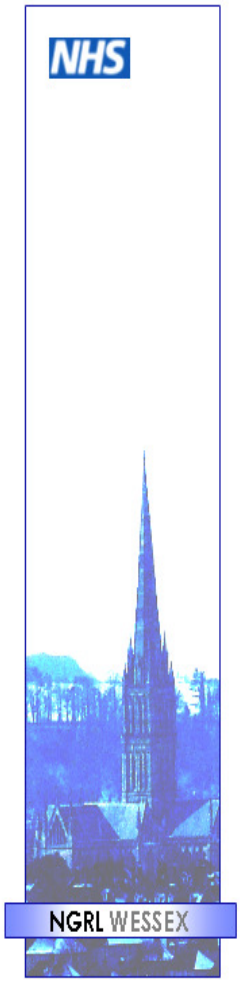
Salisbury NHS
NHS Foundation Trust



Developing NIPD for aneuploidy

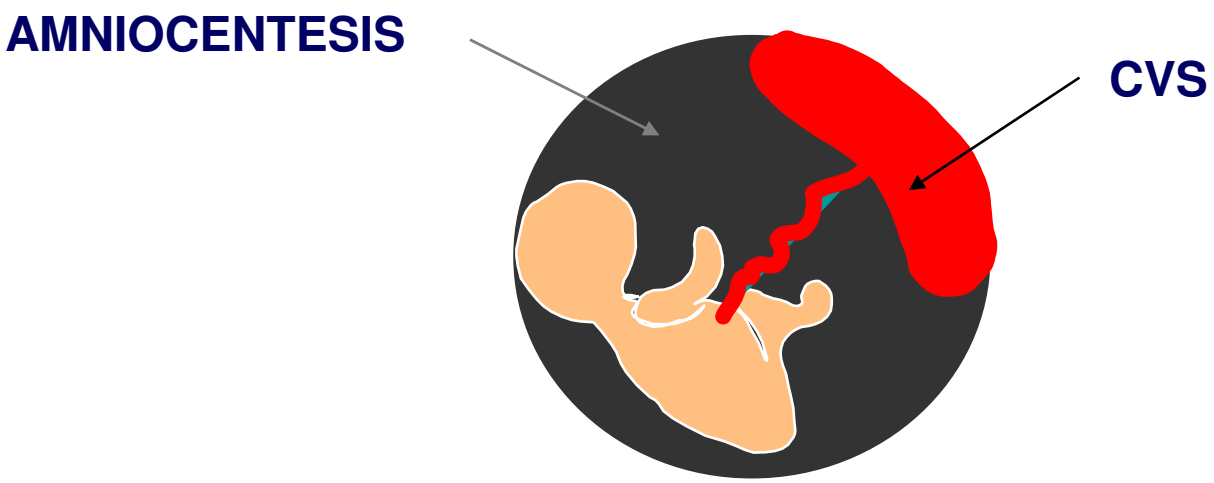
Helen White, PhD

Senior Scientist

National Genetics Reference Lab (Wessex)

DH Department of Health

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:

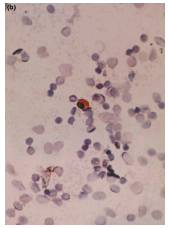
~700,000 pregnant women a year underwent antenatal screening

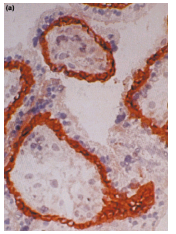
20,000 amniocentesis and 5,200 CVS were performed

Estimated associated procedural related pregnancy loss of ~250

Other sources of fetal tissue for non-invasive prenatal diagnosis

Fetal cells in maternal circulation





erythroblasts
trophoblastic cells
leucocytes

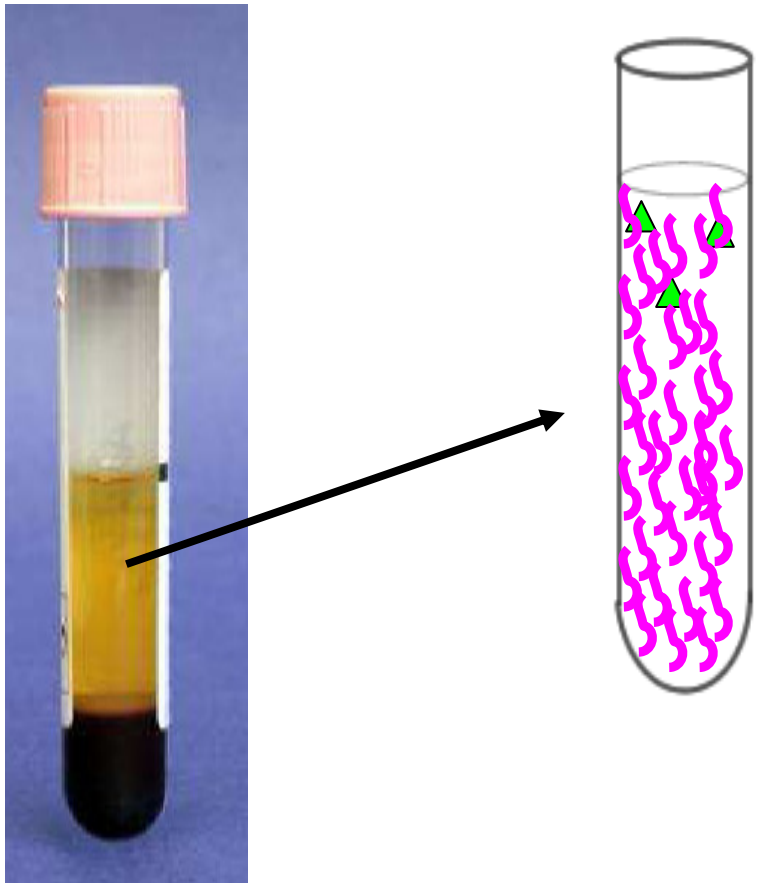
Difficult to isolate and persist for years after pregnancy

