NEXT GENERATION SEQUENCING IN NONINVASIVE PRENATAL DIAGNOSIS

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Overview

- Next Generation Sequencing
- NGS in non-invasive prenatal diagnosis (NIPD)

Background

Background

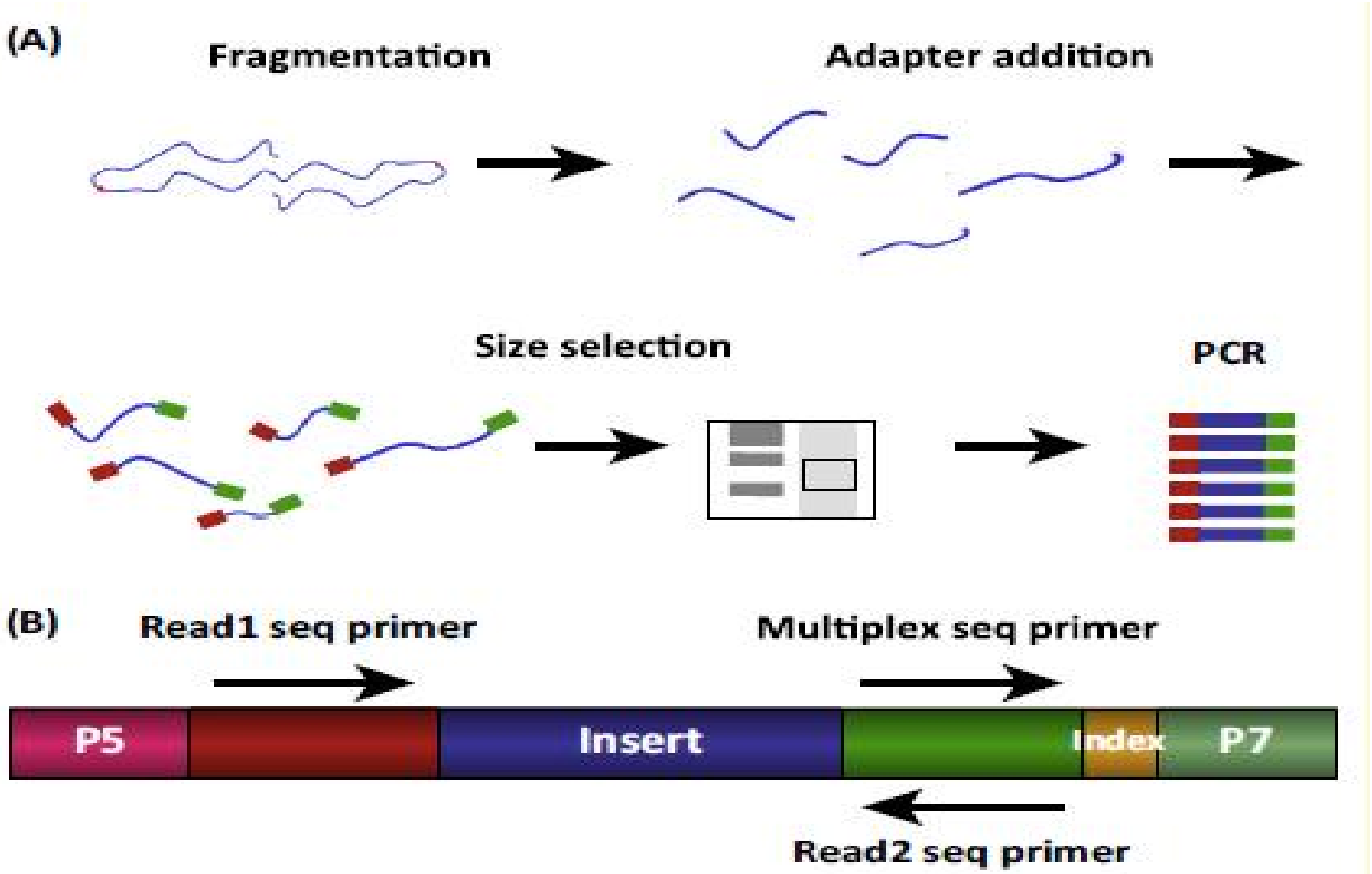
- In the 1970s, Sanger and colleagues and Maxam and Gilbert developed methods to sequence DNA by chain termination and fragmentation techniques, respectively.
- Sanger sequencing has been the prevailing DNA sequencing method for 30 years.
- Sanger technique ultimately enabled the completion of the first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

