Non Invasive Prenatal Testing (NIPT)

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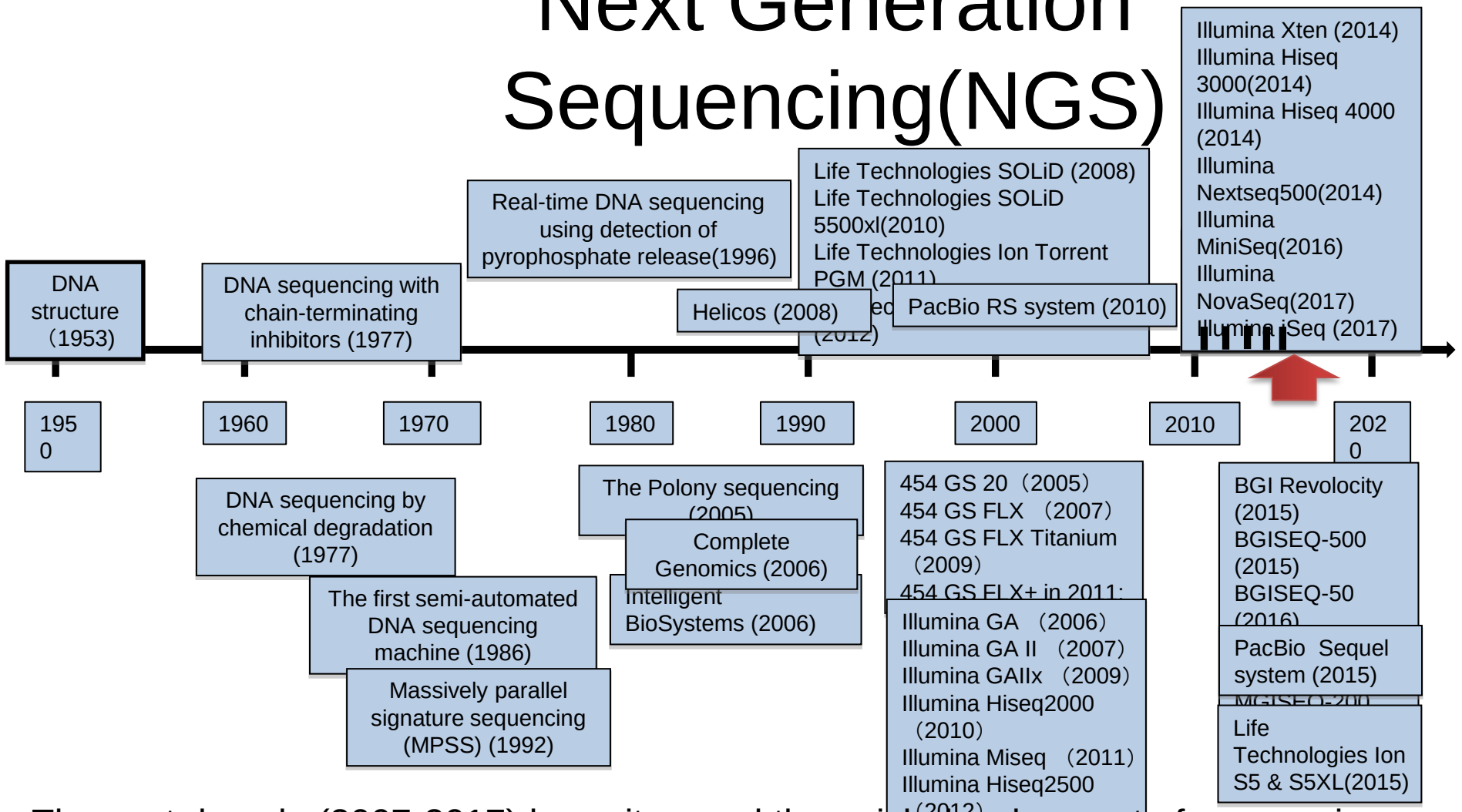
April, 2019

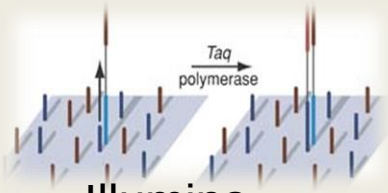
Next Generation Sequencing(NGS)



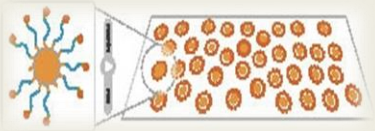
The past decade (2007-2017) has witnessed the quick development of sequencing technology as well as the fast transfer of this new technology from basic science research to practical clinical application *(from dream to reality)*