Cell free fetal nucleic acid in the maternal circulation

Originates from trophoblast and detectable from 5 weeks' gestation

Both DNA and RNA cleared from circulation within 30 minutes of delivery

Extraction of cell free fetal nucleic acids from maternal plasma



Cell free maternal DNA (96.6%)

Cell free fetal DNA (3.4%)

Amount of cf fetal DNA extracted is equivalent to 25 genomes / ml plasma

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 extremely accurate copy number ‘counting’

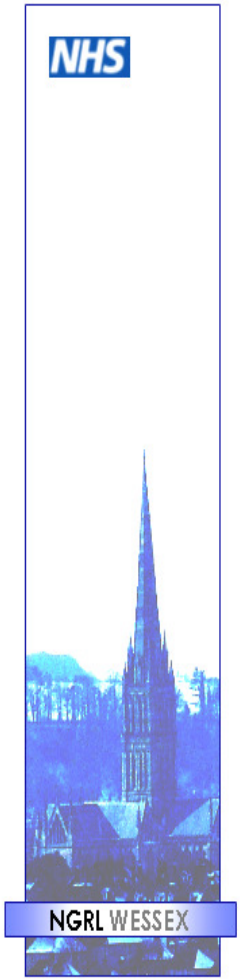
- **Recent major breakthroughs**

  (Quantitative analysis of Single Nucleotide Polymorphisms in **fetal specific** mRNAs)

  Digital PCR of cfRNA and cfDNA

  Massively parallel sequencing of cfDNA

  Epigenetic analysis

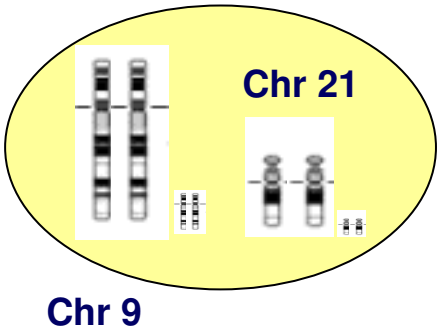


Massively parallel sequencing

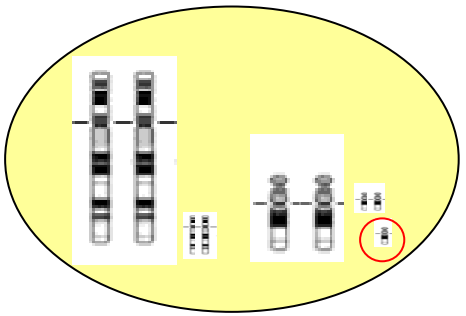
Digital quantification of DNA

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*§

*Department of Bioengineering, Stanford University and Howard Hughes Medical Institute, 318 Campus Drive, Clark Center, Room E300, Stanford, CA 94305; †Division of Maternal and 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)

- Shotgun sequenced plasma DNA samples from 18 women:

  9 trisomy 21

  2 trisomy 18

  1 trisomy 13

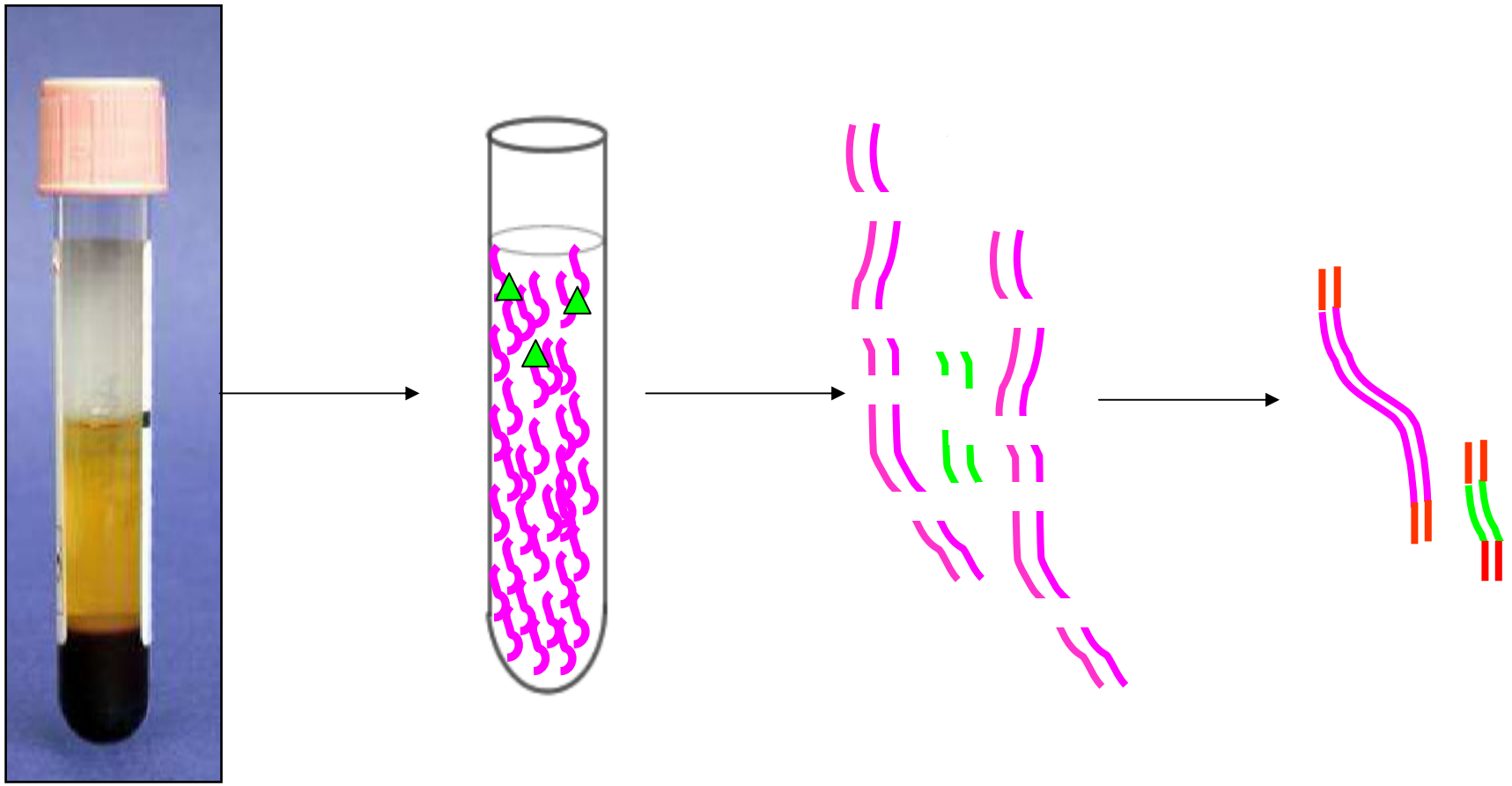
  6 normal

  and 1 genomic DNA sample from a male control

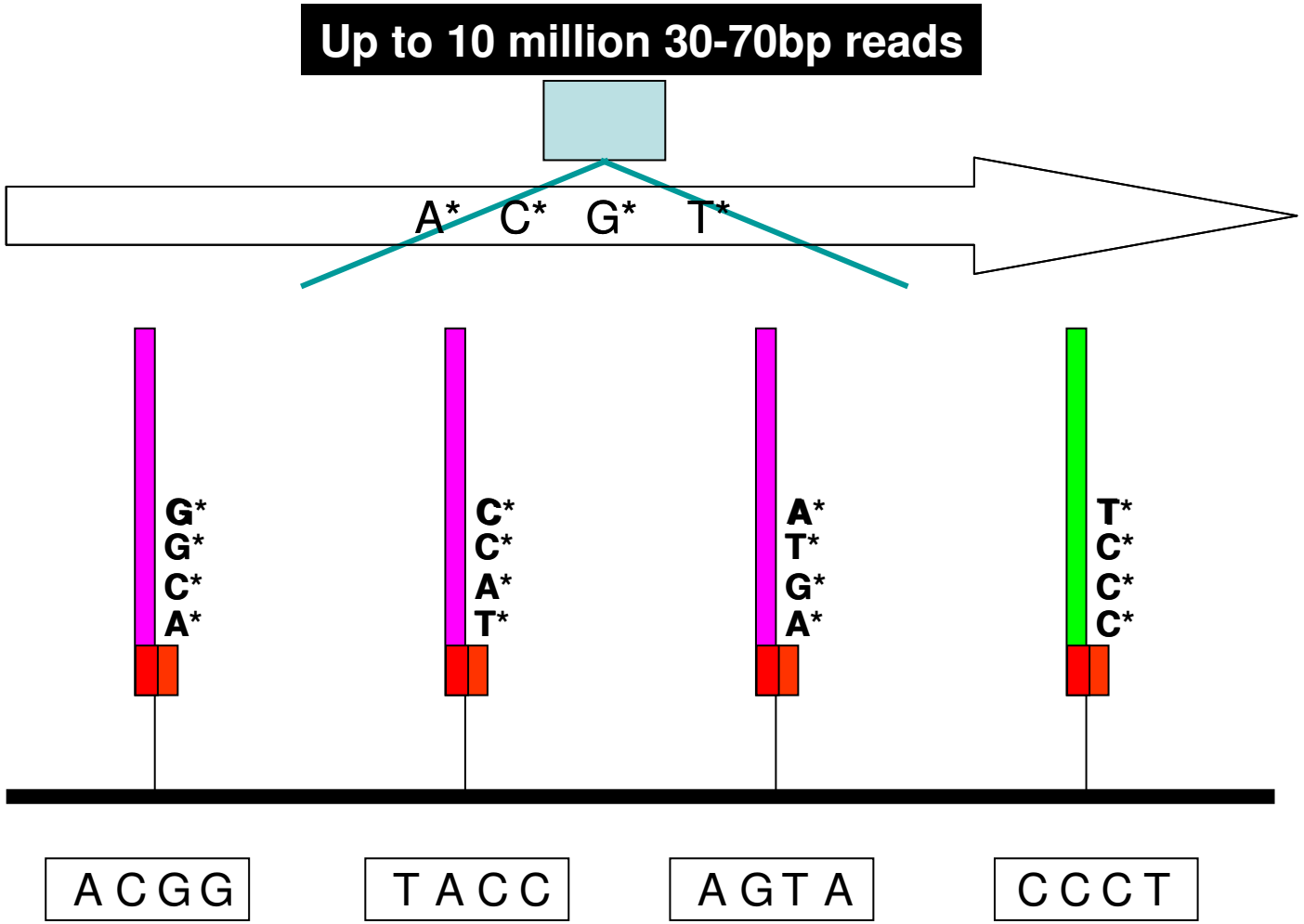
- Gestational age 10 – 35 weeks (earliest trisomy case 14 weeks)
- 5 million sequencing reads for each patient
- Compared density of reads on each chromosome to those obtained from a normal genomic DNA sample
- Also compared density of Chr 21 reads from disomy and trisomy 21 samples
- Coverage of Chr 21 sequences in trisomy 21 was 4 – 18% higher than disomic cases

