# The Paradigm Shift of the Lab Test: NGS vs Precision Medicine

**Joseph. W. O. Tam, CEO DiagCor Bioscience Ltd**

Abstract:

The cost of whole genome DNA sequencing has come down from hundreds of million to $999 and is expected to go down to below $20, which led Daniel H Farkas to predict "DNA sequencing will be the Ultimate Laboratory Test" in 2014 and subsequently the Obama's Precision Medicine initiative in 2015. This initiative has created huge excitements around the world, especially in China, where thousands of NGS startups to such Gold rushing exercise. On the other hands, 23andMe, a front runner of the genetic-testing company working on this cutting-edge technology already has millions of customers, has abundance the whole team of NGS scientists at the center of activities.

What might be the impact of this NGS paradigm shift?

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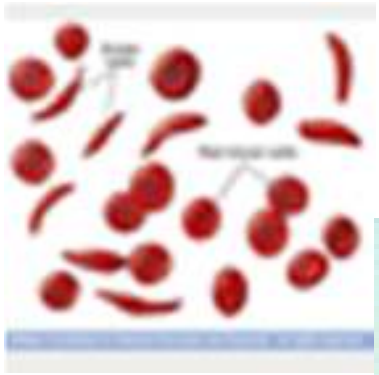
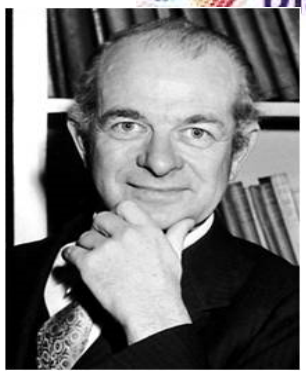
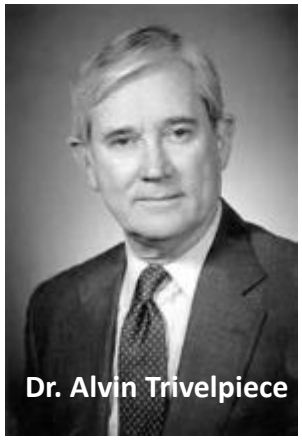


1949 HB-S Sickle Cell Is a molecular Disease



1978 Use DNA polymorphism to detect Sickle Gene

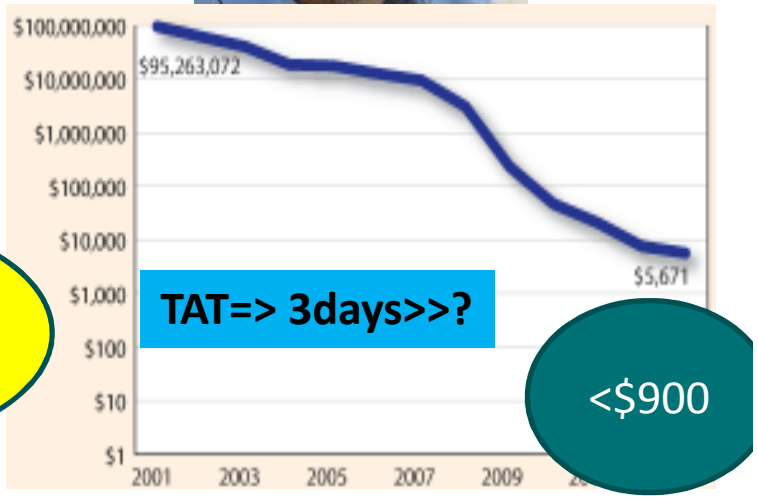



HGP (1990-2003) took 14 years ; 20 universities , research centers and company (3bUSD +++)

1000 genome Project

The Ultimate Lab Test

2nd Genome Project (HOOD)



# The Development of DNA Sequencing

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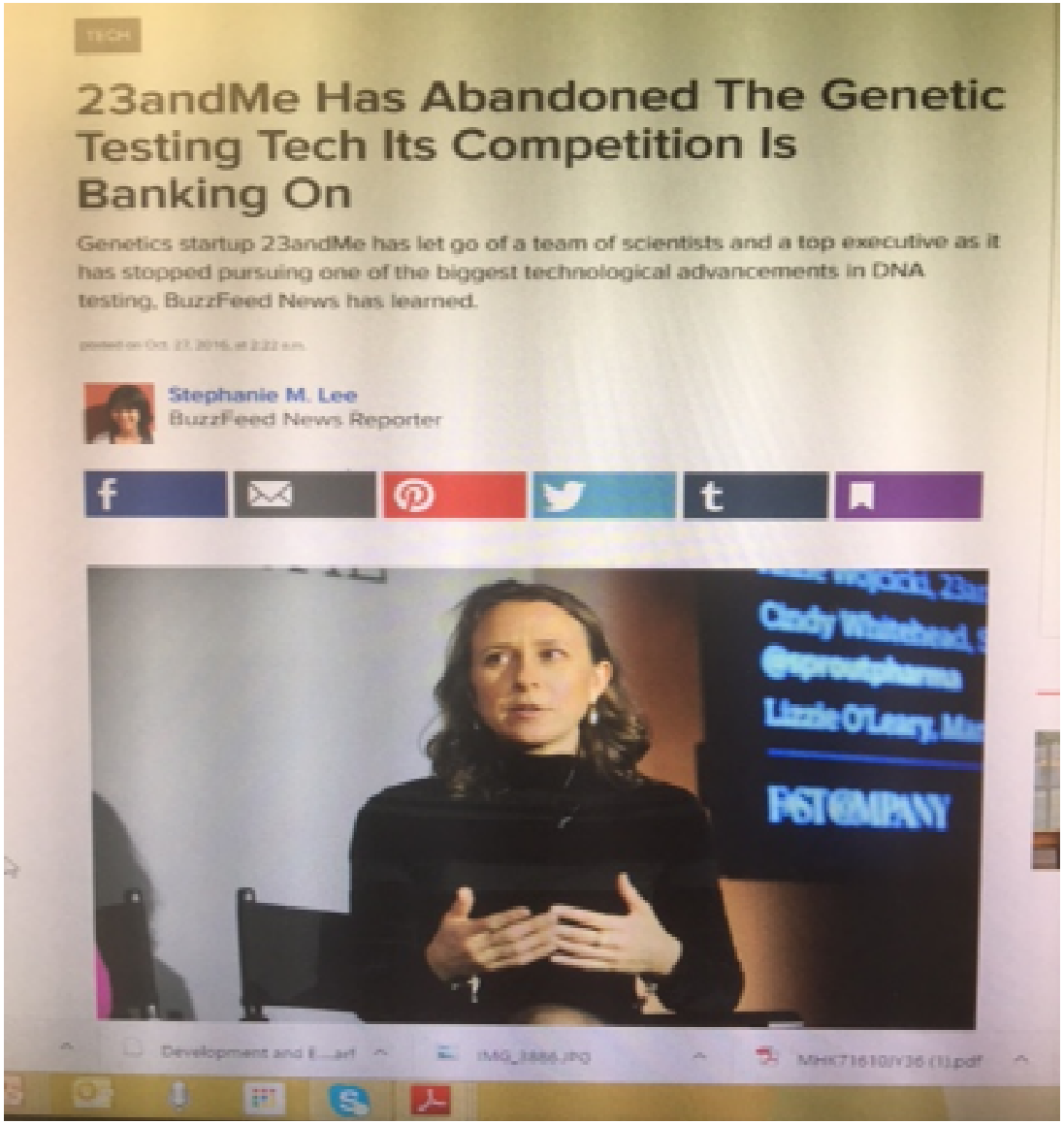
2014 Dr. Daniel H Farkas declared
**"DNA Sequencing will be the Ultimate Lab Test "**




2015 Obama and then China
**Precision Medicine Initiative**

**Veritas ; Helix ,**
**Color etc .**
Offer $999 WGS
$299; $99 Target Seq

**Nanopore etc. => POCT equip**

**=> => $20/test**

**1M genome project =>**

**Do we need $1b ?**

- What would the impact of $990 WGS test ?

# The Complexities of the human genome ($3x10^9$bp)

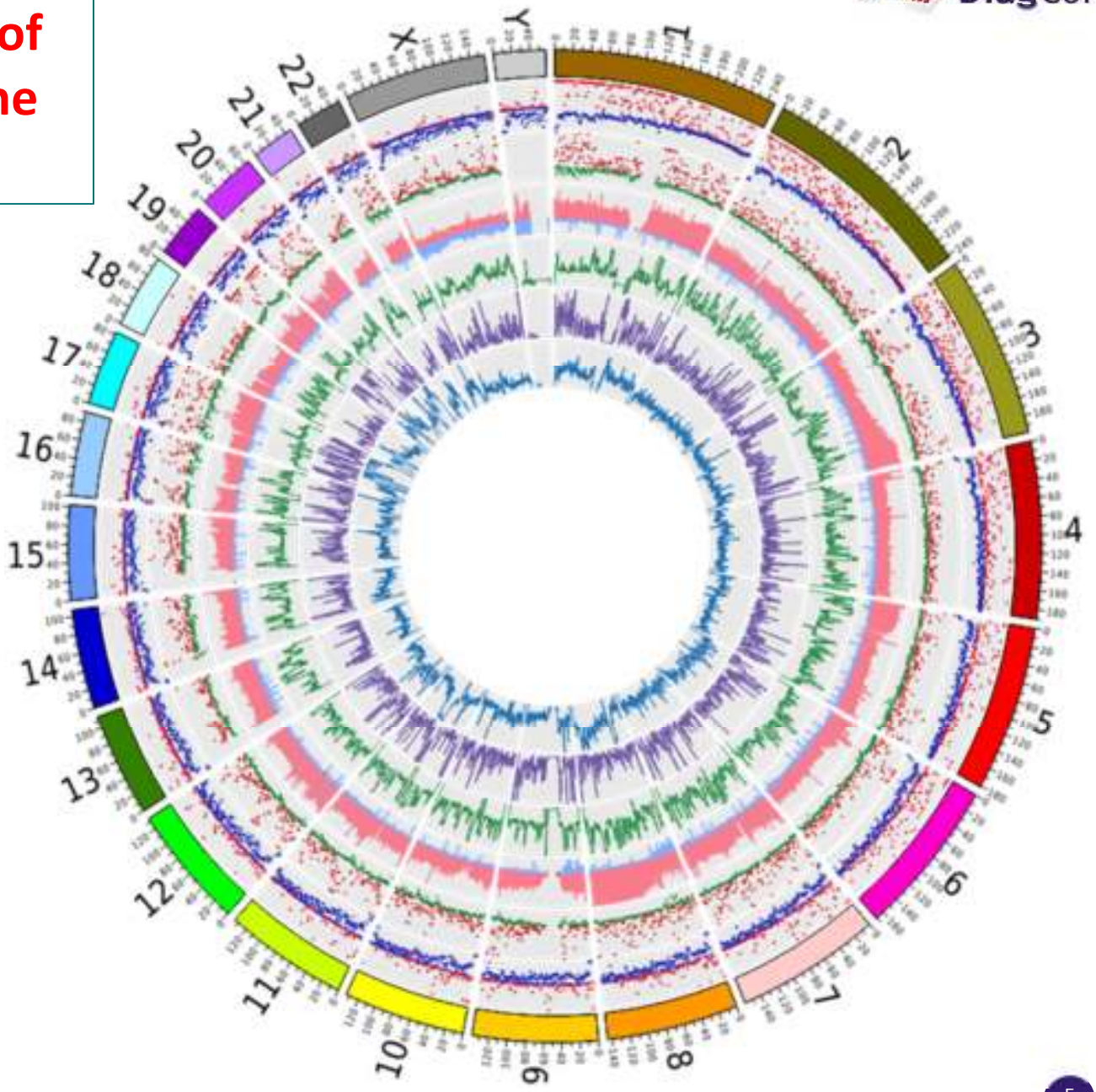



**Image key**

- Hyper Methylation
- Hypo Methylation
- Over Expression
- Under Expression
- CNV Gain
- CNV Loss
- Non Coding Variants
- Coding Mutations
- Structural Mutations

