# CLINICAL NEXT GENERATION SEQUENCING

M Ataei, PhD
Tehran Medical Genetics Laboratory
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# Overview



- Background
- NGS in non-invasive prenatal diagnosis (NIPD)

# Background

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- In the 1970s, Sanger and colleagues and Maxam and Gilbert developed methods to sequence DNA by chain termination and fragmentation techniques, respectively.
- Sanger sequencing has been the prevailing DNA sequencing method for 30 years.
- Sanger technique ultimately enabled the completion of the first human genome sequence in 2004.

# Cont'

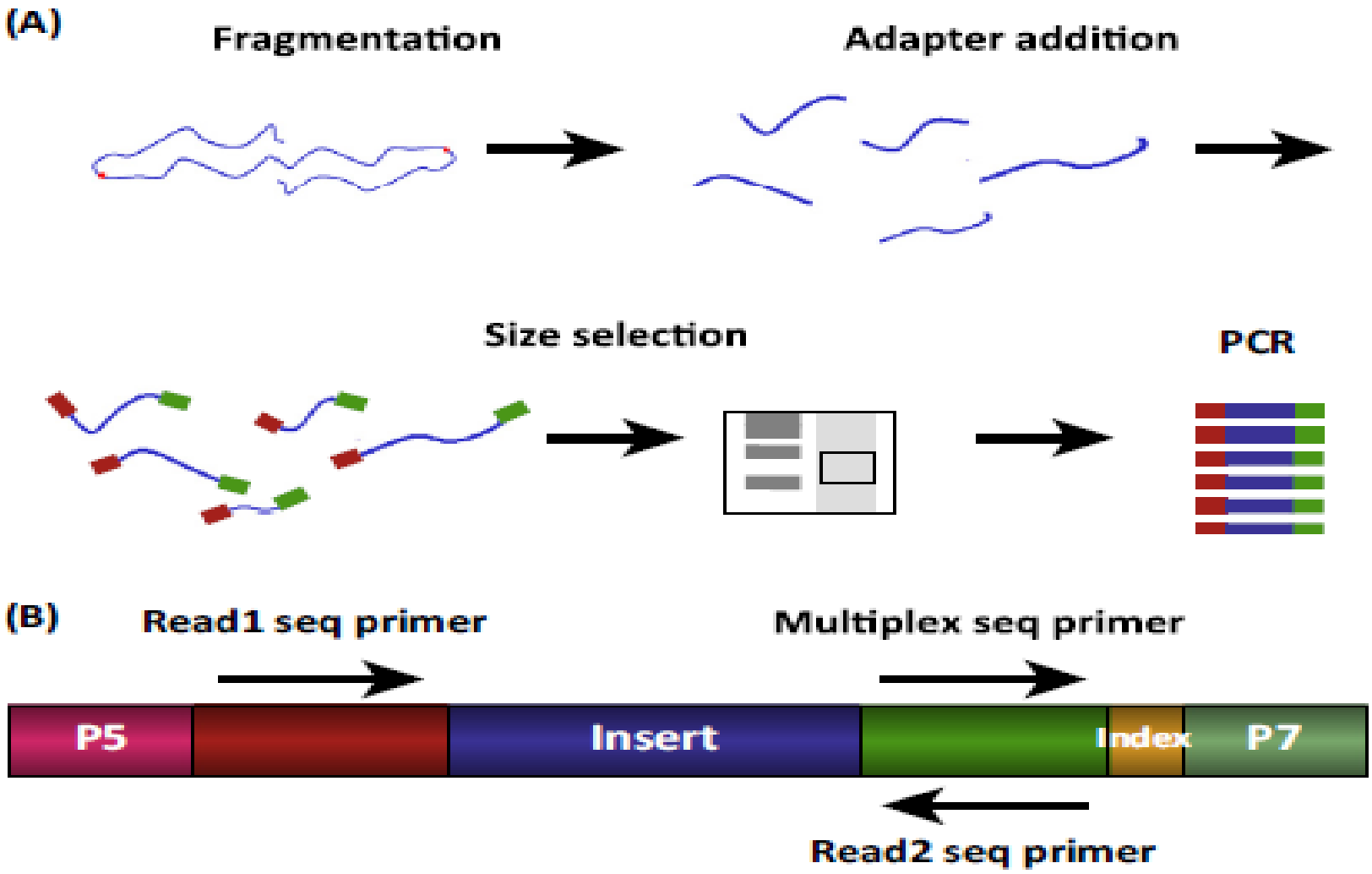


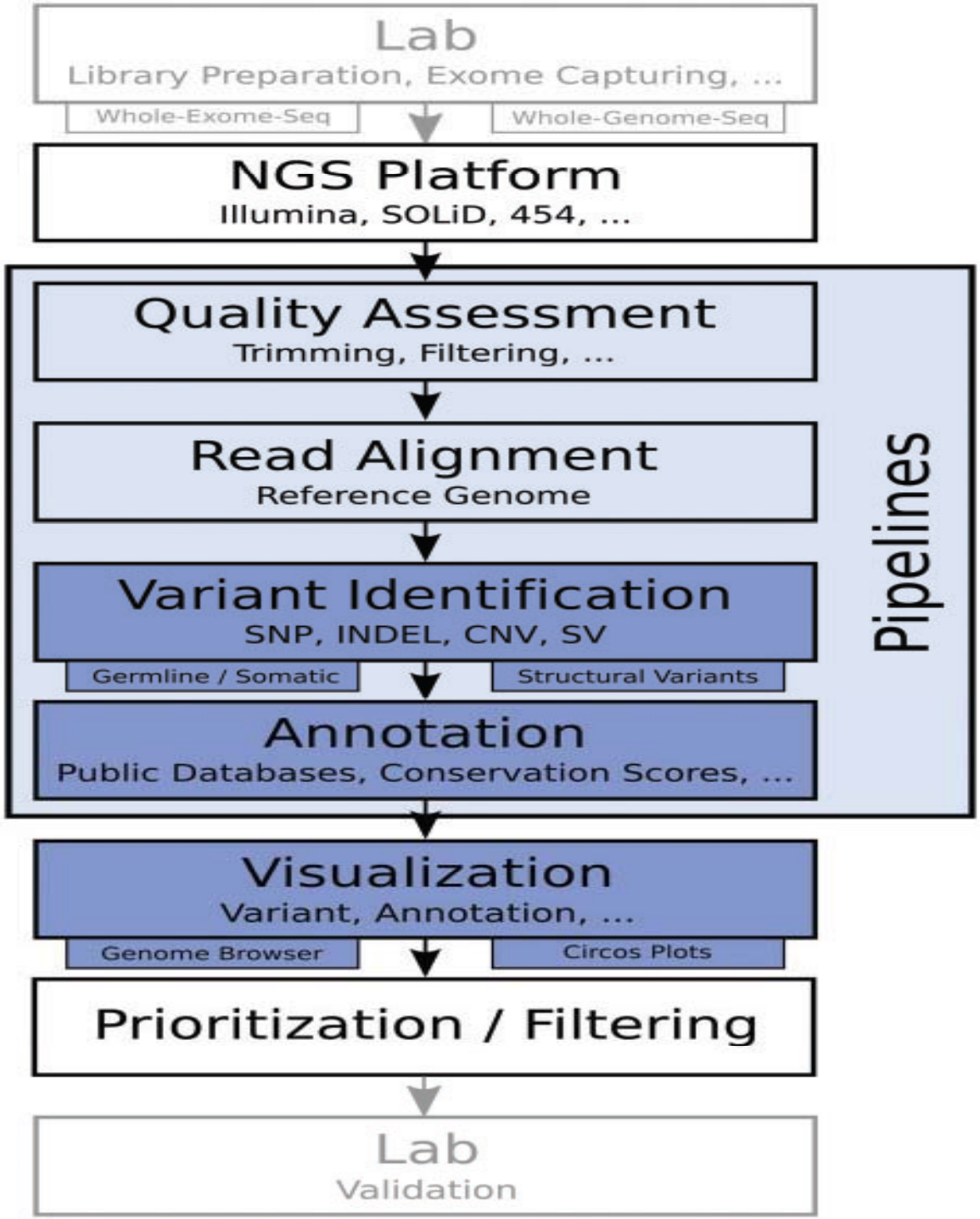
- In the same year (2004) the National Human Genome Research Institute (NHGRI) initiated a funding program with the goal of reducing the cost of human genome sequencing to US$1000 in ten years.