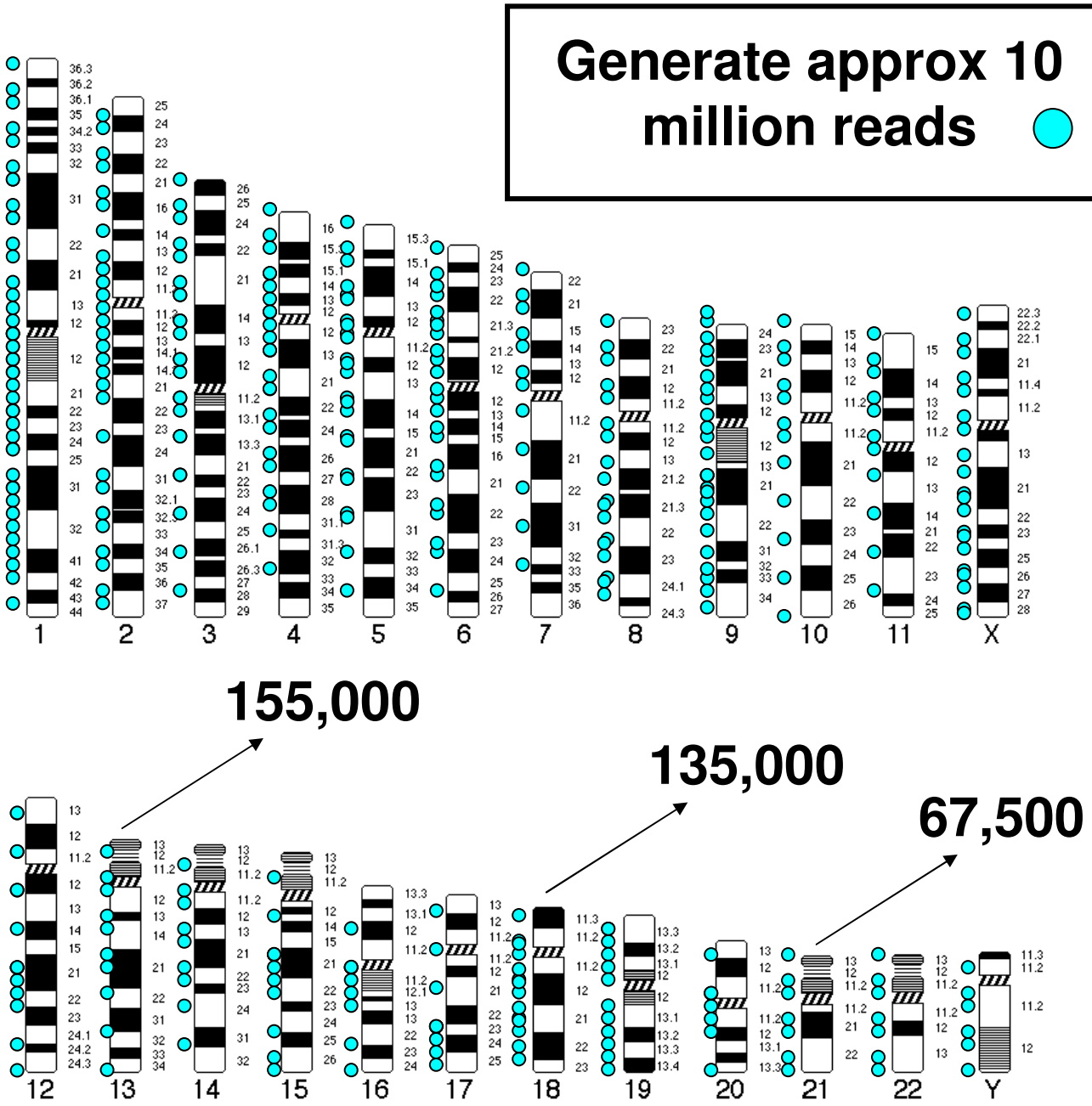
Fan et al., PNAS Oct 2008

Shot gun sequencing

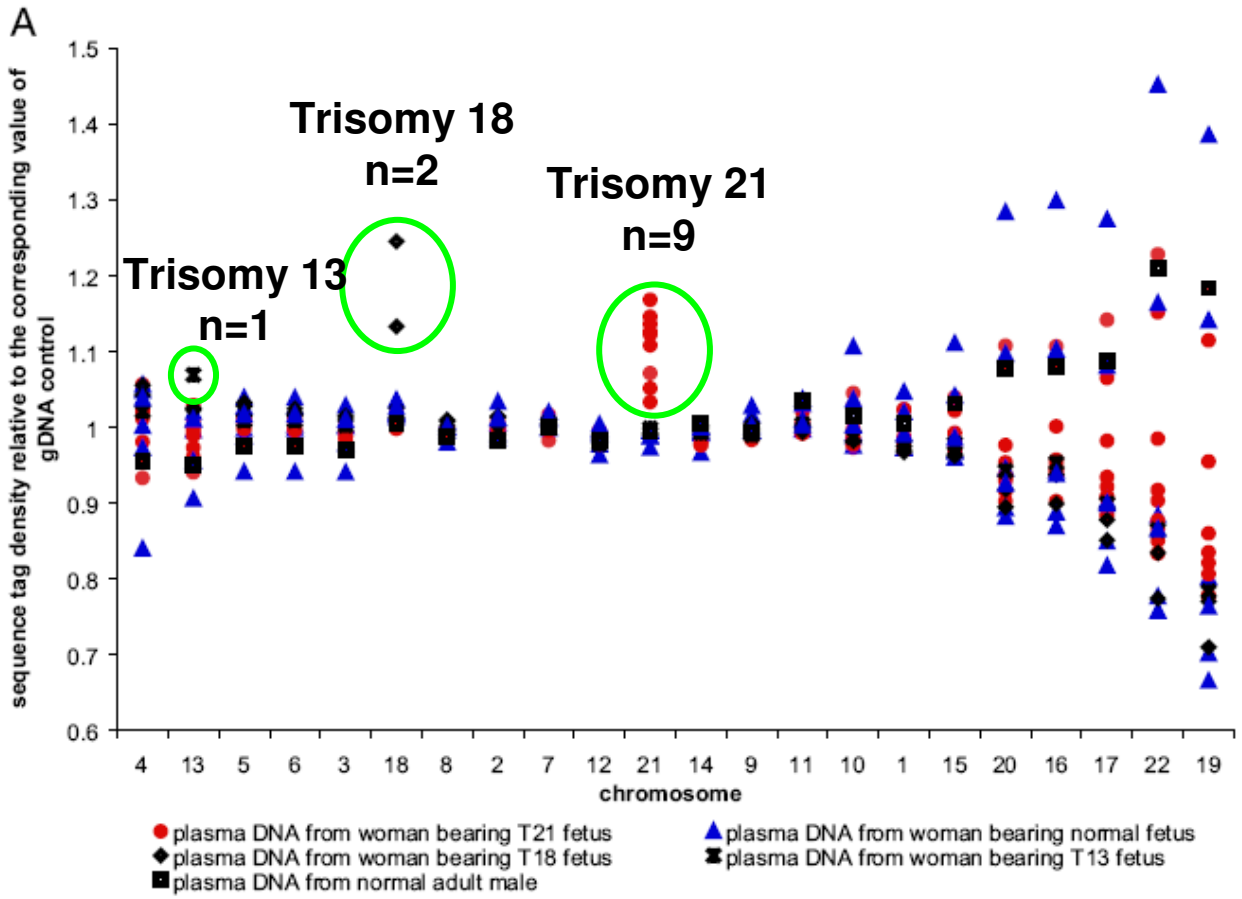


Sequencing by Synthesis




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