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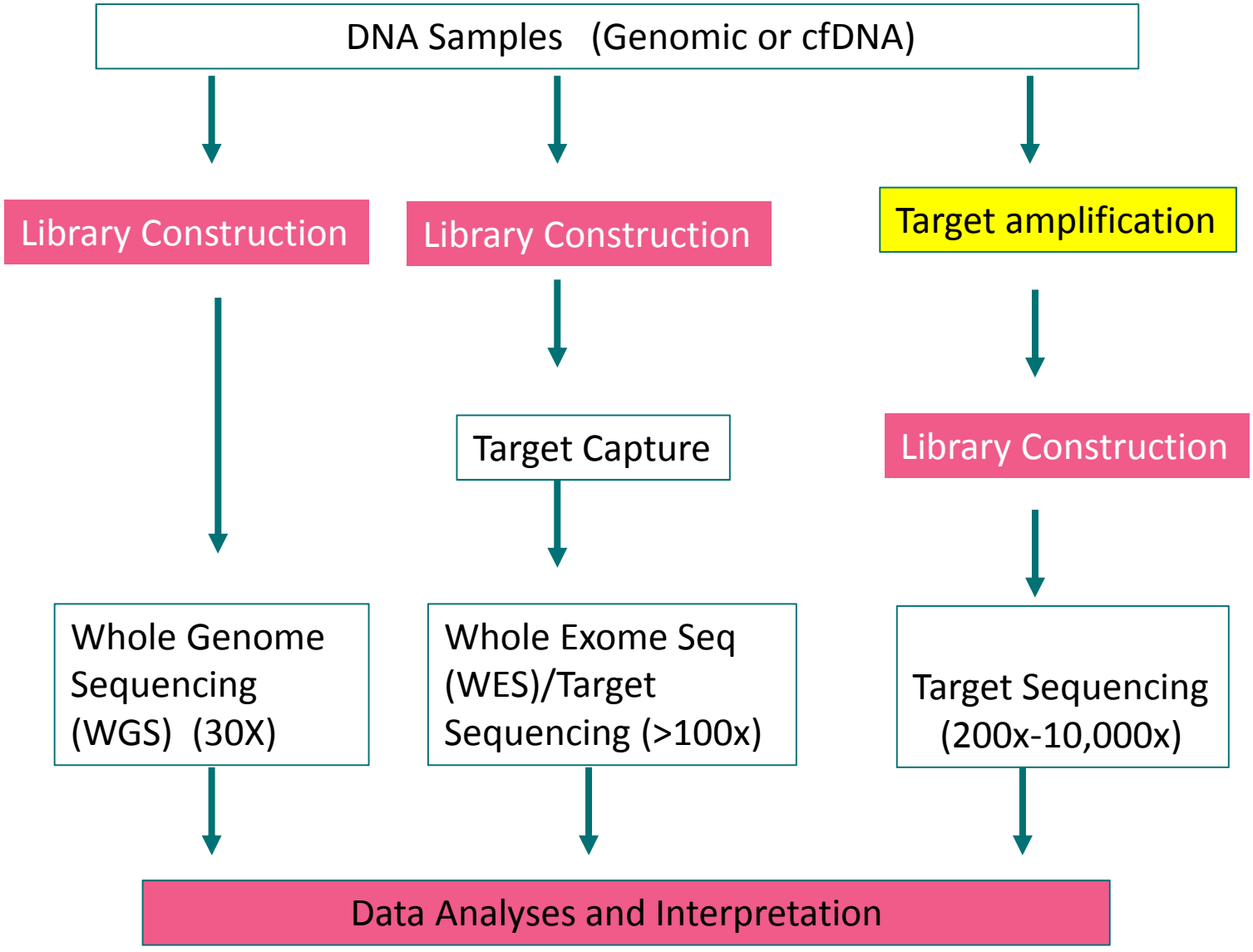
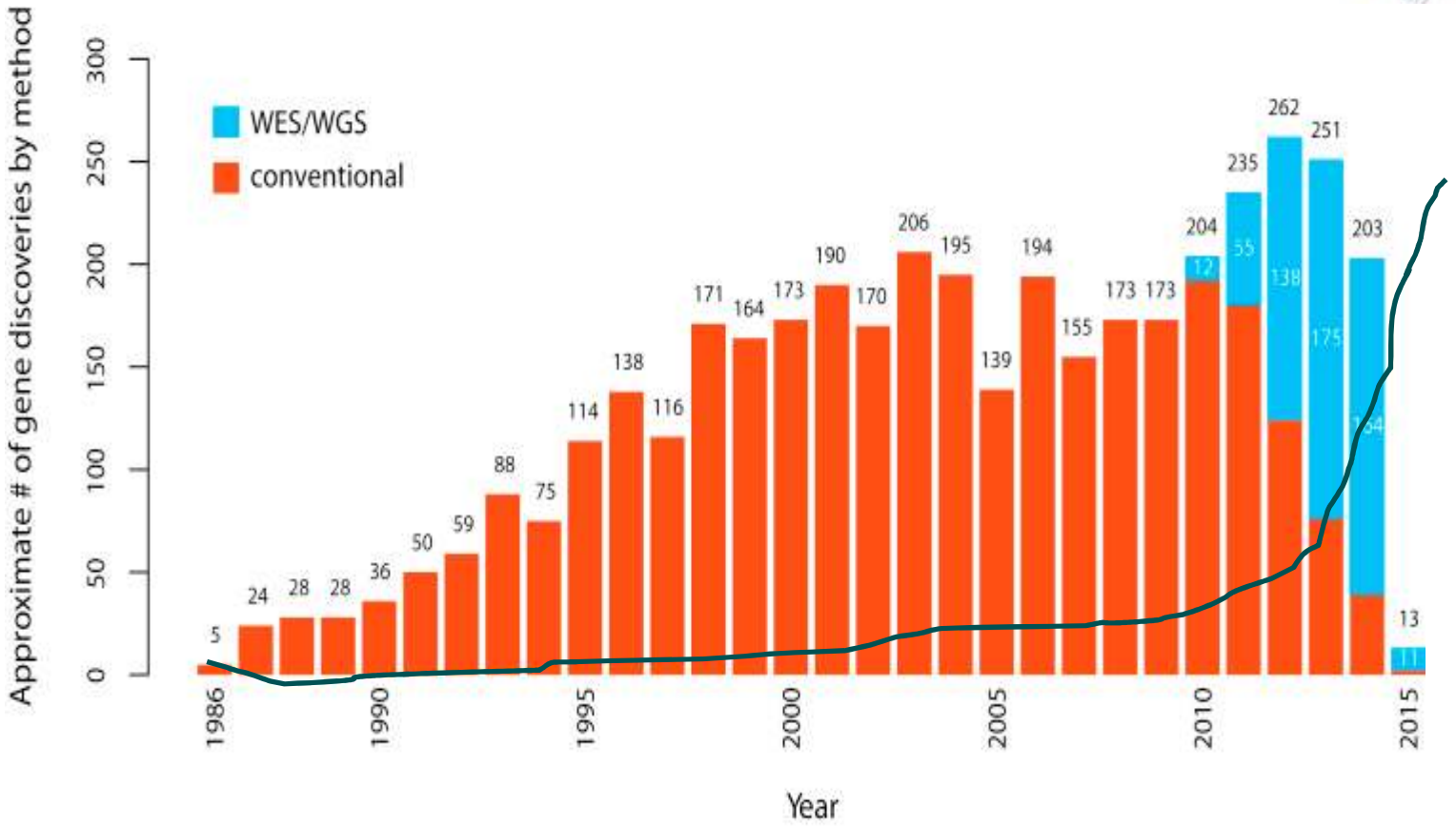
- **Is DNA going to be the ONLY Ultimate Lab Test?**

- **What we can do with NGS?**

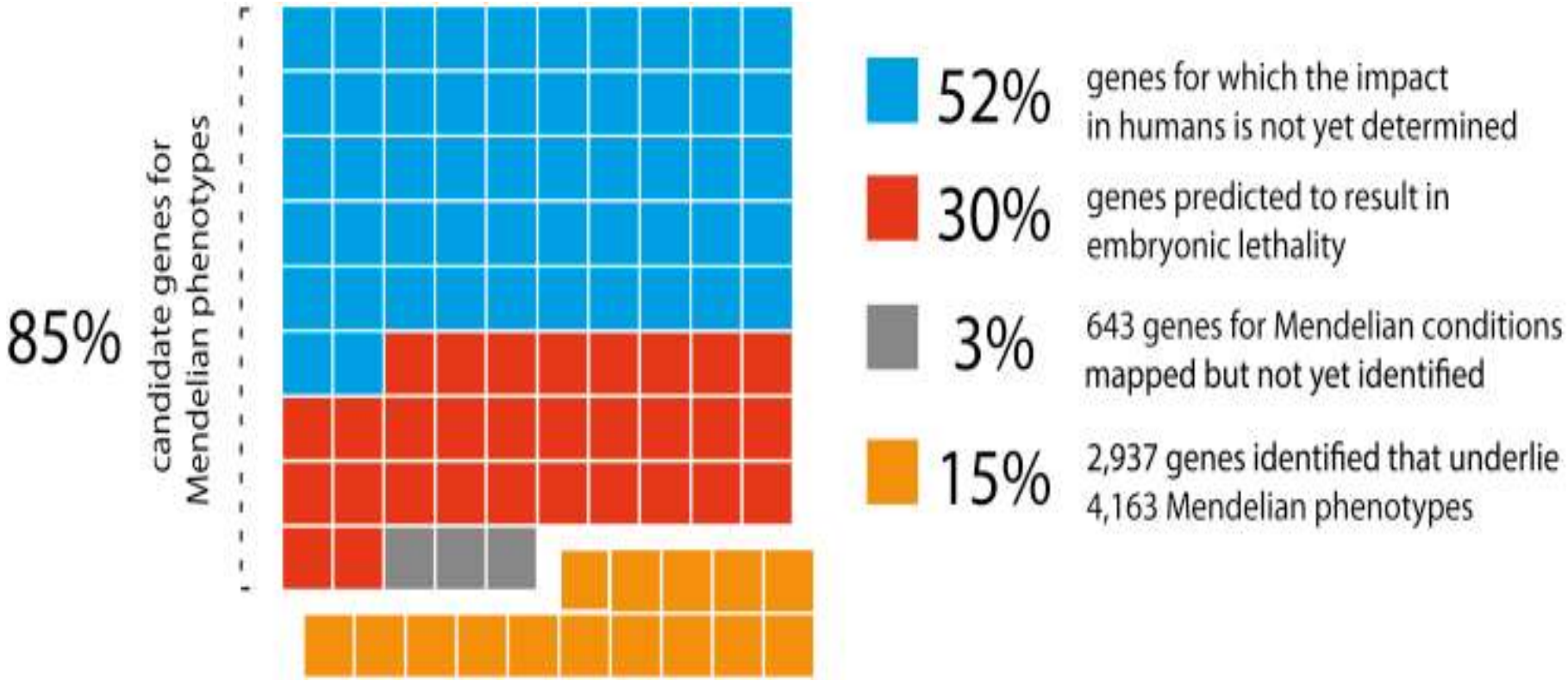

DNA Samples (Genomic or cfDNA)
Library Construction
Library Construction
Target amplification
Target Capture
Library Construction
Whole Genome Sequencing (WGS) (30X)
Whole Exome Seq (WES)/Target Sequencing (>100x)
Target Sequencing (200x-10,000x)
Data Analyses and Interpretation




