Developing NIPD for fetal aneuploidy

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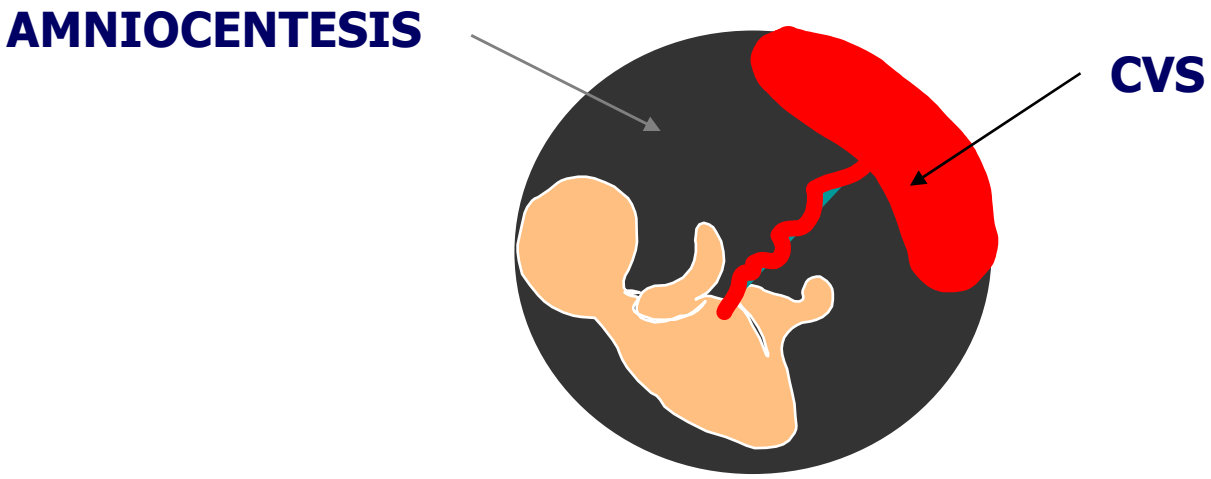
Outline of talk

- Current prenatal screening for aneuploidy
- How can cell free fetal nucleic acids be used for DS testing?
- New non-invasive techniques for detection of DS
  - Quantitative SNP analysis from cffRNA
  - Shot gun sequencing of cfDNA
  - Epigenetics
  - Digital PCR
- RAPID: Plans to develop NIPD for aneuploidy

Current prenatal screening for aneuploidy



- Prenatal screening for is offered to all pregnant women
- Undertaken in two phases:

  screening and risk assessment

  invasive prenatal diagnosis of high risk cases

- Gold standard for diagnosis of chromosomal abnormalities is karyotyping

Current prenatal screening for aneuploidy

Due to a small but significant risk to the pregnancy, many women are reluctant to opt for these procedures

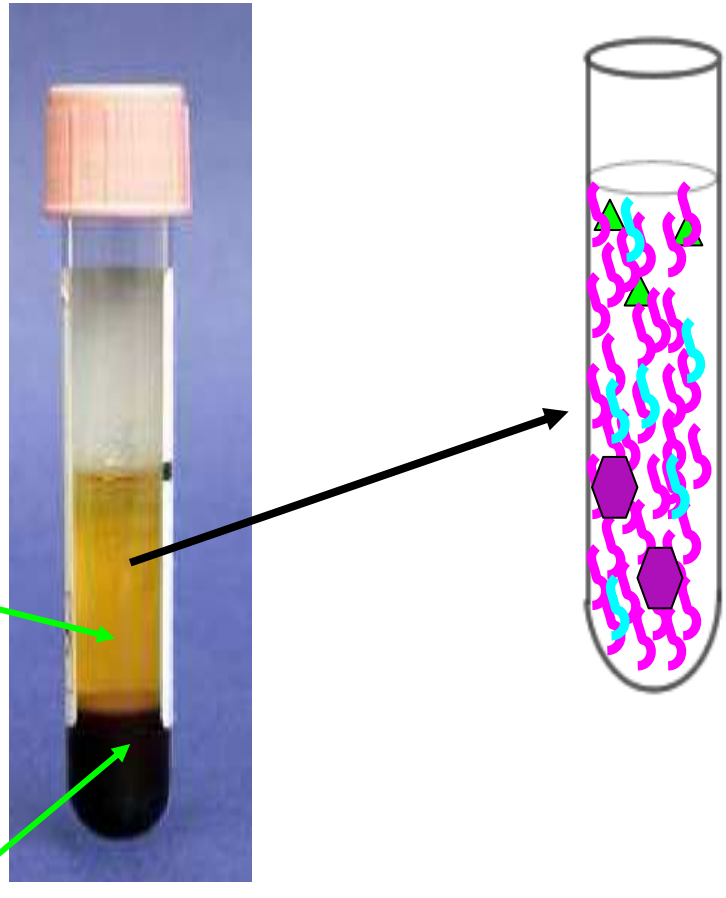
In 2006-7:

~700,000 pregnant women a year underwent antenatal screening

20,000 amniocentesis and 5,200 CVS were performed

Estimated associated procedural related pregnancy loss of ~250

Extraction of cell free fetal nucleic acids from maternal plasma



Cell free maternal DNA (96.6%)

Cell free fetal DNA (3.4%)