Results of shotgun sequencing of maternal plasma DNA



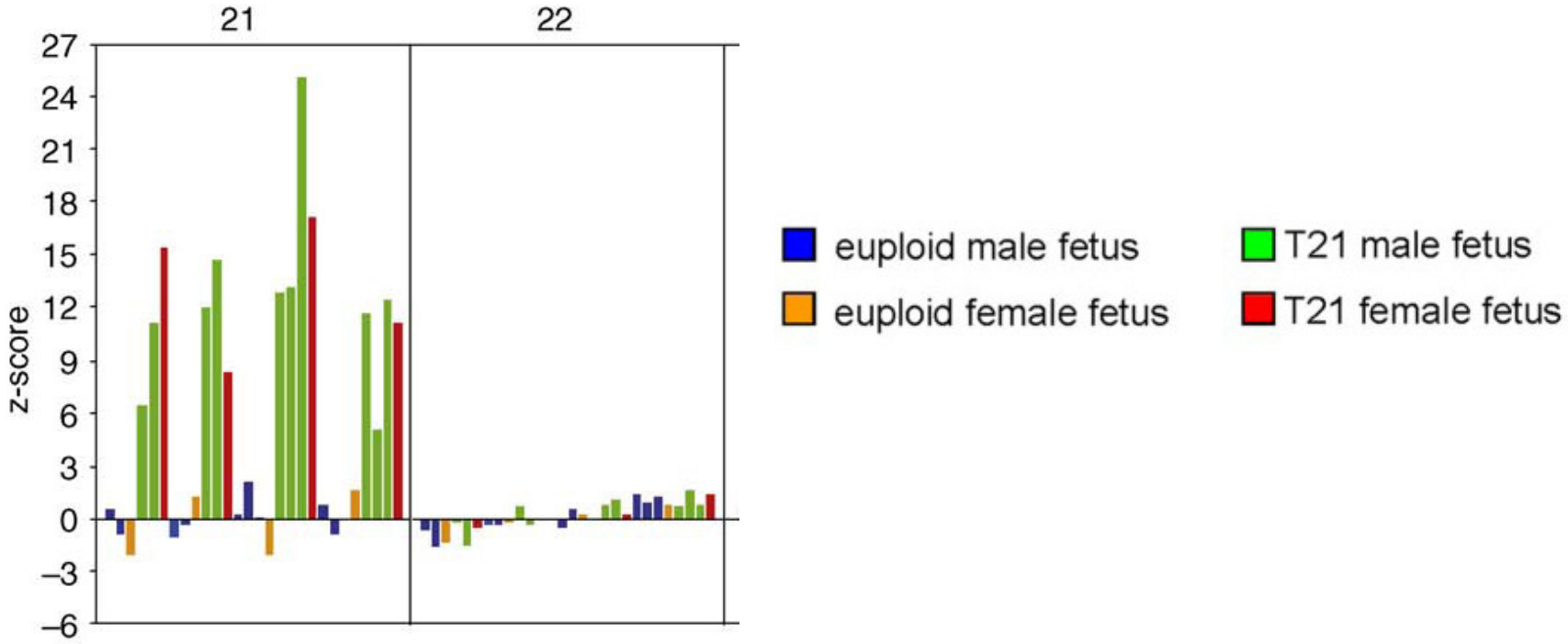
Fan et al., PNAS Oct 2008

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

Sequenced maternal plasma: 14 trisomy 21 and 14 normal cases correctly identified