**Approximate Number of Gene Discoveries Made by WES and WGS versus Conventional Approaches since 2010**

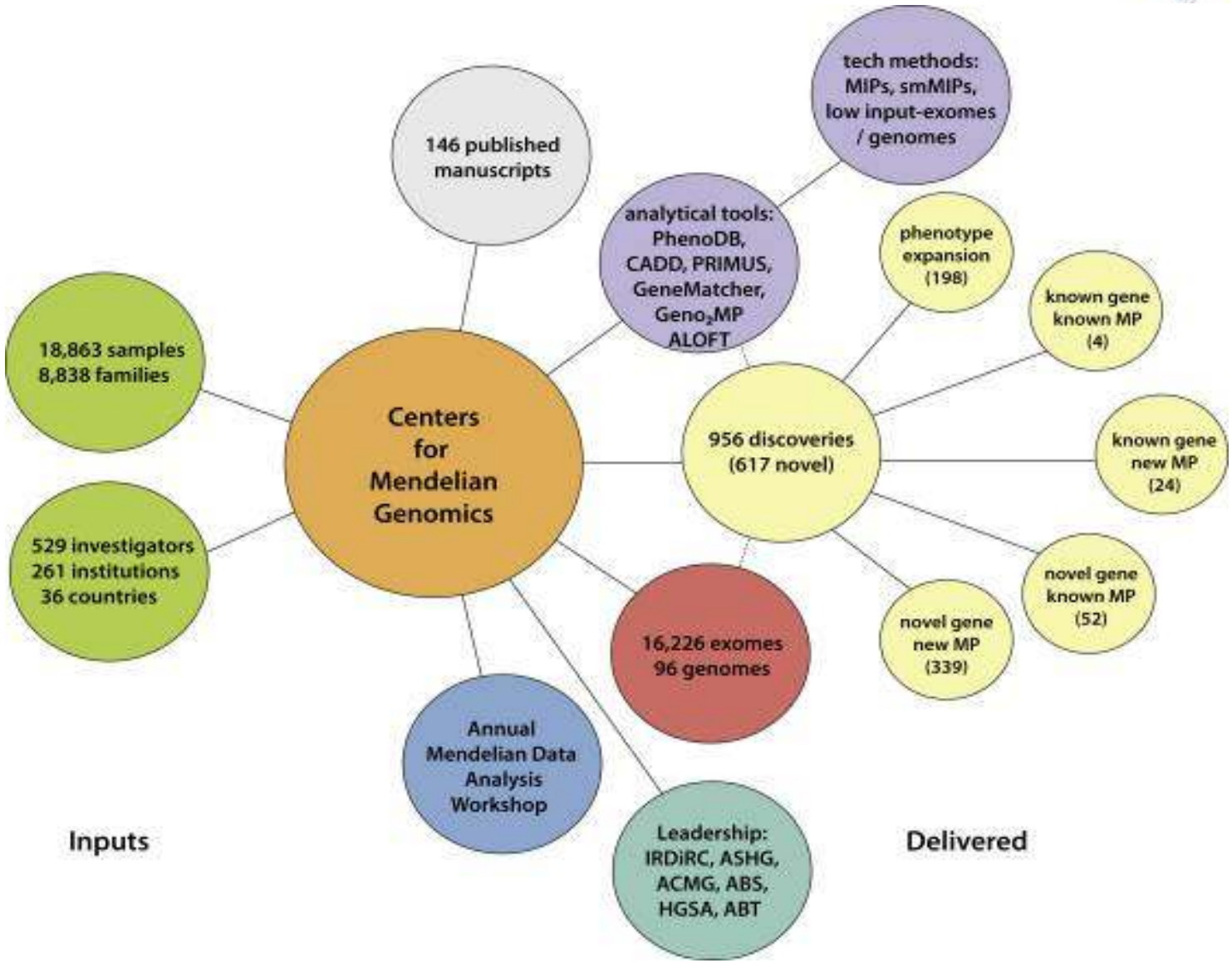
**Fusion gene discovery has increased very rapidly**

Am. J. Hum. Genet. 97, 199–215, 2015.




**Relationship between Human Protein-Coding Genes and Mendelian Phenotypes**

Am. J. Hum. Genet. 97, 199–215, 2015.

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8836 families; 18863 samples; 529 investigators 361 Institutes 36 countries

| Discovery Type | | Evidence of Causality | | |
|---|---|---|---|---|
| | | **conservative** | suggestive | Total |
| Known | known gene; explained, known phenotype | 320 | 9 | 339 |
| Novel | phenotype expansion | 174 | 24 | 198 |
| known gene; unexplained, known phenotype | | 4 | 0 | 4 |
| known gene; new phenotype | | 17 | 7 | 24 |
| novel gene; unexplained, known phenotype | | 25 | 27 | 52 |
| novel gene; new phenotype | | 107 | 232 | 339 |
| **Total novel** | | **327** | **290** | **617** |
| **Total number of discoveries** | | **647** | **309** | **956** |

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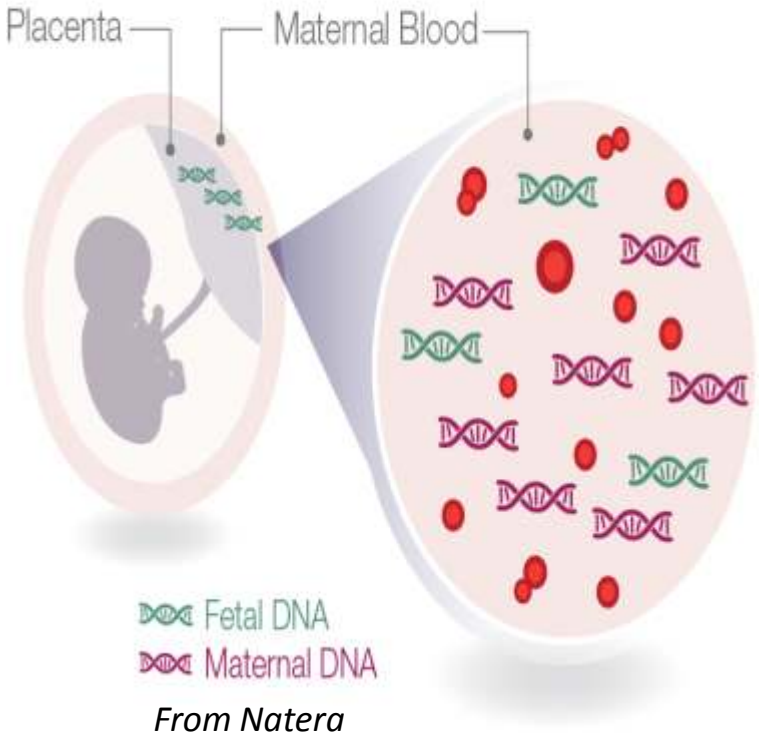
# OMIM Phenotypes for which the Molecular Basis Is Known

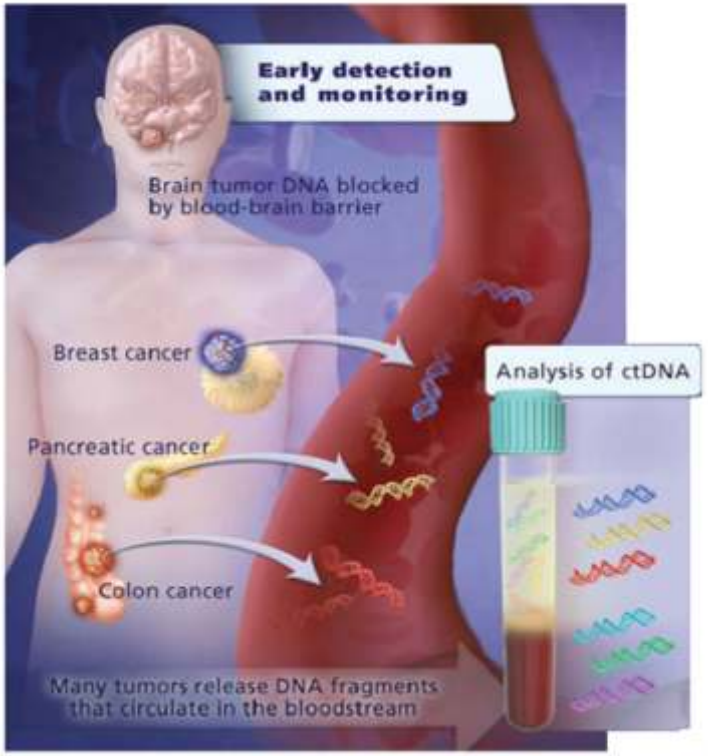
| Inheritance Pattern | January 2007 | July 2013 | 2015 | 2016 |
|---|---|---|---|---|
| Autosomal | 1,851 | 3,525 | | |
| X Linked | 169 | 277 | | |
| Y Linked | 2 | 4 | | |
| Mitochondrial | 26 | 28 | | |
| Total | 2,048 | 3,834 | 4,163 | 4,864 |

## The deep NGS application (100-10,000x):

(1) Targeted Genes mutations for CDX
(2) Targeted Genes Mutation for cfDNA:
    Liquid Biopsy for
      (i) Screening
      (ii) Diagnosis
      (iii) monitoring