- Development of NGS technologies

# Basic principles of NGS library preparation






Lab
Library Preparation, Exome Capturing, ...
Whole-Exome-Seq
Whole-Genome-Seq
NGS Platform
Illumina, SOLiD, 454, ...
Quality Assessment
Trimming, Filtering, ...
Read Alignment
Reference Genome
Variant Identification
SNP, INDEL, CNV, SV
Germline / Somatic
Structural Variants
Annotation
Public Databases, Conservation Scores, ...
Pipelines
Visualization
Variant, Annotation, ...
Genome Browser
Circos Plots
Prioritization / Filtering
Lab
Validation

# Improvements made by NGS

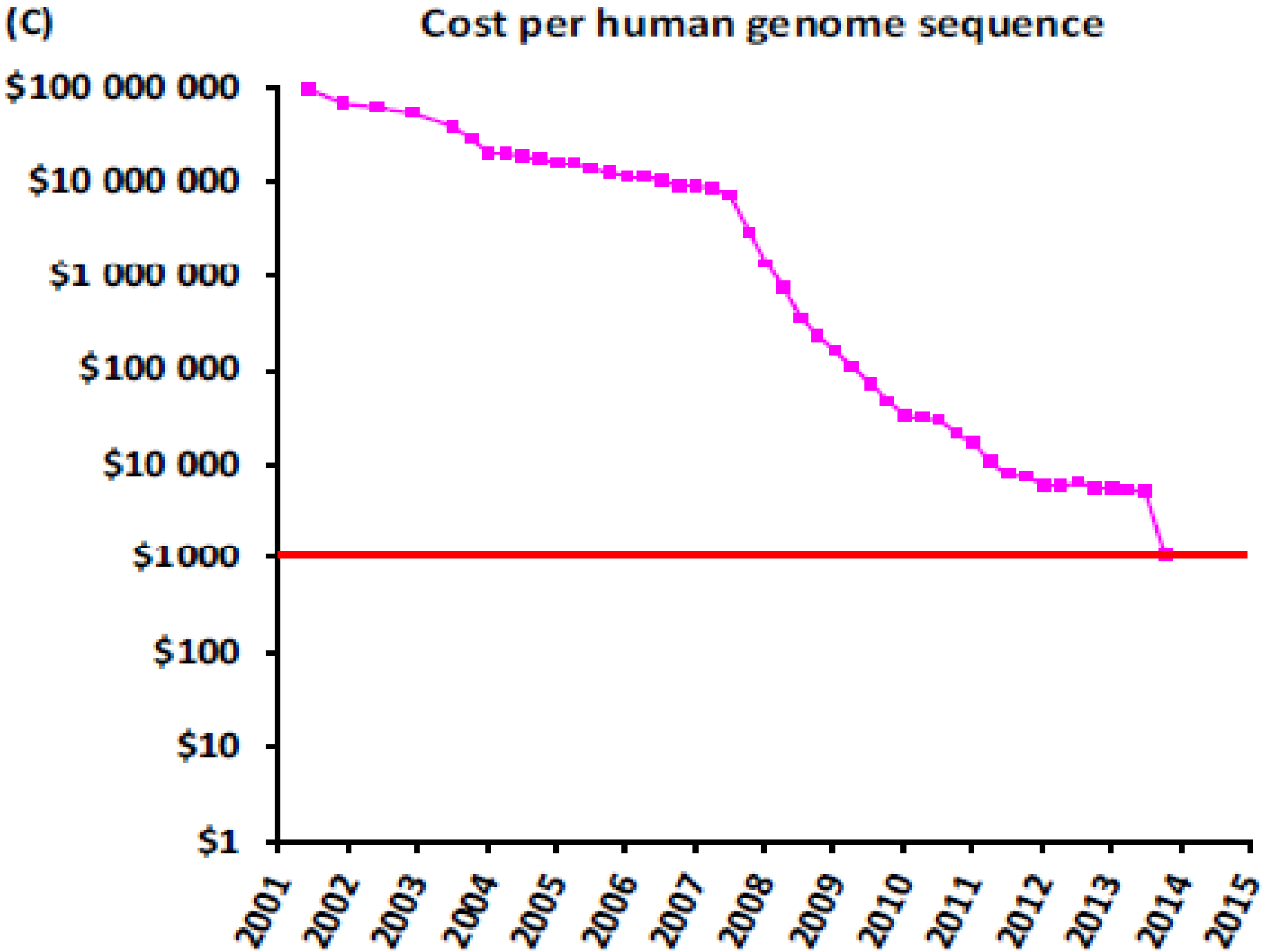


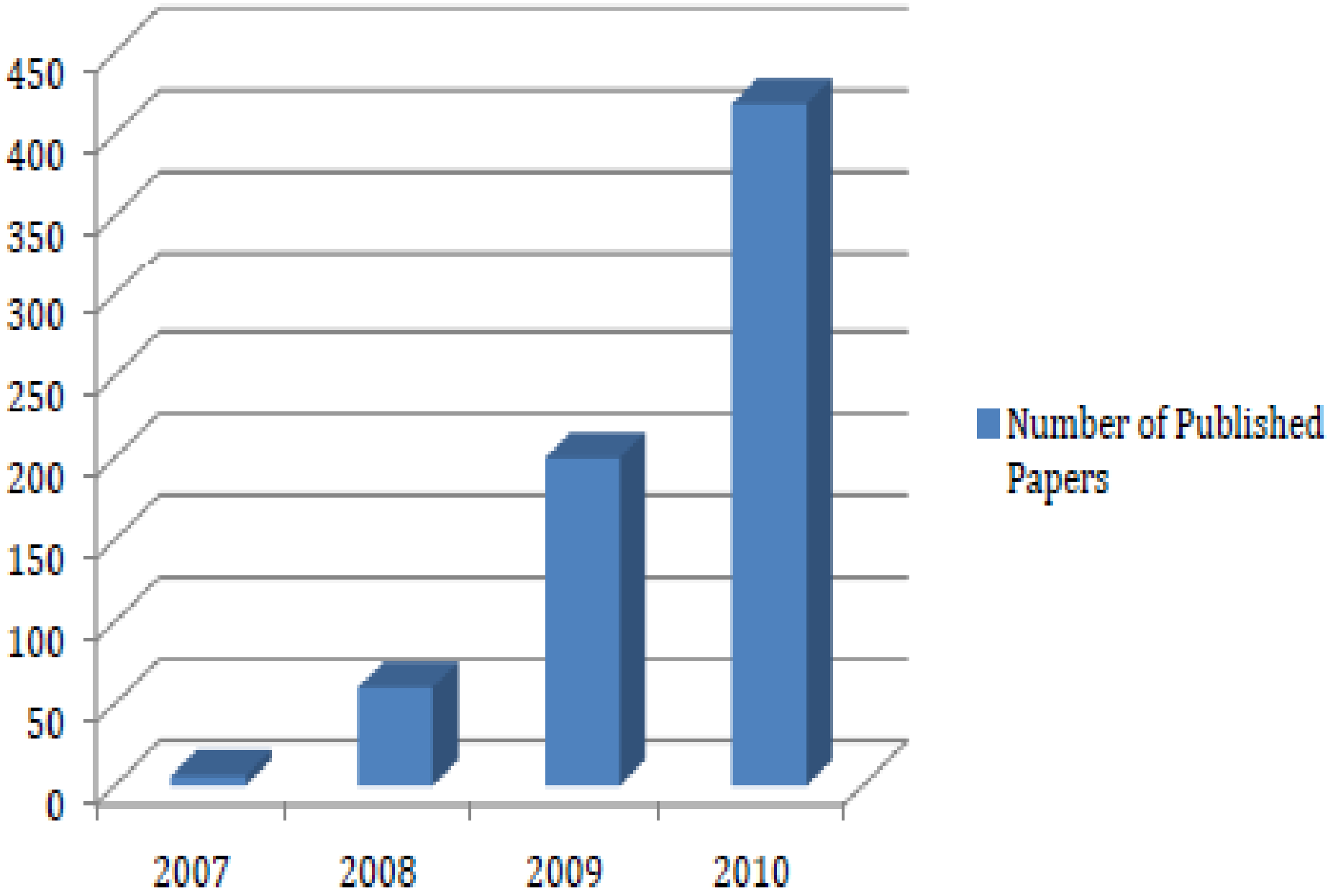
- First, rely on the preparation of NGS libraries in a cell free system.
- Second, thousands-to-many-millions of sequencing reactions are produced in parallel.
- Third, without the need for electrophoresis; base interrogation is performed cyclically and in parallel.

