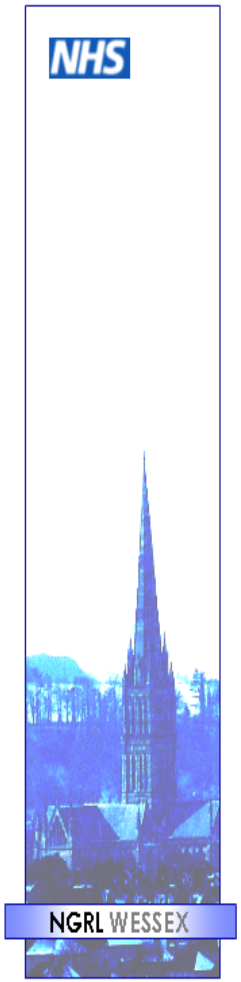
Salisbury NHS
NHS Foundation Trust



Non-invasive testing for Down syndrome - a simple blood test?

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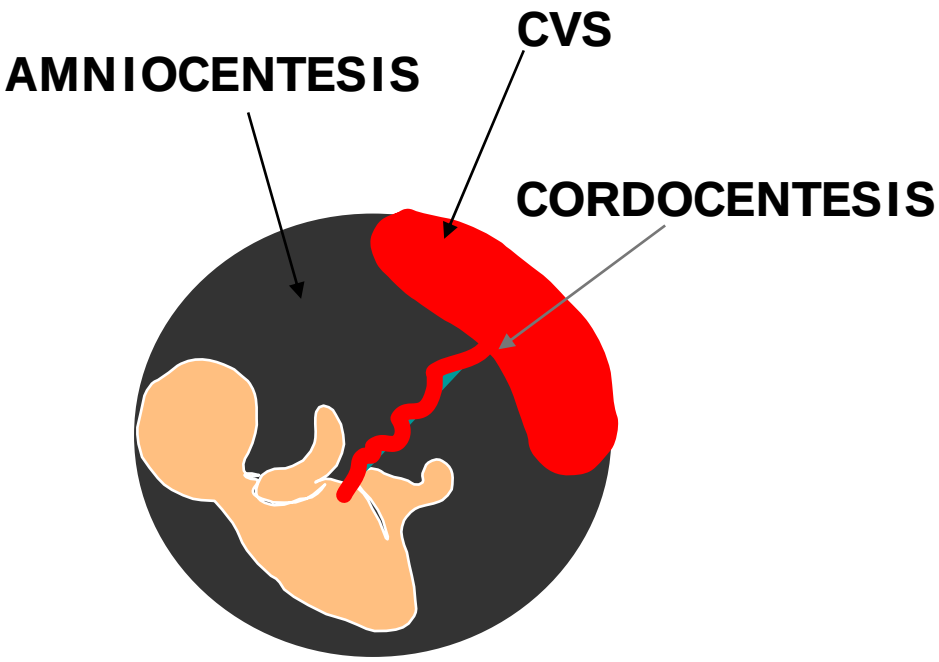
Senior Scientist

National Genetics Reference Lab (Wessex)

DH Department of Health

Current prenatal diagnosis requires invasive procedures



Aneuploidy

Single gene disorders

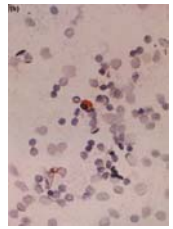
Haemoglobinopathies

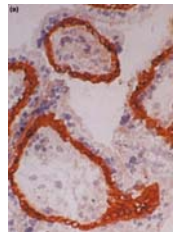
1% risk of miscarriage

Not possible before 11 weeks'

Other sources of fetal tissue for PND

Fetal cells in maternal circulation





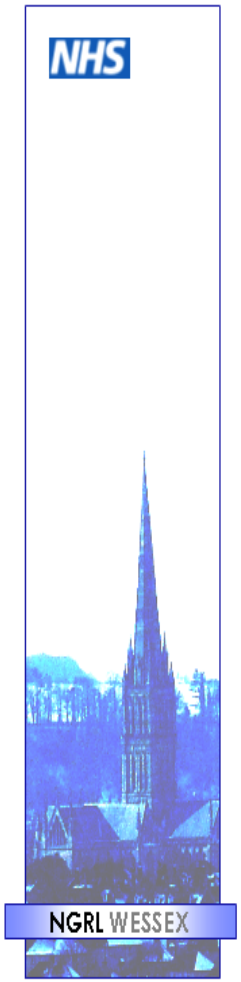
erythroblasts
trophoblastic cells
leucocytes