*From Natera*




From GATC Bio

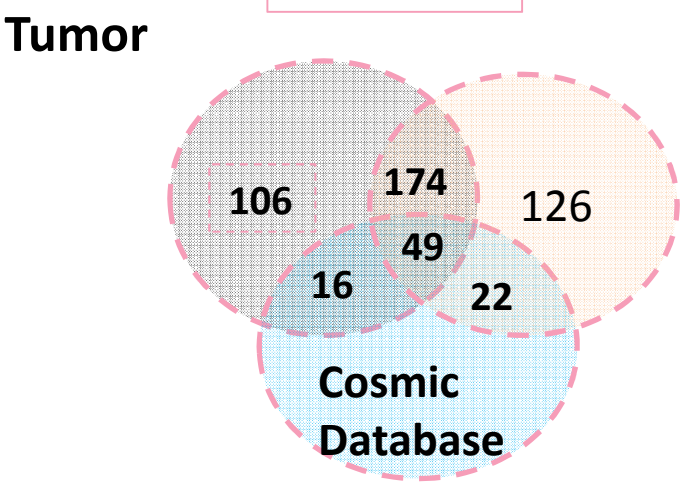
# Origins of cfDNA

1) **Preventive measure**

2) **Treatment & Prognostic measure (CDx)**

# Mutations found within tumor mass and plasma sample

## Liver CA

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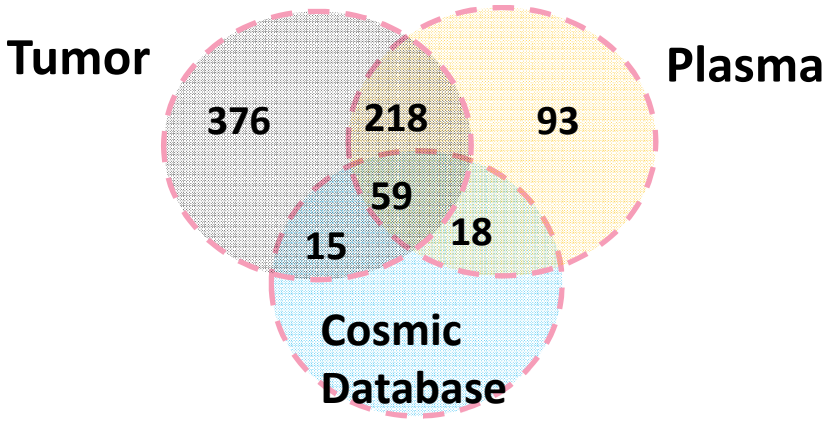


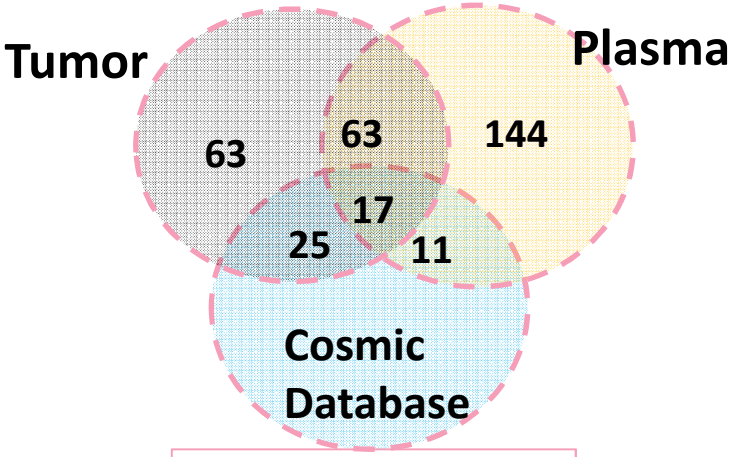


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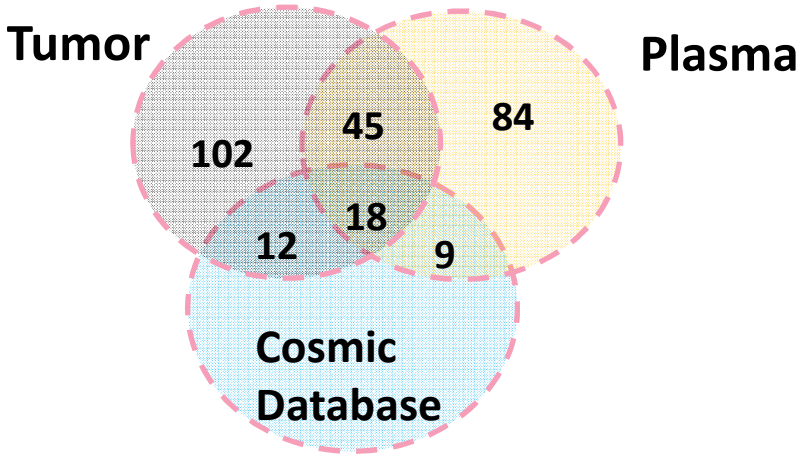
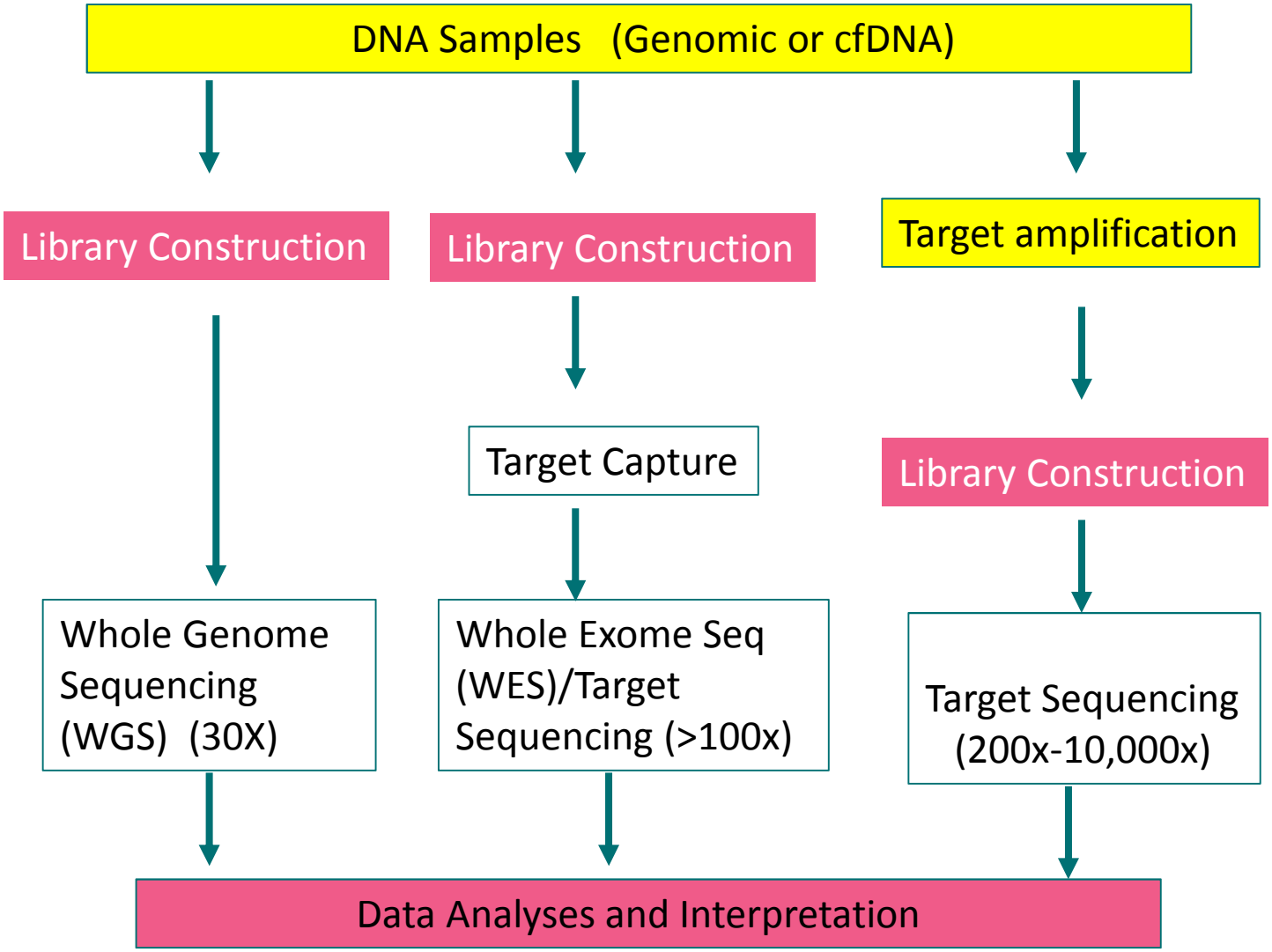



## NSCLC CA

From GATC Bio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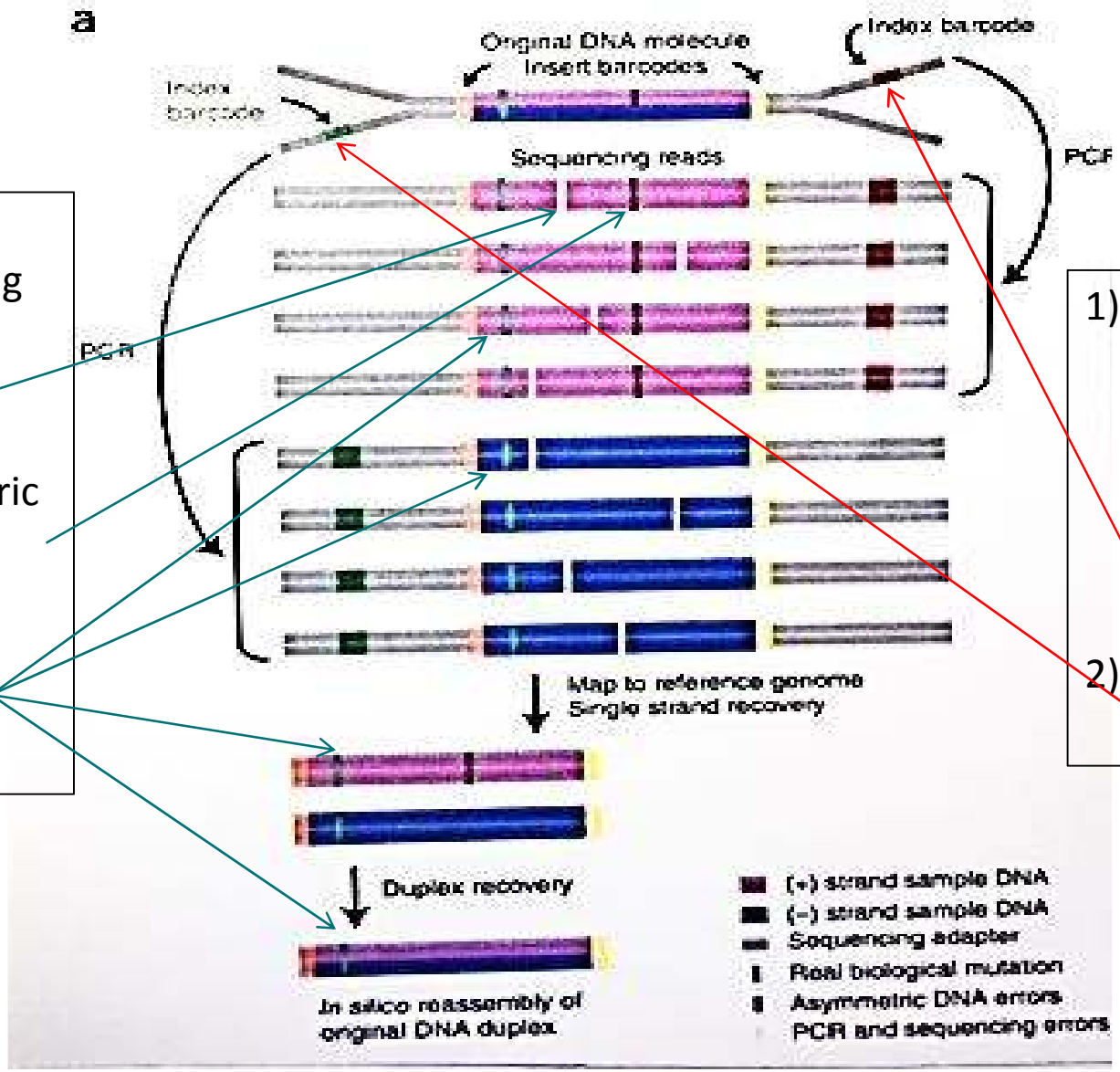
Target Sequencing (200x-10,000x)
Data Analyses and Interpretation

