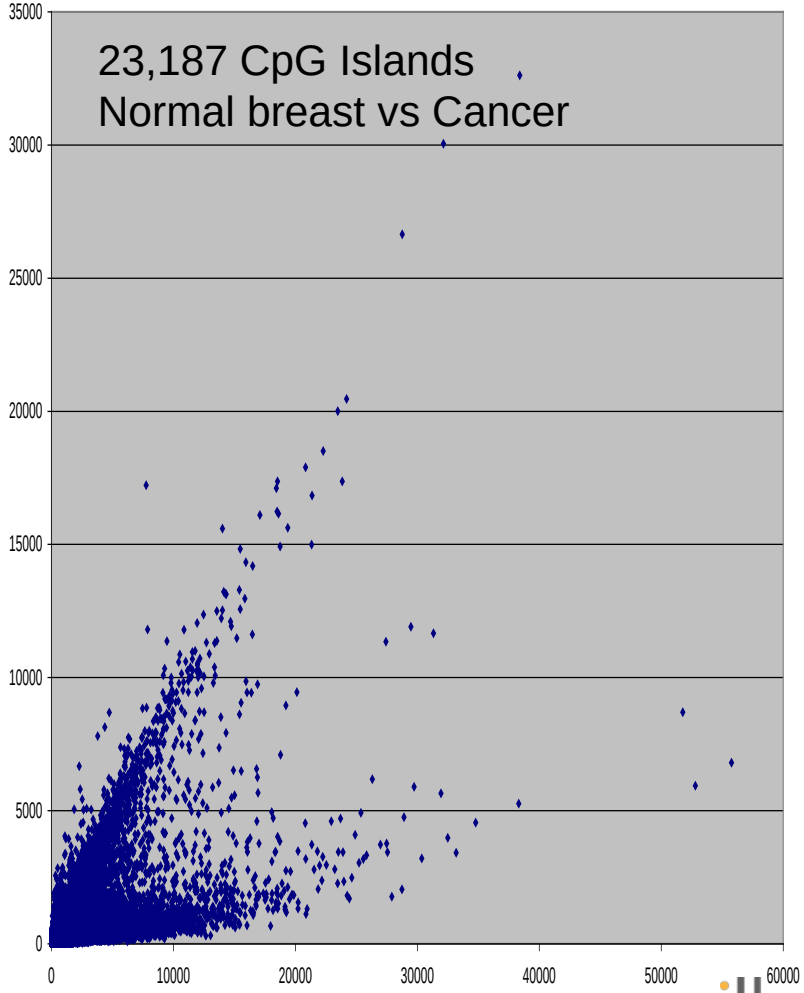
Lister, R., et al., (2008) Highly integrated single-base resolution maps of the epigenome in Arabidopsis. Cell. 133(3), 523-536.

# Average CpG Island Methylation

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# What is Targeted Resequencing?

- **What is targeted resequencing?**
  - Target Enrichment, Sequence Capture, Genome Partitioning, Genomic Capture, Target Capture, Targeted Pullout
- **Focus on subset of the genome**
  - Remaining genomic material discarded
- **Up to several Mb size candidate genomic regions**
- **Find exact disease causing genes and mutations**
- **Understand complex human traits**

