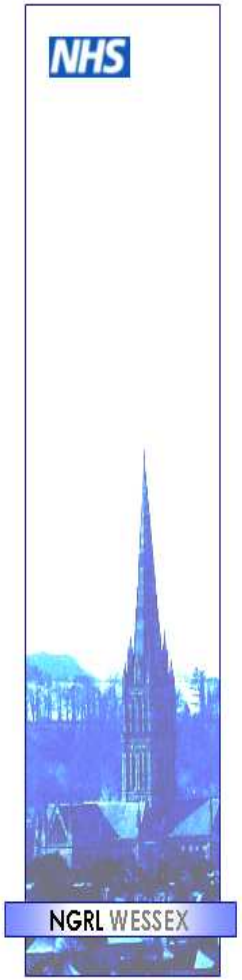
Salisbury NHS
NHS Foundation Trust



Non-invasive prenatal detection of Down syndrome

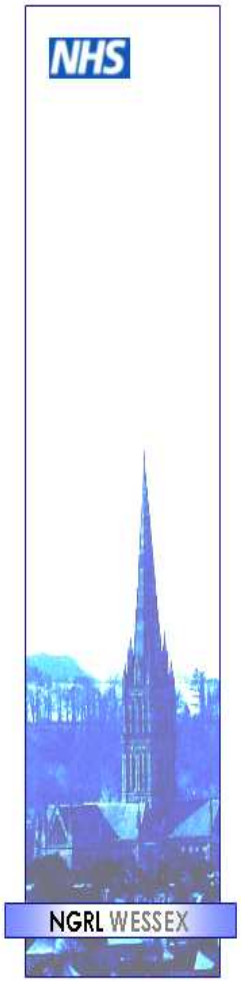
Helen White, PhD

Senior Scientist

National Genetics Reference Lab (Wessex)

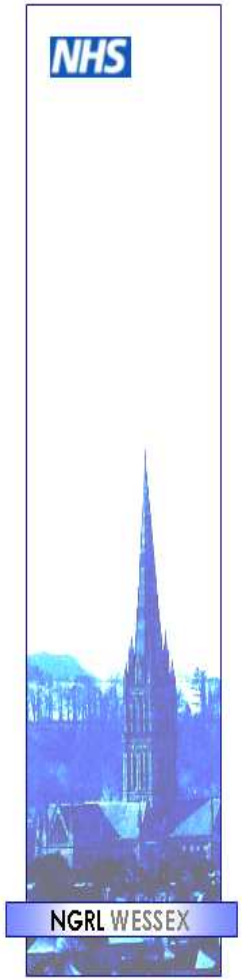
DH Department of Health

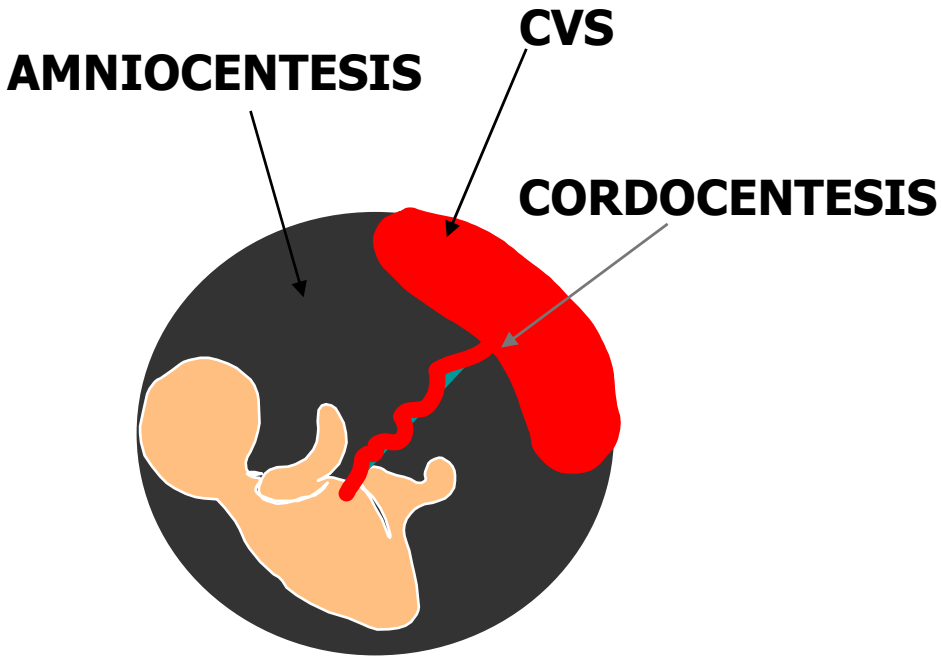
Outline of talk



- Current practice for prenatal DS diagnosis
- Cell free fetal nucleic acids in blood of pregnant women
  - o What are they?
  - o How can they be used for non invasive DS testing?
  - o The future..

Current prenatal diagnosis requires invasive procedures





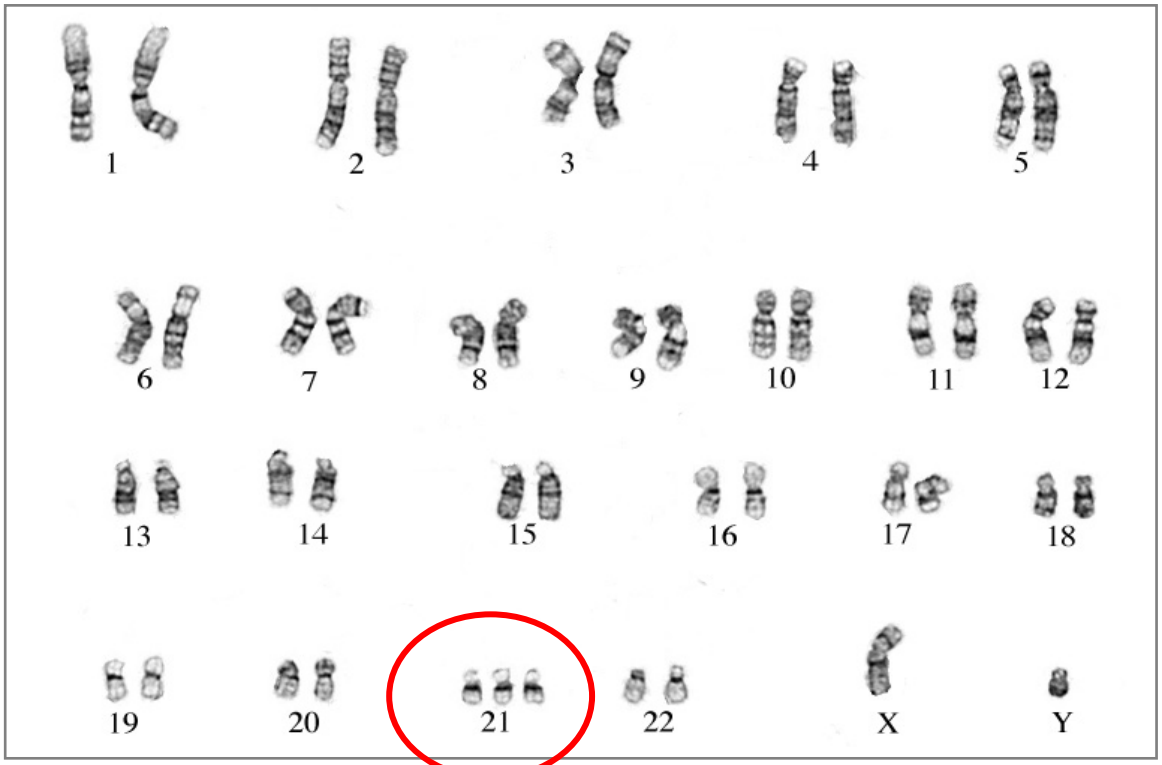
1% risk of miscarriage

Not possible before 11 weeks' gestation

Current prenatal diagnosis requires invasive procedures

Cells are cultured from amniotic fluid or chorionic villus samples

Gold standard test is chromosome analysis by karyotyping

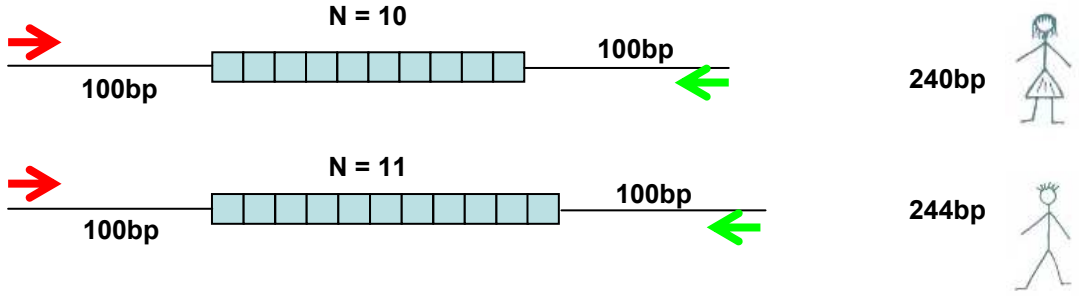


Reporting times:	CVS	7.9 days WRGL	(UK Average 14.8 days)
	AF	7.2 days WRGL	(UK Average 13.5 days)

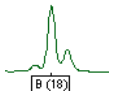
Current prenatal diagnosis requires invasive procedures

Cells are cultured from amniotic fluid or chorionic villus samples

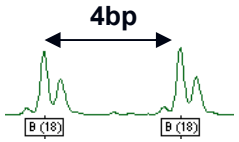
DNA extracted an analysed by QF-PCR: reporting time 24 - 72 hours



Uninformative

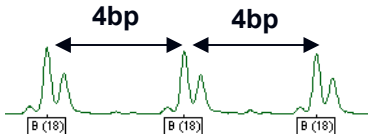


Normal (two alleles)



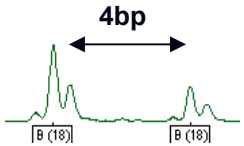
1 : 1

Trisomy (three alleles)



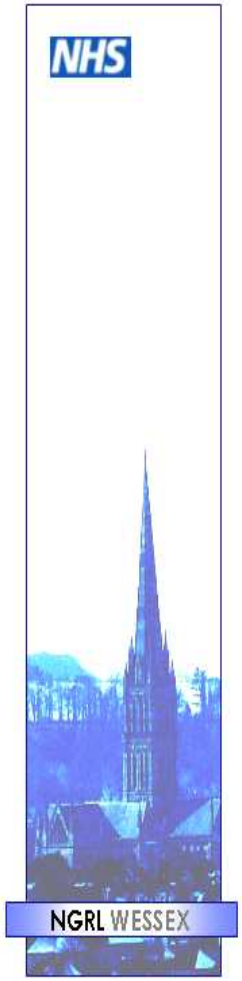
1 : 1 : 1

Trisomy (alleles in 2:1 ratio)