Cost for one human genome dropped from $3,000,000,000 (Human genome project) to

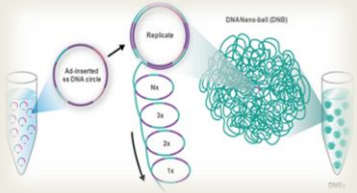
Sequencing technologies



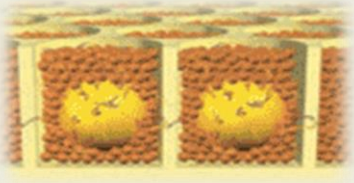
Illumina



SOLiD (ABI)

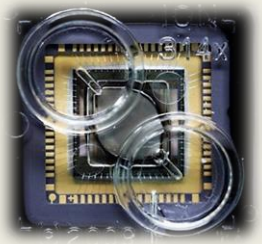


Complete Genomics



454 (Roche)

Optical Sequencing



PGM/Proton

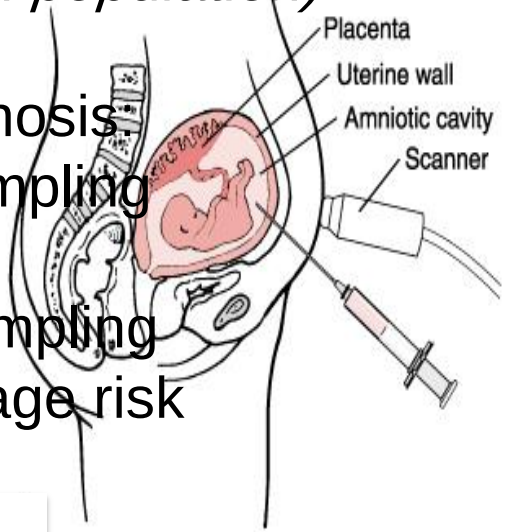
Semi-conductor Sequencing

Key characteristics:

- Massively parallel analysis
- High throughput
- Reduced cost
- Short read length
- Higher error rate than 1st generation

NIPT and NGS

- NIPT: noninvasive prenatal testing *(screen the high-risk subgroup from general population)*

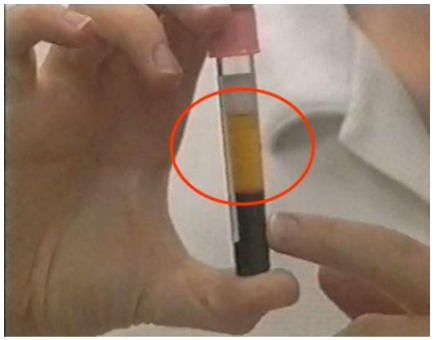


- Invasive prenatal diagnosis
  - chorionic villus sampling
  - Amniocentesis
  - umbilical blood sampling
  - 0.5%-1% miscarriage risk

- NIPT refers to a wide scope of all prenatal cares in a noninvasive or minimal invasive way. Yet currently, **NIPT is mostly related to the cell free DNA-based testing of genetic diseases, especially chromosomal aneuploidies**.

Cell-free fetal DNA and NIPT

- Short DNA fragments ~145-200bp, circulating in maternal peripheral blood and originating from placental trophoblasts.
- Can be observed since the 5th gestation week (GA)
- Content is proportional to GA and inversely proportional to maternal BMI
- Averagely 10%, diverse among individuals

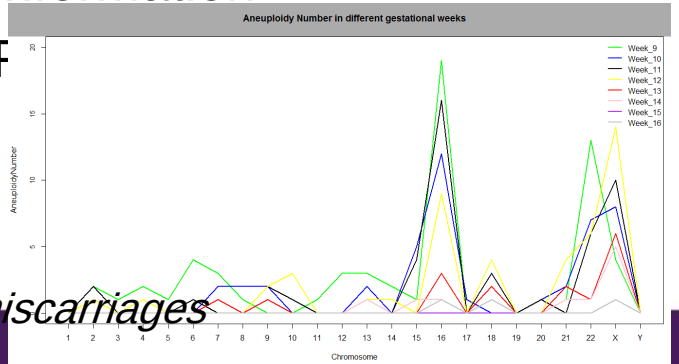


Lo YM et al., Lancet, 1997; 350: 485-87; Alberry M. et al. Prenat Diagn. 2007 May;27(5):415-8.; Zhou Y