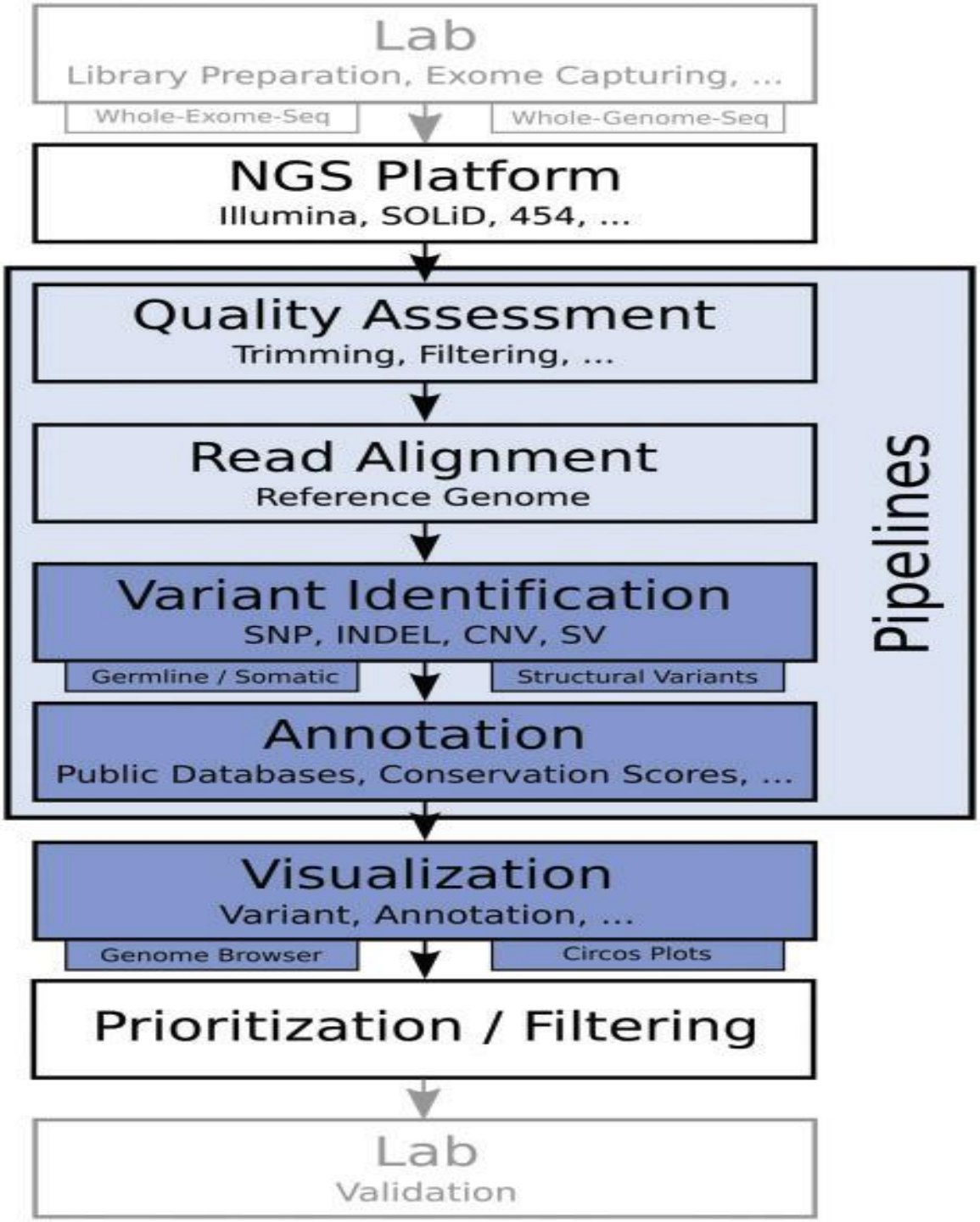
Cont'