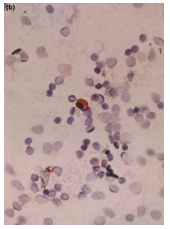


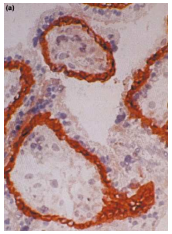
2 : 1

Other sources of fetal tissue for non-invasive prenatal diagnosis



Fetal cells in maternal circulation





erythroblasts

trophoblastic cells

leucocytes

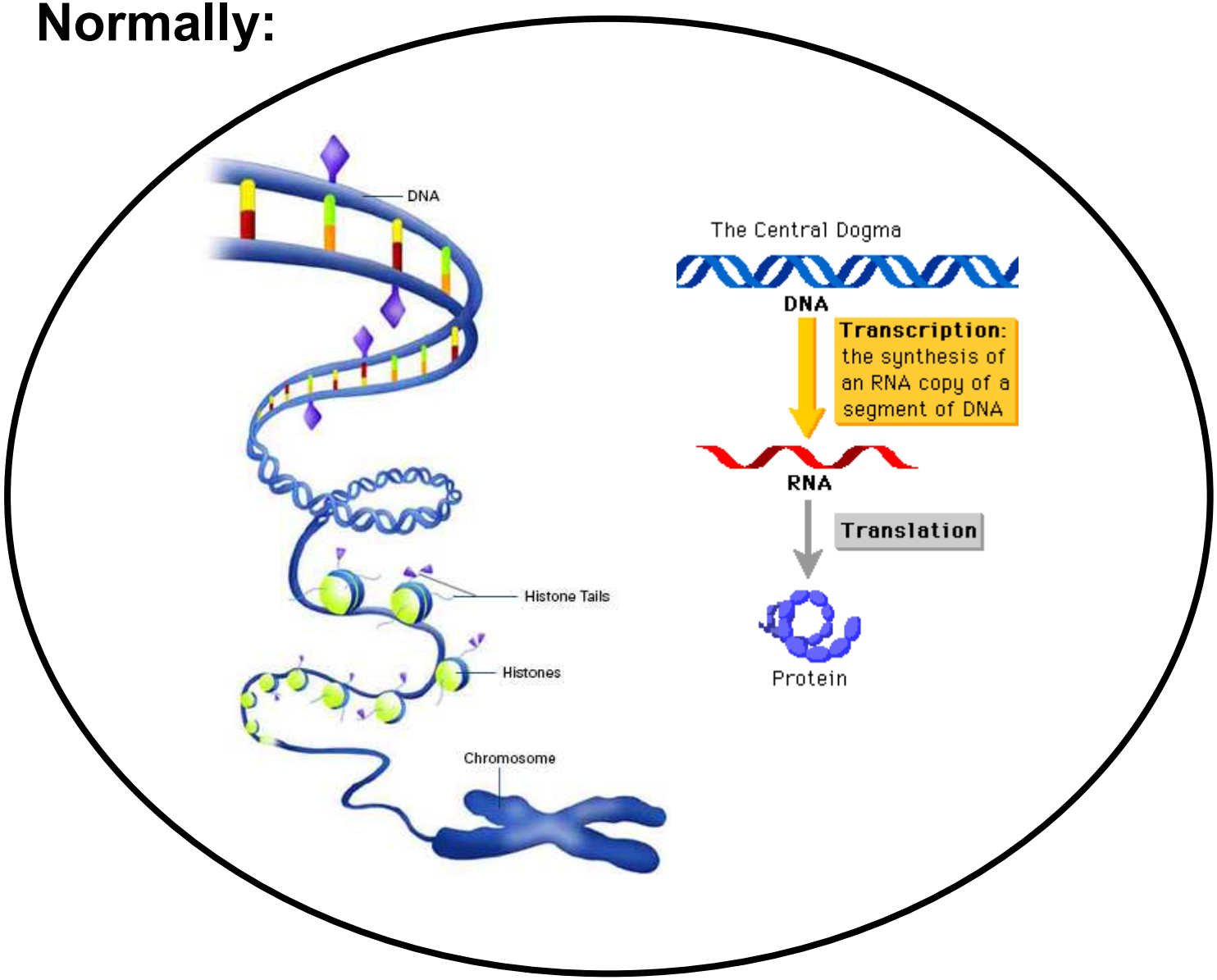
Difficult to isolate

Very low abundance

Persist for years after pregnancy

Cell free fetal nucleic acids from maternal plasma

Normally:

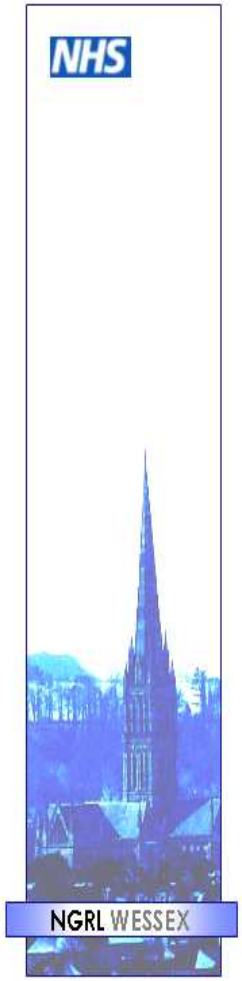


NHS

NGRL WESSEX

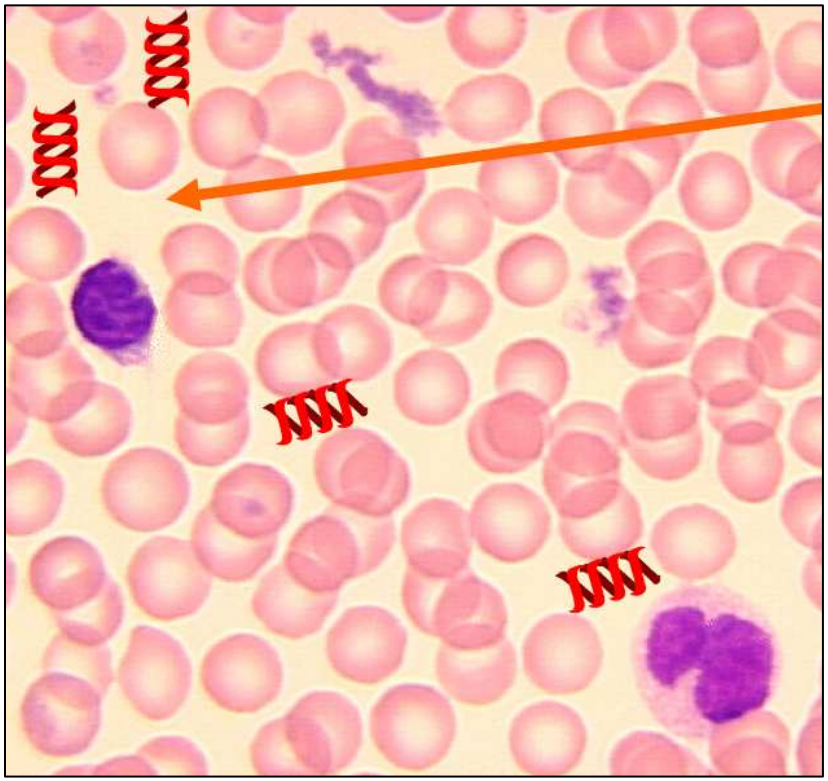
Cell free fetal nucleic acids from maternal plasma

But:



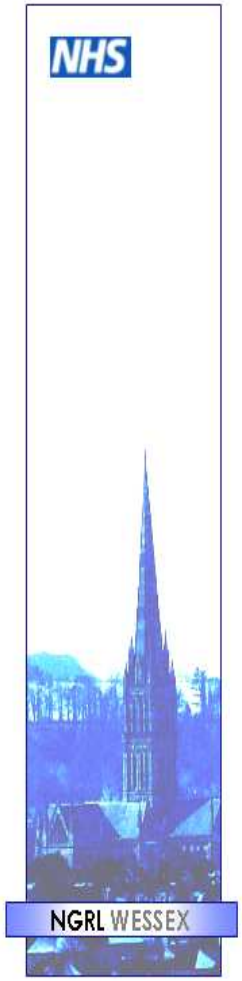
1977: Small quantities of free DNA observed in cancer patients