1) PCR and Sequencing Errors (random)

2) Asymmetric DNA error

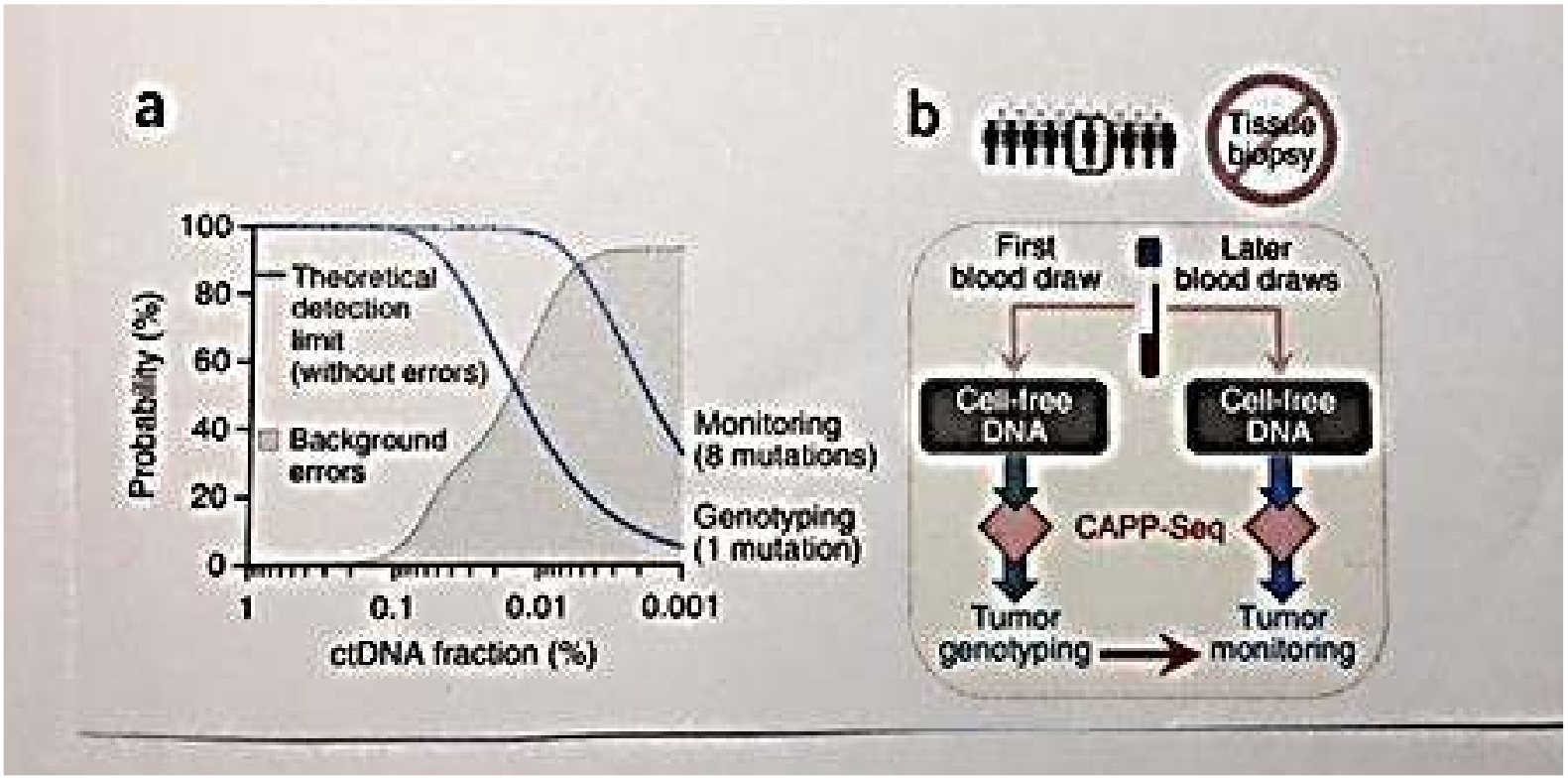
3) Real Mutation

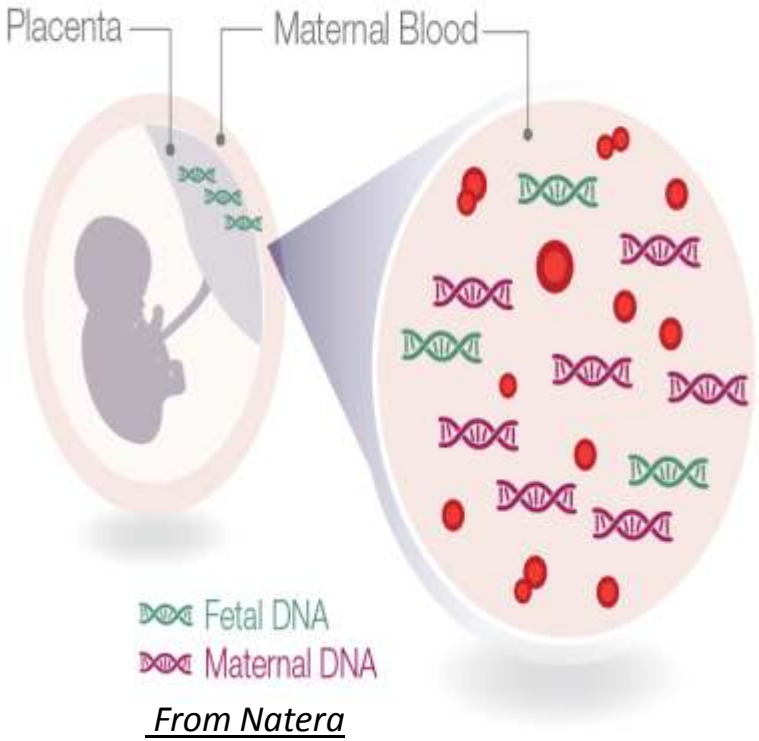



1) + strand Unique Marker Index Barcodes (UMIs)

2) - strand UMIs

Newman AM et.el. Nature Biotechnology: 28 March, 2016.
Integrated digital error suppression for improved detection of circulating DNA
( iDES-CAPP-Seq by Stanford U)



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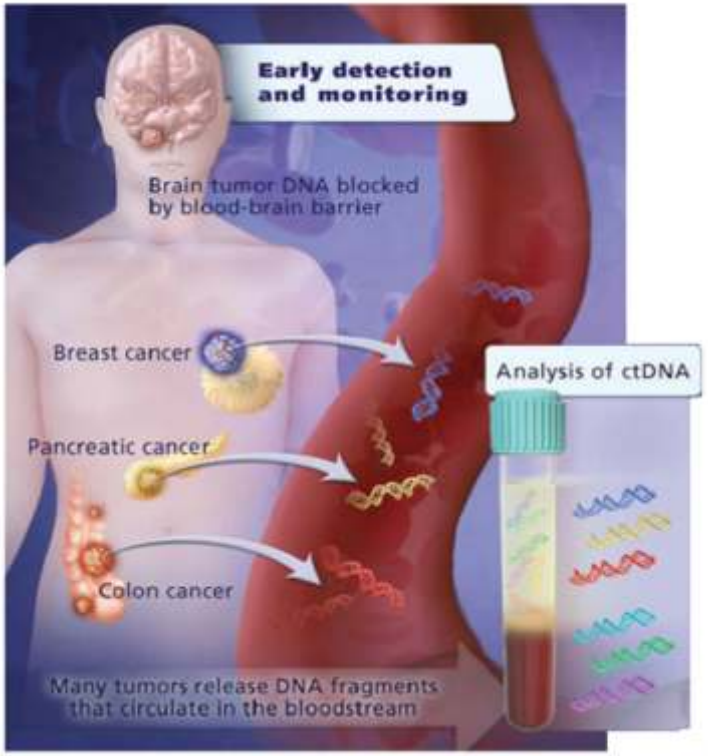


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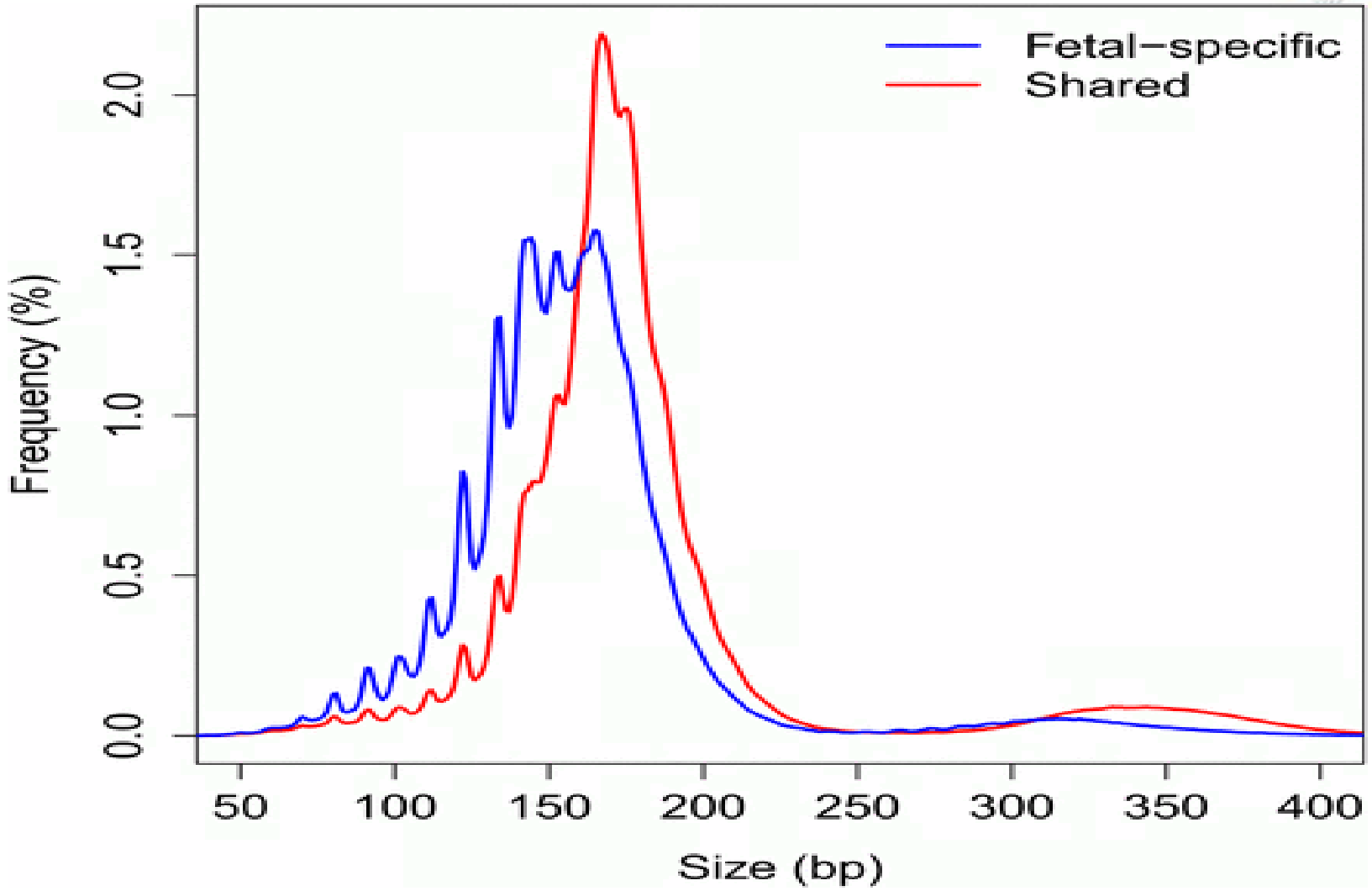