Difficult to isolate and persist for years after pregnancy

Cell free fetal DNA in the maternal circulation

Detectable from 5 weeks' gestation
Cleared from circulation within 30 minutes of delivery
3 – 6% of total circulating cell free DNA
Originates from trophoblast

NIPD for detection of aneuploidy



The ability to use NIPD to detect fetal aneuploidies, particularly trisomy 21, represents a major breakthrough in prenatal diagnosis

- **Major technical challenge**

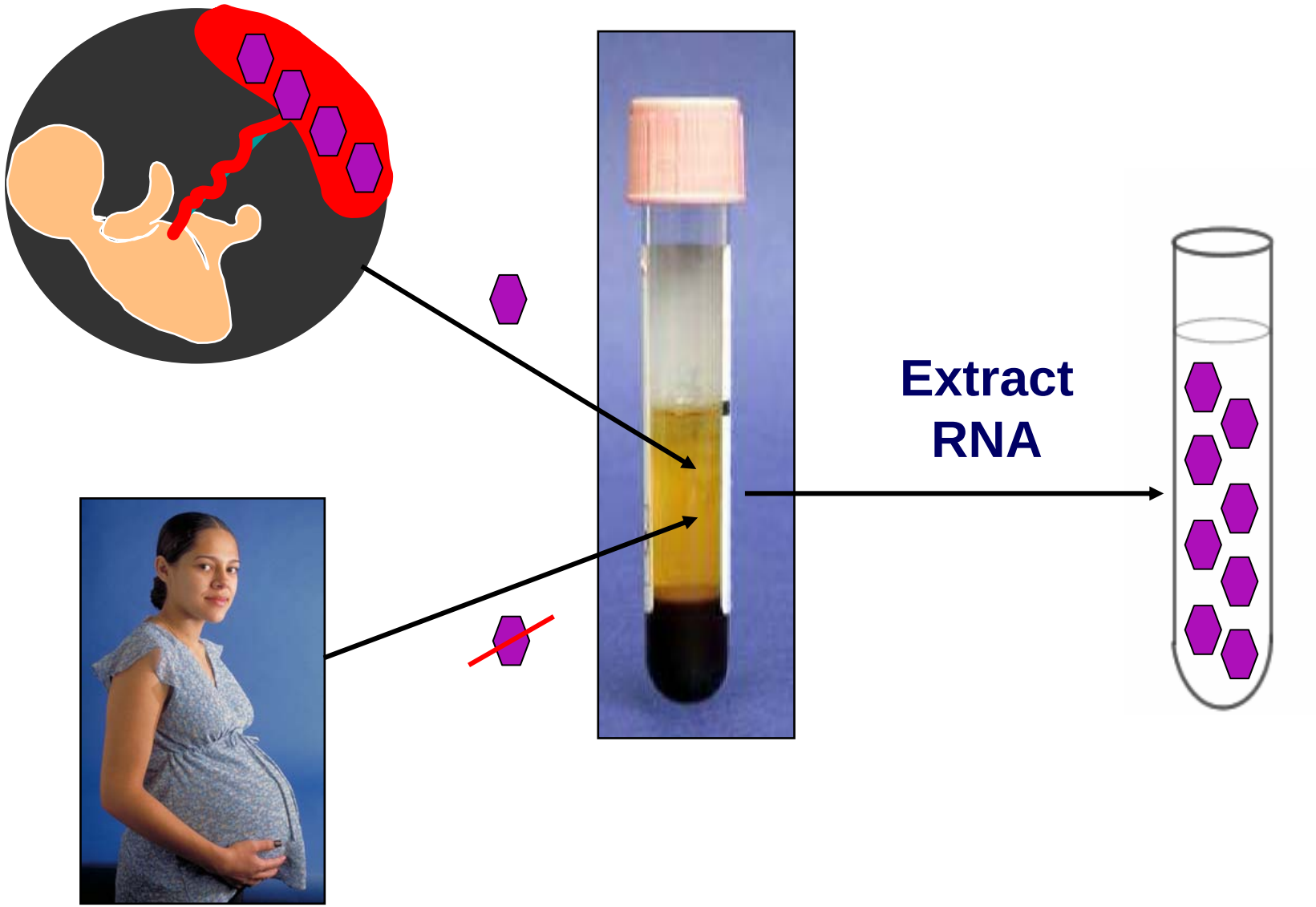
Background of cell free maternal DNA mean direct quantification of fetal chromosome copy number is not yet feasible