- 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



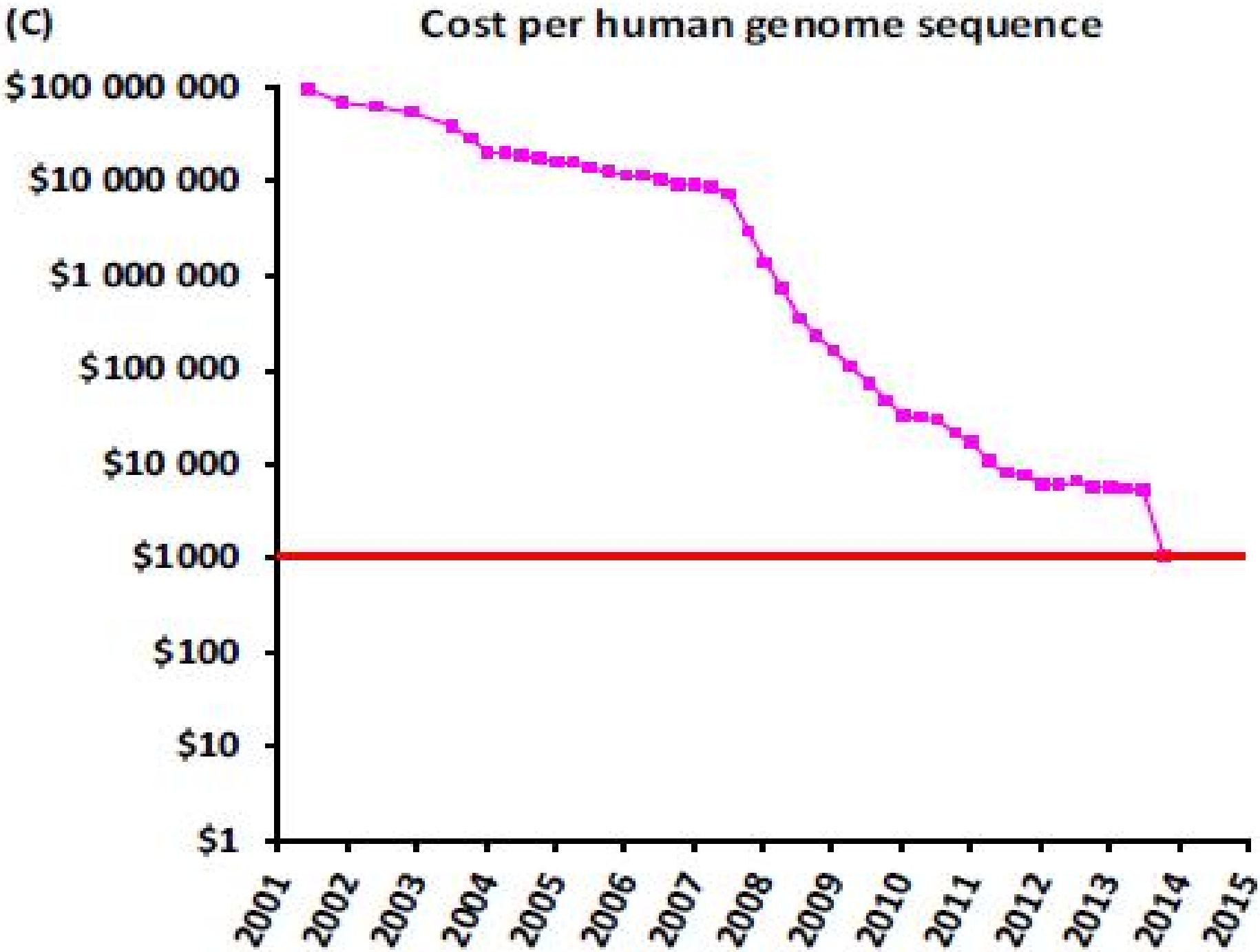

Lab
Library Preparation, Exome Capturing, ...
Whole-Exome-Seq
Whole-Genome-Seq
NGS Platform
Illumina, SOLiD, 454, ...
Quality Assessment
Trimming, Filtering, ...
Read Alignment
Reference Genome