# Background



- Over the last few years, advances in next-generation sequencing technologies have decreased the cost of sequencing per base pair about 10-fold
- Improved accuracy
- Greatly increased the speed of generating sequence data.
- Currently, Illumina, which offers the highest throughput and the lowest per-base cost, is the leading NGS platform.


(C)
Cost per human genome sequence
$100 000 000
$10 000 000
$1 000 000
$100 000
$10 000
$1000
$100
$10
$1
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015


450
400
350
300
250
200
150
100
50
0
2007
2008
2009
2010
Number of Published Papers

# NGS applications



- DNA sequencing
- RNA sequencing
- Protein–DNA interactions
- Epigenetic  studies

# NGS types for genomic studies



- Whole Genome Sequencing (WGS)
- Whole Exome Sequencing (WES)
- NGS panels

# WGS limitations



- Huge quantities of information
- Depth of sequence coverage that is lower than that achieved by whole-exome sequencing
- Novel candidate variants to disease phenotype are challenging.
- Multiple pseudogenes
- Repetitive regions

# WES limitations



- Large duplication and deletion
- Balanced translocation
- Inversions
- Ploidy changes
- Uniparental disomy
- Methylation alterations
- Repetitive DNA, including trinucleotide repeats
- Copy-number variants
- Mitochondrial mutations

# Applications



- Many patients with genetic diseases are not given a specific diagnosis despite applying the standard practice.
- Different types of inheritance in Mendelian diseases
- Deafness, blindness, Neuromascular disorders, Metabolic disorders,…
- Individualized medicine
- Reported detection rates for deleterious mutations range from 25 to 50%

# NGS in NIPD

- A long desired goal of research in prenatal medicine

NIPT

Non invasive

Prenatal Test

# NGS platforms for NIPD



- Illumina Genome Analyzer/Illumin a HiSeq 2000 Genome Analyzer
- Applied Biosystems (ABI) SOLiD Analyzer
- Helicos Heliscope
- IonTorrent (Life Technologies)

- These platforms are all short-read platforms.
- cffDNA shows a fragmentation pattern of around 146 bp.

# Companies offering NIPD



- Sequenom, Inc (San Diego, CA, USA)
- Verinata Health, Inc (Redwood City, CA, USA)
- Ariosa Diagnostics, Inc (San Jose, CA, USA)
- Natera, Inc (San Carlos, CA, USA)