Dennis LM LO et al. PNAS 2016

# Noninvasive fetal whole genome analysis

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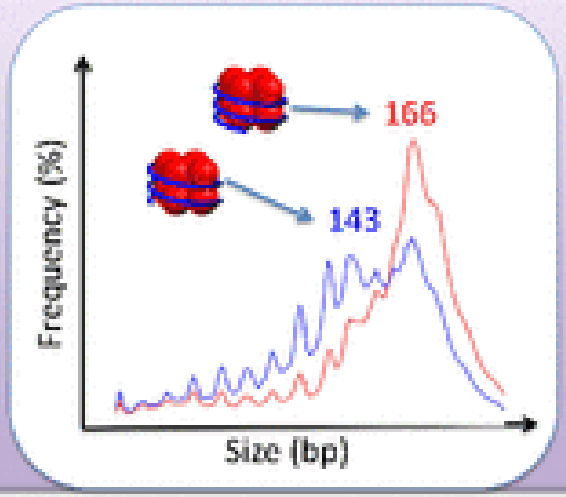
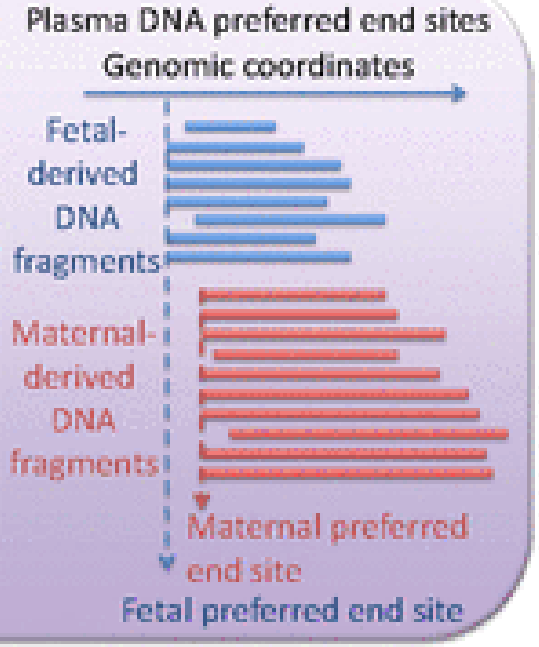
| | 1st generation[a] | 2nd generation |
|---|---|---|
| Sequencing Depth | 53× – 78× | 195× – 270× |
| **De Novo Mutation** | | |
| *Sensitivity* | 38.6% | 81% – 85% |
| *PPV* | 0.438% | 62% – 74% |
| **Maternal Inheritance** | | |
| *Principle* | Haplotype-based by RHDO* | SNP-based by GRAD* |
| *Resolution* | 300k – over 1M | Single nucleotide |

**Plasma DNA Fragmentation Pattern**

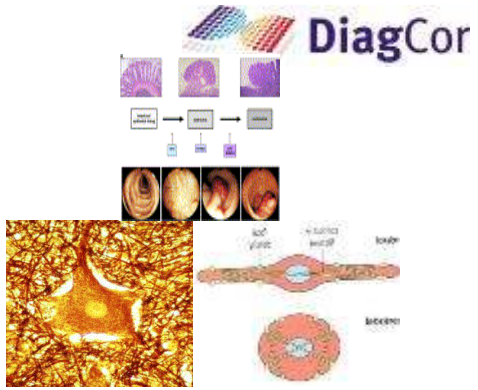
1. Nucleosomal pattern
2. Size difference between maternal- and fetal-derived fragments

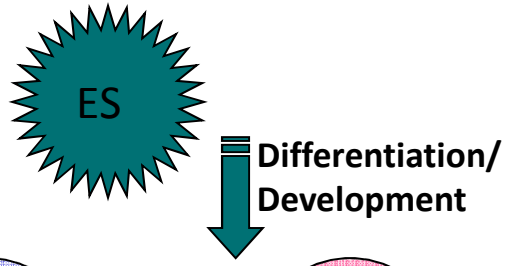


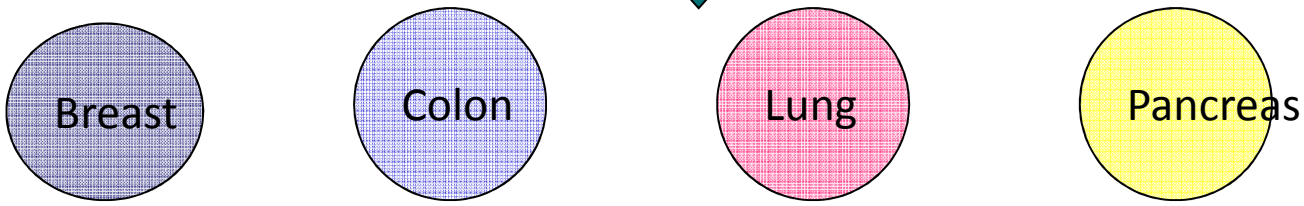

Dennis LM LO et al. PNAS 2016

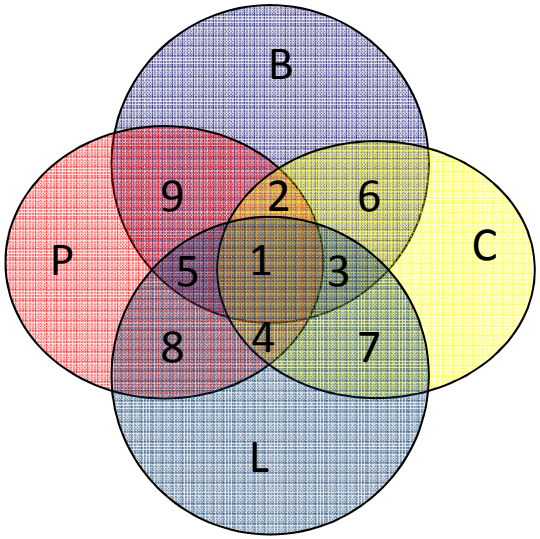
# Cell commitment




organ Specific Stem-cells



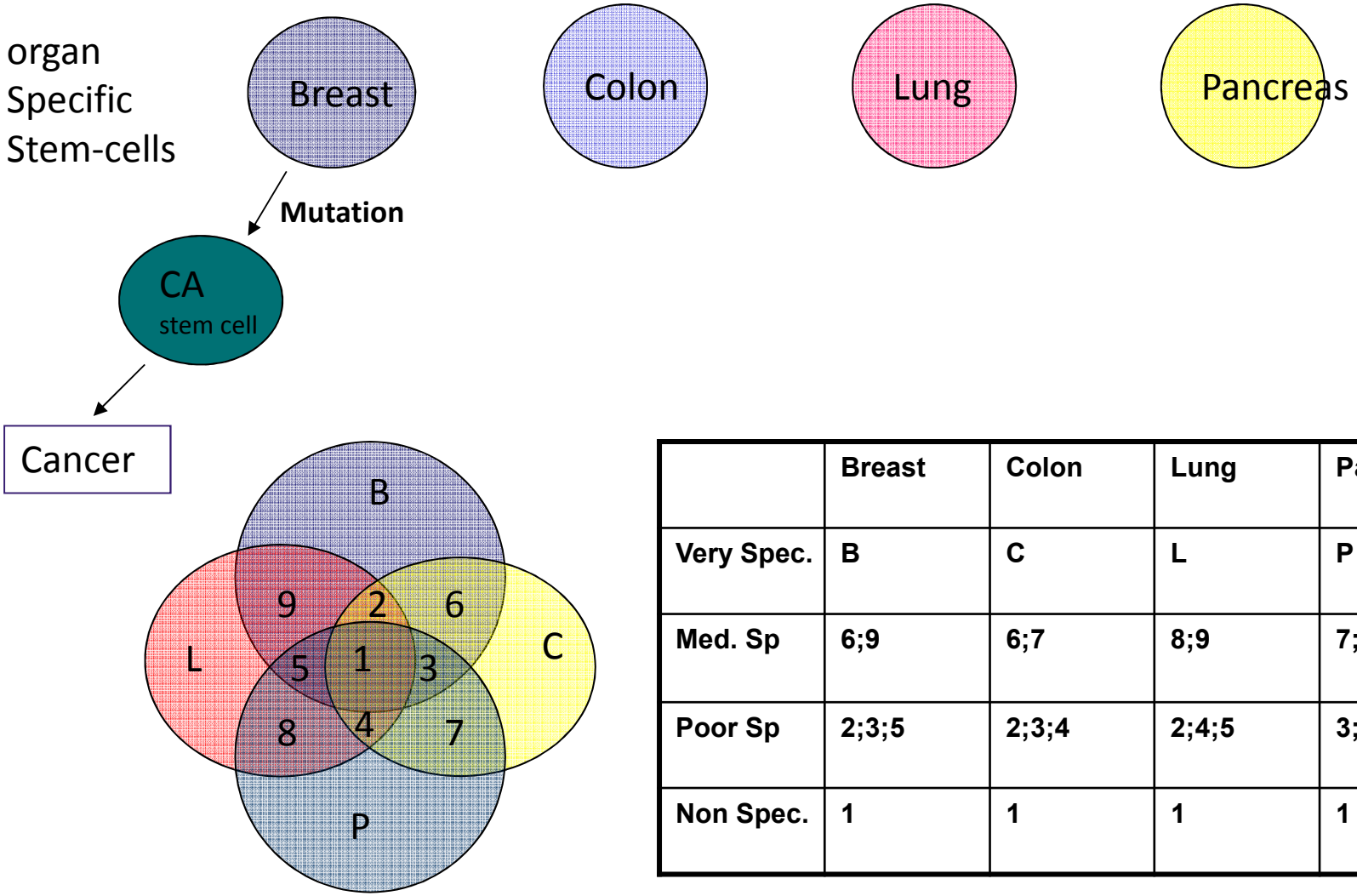




**Gene Expression studies**

**(i.e. High density microArrays)**

| gene | Breast | Colon | Lung | Pancreas |
|---|---|---|---|---|
| **1** | **Common to all- very essential for cell function** - House keeping genes etc. (i.e. DNA polymerase) | | | |
| **B,C,L,P** | **Tissue specific genes only express in the given cell** | | | |
| **By doing similar studies with Normal-cancer cells, specific "expression profiles" can be used for definitive and accurate Diagnosis, prognosis and disease management** | | | | |