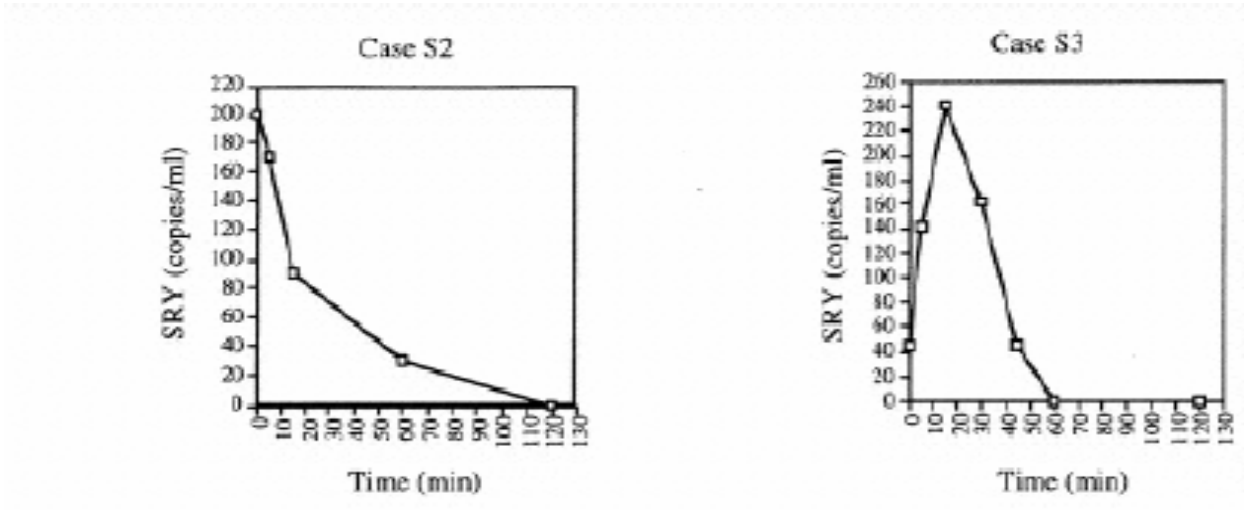
Amount of cell free fetal DNA extracted is equivalent to 25 cells / ml plasma

Cell free maternal RNA

Cell free fetal RNA

Clearance of cell free fetal nucleic acids after delivery

ffDNA



ffRNA

How can cell free fetal nucleic acids be used for non-invasive Down syndrome testing?

- **Major technical challenge**

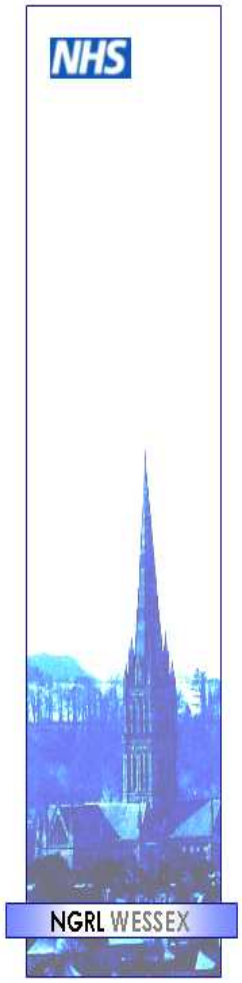
  Background of cell free maternal **DNA** means direct quantification of fetal chromosome copy number is problematic and technically demanding

  Ideally need:

    targets that are free from maternal background interference

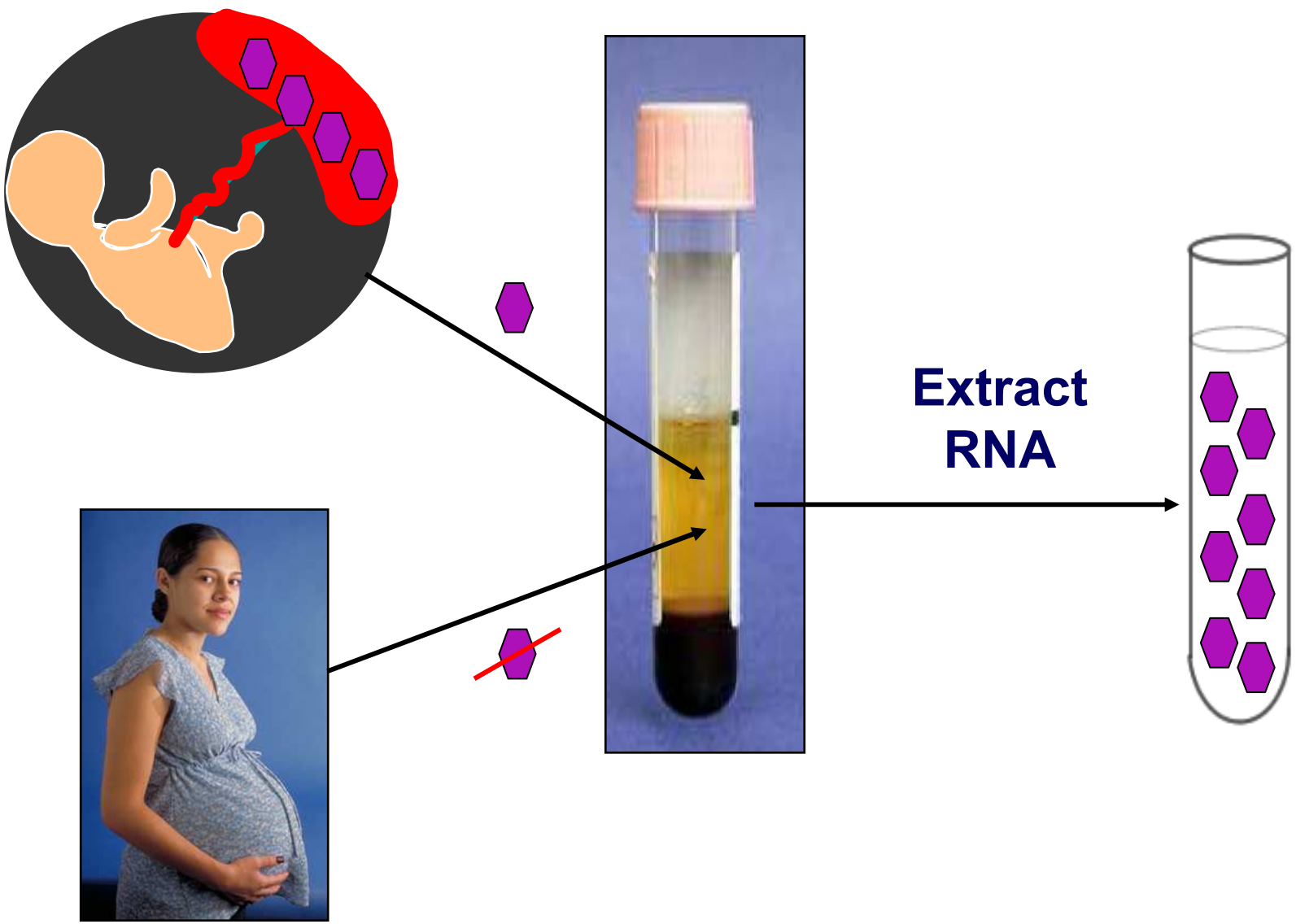
and / or

    technologies that enable accurate & large scale copy number ‘counting’