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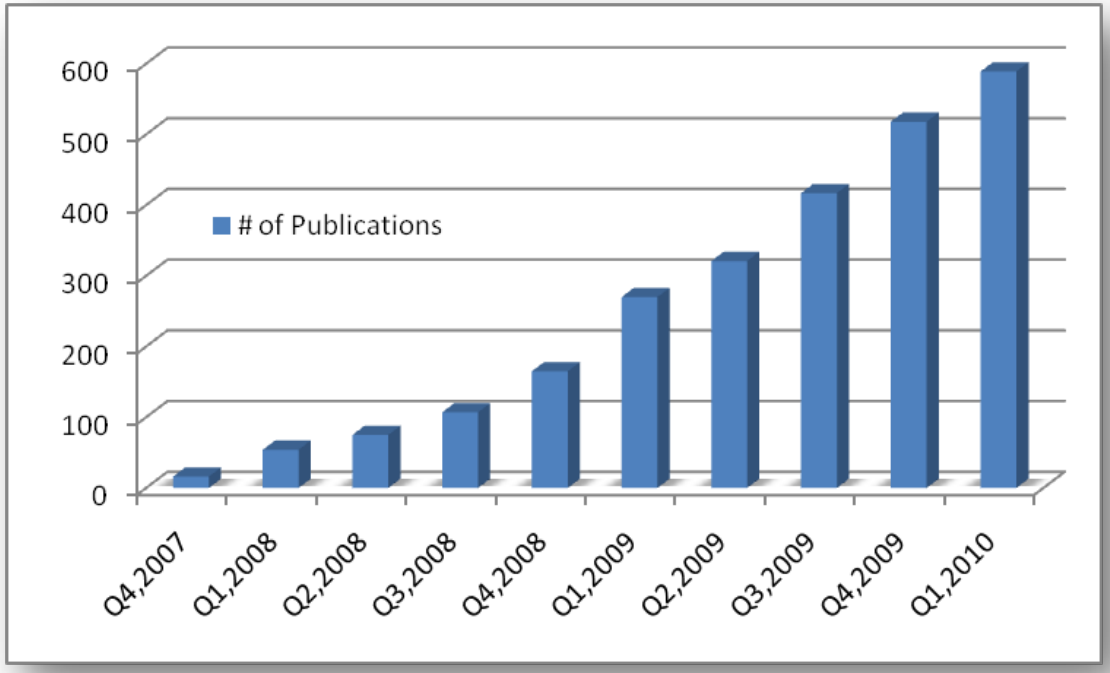
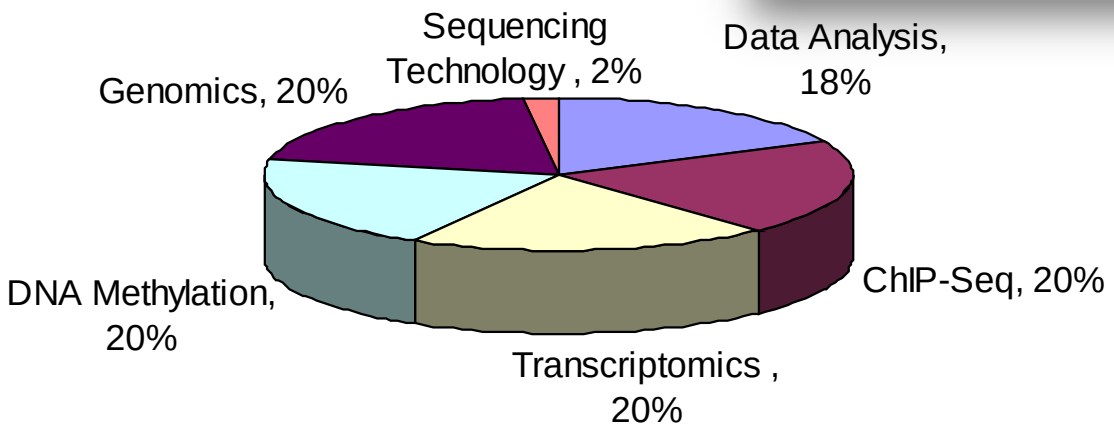
# Genetic diagnosis by whole exome capture and massively parallel DNA sequencing www.pnas.orgcgidoi10.1073pnas.091067210

- Whole-exome sequencing coupling whole exome arrays to the Illumina DNA sequencing platform.
- The ability to capture approximately 95% of the targeted coding sequences with high sensitivity and specificity for detection of homozygous and heterozygous variants
- We illustrate the utility of this approach by identification of a rare mutation in a patient that led to an unexpected clinical diagnosis of the **Bartter syndrome**
- “Because the cost of sequencing on the Illumina platform is potentially considerably lower, we adapted hybrid capture using the NimbleGen 2.1M Human Exome Array to the Illumina DNA sequencing platform”

# Illumina Sequencing Publications:

| | |
|---|---|
| nature | 93 |
| Science | 25 |

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|---|---|---|---|

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- Read Length:150bp

**HiScanSQ**

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- 250M Reads
- Read Length:100bp

**Genome Analyzer$_{IIx}$**

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- 250M Reads
- Read Length:100bp

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