1997: Cell free DNA isolated from the plasma of pregnant women

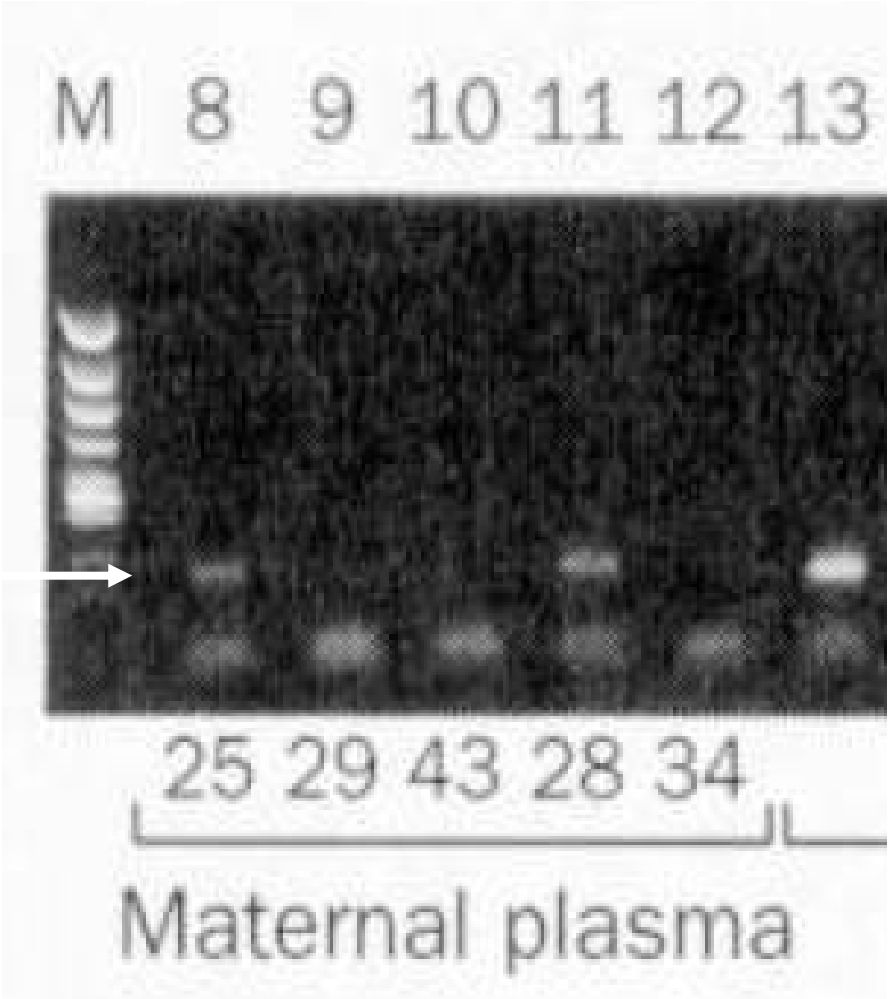


Plasma

Detection of male specific sequences in maternal plasma

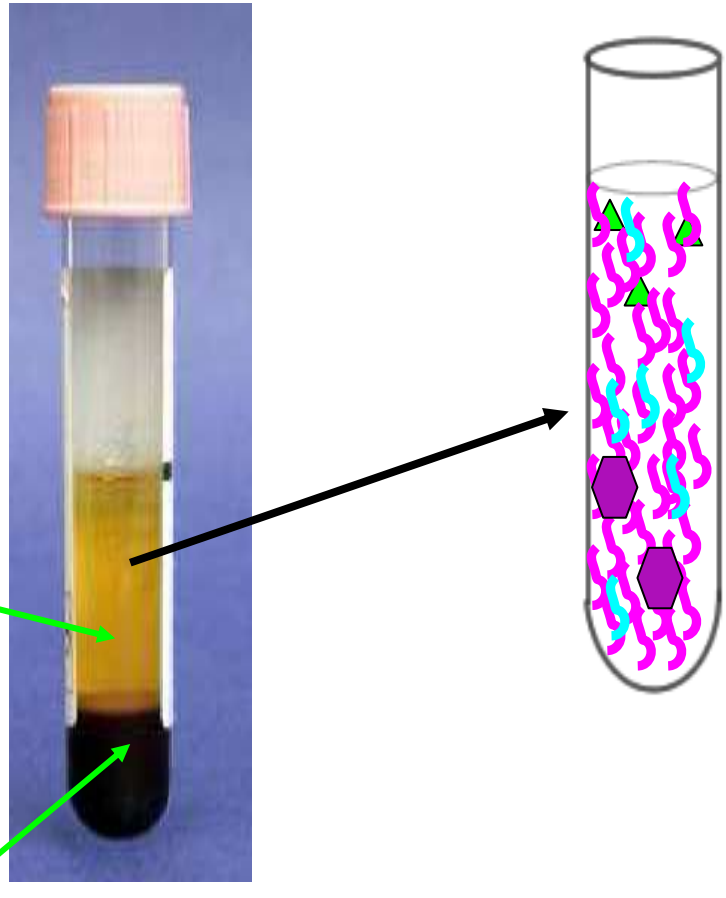


Y chromosome



Lo et al. *Lancet* 1997; 350:485

Extraction of cell free fetal nucleic acids from maternal plasma



Cell free maternal DNA (96.6%)

Cell free fetal DNA (3.4%)

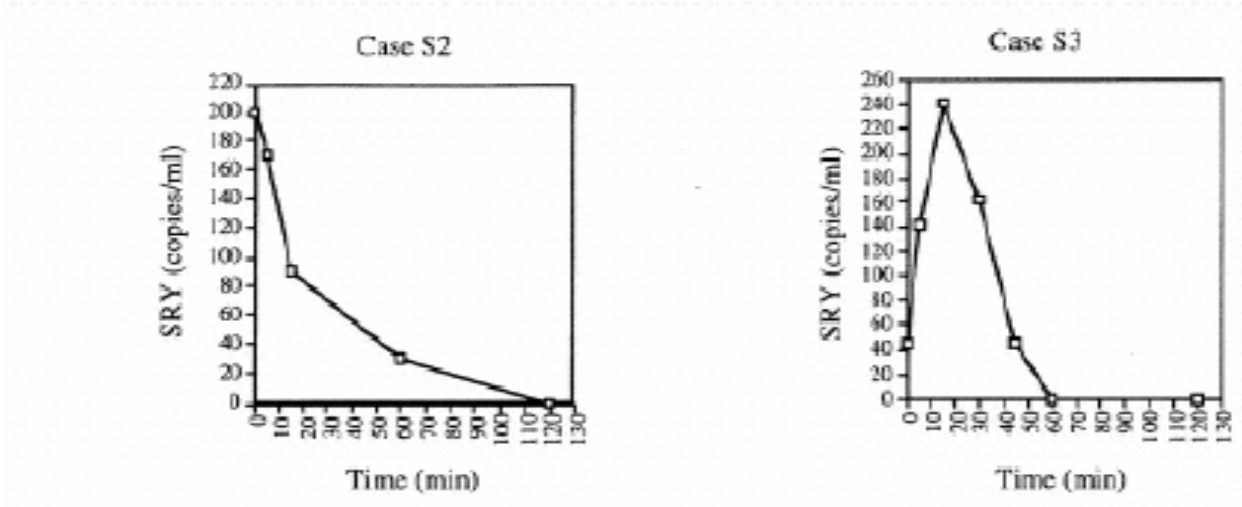
Amount of cell free fetal DNA extracted is equivalent to 25 cells / ml plasma

Cell free maternal RNA

Cell free fetal RNA

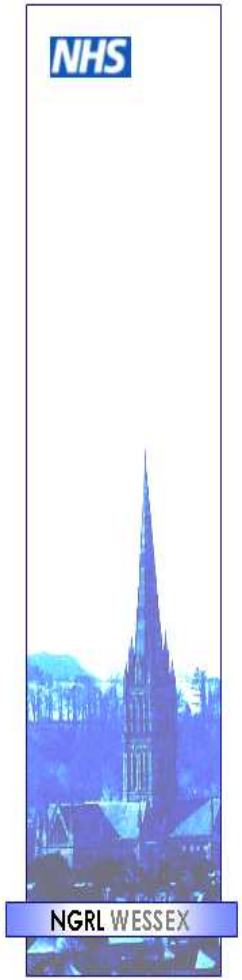
Clearance of cell free fetal nucleic acids after delivery

ffDNA



ffRNA

Summary: what are cell free nucleic acids



Cell free fetal DNA (cffDNA)

- cff DNA can be detected in plasma from pregnant women
- cff DNA only makes up about 5% of total cell free DNA extracted – most comes from the mother
- cff DNA derived from the placenta
- Can be detected from as early as 5 weeks gestation
- Rapidly cleared after delivery

Cell free fetal RNA (cffRNA)

- cff RNA can be detected in plasma from pregnant women
- cfRNA can be fetal specific, maternal specific or expressed in both the fetus and the mother
- Can be detected early in pregnancy
- Rapidly cleared after delivery

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

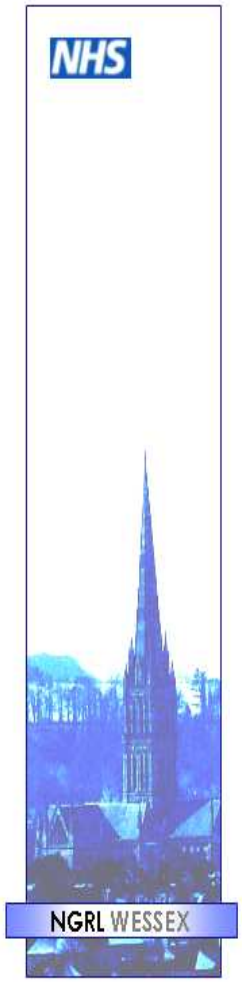
  and / or

  technologies that enable extremely accurate copy number ‘counting’

- **Recent major breakthroughs**

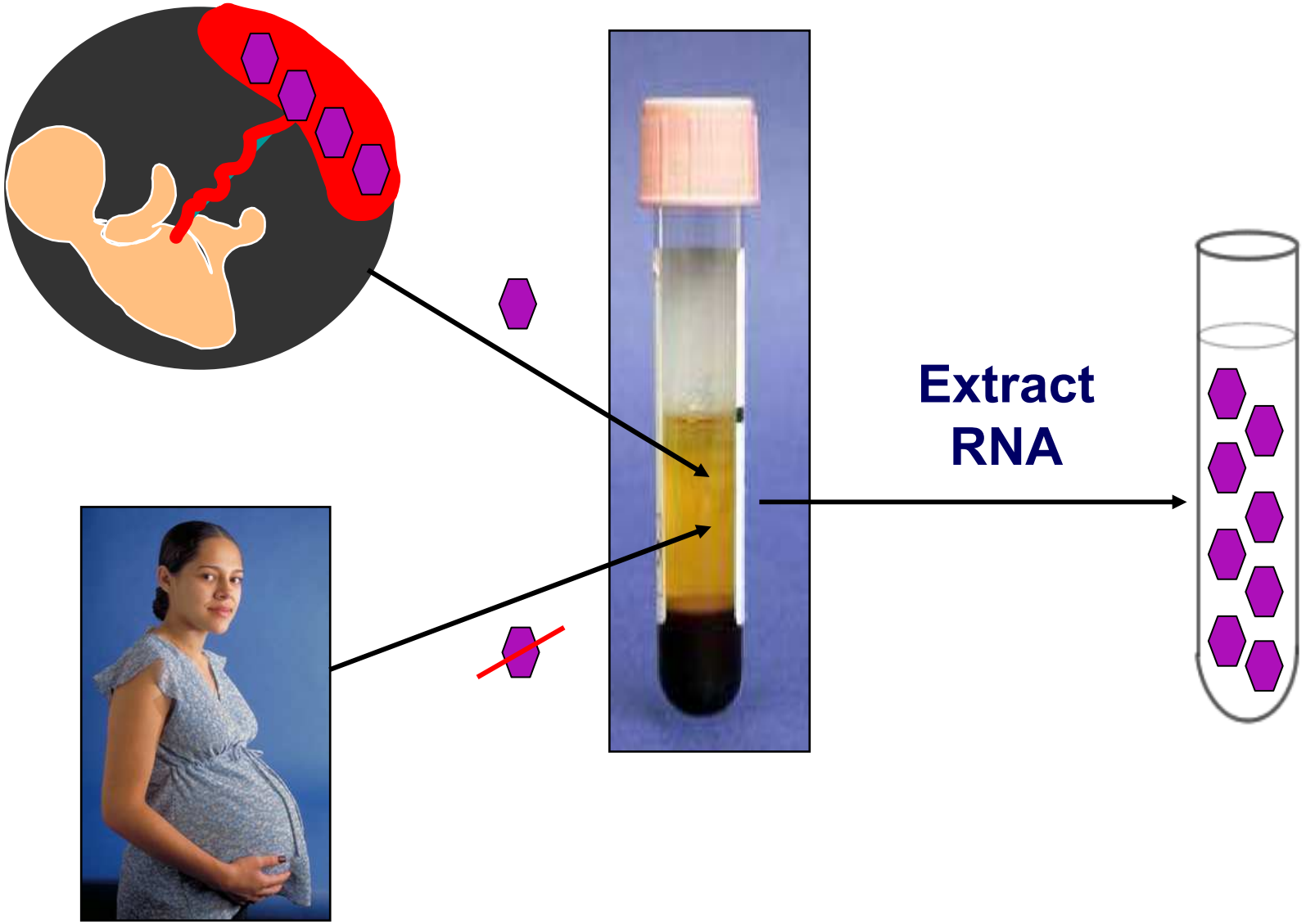
  Quantitative analysis of Single Nucleotide Polymorphisms in **fetal specific** RNA