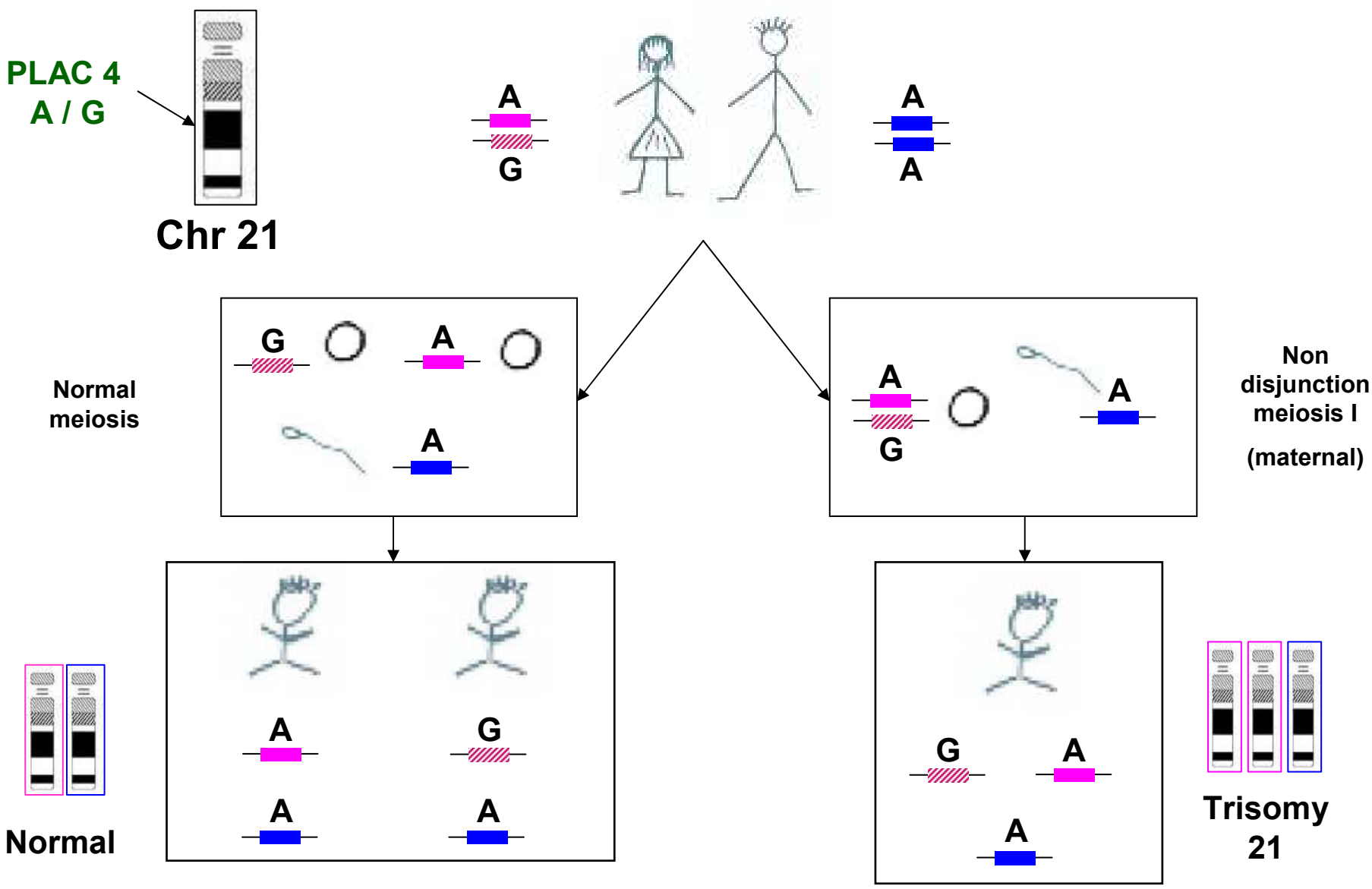


Quantitative analysis of Single Nucleotide Polymorphisms in fetal specific mRNAs

Quantitative analysis of SNPs in fetal specific mRNA



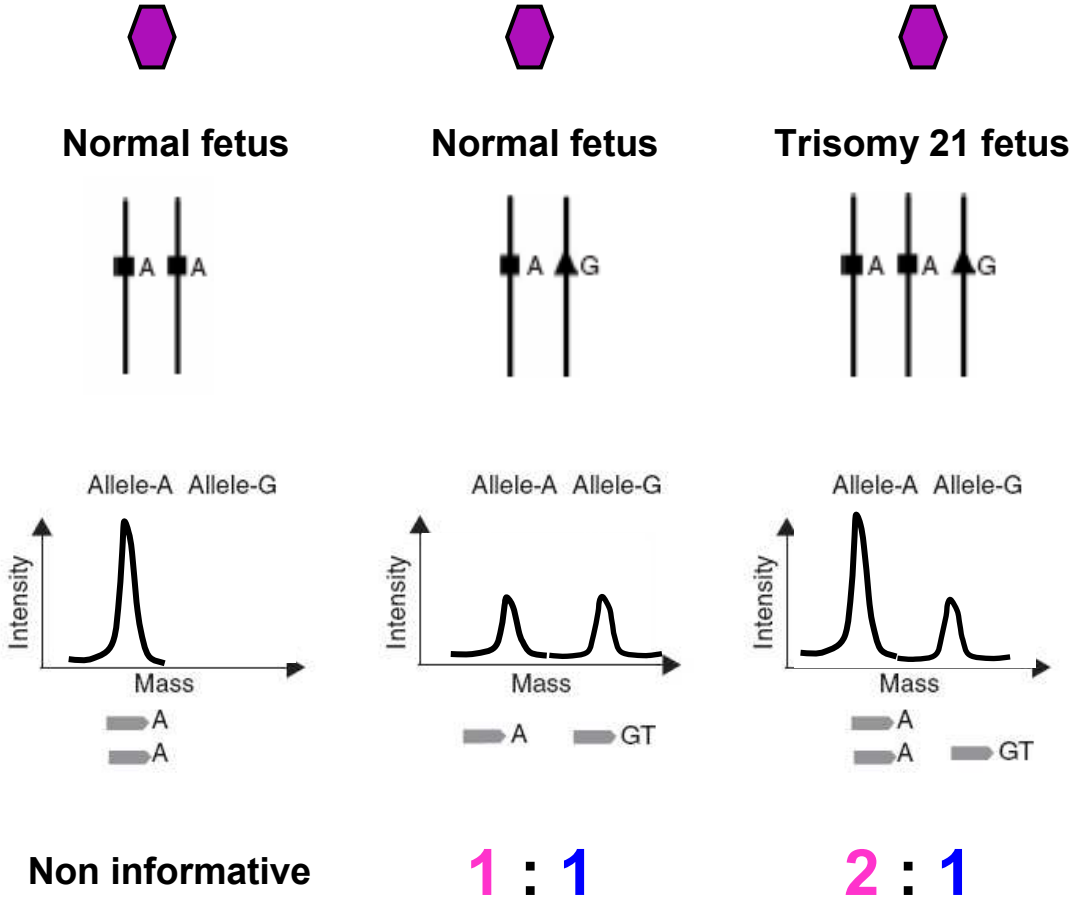
Quantitative analysis of SNPs in fetal specific mRNA



Quantitative analysis of SNPs in fetal specific mRNA

Analysis by MALDI-TOF (mass spectrometry)

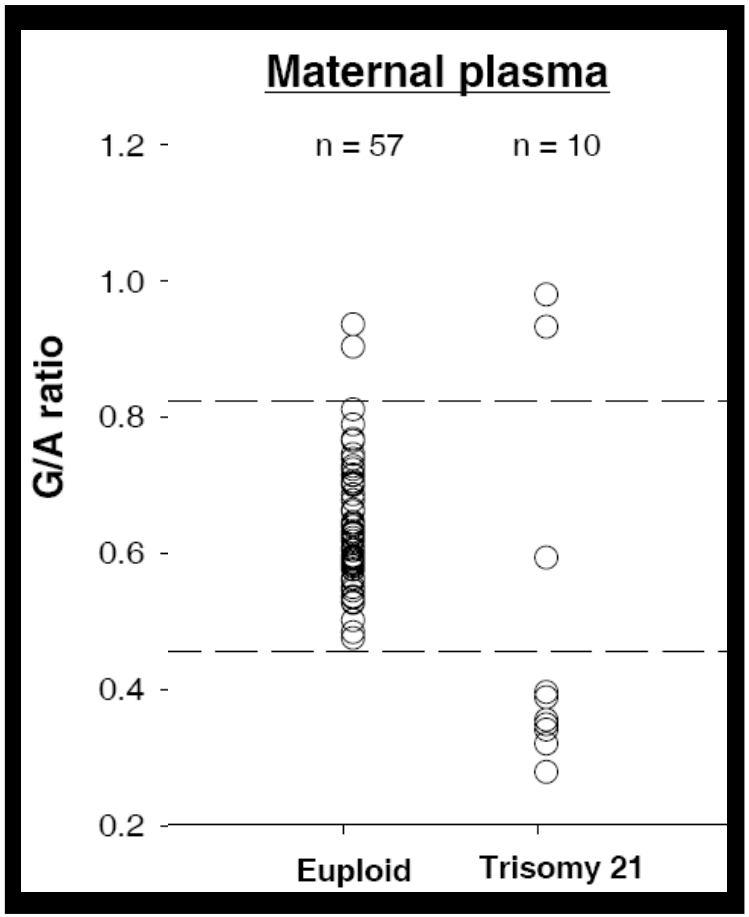
- PLAC4 mRNA (⬢) is derived exclusively from fetal chromosome 21
- PLAC4 mRNA expressed in the placenta and is found in the plasma of pregnant women