Variant Identification
SNP, INDEL, CNV, SV
Germline / Somatic
Structural Variants
Annotation
Public Databases, 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
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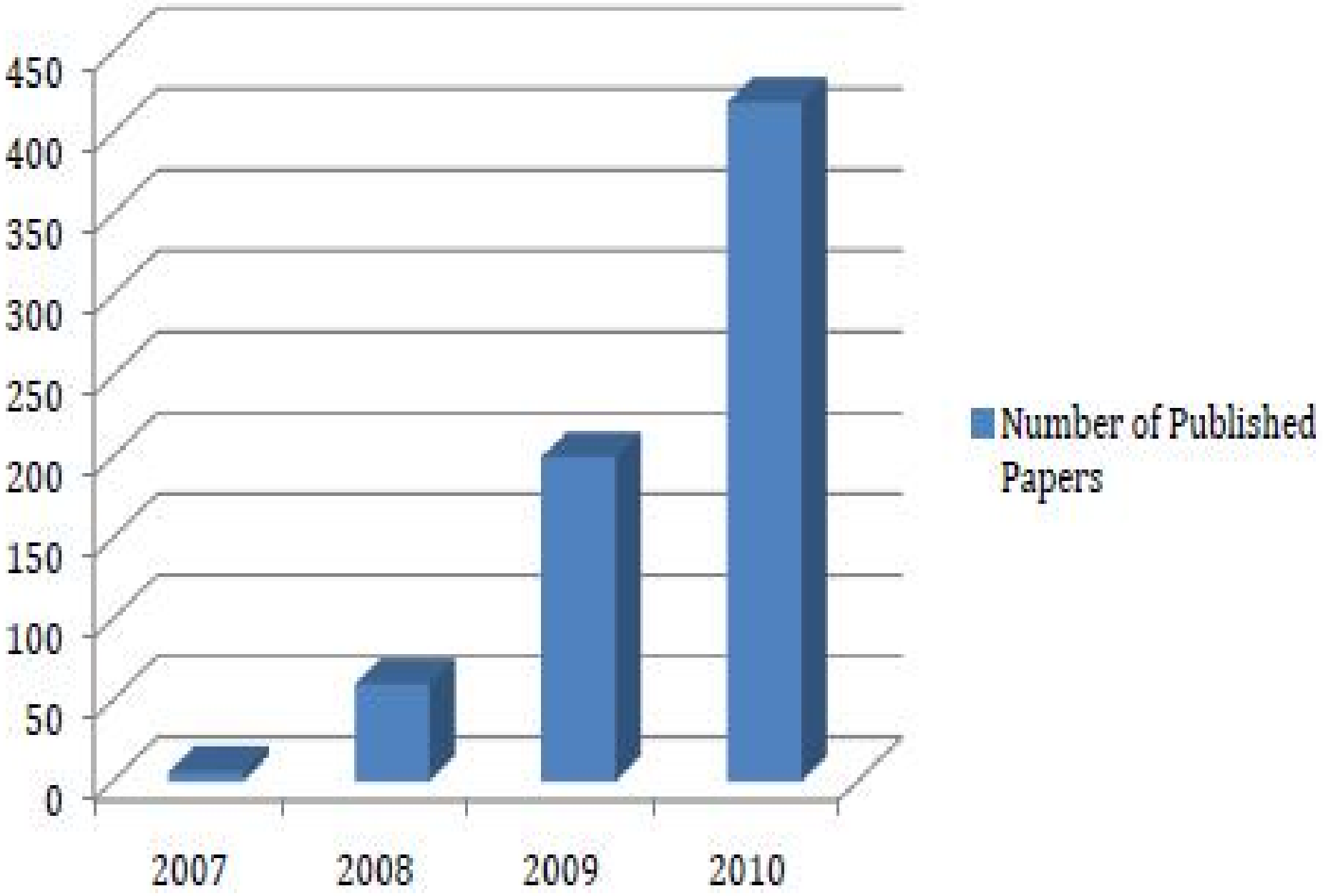
2011
2012
2013
2014
2015