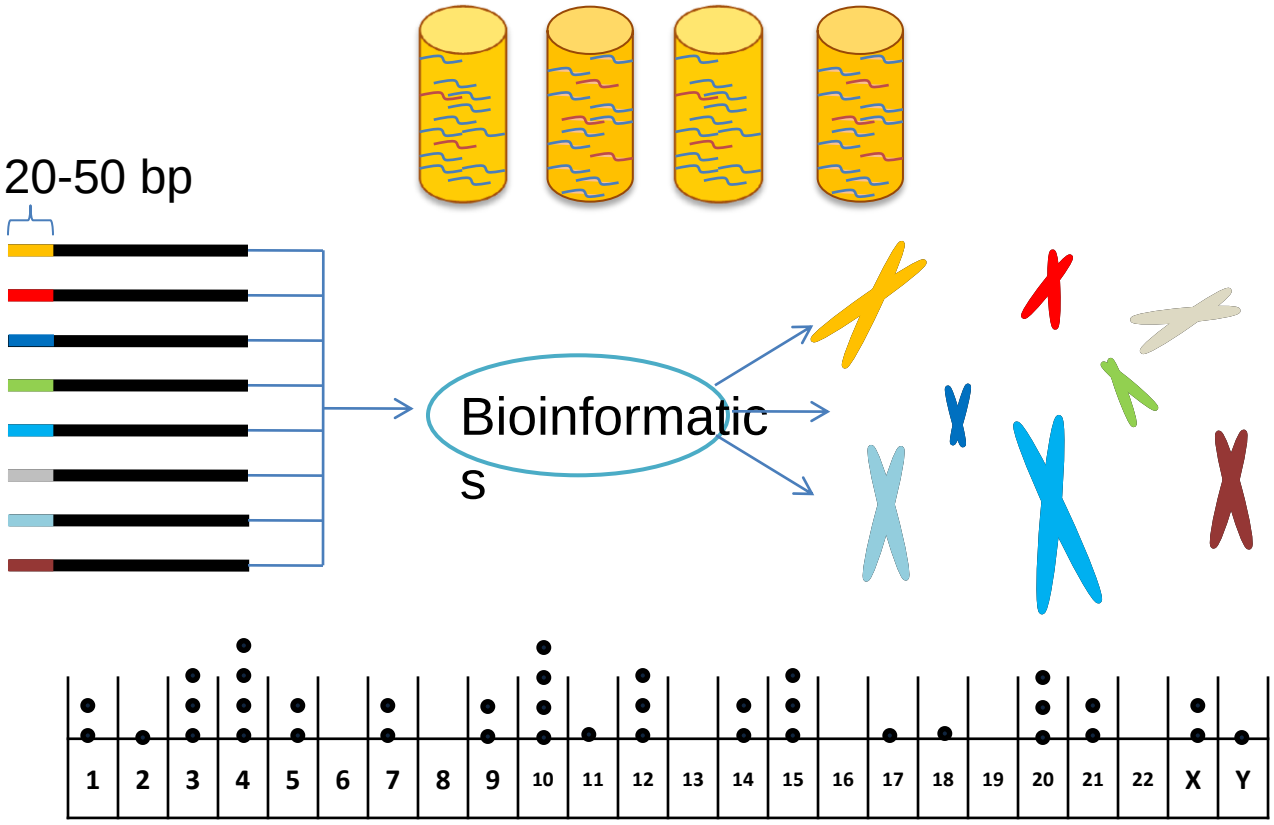
Sequencing strategies for NIPT

- **Whole genome sequencing**
  - Non-polymorphism unique region in the human genome(>95%)
  - Detect aneuploidies in 24 chromosomes and smaller deletion/duplication parallel
  - Discriminate standard T21, partial T21 and mosaic T21
  - Fetal fraction estimated by chromosomal Y specific or chromosomal specific sequence

- **Target region sequencing**
  - Polymorphism region in the human genome(2000-10,000 SNPs)
  - Can detect aneuploidies at selected chromosomes and regions (21,18,13,X,Y)
  - Fetal fraction estimated by chromosomal Y specific or father-inherited SNP ratio information
  - F