  Massively parallel sequencing of cfDNA

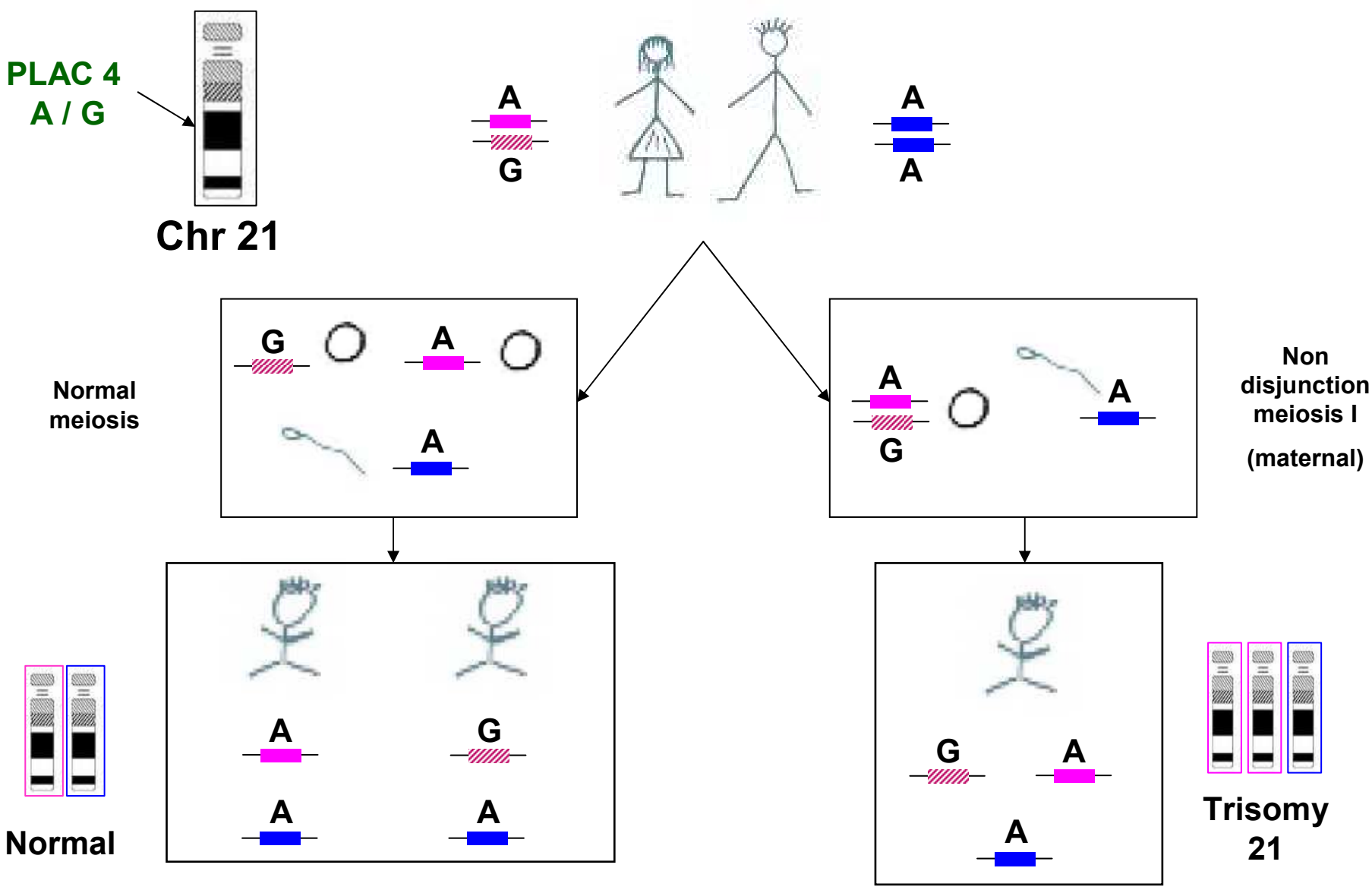


Quantitative analysis of Single Nucleotide Polymorphisms in fetal specific mRNAs

Quantitative analysis of SNPs in fetal specific mRNA



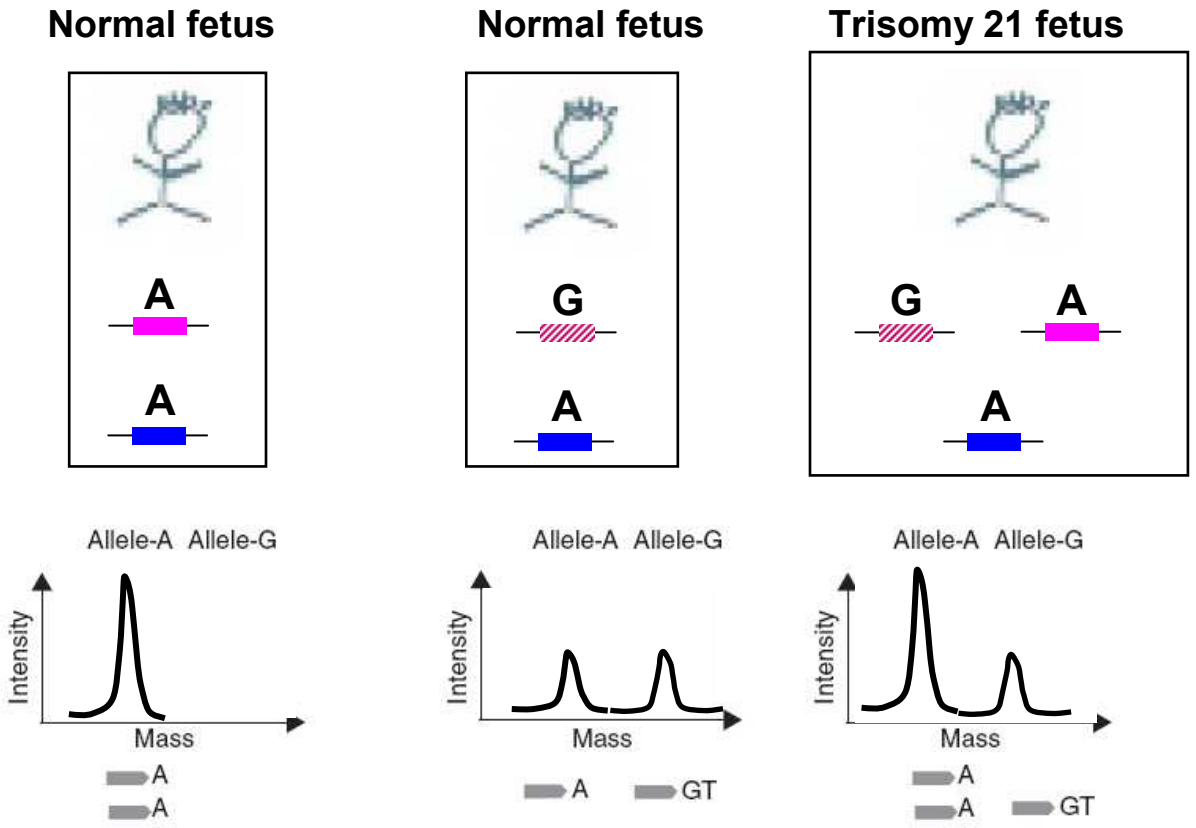
Quantitative analysis of SNPs in fetal specific mRNA