Lo et al., Nature Med & PNAS, 2007

Quantitative analysis of SNPs in fetal specific mRNA

Analysis by MALDI-TOF (mass spectrometry)



- Correctly diagnosed fetal trisomy 21 in **90%** of +21 cases (n=10)
- Excluded diagnosis of trisomy 21 in **96.5%** of normal controls (n=57)

- Sensitivity: 90%
- Specificity: 96.5%

Lo et al., Nature Med & PNAS, 2007

Quantitative analysis of SNPs in fetal specific mRNA

- **ADVANTAGES**

  • Diagnostic sensitivity and specificity using one marker are comparable to multiple marker screening tests for DS currently in practice (if informative)

  • Test is insensitive to gestational age and can be offered early in pregnancy

  • Target free of maternal background

  • Compatible with high throughput screening

- **DISADVANTAGES**

  • Fetus has to be informative for SNP analysed

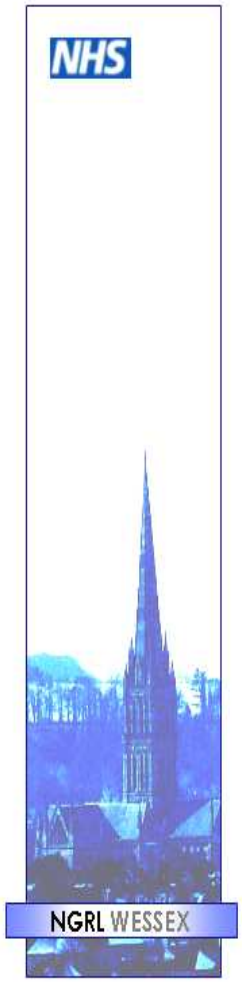
  • RNA can be unstable – implications for sample collection

- **FUTURE REQUIREMENTS**

  • Identification of more polymorphic loci to increase informative cases

  • Multi centre large scale validation required

  • Expand testing to include fetal specific transcripts from chromosomes 18 & 13

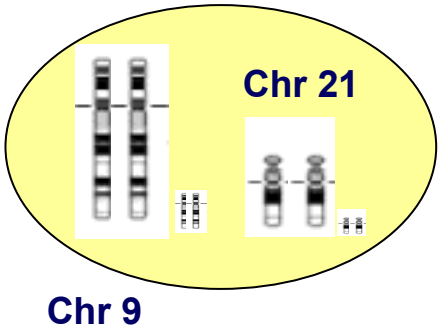


Shot gun sequencing

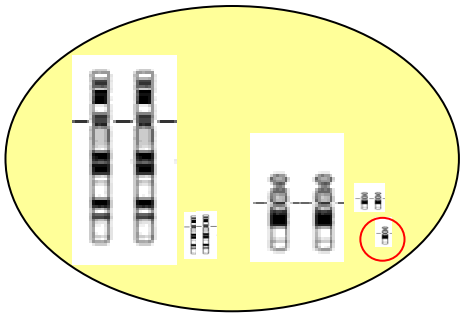
A method for digital quantification of DNA

DNA testing preferable: is universal i.e. polymorphism independent

- Only 3-6% of the cell - free DNA fraction is fetal
- Expected enrichment of chromosome 21 lies within the range of 1.5% to 3%



$$\frac{\text{Total amount of Chr 21 (0.94 + 0.06)}}{\text{Total amount of Chr 9 (0.94 + 0.06)}} = 1$$



$$\frac{\text{Total amount of Chr 21 (0.94 + 0.09)}}{\text{Total amount of Chr 9 (0.94 + 0.06)}} = 1.03$$

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*§

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

- Shotgun sequenced plasma DNA samples from 18 pregnant women:

  9 trisomy 21

  2 trisomy 18

  1 trisomy 13

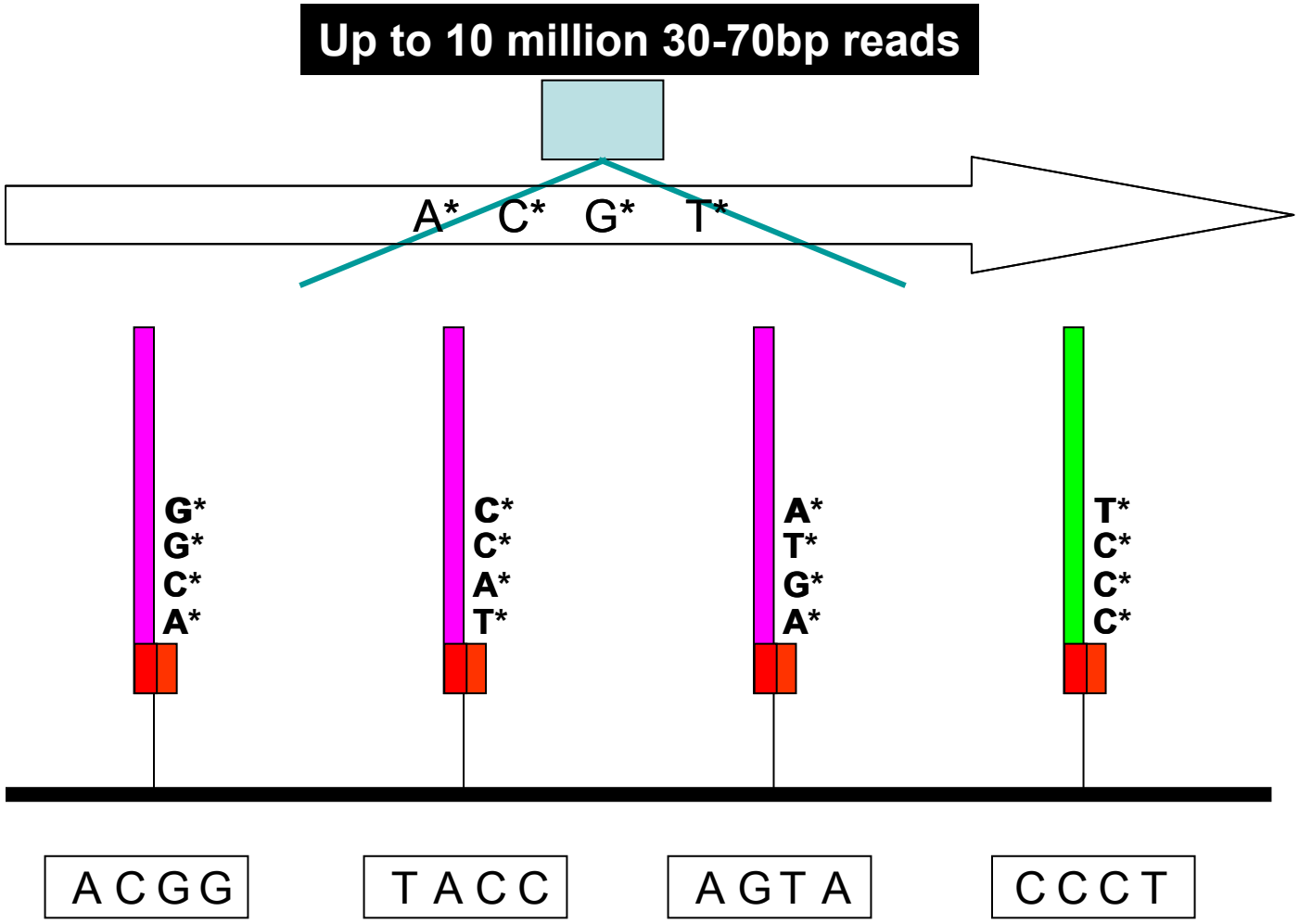
  6 normal

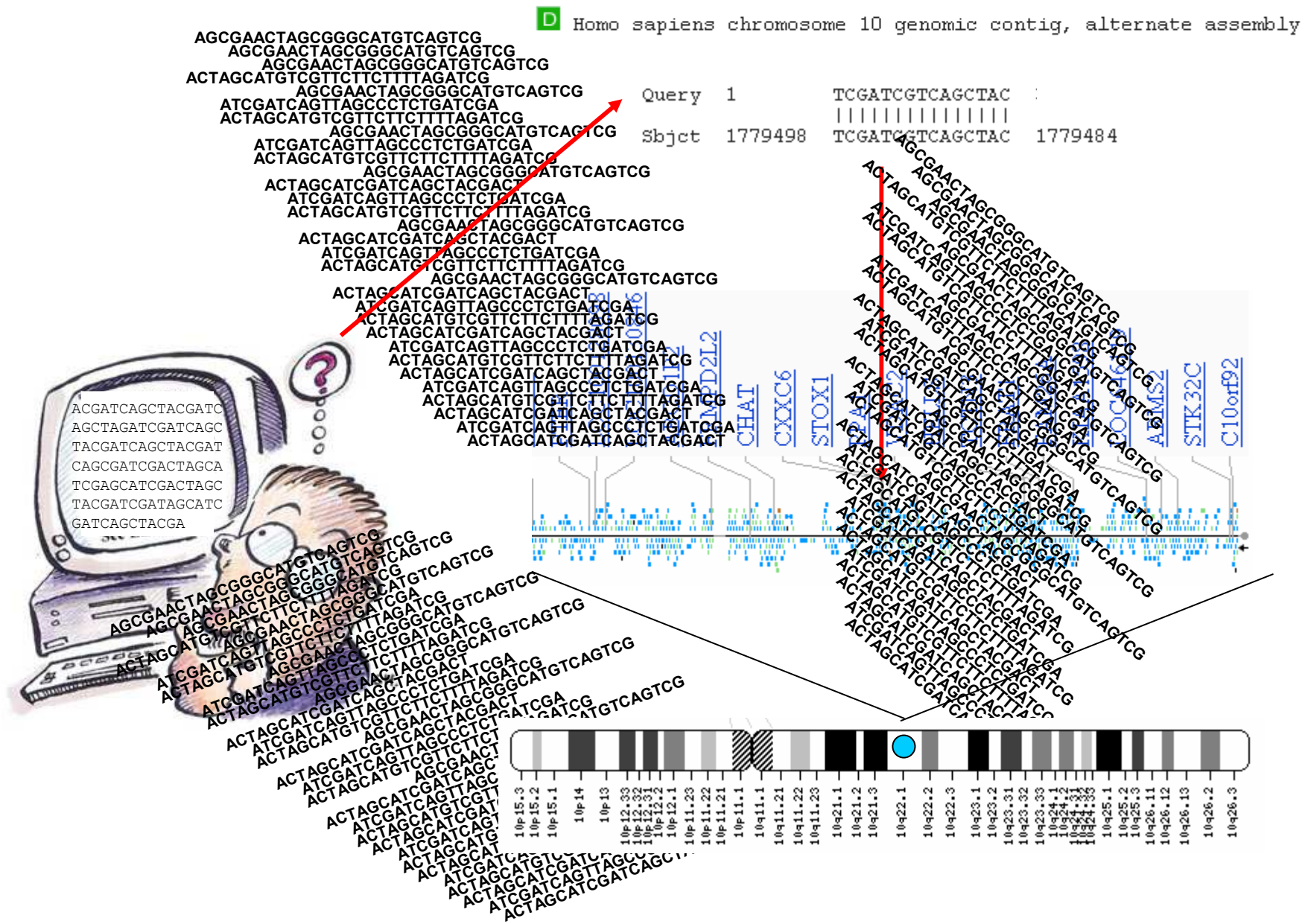
  1 genomic DNA sample from a male control