organ
Specific
Stem-cells

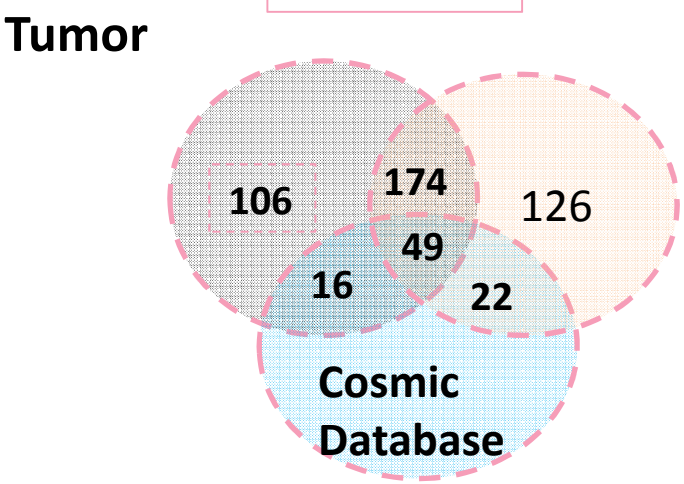



| | Breast | Colon | Lung | Pancreas |
|---|---|---|---|---|
| Very Spec. | B | C | L | P |
| Med. Sp | 6;9 | 6;7 | 8;9 | 7;8 |
| Poor Sp | 2;3;5 | 2;3;4 | 2;4;5 | 3;4;5 |
| Non Spec. | 1 | 1 | 1 | 1 |

DiagCor

# Mutations found within tumor mass and plasma sample

## Liver CA

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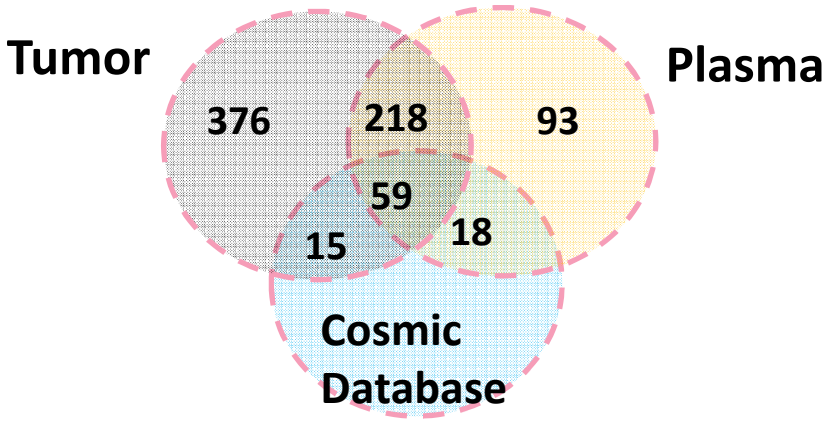


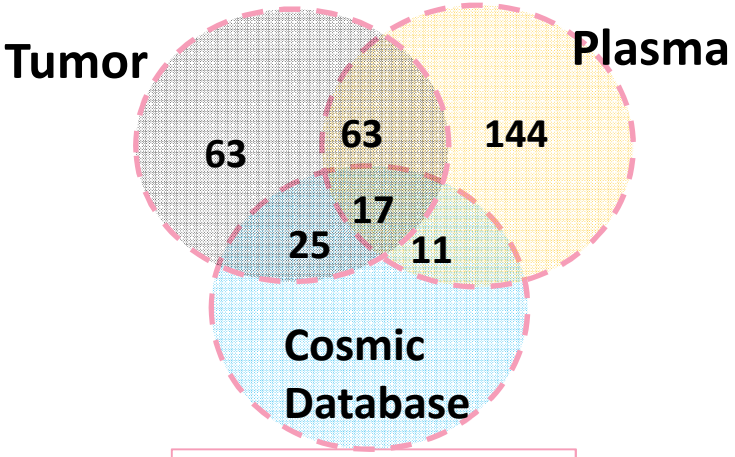


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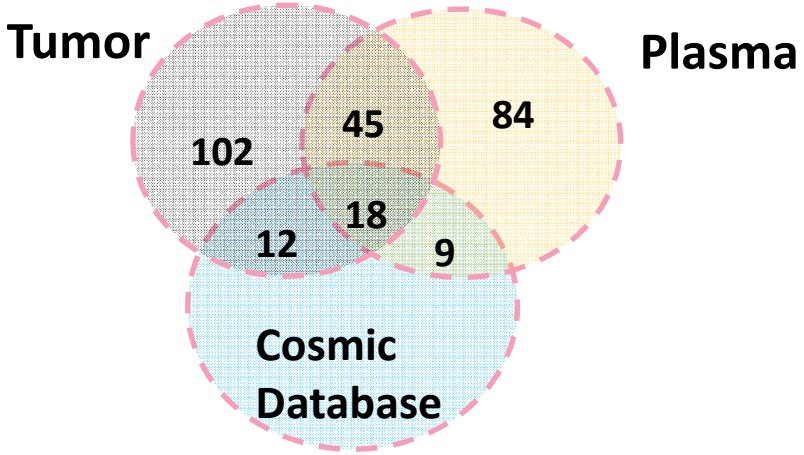
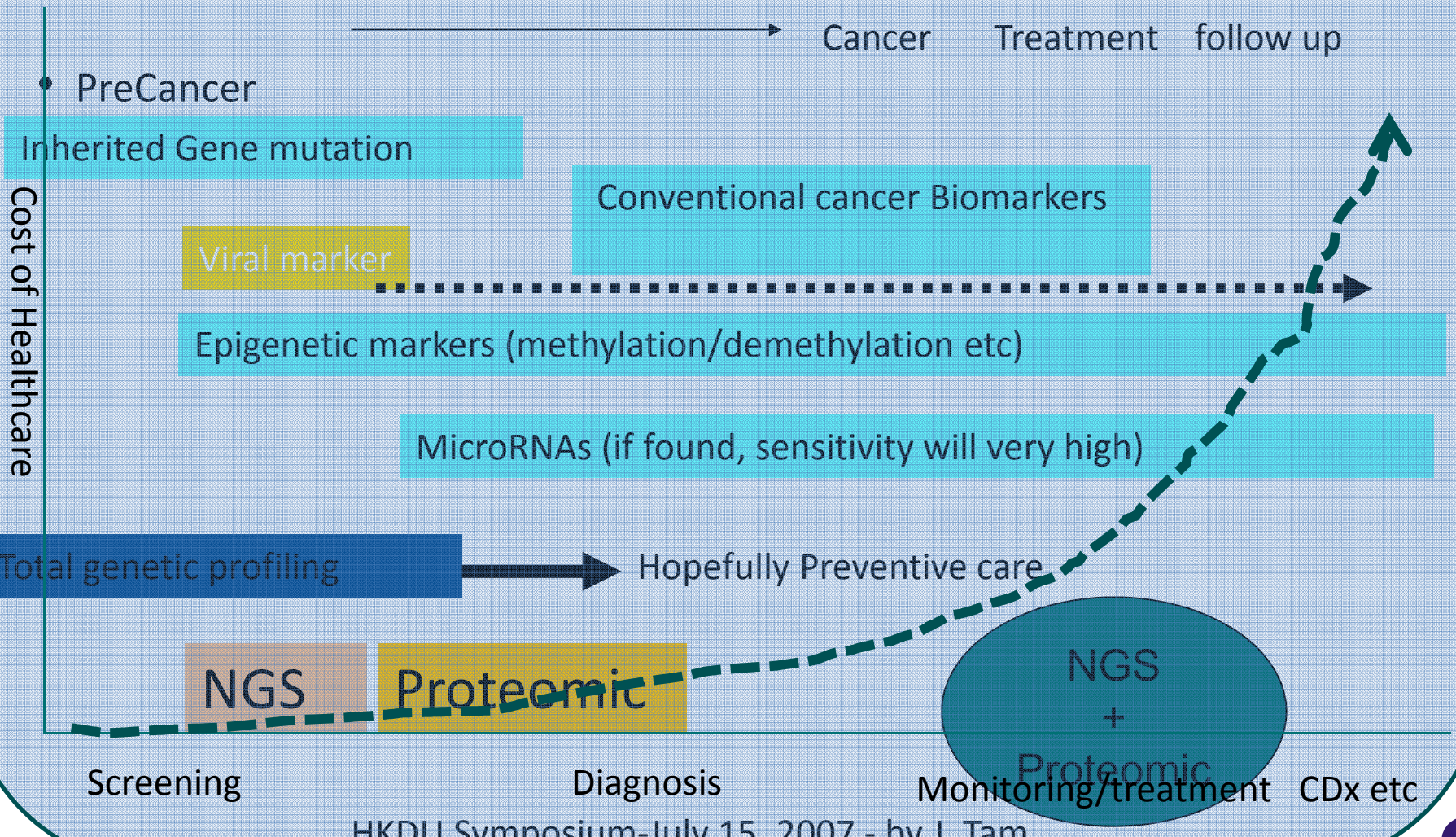