Chiu, PNAS, Dec 2008

Shot gun sequencing

- **ADVANTAGES**
  - Successful proof of principal study for detection of major trisomies; 13, 18 and 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

RAPID: Role of NGRL (Wessex)

- Develop NIPD for DS testing in collaboration with ICH / GOSH using

  - targeted new generation sequencing
  - (MALDI-TOF mass spectrometry)
  - digital PCR

- Define Down Syndrome (DS) test analytical sensitivity and specificity

- Develop prototype reference materials in collaboration with NIBSC & NGRL (M)

- Produce standardised protocols in collaboration with GOSH & NGRL (M)

- Participate in a model-based economic evaluation to assess incremental cost-effectiveness of NIPD versus current testing methodology

Targeted new generation sequencing assays

- Combination of next generation sequencing and relative chromosome dosage analysis

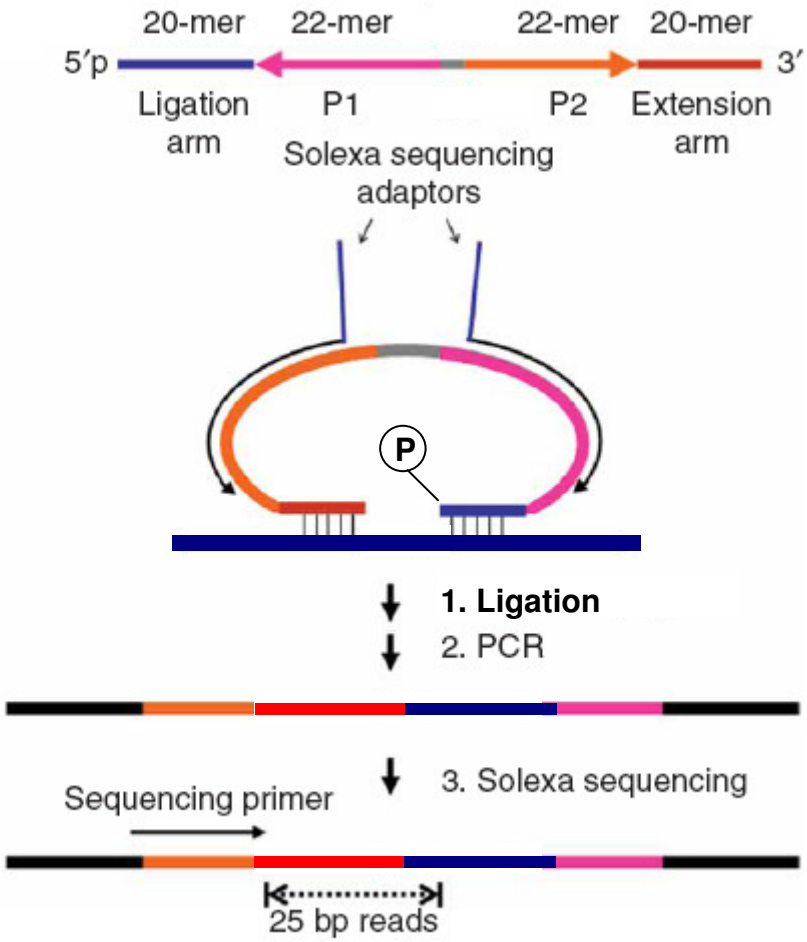
- 'Trapping' specific sequences on chromosomes 21, 18 and 13 and comparing against sequences on other autosomes (multiplexed)

- Analyse data by comparing copy numbers of sequences on 21, 18 and 13 with those from autosomes

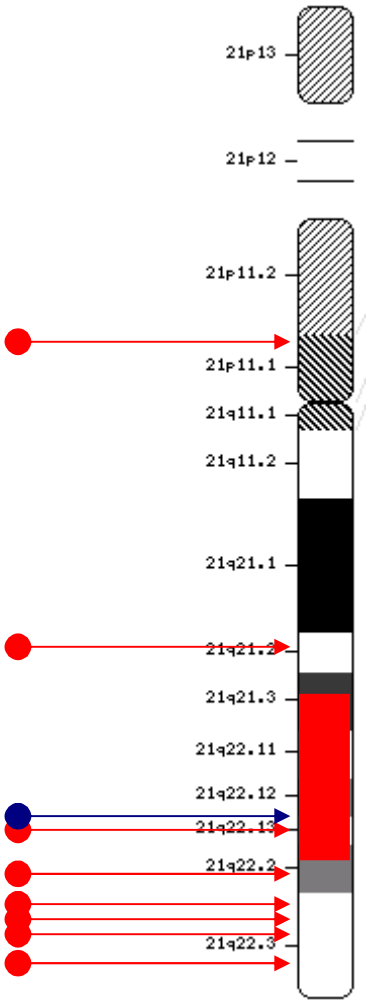
- Developing several strategies: 'MLPA' and padlock probes