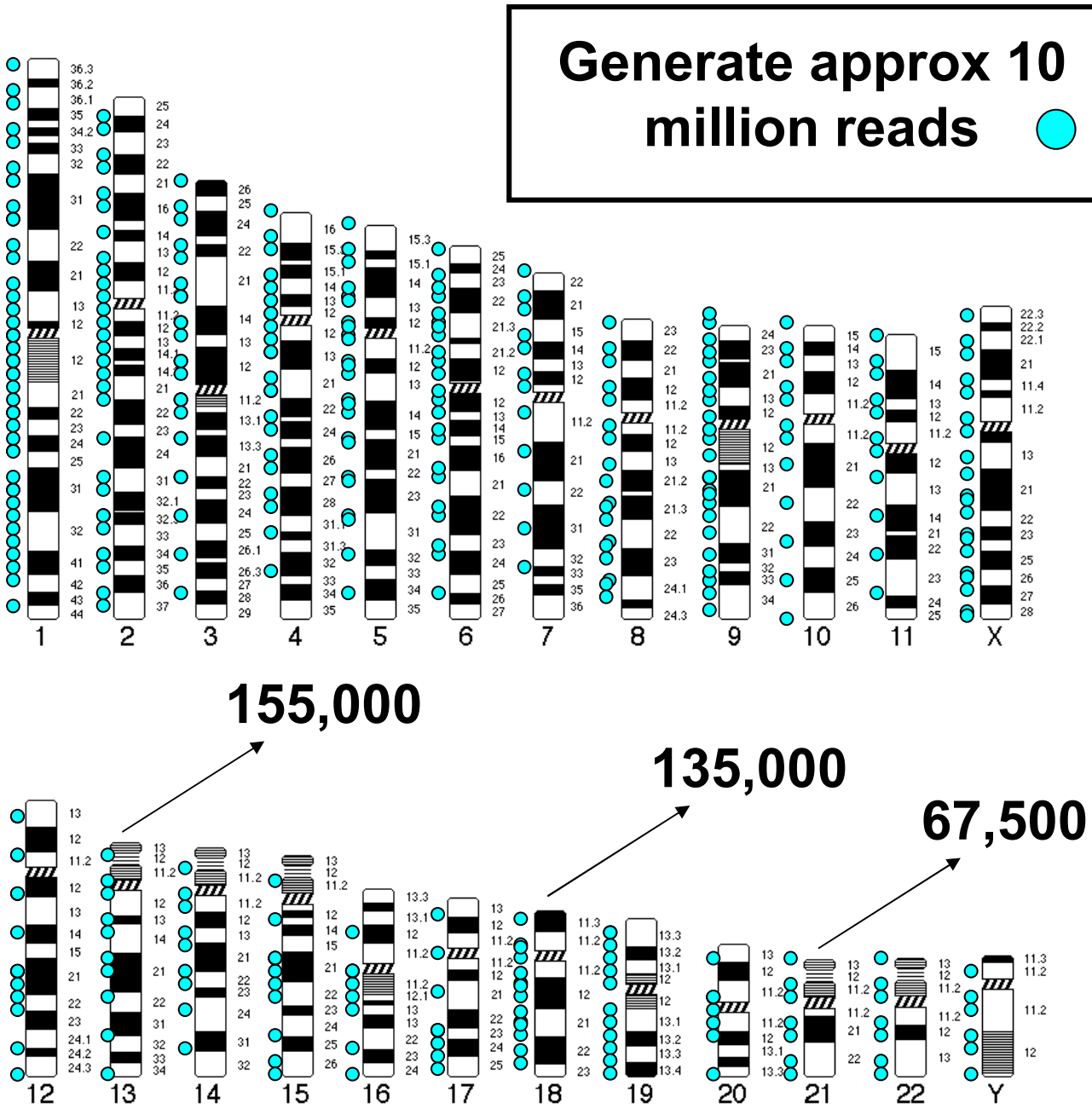
- Gestational age 10 – 35 weeks (earliest trisomy case 14 weeks)

Fan et al., PNAS Oct 2008

Sequencing by Synthesis




Homo sapiens chromosome 10 genomic contig, alternate assembly
Query 1 TCGATCGTCAGCTAC
Sbjct 1779498 TCGATCGTCAGCTAC 1779484
CHAT
CXXC6
STOX1
AAMS2
STK32C
C10orf92
ACGATCAGCTACGATC
AGCTAGATCGATCAGC
TACGATCAGCTACGAT
CAGCGATCGACTAGCA
TCGAGCATCGACTAGC
TACGATCGATAGCATC
GATCAGCTACGA