Need targets that are free from maternal background interference

- **Recent major breakthrough**

Quantitative analysis of SNPs in **fetal specific** mRNA transcripts

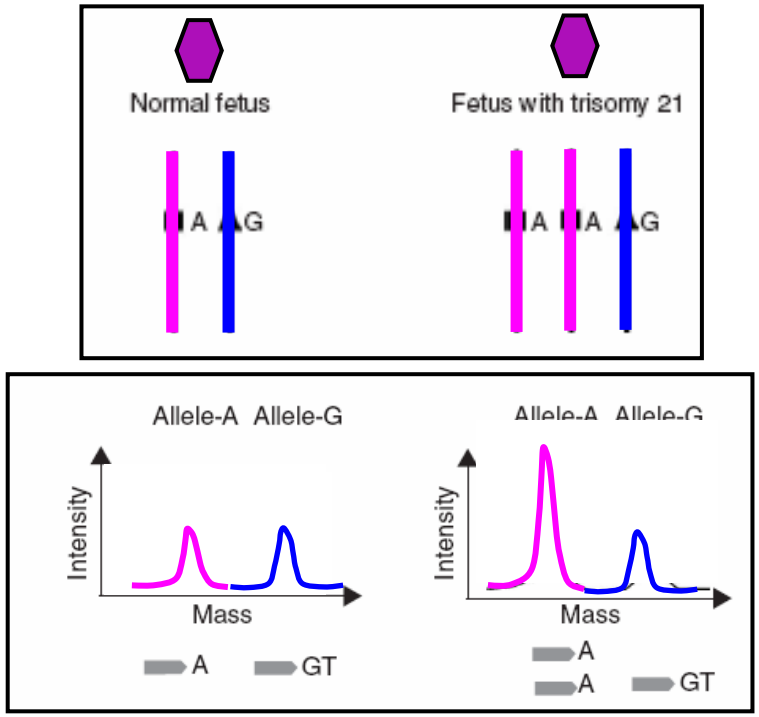
Quantitative analysis of SNPs in fetal specific mRNA



Quantitative analysis of SNPs in fetal specific mRNA

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

Normal fetus



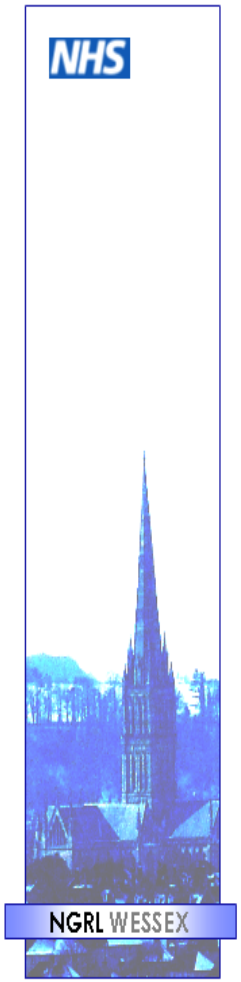
Trisomy 21 fetus

1 : 1

2 : 1

- Correctly diagnosed fetal trisomy 21 in **90%** of +21 cases (n=10)
- Excluded diagnosis of trisomy 21 in **96.5%** of chromosomally normal controls (n=57)
- Fetus has to be informative for SNP analysed

Advantages of testing cf fetal mRNA for DS testing and challenges for the future



- Transcripts are **fetal specific** and therefore independent of maternal contribution allowing copy numbers to be accurately quantified