Principals of Sequencing-Based NIPT



Plasma DNA extraction

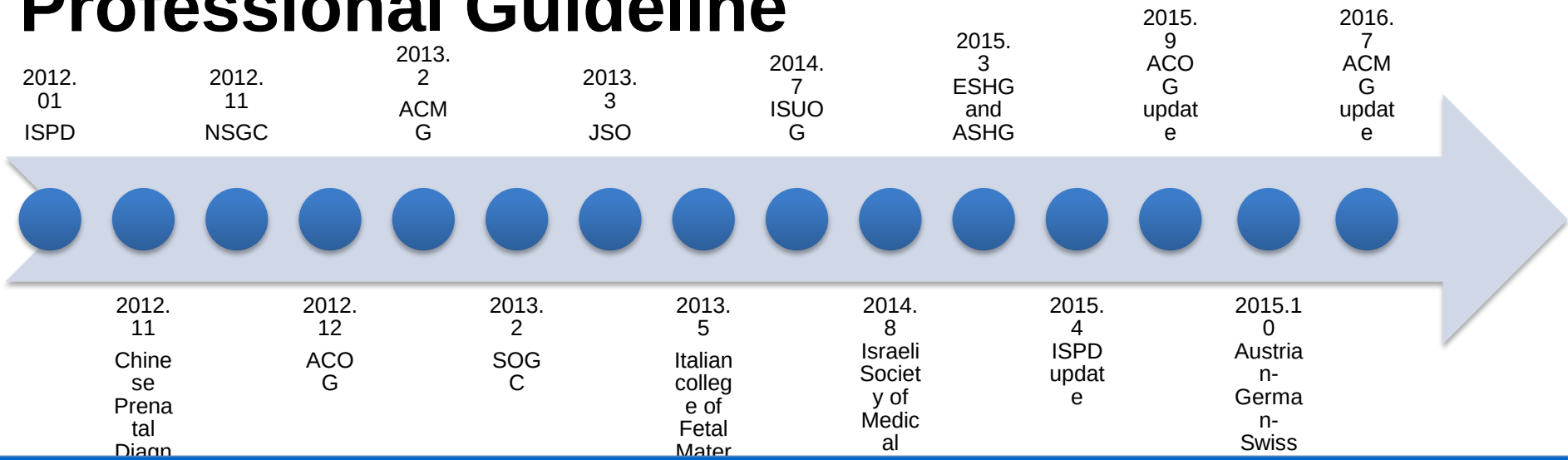
Sequencing & alignment

Chromosome counting

Coverage computation

$$\%chrN = \frac{\text{Unique fragments for chrN}}{\text{Total unique fragments}}$$