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

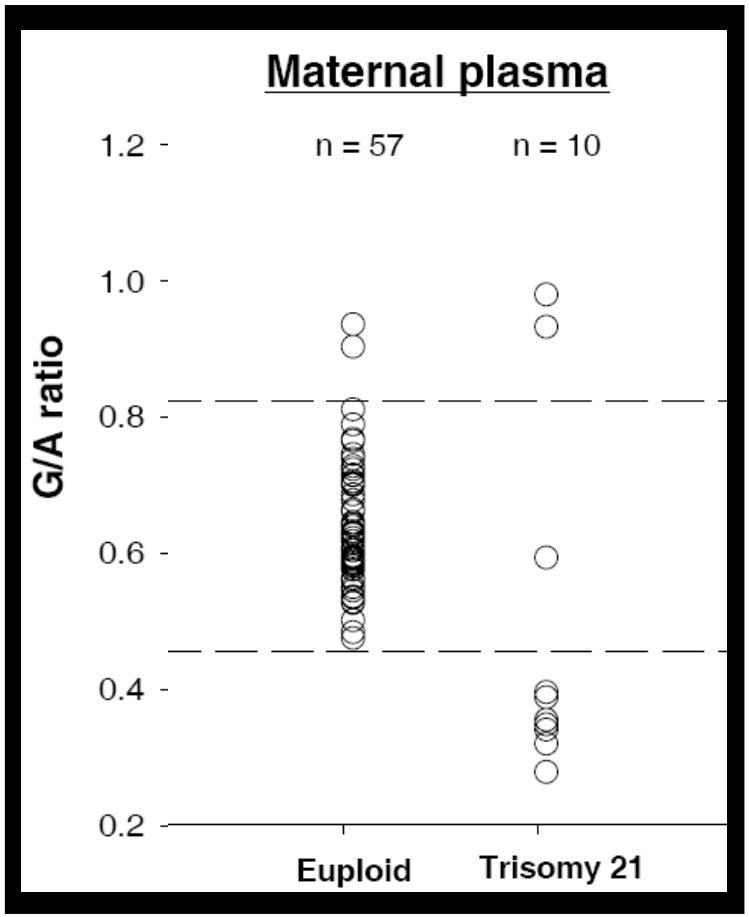


Non informative **1 : 1** **2 : 1**

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%

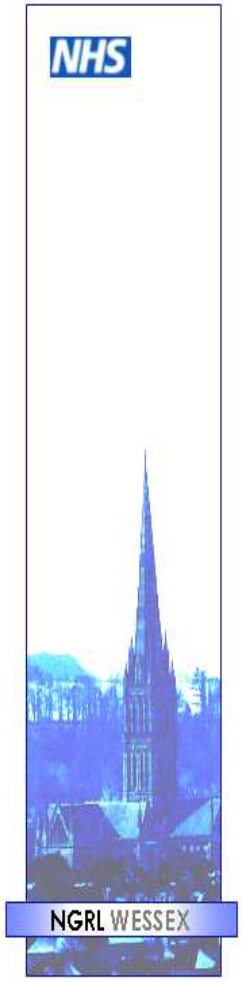
Lo et al., Nature Med & PNAS, 2007

Quantitative analysis of SNPs in fetal specific mRNA

- **ADVANTAGES**
  - Diagnostic sensitivity and specificity using one marker are high
  - Test is insensitive to gestational age and could be offered early in pregnancy
  - Target free of maternal background
- **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 – population dependent
  - Multi centre large scale validation required
  - Expand testing to include fetal specific transcripts from chromosomes 18 & 13

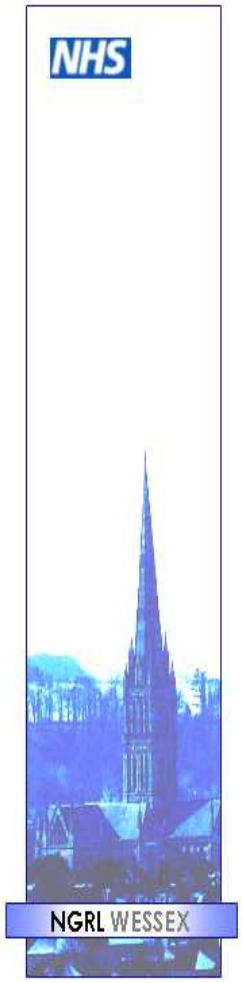
Quantitative analysis of SNPs in fetal specific mRNA

Sequenom Inc, SEQureDx(TM) Technology



- Developed quantitative RNA SNP test analysing multiple (>10) SNPs on chr 21:
  - increases test coverage to **greater than 95%** in the US population
- Complete concordance with clinical results in 619 samples tested to date
- Initiating multi-site 5000-sample laboratory developed test (LDT) validation study
- Acquired Center for Molecular Medicine, a CLIA-certified molecular diagnostics lab and anticipate commercial launch of **primary screening test** in **June 2009**
- Sponsoring RNA Noninvasive Aneuploidies ("RNA") study:
  - multi-center, prospective study involving 10,000 samples from first and second trimester pregnancies using the SEQureDx technology, managed and analysed by an independent third-party
- Identified novel markers for Trisomy 18 that have passed initial selection criteria

Press release: Sequenom, Inc Sept. 23, 2008



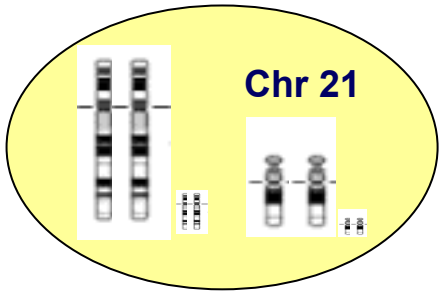
Massively parallel whole genome sequencing

An method for digital quantification of DNA

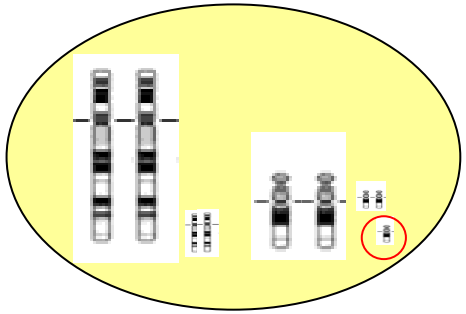
DNA testing preferable: is universal i.e. polymorphism independent

PROBLEM:

- 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%
- Relative chromosome dosage: can we compare amount of chr 21 present with amount of another autosome?

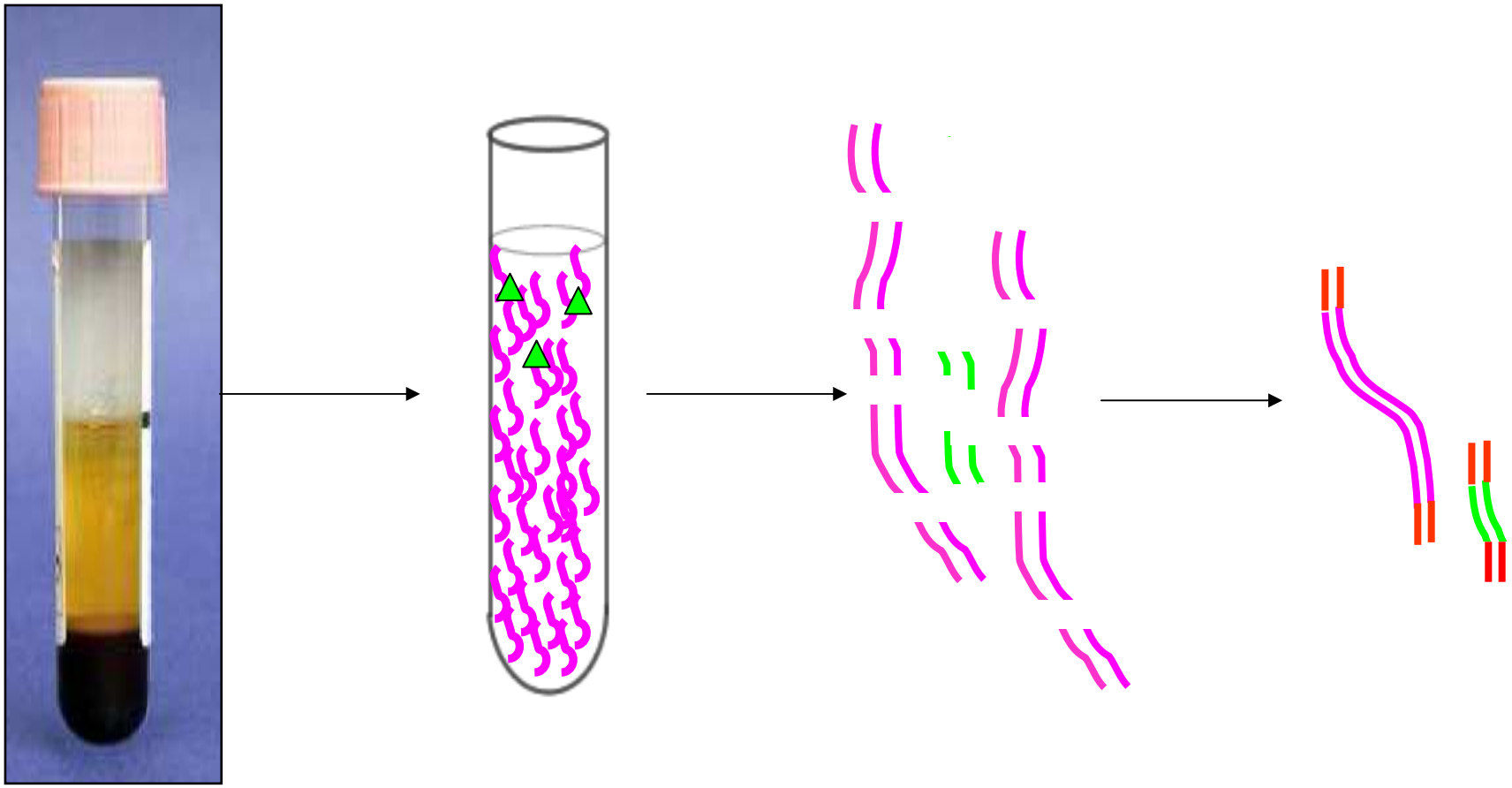


$$\frac{\text{Total amount of Chr 21 (0.94 + 0.06)}}{\text{Total amount of Chr 9 (0.94 + 0.06)}} = 1$$