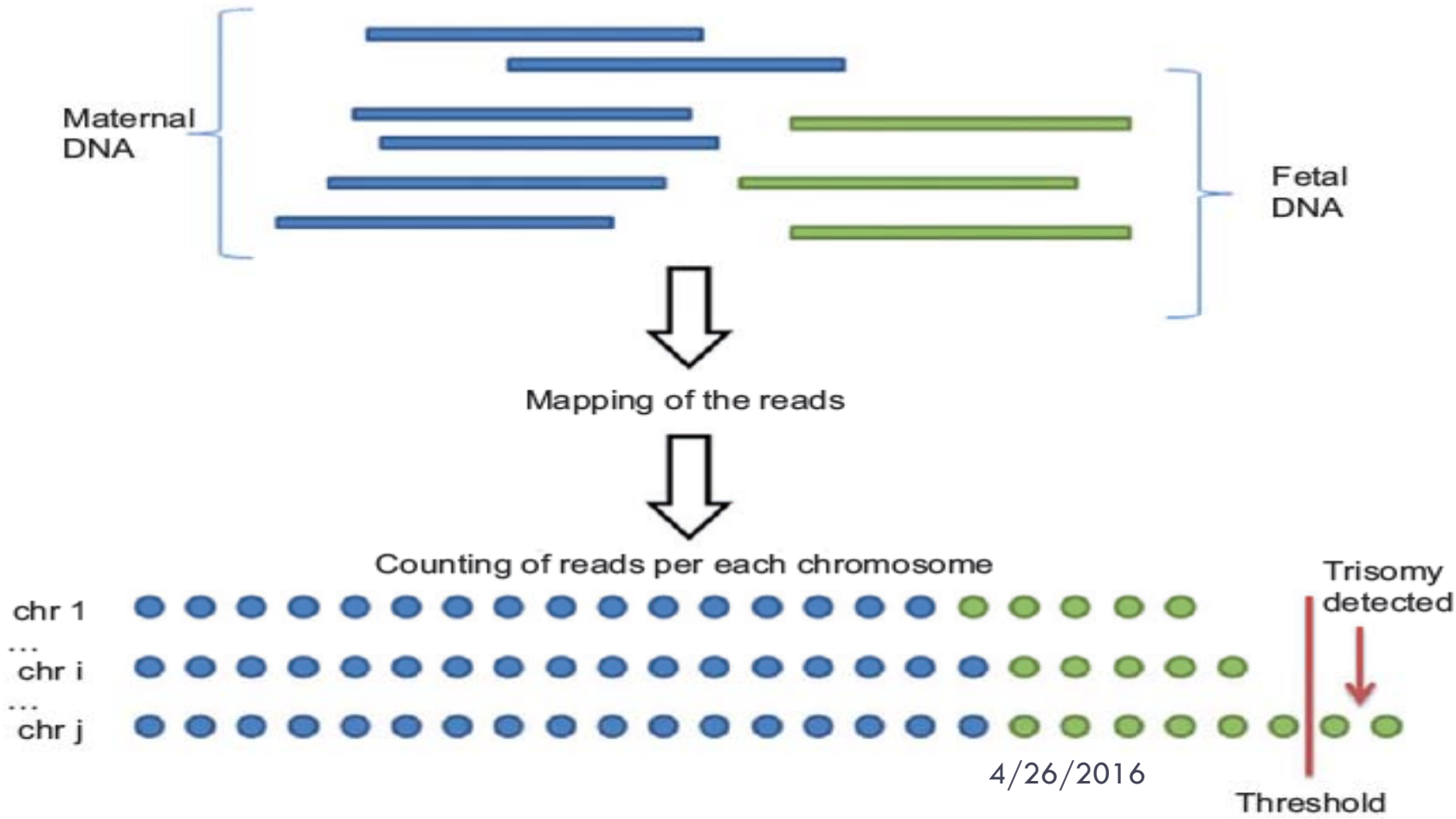
# Cont'



- Whole genome and targeted sequencing
- Both approaches are capable of detecting the most common fetal aneuploidies in the population
- Targeted sequencing is more cost effective

# Counting of reads per each chromosome instead of genotyping





# NIPD in Mendelian disorders



- At the moment, non-invasive methods do not detect other genetic abnormalities, such as SNP and indel variations causing Mendelian disorders, and invasive procedures are thus still required.
- Non-invasive methods of fetal genotyping are currently in development, and accurate screening of both dominant and recessive single gene disorders may be possible in clinical practice in the near future.

# The end goal is simple



To offer patients information early in pregnancy about fetal genomes without incurring procedural risks. This will allow patients an opportunity to make informed reproductive and pregnancy management decisions based on precise fetal genomic information

# Thank you