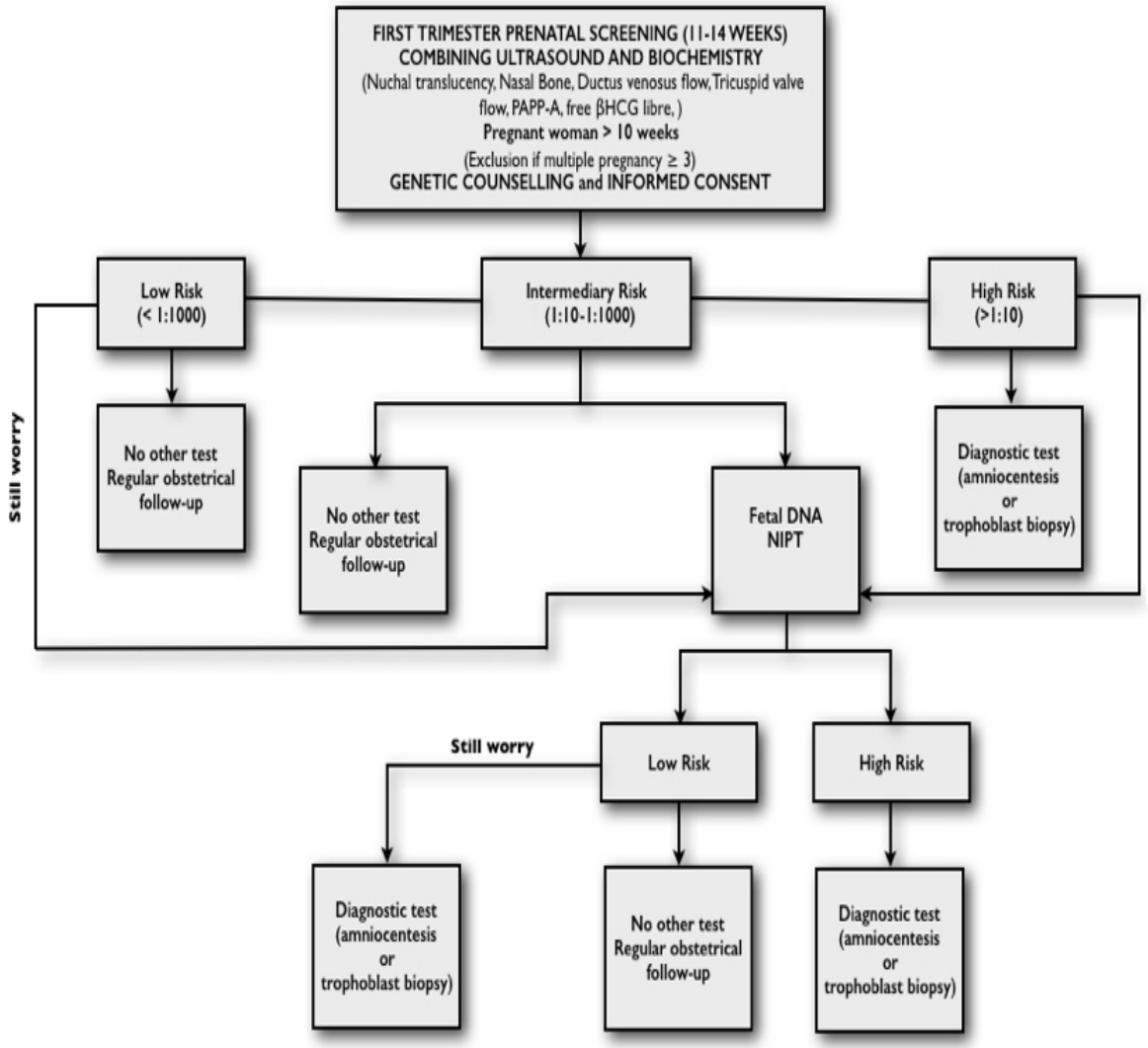
Professional Guideline



ACOG（American College of Obstetricians and Gynecologists）	ISPD（International Society for Prenatal Diagnosis ）	ESHG-ASHG（European Society of Human Genetics- American Society of Human Genetics）	ACMG（American College of Medical Genetics and Genomics）
2015.6 26 online published	2015.6 4 online published	2015.3.18 online published	2016.7 online published
Recommended: • Not applied in micro-deletion/duplication syndrome • Need more	Recommended: • Only include disease with clinical significance • Equip with trained	Recommended: • Not to expand NIPT to other syndrome: not deny the potential value • Target region based methods has limitations	Recommended: • NIPS is the most sensitive screening option for traditionally screened aneuploidies • Restrict only in disease with severe clinical significance • Not to expand NIPS to other chromosome aneuploidy, need more validation

Non invasive prenatal screening using the detection of fetal DNA circulating in maternal blood