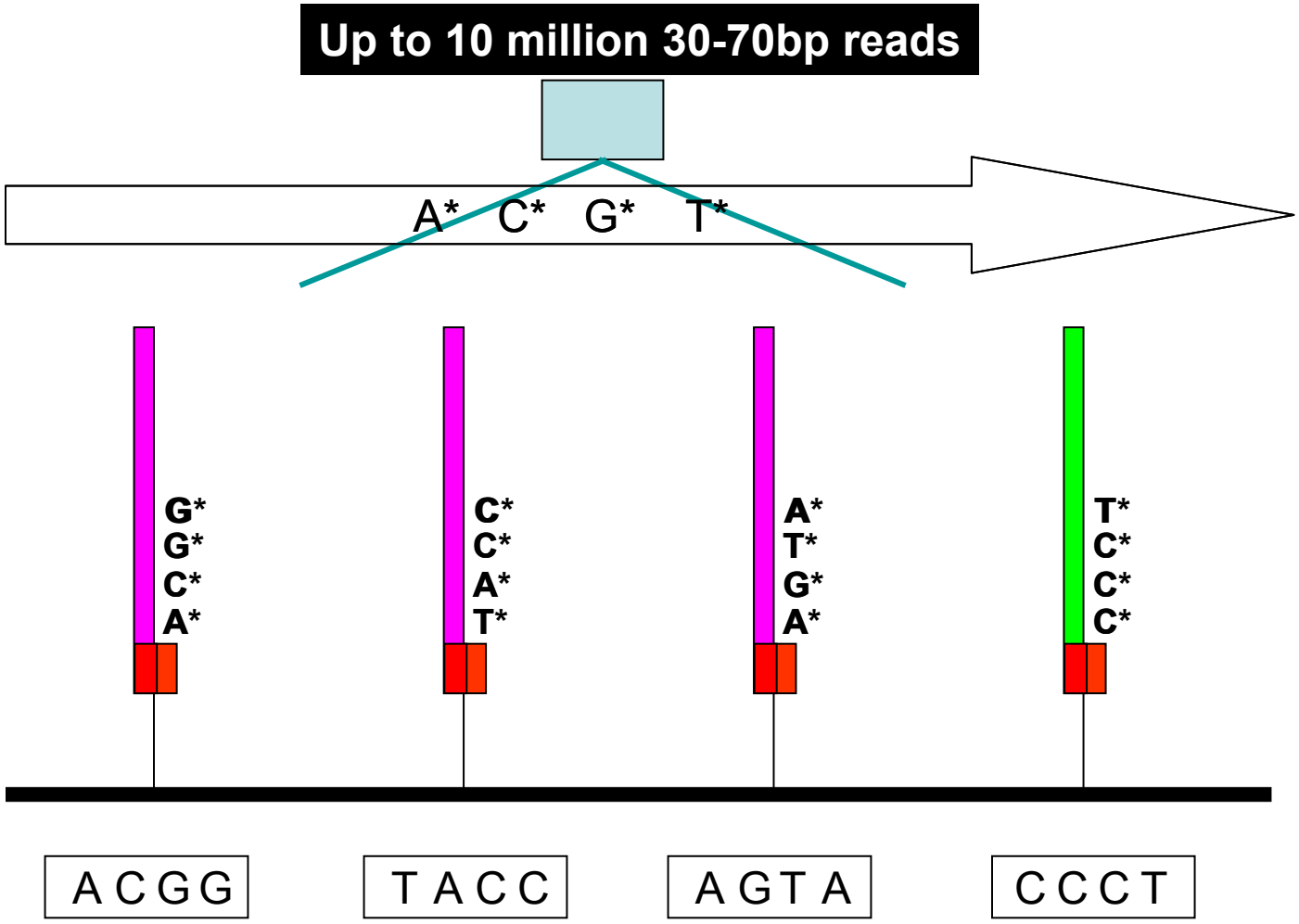


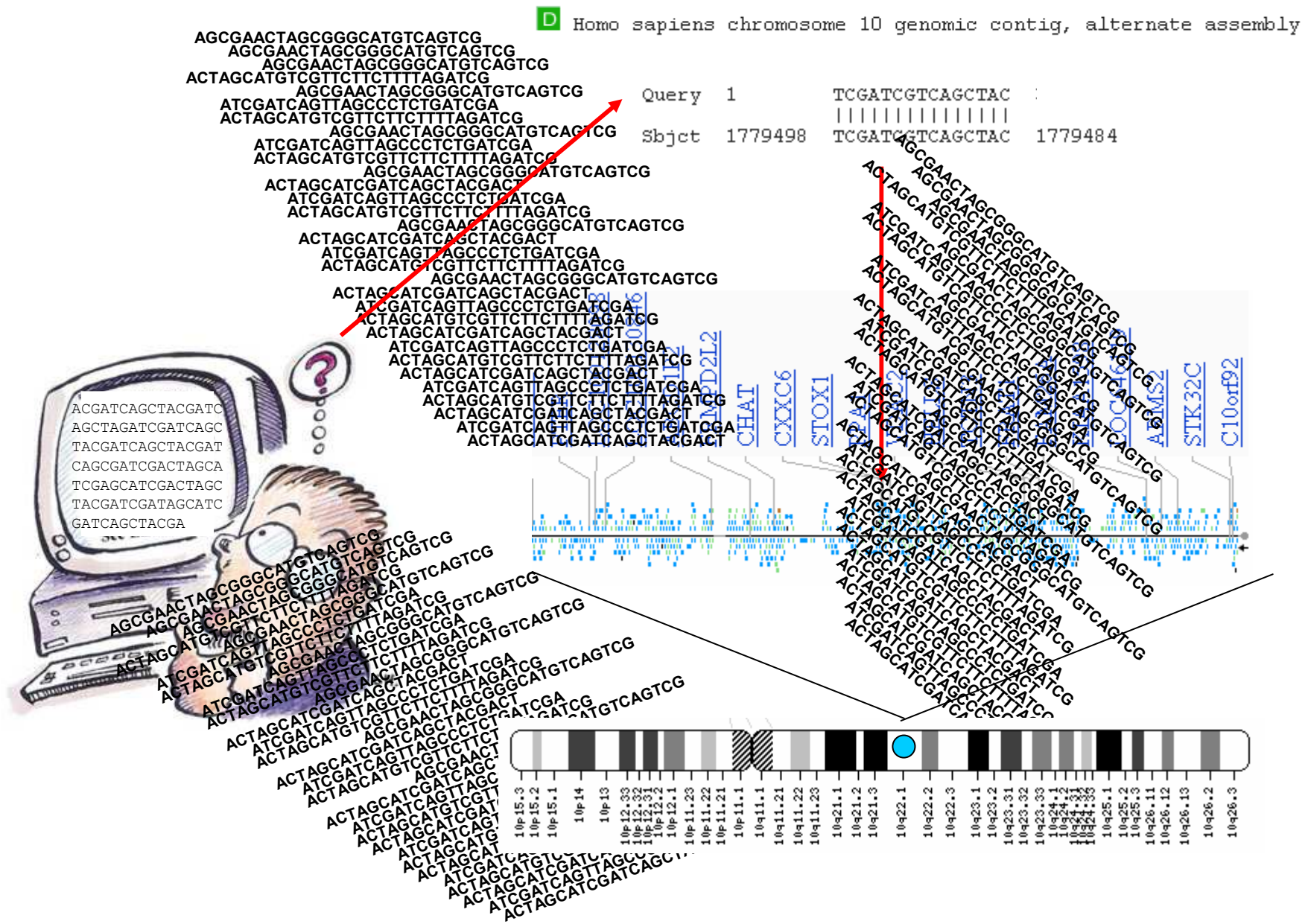
$$\frac{\text{Total amount of Chr 21 (0.94 + 0.09)}}{\text{Total amount of Chr 9 (0.94 + 0.06)}} = 1.03$$

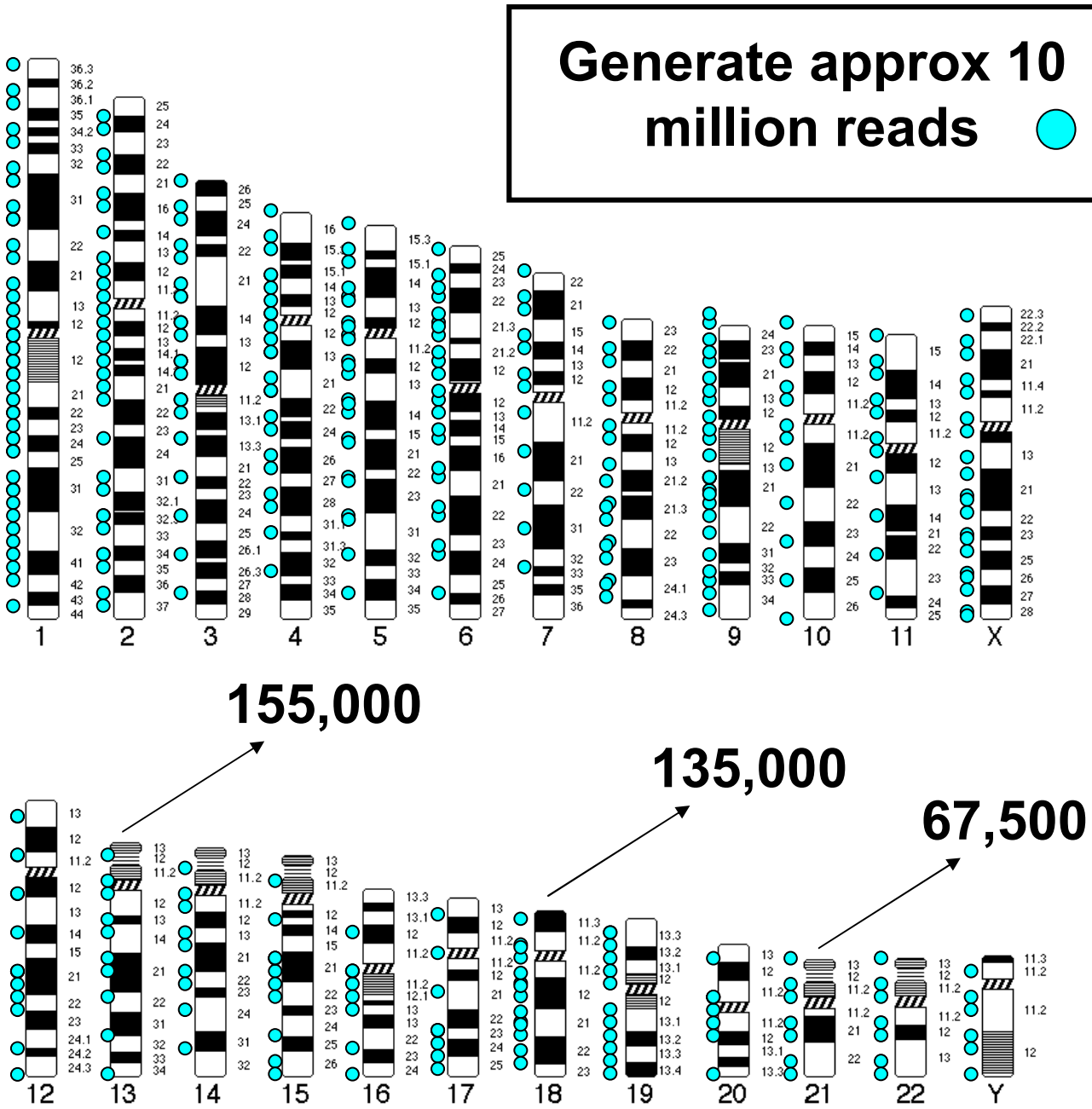
Massively parallel whole genome sequencing