## NSCLC CA

From: GACT Biotech

# Cancer Screening



**Diagnosis:**

**(i) Physical Exam; imaging**

**(ii) Biomarkers**

**(a) gene pdts & *metabolites***

**(b) gene pdts- *mRNA, miRNAs***

**(iii) Genome Markers**

**(a) inherited mutations**

**(b) Viral invasions**

**(c) Sporadic Changes:**

**Mutagen induced mutations**

**epigenetic (=> lost function)**

**(due to aging etc…)**

**Screening, Prognosis, monitoring treatment**

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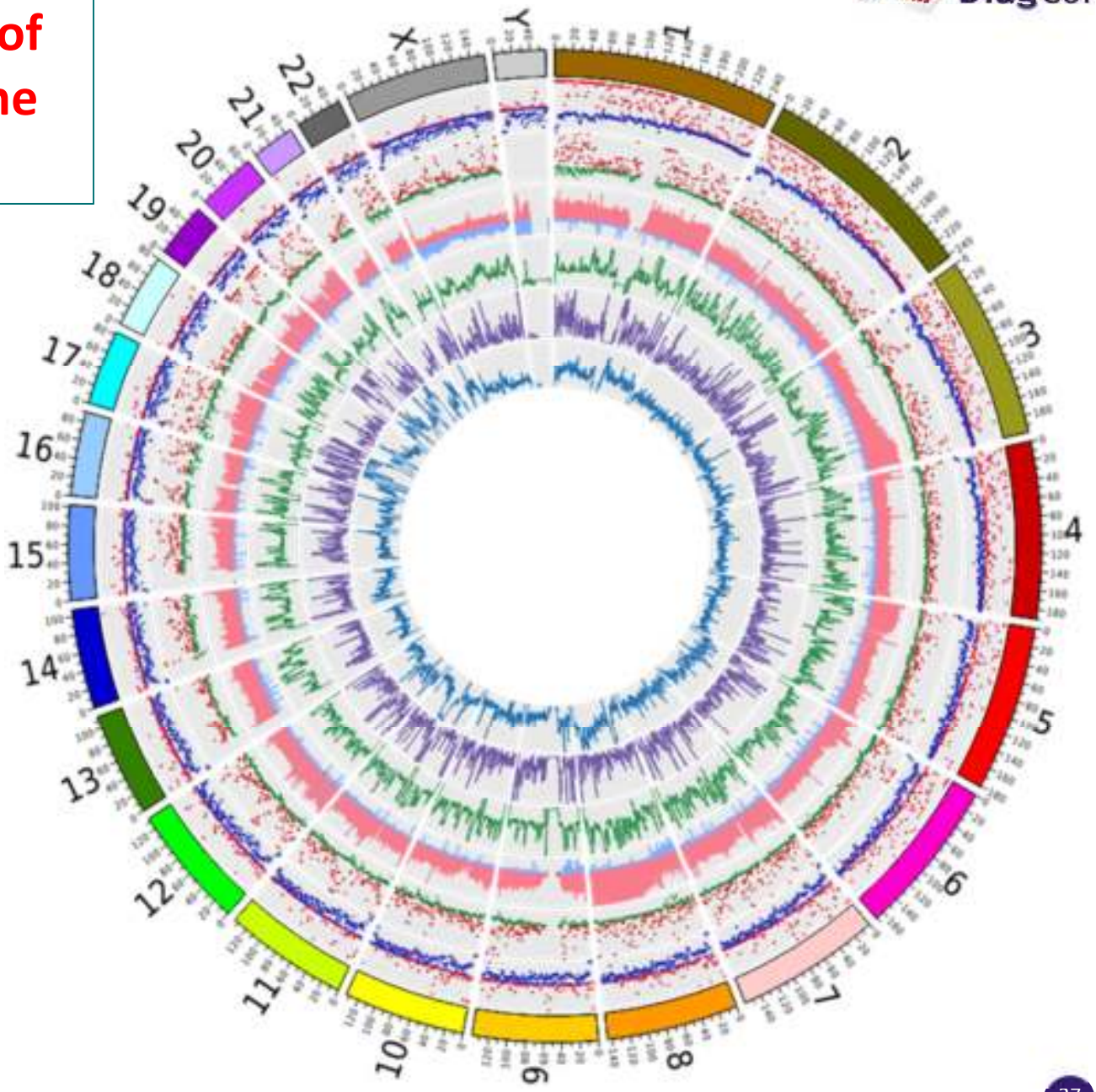


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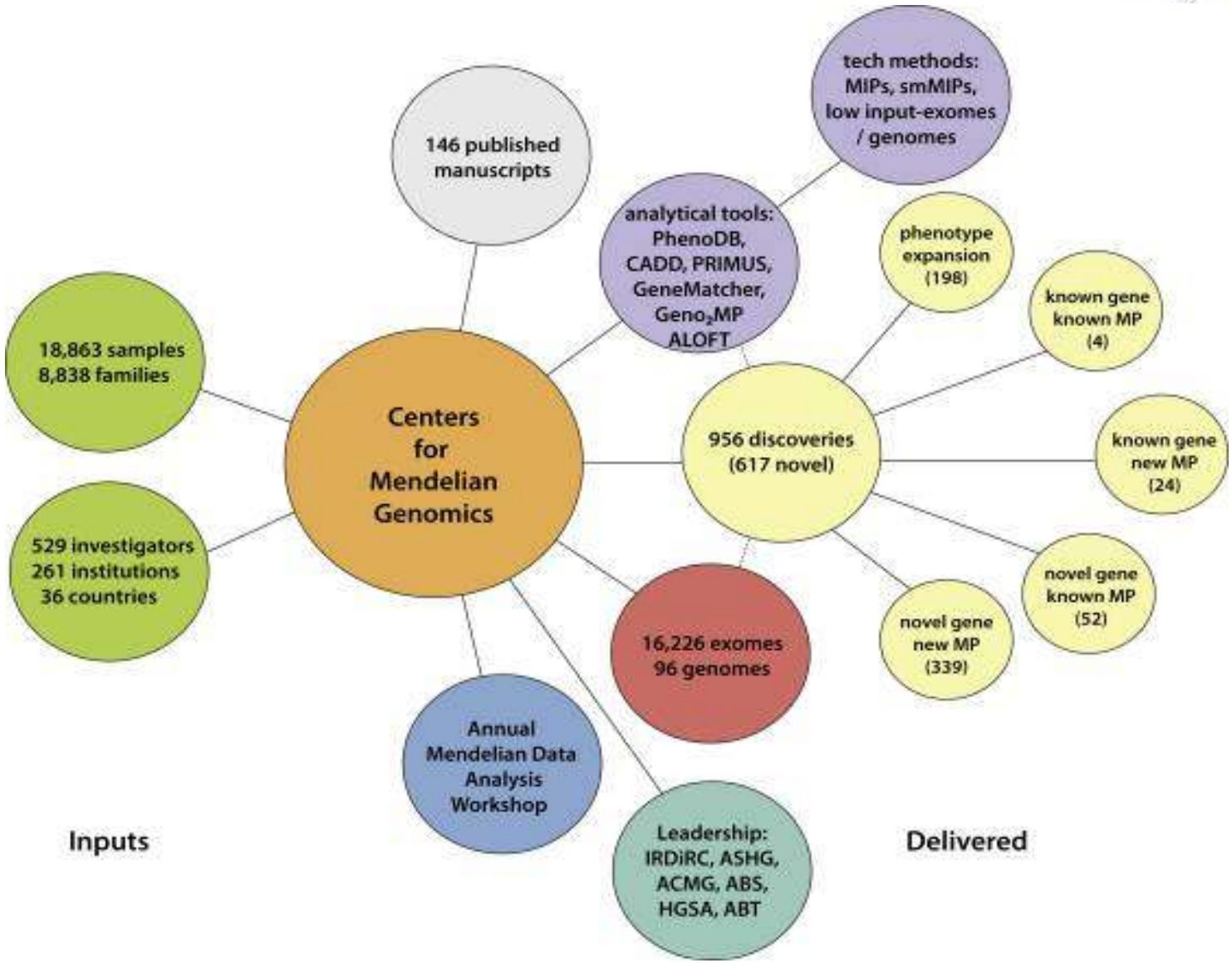



Am. J. Hum. Genet. 97, 199–215, 2015.

- What we need to do to achieve
  Precision Medicine => Personalized Medicine ?

We must couple DNA and protein /metabolites to gain a comprehensive Picture :

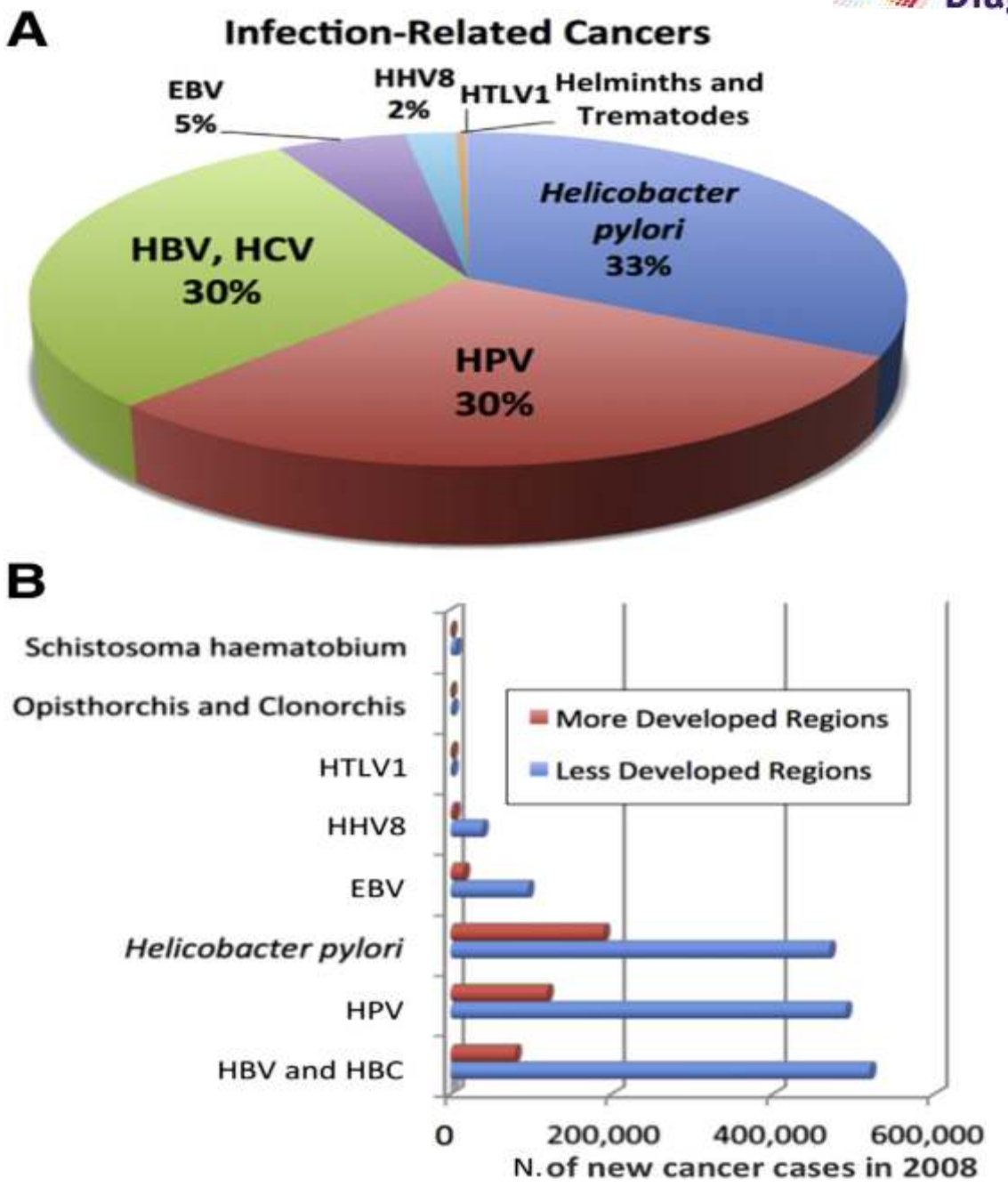
**Genomic & Proteomic => (Big Data)**
**The Complex Genome Project**

**2nd Genome Project**
**(DNA and proteomic)**
**(HOOD 2014)**

**In the mean time**

**Please consider**

**The immediate need of the Poor's Healthcare**



# DiagCor

**MDx** is in our DNA