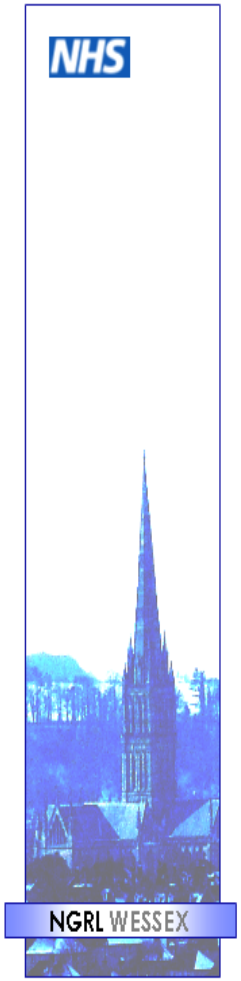
- Diagnostic sensitivity and specificity from this study using one marker are comparable to multiple marker screening tests for DS

- Test appears to be insensitive to gestational age and can be offered early in pregnancy

- Identification of more polymorphic loci to increase number of informative cases

- Expand testing to include other common fetal aneuploidies when fetal specific transcripts from chromosomes 18 and 13 are identified

Preliminary Study – NGRL (Wessex)



- **Ethical approval granted 2007 for technical study**

- **100 blood samples from women carrying pregnancies of a variety of gestational ages will be collected so that technical parameters can be determined for the various assays outlined below**

- **Collection of maternal blood samples started July 2007**

  - Assessment of extraction techniques for free foetal DNA and RNA

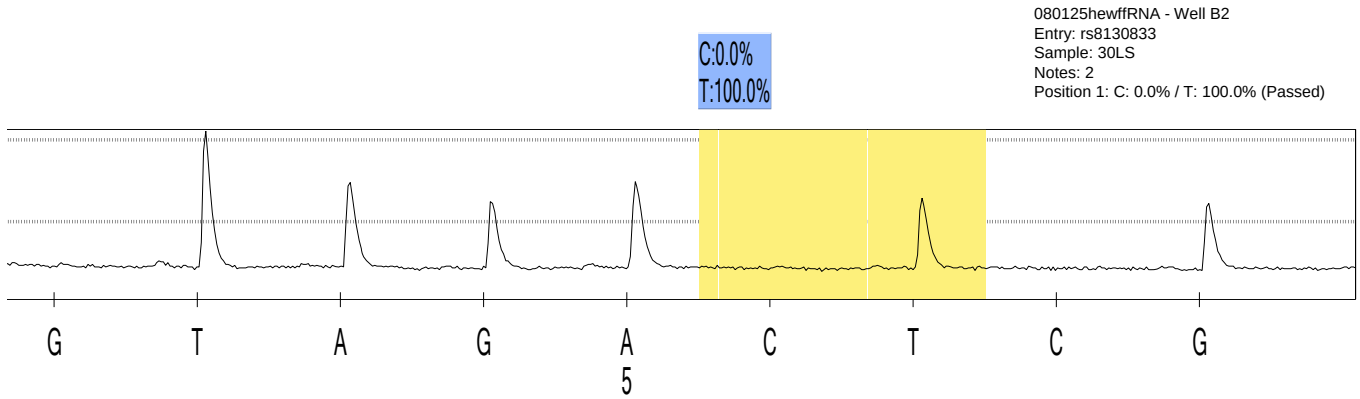
  - Design, optimisation and evaluation of allele quantification assay for mRNA SNPs for detection of trisomy 21

  - Optimisation and evaluation of assays for Rhesus D and fetal sexing

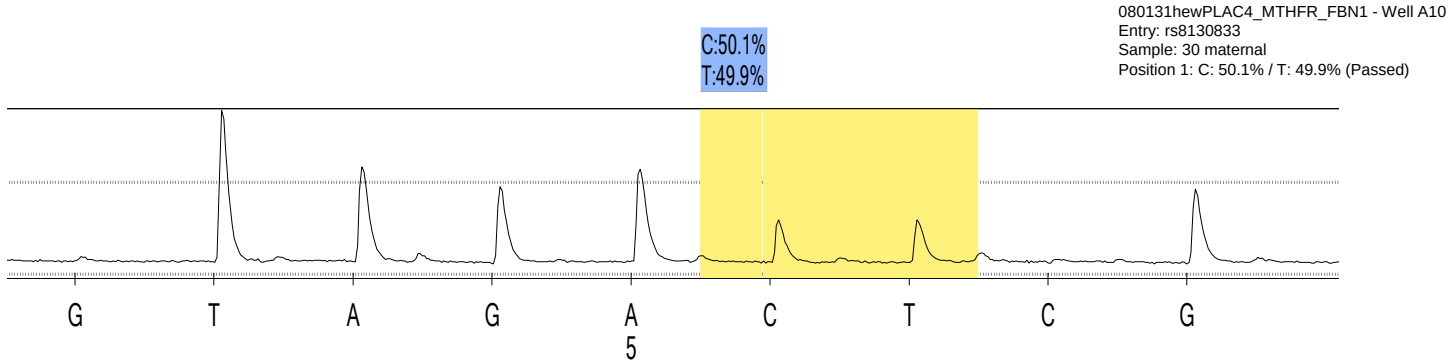
Pyrosequencing assay using free fetal RNA