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

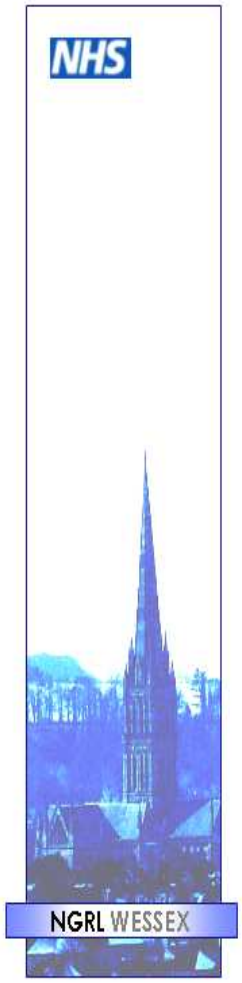

Generate approx 10 million reads
155,000
135,000
67,500
1
2
3
4
5
6
7
8
9
10
11
X
12
13
14
15
16
17
18
19
20
21
22
Y

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 pregnant women:

  9 trisomy 21

  2 trisomy 18

  1 trisomy 13

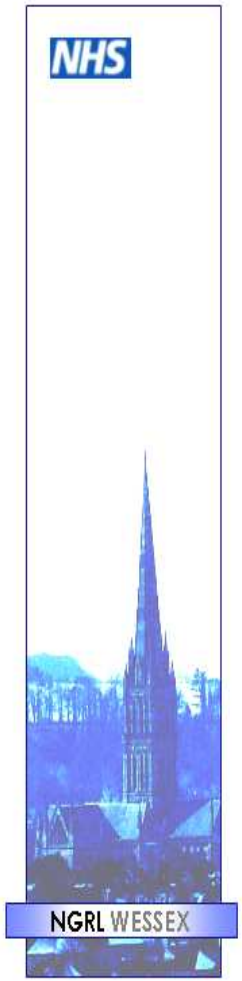
  6 normal

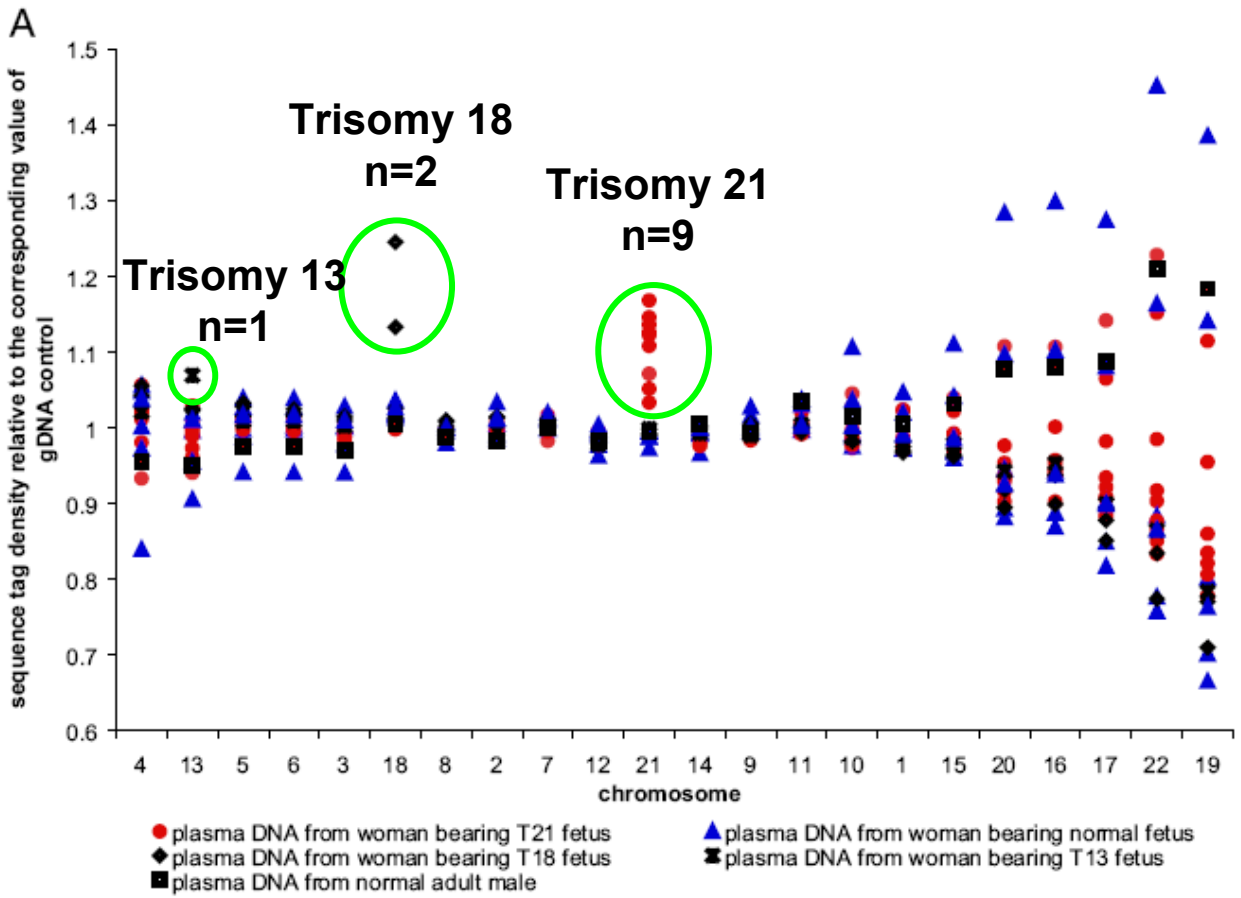
  and 1 genomic DNA sample from a male control

- Gestational age 10 – 35 weeks (earliest trisomy case 14 weeks)
- 5 million sequencing reads for each patient
- 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

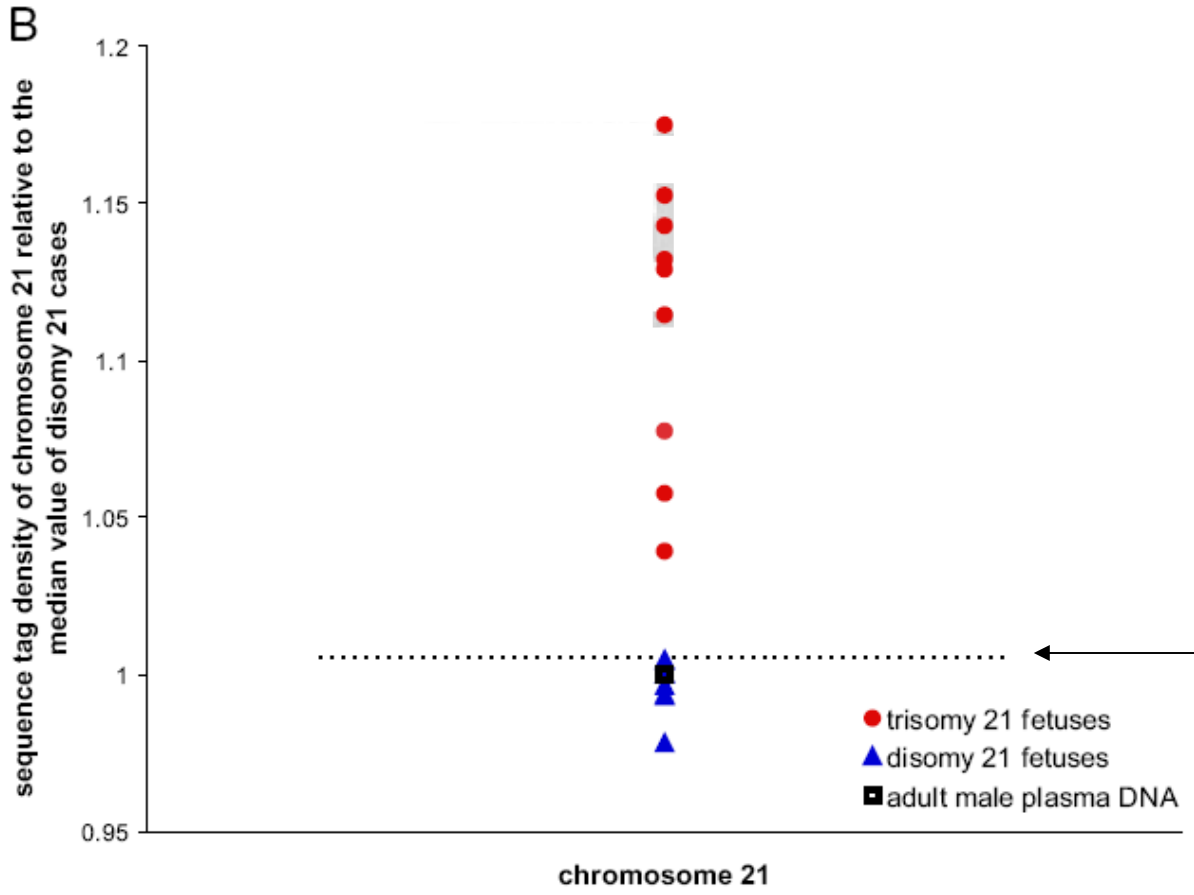
Results of shotgun sequencing of maternal plasma DNA





Fan et al., PNAS Oct 2008

Results of shotgun sequencing of maternal plasma DNA



Upper 99% confidence limit for normal samples

Fan et al., PNAS Oct 2008

Massively parallel whole genome 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**
  - Prohibitively expensive and slow
  - Large amount of data processing and interpretation is complex
  - In current form cannot be adapted to high throughput screening
- **FUTURE REQUIREMENTS**
  - Technological development required to produce machines and workflow protocols that could cope with a high throughput of samples (700,000 / year)
  - Likely that targeted approaches will be more successful

Summary: How can free fetal nucleic acids be used for DS testing?

- Both cf fetal RNA and DNA have been used successfully to detect DS
- New technologies need to be validated in large UK patient cohorts to determine accuracy
- The limits of gestation for testing using all techniques need to be determined
- Laboratory standards need to be developed
- Have the potential to replace current Down syndrome screening tests with a test that would be diagnostic
- Tests unlikely to replace invasive testing / current screening for some time although Sequenom may offer primary screening / diagnostic test in the US from June 2009
- Important to ensure that women and healthcare professionals understand the changes and women fully understand the implications of these tests
- NIHR funding secured to evaluate NIPD in more detail and determine the infrastructure and resources that will be required for timely implementation into NHS practice

The future..

"Nobody uses crystal balls anymore!"