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

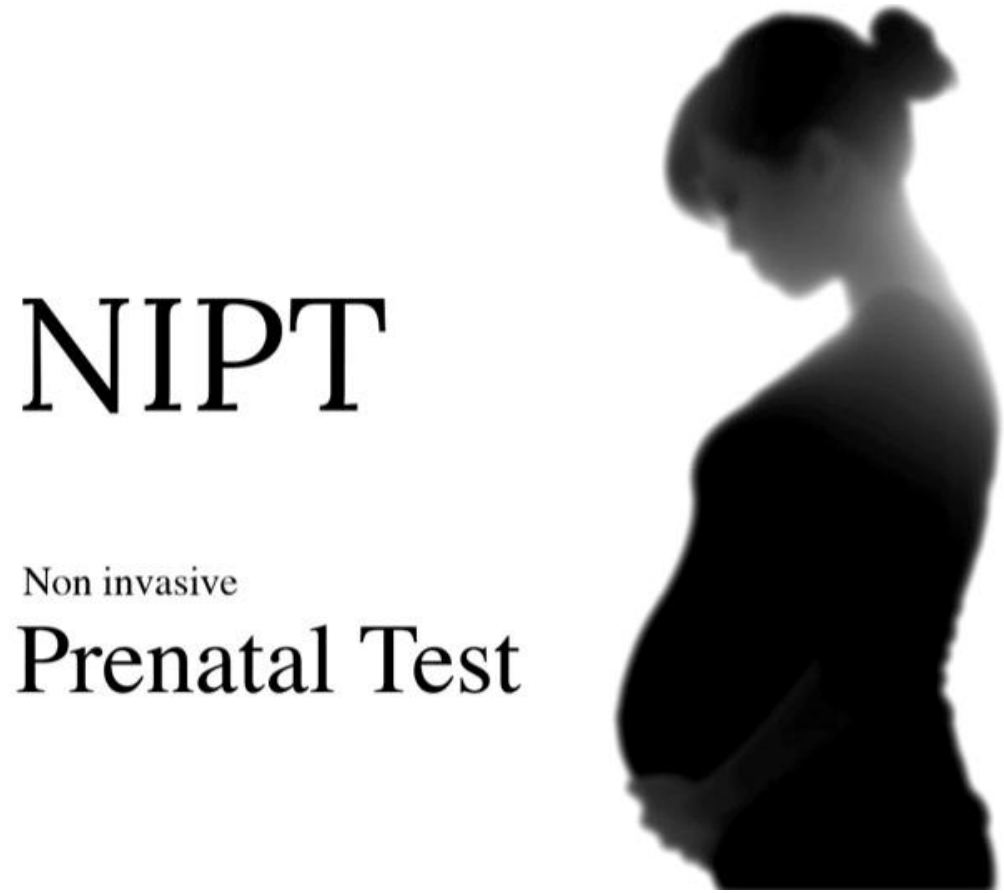
- Large duplication and deletion
- Balanced translocation
- Inversions
- Ploidy changes
- Uniparental disomy
- Methylation alterations
- Repetitive DNA, including trinucleotide repeats
- Copy-number variants
- Mitochondrial mutations

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



Discovery of free fetal DNA

Lancet. 1997 Aug 16;350(9076):485-7.

Presence of fetal DNA in maternal plasma and serum.

Lo YM[1], Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS.

Author information

Abstract

BACKGROUND: The potential use of plasma and serum for molecular diagnosis has generated interest. Tumour DNA has been found in 'the plasma and serum of cancer patients, and molecular analysis has been done on this material. We investigated the equivalent condition in pregnancy-that is, whether fetal DNA is present in maternal plasma and serum.

METHODS: We used a rapid-boiling method to extract DNA from plasma and serum. DNA from plasma, serum, and nucleated blood cells from 43 pregnant women underwent a sensitive Y-PCR assay to detect circulating male fetal DNA from women bearing male fetuses.

FINDINGS: Fetus-derived Y sequences were detected in 24 (80%) of the 30 maternal plasma samples, and in 21 (70%) of the 30 maternal serum samples, from women bearing male fetuses. These results were obtained with only 10 microL of the samples. When DNA from nucleated blood cells extracted from a similar volume of blood was used, only five (17%) of the 30 samples gave a positive Y signal. None of the 13 women bearing female fetuses, and none of the ten non-pregnant control women, had positive results for plasma, serum or nucleated blood cells.

INTERPRETATION: Our finding of circulating fetal DNA in maternal plasma may have implications for non-invasive prenatal diagnosis, and for improving our understanding of the fetomaternal relationship.

- Originally, the percentage of ffDNA fraction 3%-6%
- Recent studies, 10%-20%

- Two big challenges: discrimination and enrichment

The first reports in 2008

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 [b]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

Contributed by Charles R. Cantor, October 22, 2008 (sent for review September 29, 2008)

PNAS

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

Communicated by Leonard A. Herzenberg, Stanford University School of Medicine, Stanford, CA, August 22, 2008 (received for review July 13, 2008)

We directly sequenced cell-free DNA with high-throughput shotgun some of origin and enumeration of fragments per chromosome.