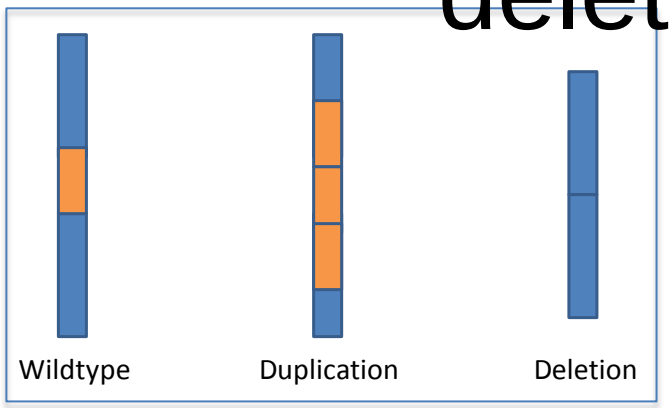
Proposed algorithm

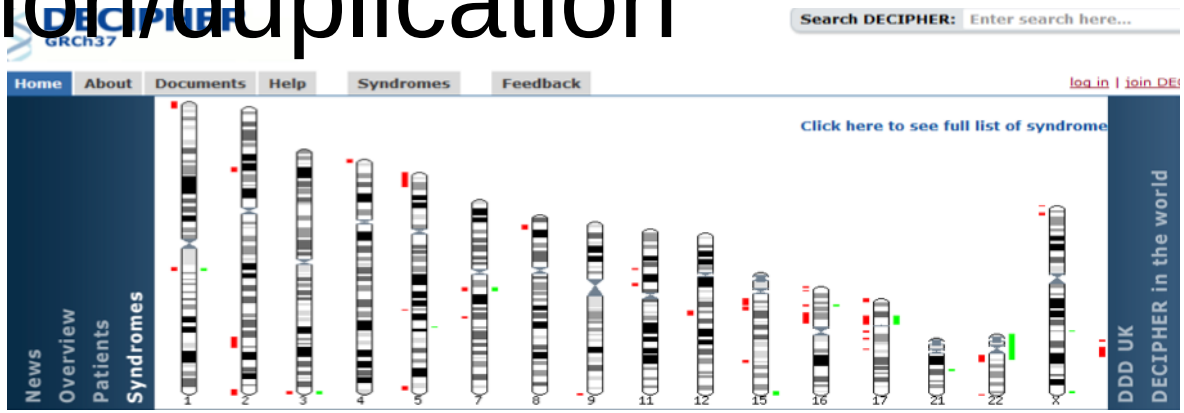


Challenges in current NIPT service

- **Simpler processing** (Automation-software-figure)
- **Easier explanation** (Z score/risk assessment-figure)
- **Faster turnaround time**（1-3-5-7-10 days)
- **Cheaper** (1000-500-300-100 USD)
- **Flexible throughput** (3456-768-196-16-1 samples per run)
- **Regulation** (CE/FDA/CAP/CLIA)
- **Easy training** (nurses/doctors/bioinformatics guys)
- **Social education** (Pregnant women/Family member)

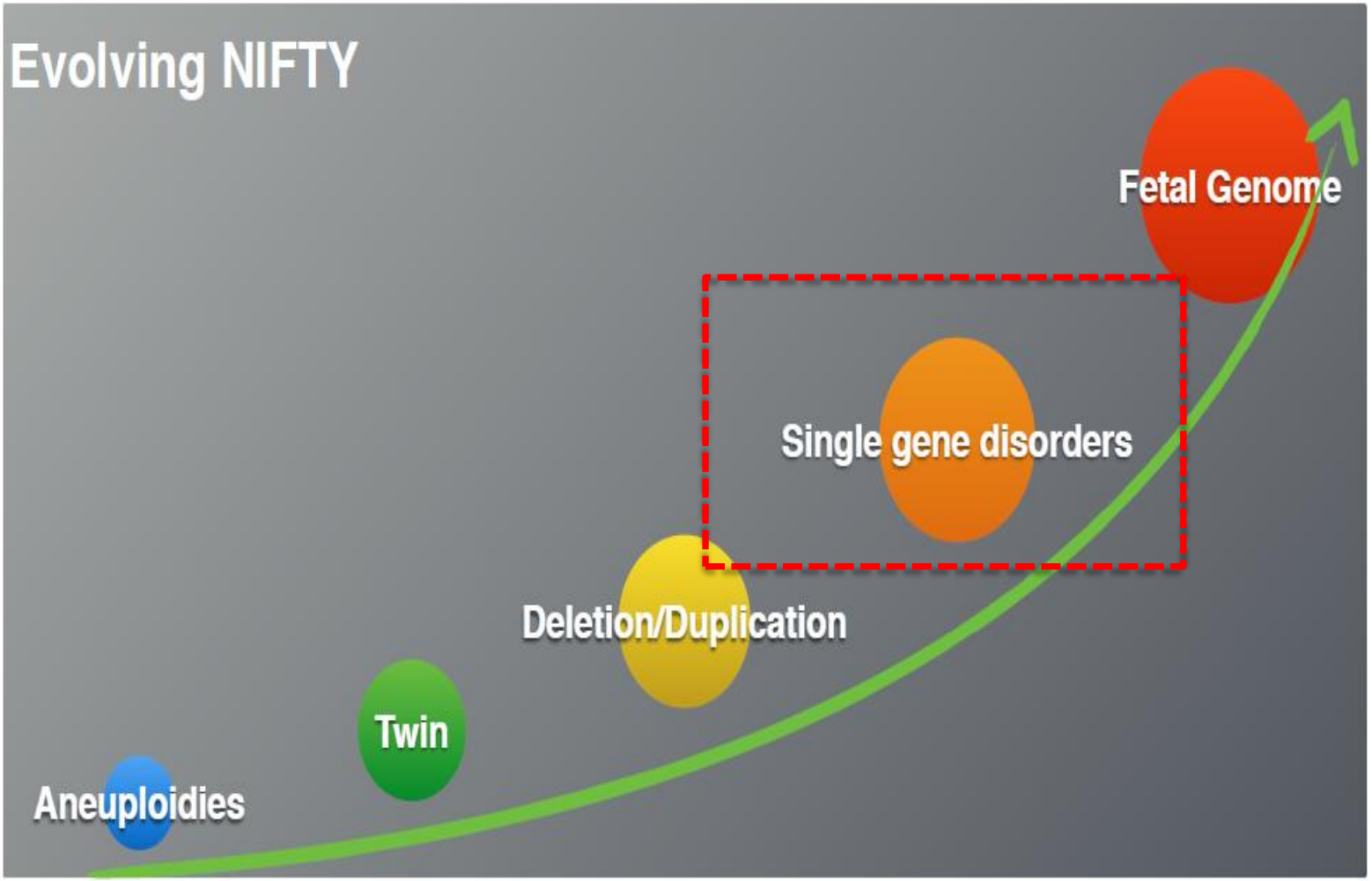
NIPT detecting fetal micro-deletion/duplication