Sample 30: 8 weeks 6 days; 5 hours 15 minute blood transit time

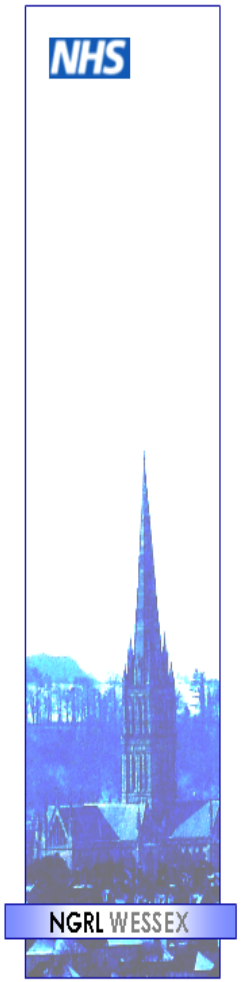
Cell free fetal RNA sample: Not informative T/T



Maternal DNA sample: genotype C/T (allele ratio 1.0)



RAPID : Reliable Accurate Prenatal non - Invasive Diagnosis: an integrated project to refine and implement safer antenatal testing



NIHR programme grant (2008 - 2013) co-ordinated by Dr Lyn Chitty:

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 protocols for NIPD for:

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

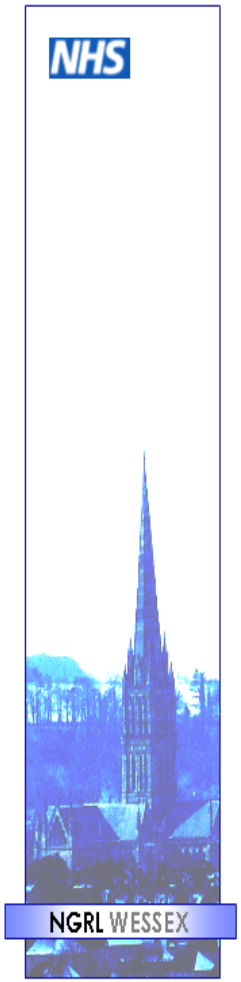
2) Evaluate NIPD to:

- Determine cost effectiveness
- Determine couples' choices, preferences and needs
- Consider wider ethical, legal and social issues
- Develop competences for health professionals

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

RAPID: Role of NGRL (Wessex)

Co applicants Helen White & John Crolla



• Define Down Syndrome (DS) test sensitivity and specificity and evaluate new polymorphic markers (300 DS and 300 normal controls)

- women identified as high risk and undergoing invasive procedure

- compare allele ratio from maternal plasma sample to fetal cells cultured from AF or CVS.

• Undertake pilot feasibility studies for DS testing with new technologies

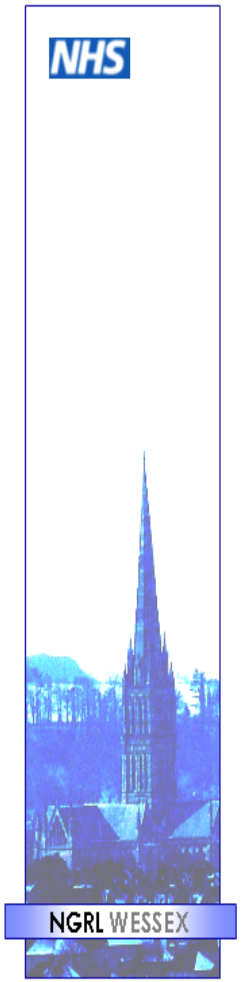
- mass spectrometry

- digital PCR

• Undertake population based feasibility study of NIPD for DS testing

- Salisbury
- King's College London Hospital
- University College London Hospital

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



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