- Investigating different data analysis methodologies

Padlock probes: trapping sequences



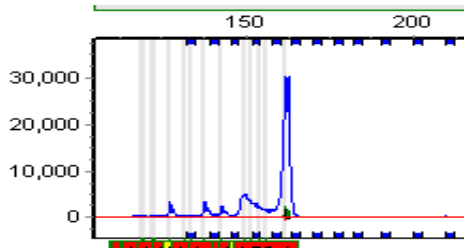
Genomic locations of chromosome 21 padlock probes



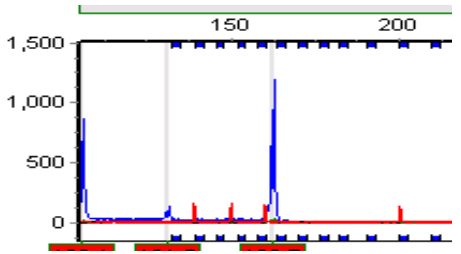
Down's critical region

Ligated Probe: 165bp

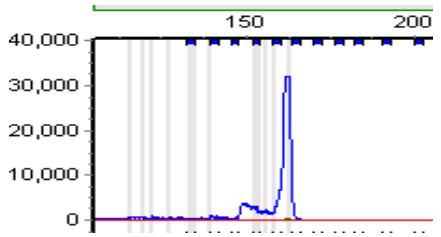
Male
Genomic DNA



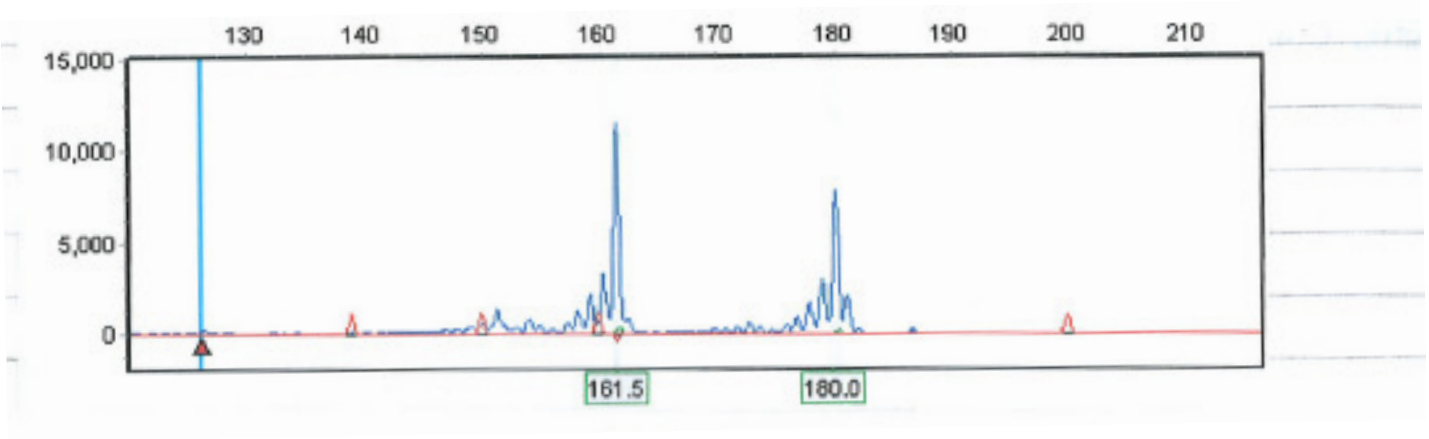
Female
Circulating DNA



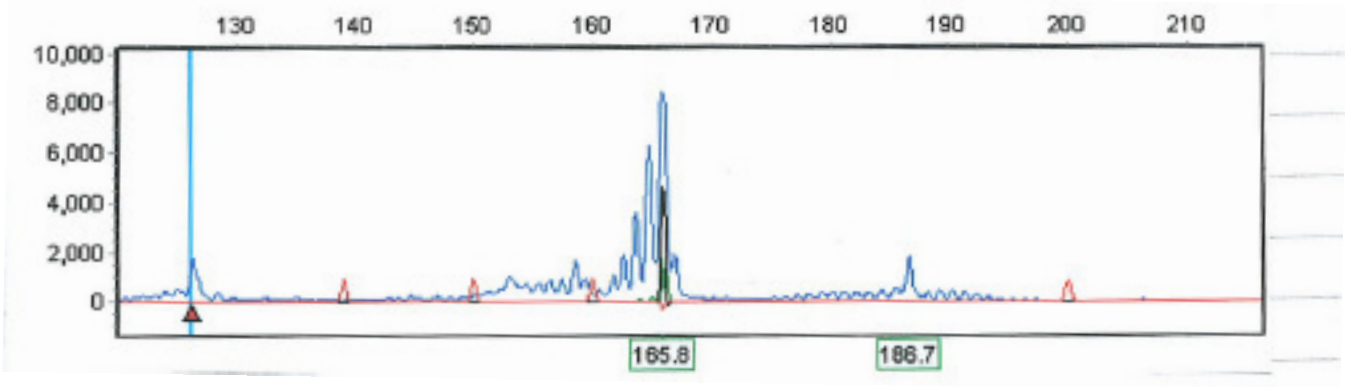
Male
CirculatingDNA



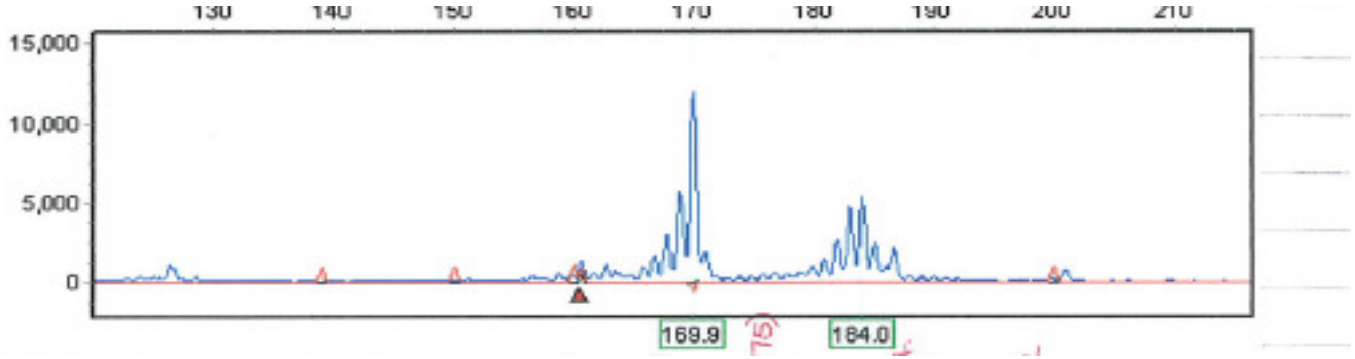
Chr 21



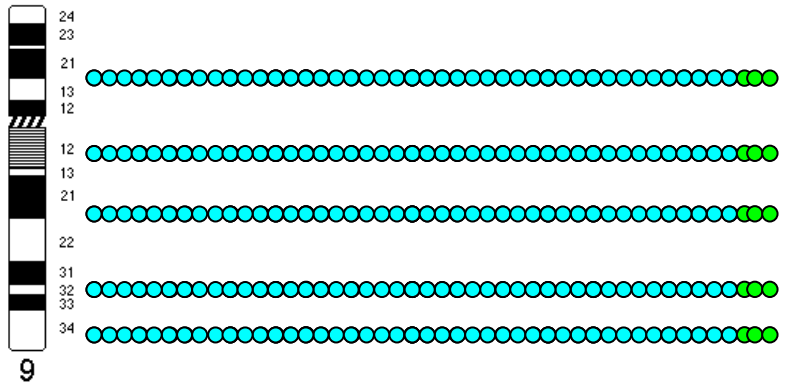
Chr 18

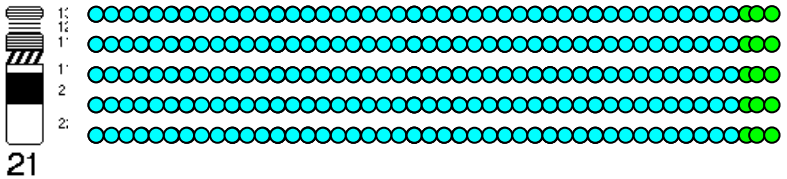


Chr 13

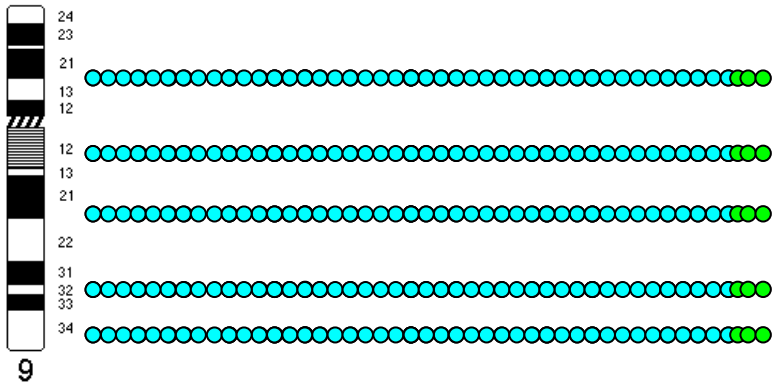


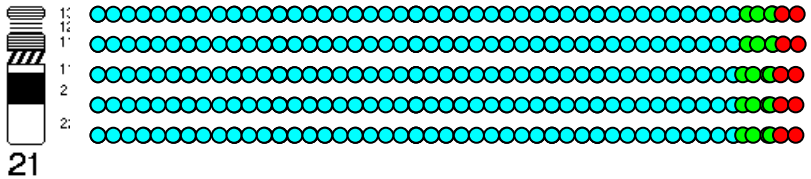
Mother and diploid fetus





Mother and trisomy 21 fetus





Illumina Genome Analyser

- 8 lanes
- Approx 10 million mappable reads per lane
- 80 million mappable reads per run
- ID tags allow 12 patients per lane
- 96 patient samples per run
- Consumable cost per run c. £7 - 10K
- Cost per patient c. £100

HiSeq 2000 now released - 10X higher capacity

Targeted next generation sequencing

- **ADVANTAGES**

  - Polymorphism independent and could be used in all pregnancies
  - Has potential to be expanded to cover microdeletion / duplications, other loci in targeted fashion
  - Data analysis simplified and cost reduced
  - Adaptable to high throughput analysis

- **DISADVANTAGES**

  - Proof of principle required
  - Need to know more about free fetal DNA composition

- **FUTURE REQUIREMENTS**

  - Large scale validation required

Summary

- New technologies need to be validated for analytical and clinical validity in large UK patient cohorts
- The limits of gestation for testing using all techniques need to be determined
- Need for standardised protocols and control materials
- Potential to replace current DS screening tests with a diagnostic test
- Unlikely to replace invasive testing / current screening for some time
- Important to ensure that women and healthcare professionals understand the changes and women fully understand the implications of these tests

Acknowledgements

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Prof Lyn Chitty

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www.ngrl.org.uk/Wessex

www.rapid.nhs.uk