Intellectual property issues

Multiple patents covering new products, methods and applications

Sequenom:

Exclusive licence for ALL uses of NIPD technology (original ISIS European patent)

Owns IP for relevant technology platforms MALDI-TOF mass spectrometry, digital PCR

Fluidigm:

Secured co-exclusive licenses (unidentified partner) for Stanford University inventions including combined use of digital PCR and high throughput sequencing

Must assume that NIPD will be covered by commercially valuable IP rights

Options when offering NIPD as a service:

- Negotiate a reasonable licence
- UK Compulsory licence provision

Although NHS may have to pay for licensing it is unlikely that this cost will be prohibitive to offering NIPD as an NHS sevice

RAPID: Reliable Accurate Prenatal non-Invasive Diagnosis - an integrated project to refine and implement safer antenatal testing (2009 – 2014)

To improve the quality of NHS prenatal diagnostic services by evaluating early non-invasive prenatal diagnosis based on cell free fetal DNA and RNA extracted from maternal plasma.

1) Confirm laboratory standards for NIPD for:

- Fetal sex determination
- Single gene disorders
- Down syndrome (DS)

2) Evaluate NIPD for those indications using the ACCE framework (Analytic and Clinical validity, Clinical utility, and Ethical, legal and social aspects)

- Evaluating cost effectiveness
- Determining couples' choices, preferences and needs
- Considering wider ethical, legal and social issues
- Developing competences for health professionals

3) Develop an implementation plan for use by commissioners to establish NIPD as an NHS service

Applicants

Principal Investigator

Lyn Chitty	Institute of Child Health, co-ordination and clinical implementation

Co Applicants

Neil Avent	University of the West of England, proteomics
Hilary Burton	PHG foundation, public health
John Crolla	NGRL (Wessex), national strategy
Helen White	NGRL (Wessex), lab evaluation
Jackie Westwood	UKGTN, commissioning
Alistair Kent	Genetics Interest Group, patient engagement
Ainsley Newson	Bristol University, ethics
Stephen Morris	Brunel University, health economics
Gail Norbury	Great Ormond Street Hospital, lab evaluation
Peter Soothill	Bristol University, implementation and policy
Peter Farndon	National Genetics Education and Development Centre, education

RAPID: Role of NGRL (Wessex) 2009 - 2012

- Define Down Syndrome (DS) test analytical sensitivity and specificity
- Undertake pilot feasibility studies for DS testing in collaboration with UCL / GOSH
  - MALDI-TOF mass spectrometry (potential collaboration with Sequenom)
  - targeted new generation sequencing
- Undertake population based feasibility study of NIPD for DS testing
  - Wessex region units (n=10)
  - King's College London Hospital,
  - University College Hospital London
  - Fetal Medicine centre in Harley Street
- 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

UK working group to review NIPD

Expert UK working group formed at the request of JCMG in 2007 facilitated by the PHG Foundation

Purpose of working group:

To identify any barriers to implementation of prenatal cell-free fetal nucleic acid testing for different applications within the UK clinical services, and specify where further work is needed in order to develop a national implementation strategy.

Steering group:

Dr Hilary Burton	PHG Foundation
Dr Lyn Chitty	UCL Institute of Child Health
Dr Tessa Homfray	St Georges Hospital Medical School
Dr Ainsley Newson	University of Bristol
Mrs Gail Norbury	Great Ormond Street Hospital
Professor Peter Soothill	University of Bristol

Working group: representatives from professional bodies and advisory groups

Report of the UK expert working group

The primary objectives of the working group were identified as:

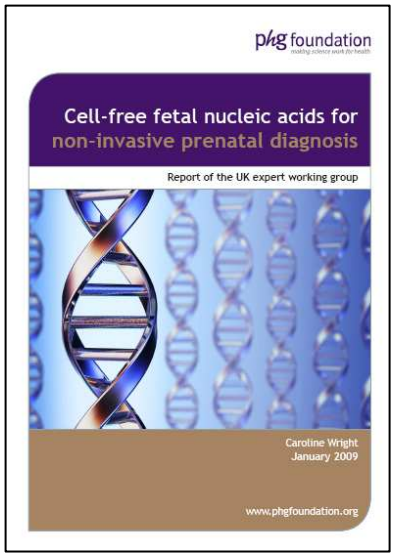
1) To review the scientific literature in addition to the ethical, legal and social implications (ELSI) and current service provision

2) Summarise unresolved issues and identify outstanding research needs;

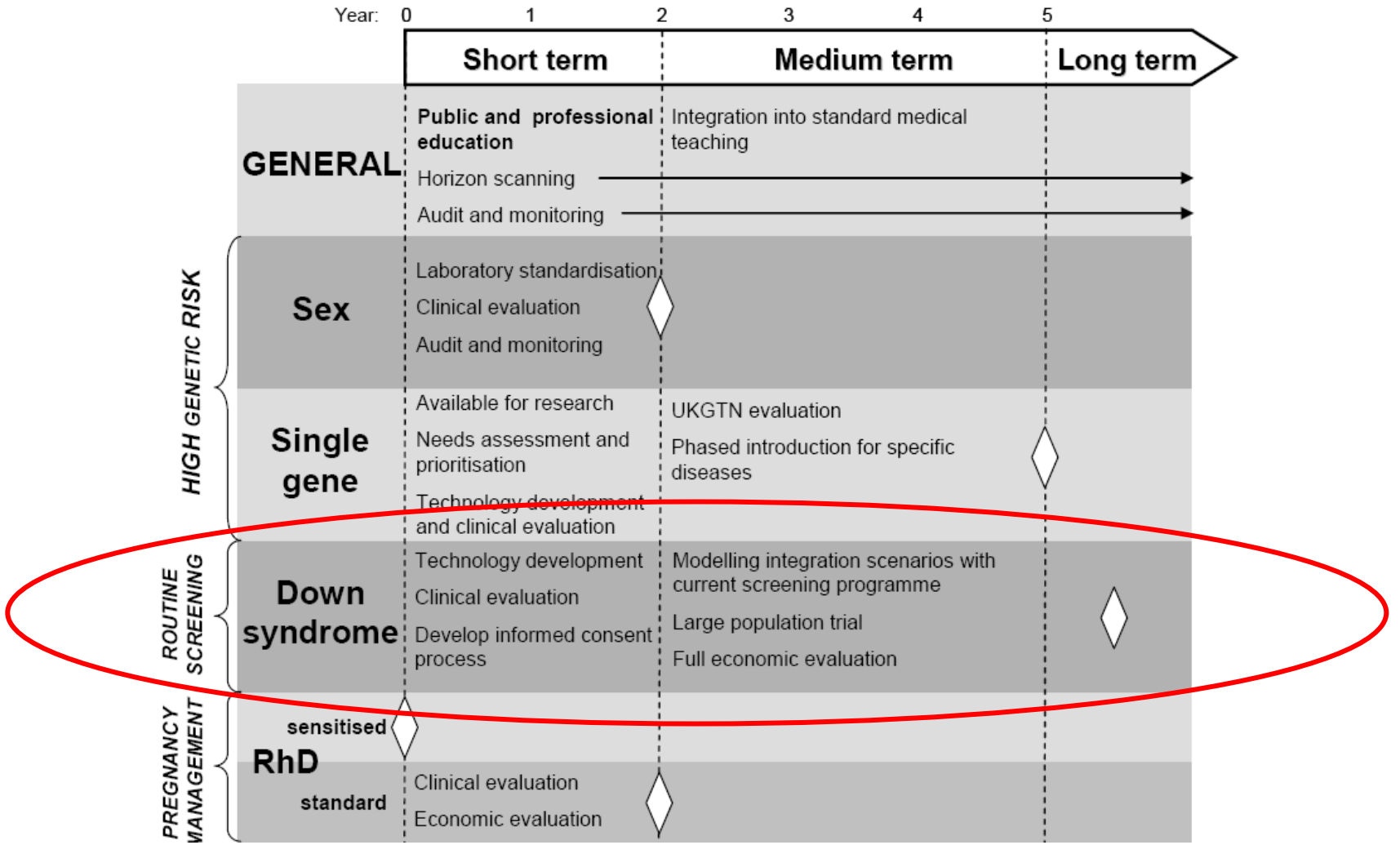
3) Make recommendations for strategies necessary for timely implementation of the technology and disseminate these findings.

These objectives were achieved by holding two workshops in May & Sept 2008.

Report will be published in mid-February



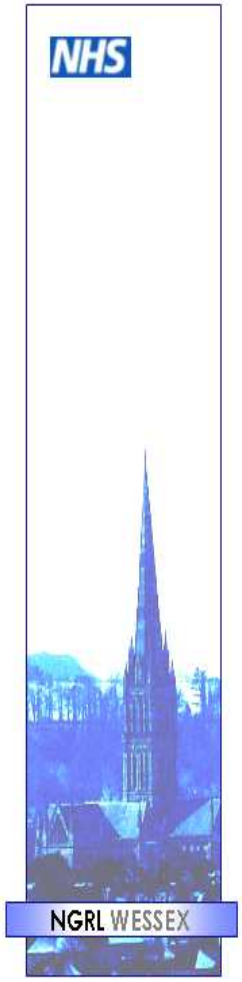
Likely timeline for implementation of NIPD across the NHS



NGRL funding — 2009 — 2012

NIHR funding (RAPID) — 2009 — 2014

Overall Summary



- Rapidly developing field

- Non invasive tests using cell free fetal nucleic acids have the potential to replace current Down syndrome screening tests with a test that would be diagnostic

- Currently unclear how tests might be used in practice:

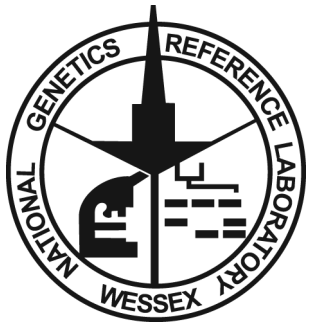
  - additional test during screening, to improve the overall risk calculation
  - intermediate test in between screening and invasive diagnosis for high risk women
  - replacement for the current biochemical screening tests
  - replacement for invasive diagnostic testing
  - replacement for both the current screening tests and invasive diagnostic testing

- Large evidence base required:

  - clinical test performance
  - population need
  - cost effectiveness
  - model of service delivery
  - improvement in quality and outcomes
  - value for money
  - patient acceptability

- Professional and public education required

More information



Lyn Chitty:	**l.chitty@ich.ucl.ac.uk**
Helen White:	**hew@soton.ac.uk**
PHG foundation:	**www.phgfoundation.org**
Sequenom:	**www.sequenom.com/Diagnostics/PrenatalDx**
NGRL (Wessex):	**www.ngrl.org.uk/Wessex**
RAPID:	**Website coming soon**

Salisbury NHS
NHS Foundation Trust