Generate approx 10 million reads
155,000
135,000
67,500
1
2
3
4
5
6
7
8
9
10
11
X
12
13
14
15
16
17
18
19
20
21
22
Y

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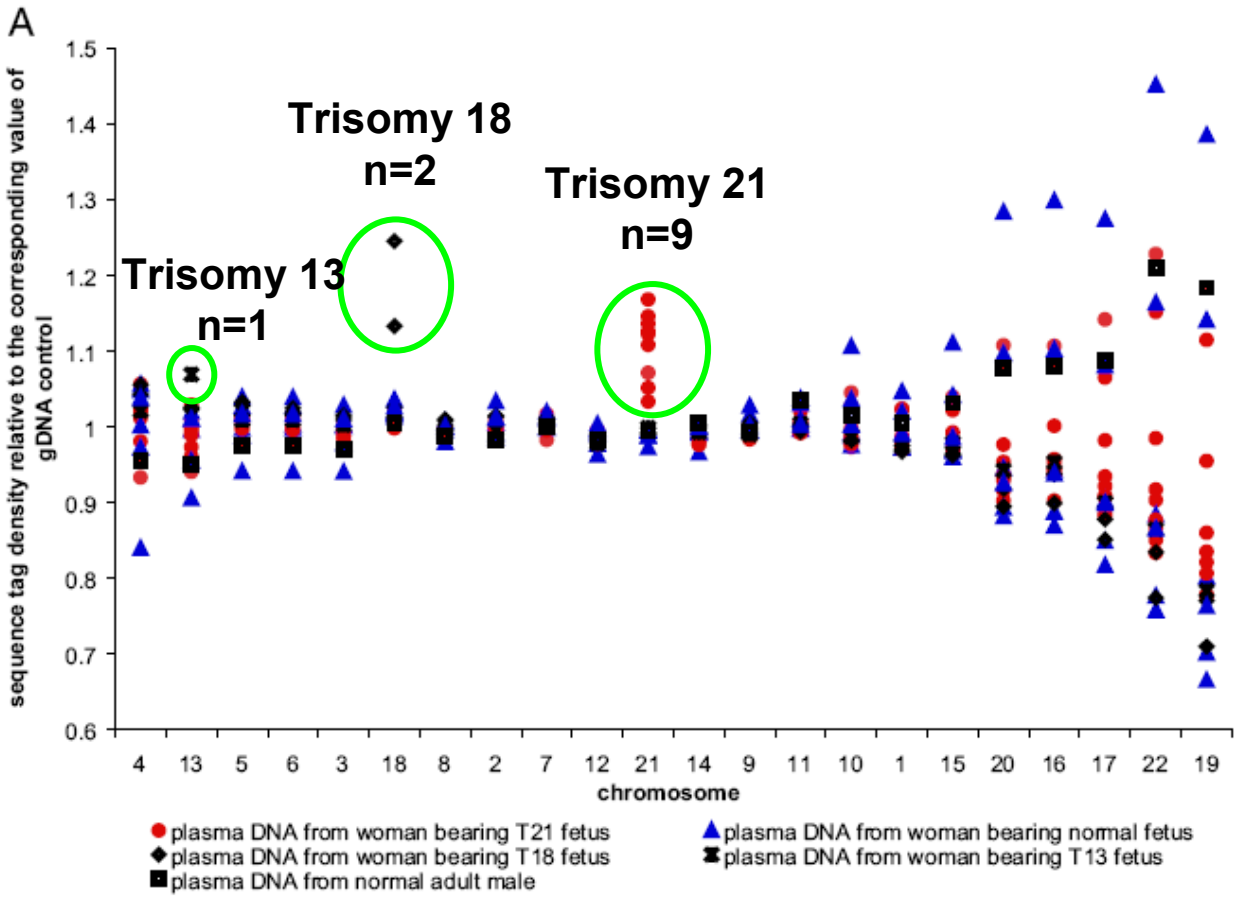
- 5 million sequencing reads for each patient
- Compared density of reads on each chromosome to those obtained from a normal genomic DNA sample
- Compared density of Chr 21 reads from disomy and trisomy 21 samples
- Coverage of Chr 21 sequences in trisomy 21 was 4 – 18% higher than disomic cases

Results of shotgun sequencing of maternal plasma DNA



Fan et al., PNAS Oct 2008

Shot gun sequencing

- **ADVANTAGES**
  - Successful proof of principal study for detection of major trisomies; 13, 18 & 21
  - Polymorphism independent and could be used in all pregnancies
  - Has potential to detect unbalanced chromosome rearrangements

- **DISADVANTAGES**
  - Expensive and large amount of data processing – interpretation.
  - In current form would not be feasible to adapt to high throughput screening

- **FUTURE REQUIREMENTS**
  - Technological development required to produce machines and workflow protocols that could cope with a high throughput of samples at reduced cost

RAPID: Plans to develop NIPD for aneuploidy

- Matched maternal blood samples and cultured fetal cells are currently being collected from women undergoing invasive prenatal testing in London and the Wessex region
- Assessment of analytical and clinical validity of tests for the non-invasive detection of DS (and trisomy 13 & 18):
  - SNP allele ratios using MALDI-TOF mass spectrometry
  - digital PCR
  - development of targetted new generation sequencing assays
- cffDNA and cffRNA will be used to evaluate NIPD tests and cultured fetal cells will be used to confirm the NIPD result
- Sensitivity and specificity of each assay will be determined

Reliable Accurate Prenatal non-Invasive Diagnosis
RAPID