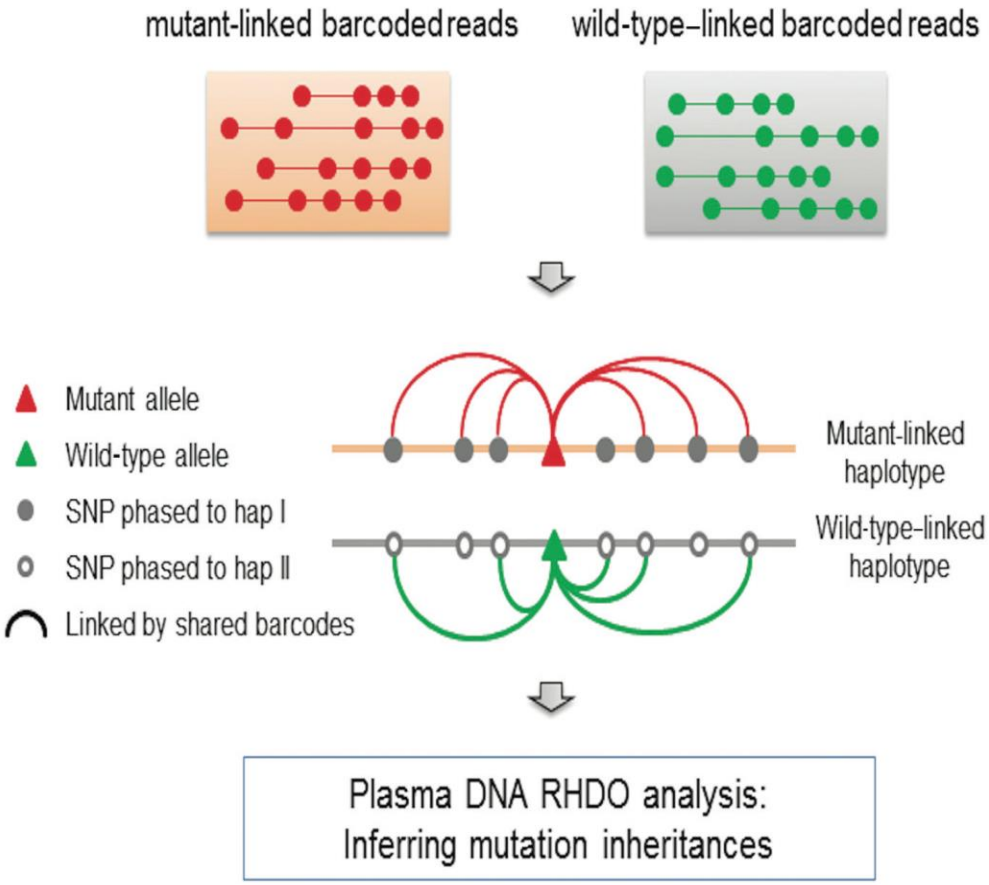
Author	Accuracy
Chen et al.2013	1311 clinical cases：Correctly identifies 3 cases of micro-deletion/duplication with 100% sensitivity. One false positive was found resulting in 99.92% specificity. No false negative results (>10M deletion/duplication)
Li et al.2016	117 clinical cases：18 CNV (>1M) were identified through microarray, 10 of 11 cases with CNV >5M were identified with NIPT; Among 7 cases with CNV >1M but <5M, NIPT identified 1 case (2.82M). Therefore, NIPT sensitivity for detecting CNV >1M was 61.1% with 5% false negative rate.
Zhao et al.2015	For 3-40M micro-deletion/duplication，17 case were identified in 18 cases. Sensitivity and specificity were 94.4% and 99.4%, respectively.
Helgeson et al.2015	In 175393 clinical cases，55 sub-chromosomal abnormality were identified with false positive rate of 0.0017%. The most common abnormality was 22q11.2, with 70.5% detection rate
Pescia et al.2016	6388 clinical cases, identifies 3 T22, 6 T7 with false positive rate of 0.71%. In other sub-chromosomal abnormality(CNV) aspect, 8 CNV were identified, including 3 false positive and 1 false negative.