PLOS ONE

RESEARCH ARTICLE

Targeted capture enrichment assay for non-invasive prenatal testing of large and small size sub-chromosomal deletions and duplications

Maria C. Neofytou[1], Kyriakos Tsangaras[2], Elena Kypri[1,2], Charalambos Loizides[2], Marios Ioannides[2], Achilleas Achilleos[2], Petros Mina[2], Anna Keravnou[1], Carolina Sismani[3], George Koumbaris[1,2], Philippos C. Patsalis[1,2]*

1 Translational Genetics Team, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, 2 NIPD Genetics Ltd, Nicosia, Cyprus, 3 Department of Cytogenetics and Genomics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

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 150 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

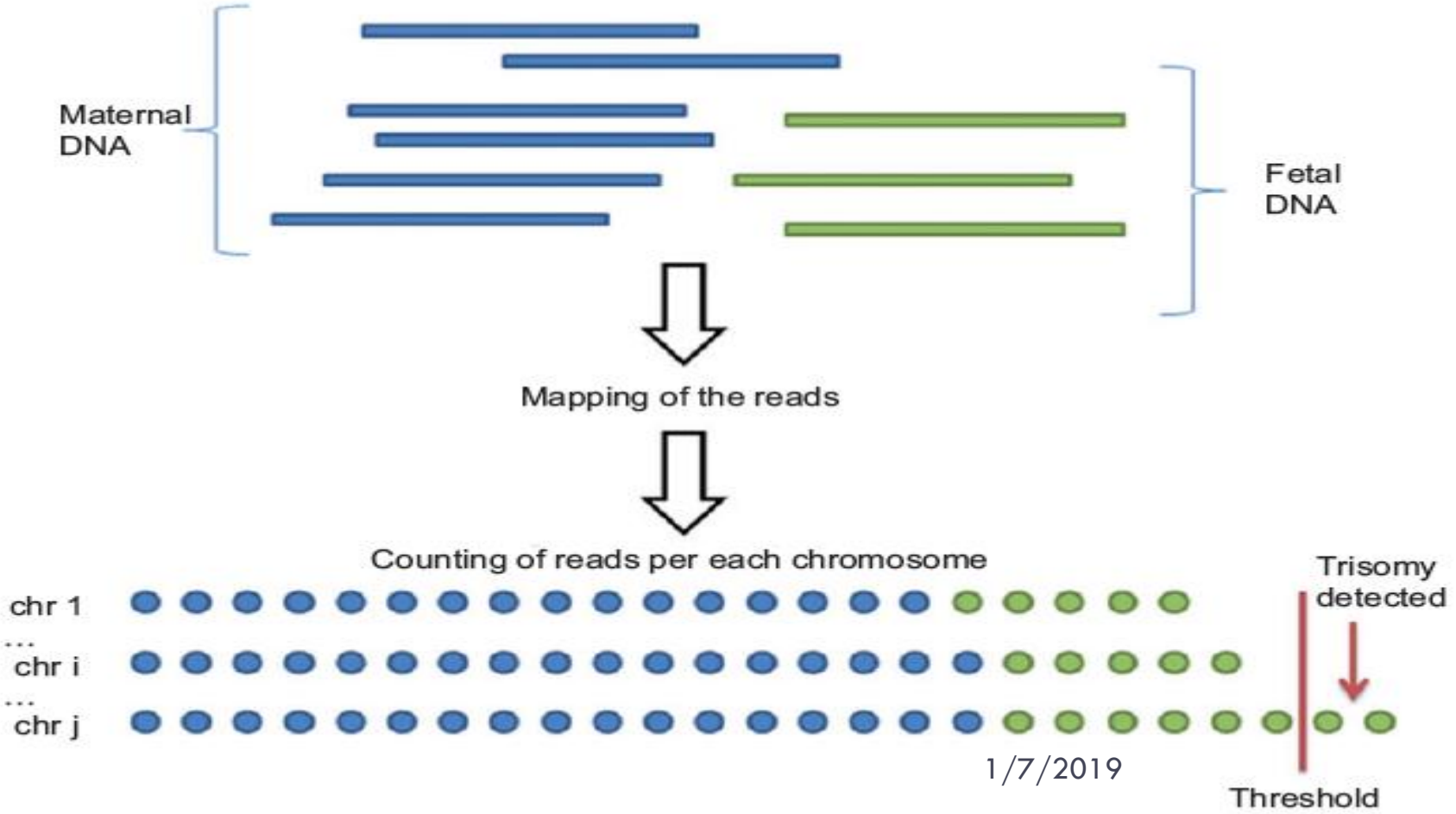
- Both approaches are capable of detecting the most common fetal aneuploidies in the population
- Detection of microdeletions (DiGeorge, 1p36 deletion, Smith-Magenis, Wolf Hirschhorn)
- Targeted sequencing is more cost effective

Targeted approach

- Targeted approaches have the potential to increase throughput and reduce cost

- Less sequencing than whole genome approaches

Counting of reads per each chromosome instead of genotyping



NIPD in Mendelian disorders

- At the moment, non-invasive methods do not detect SNP 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