Future perspective



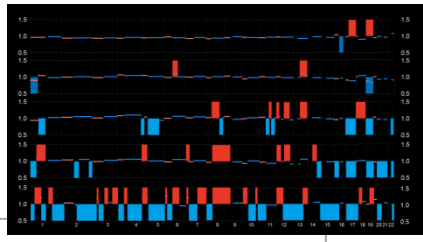
Haplotype-assisted method for NIPD

Direct haplotyping from parental DNA



Hui et al., Clin Chem. 2017 Feb;63(2):513-524.

NIPT and Maternal cancer



Amant F, et al. JAMA Oncol. 2015 Sep;1(6):814-9.

Brief Report

Presymptomatic Identification of Cancers in Pregnant Women During Noninvasive Prenatal Testing

Frédéric Amant, MD, PhD; Magali Verheecke, MD; Iwona Wlo
Nathalie Brison, PhD; Kris Van Den Bogaert, PhD; Daan Dieric
Thomas Tousseyn, MD, PhD; Philippe Moerman, MD, PhD; Ac
Patrick Neven, MD, PhD; Patrick Berteloot, MD; Katrien Putse
Peter Vandenberghe, MD, PhD; Eric Legius, MD, PhD; Joris R

- 4000 case for NIPT， 3 cases showed multiple abnorlities
- MRI image, tissue slice and genetic testing confirmation ;
- tumor tissue CNV concordant with NIPT

Bianchi, DW, et al. JAMA. 2015 Jul 14;

Preliminary Communication

Noninvasive Prenatal Testing and Incidental Detection of Occult Maternal Malignancies

Diana W. Bianchi, MD; Darya Chudova, PhD; Amy J. Sehnert,
Tracy L. Prosen, MD; Judy E. Garber, MD; Louise Wilkins-Hau
Stephen Warsof, MD; James Goldberg, MD; Tina Ziainia, MD;

- 125,426 case for NIPT， 10 cases with confirmed cancer
- NIPT give discordant results with karyotyping

Genetics inMedicine

ARTICLE | Genetics inMedicine

Article | Published: 12 April 2019

Identifying occult maternal malignancies from 1.93 million pregnant women undergoing noninvasive prenatal screening tests

Xing Ji, MD[1,2], Jia Li, PhD[3], Yonghua Huang, MD[4], Pi-Lin Sung, MD[5,6], Yuying Yuan, BS[3], Qiang Liu, BS[3], Yan Chen, PhD[3], Jia Ju, MS[3], Yafeng Zhou, PhD[3], Shujia Huang, PhD[3], Fang Chen, PhD[3], Yuan Han, BS[7], Wen Yuan, MS[7], Cheng Fan, BS[3], Qiang Zhao, PhD[4], Haitao Wu, PhD[8], Suihua Feng, MS[4], Weiqiang Liu, MD[9], Zhihua Li, MD[10], Jingsi Chen, MD[10], Min Chen, MD[10], Hong Yao, MD[11], Li Zeng, MD[12], Tao Ma, MD[13], Shushu Fan, MD[14], Jinman Zhang, MD[15,16], Ka Yiu Yuen, BS[17], So Hin Cheng, BS[17], Irene Wing Shan Chik, BS[17], Nien-Tzu Liu, MS[17], Jianyu Zhu, BS[3], Siyuan Lin, BS[17], Jeremy Cao, BS[17], Steve Tong, BS[17], Zhiyuan Shan, BS[18], Wenyan Li, MS[3], Mohammad Reza Hekmat, MS[19], Masoud Garshasbi, PhD[19,20], Javier Suela, PhD[21], Yaima Torres, MSc[21], Juan C. Cigudosa, PhD[21], F. J. Pérez Ruiz, MD[22], Laura Rodríguez, PhD[23], Mónica García, PhD[23], Janez Bernik, MS[24], Eva Traven, MS[24], Uršula Reš, MD[25], Nataša Tul, MD[26], Ching-Fong Tseng, MS[27], Depeng Zhao, MD[28], Luming Sun, MD[28], Qiong Pan, MS[29], Li Shen, MD[30], Mengyao Dai, MD[1,2], Yuying Wang, PhD[3], Jian Wang, MS[3,31], Huanming Yang, PhD[3,31], Ye Yin, PhD[3,32], Tao Duan, MD[28], Baosheng Zhu, MD[15,16], Mahesh Choolani, PhD[33], Xin Jin, PhD[3,34,35], Yingwei Chen, MD[1] and Mao Mao, MD, PhD[3]

Thank you for your attention

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