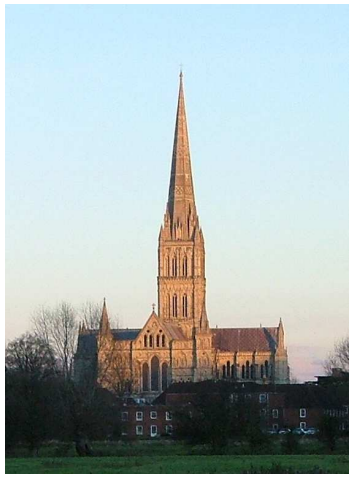
Salisbury NHS
NHS Foundation Trust

The National Genetics Reference Lab (Wessex):

who are we and what do we do?



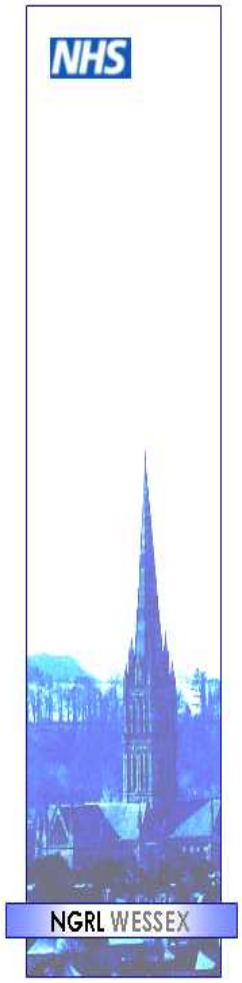
Helen White, PhD

Senior Scientist

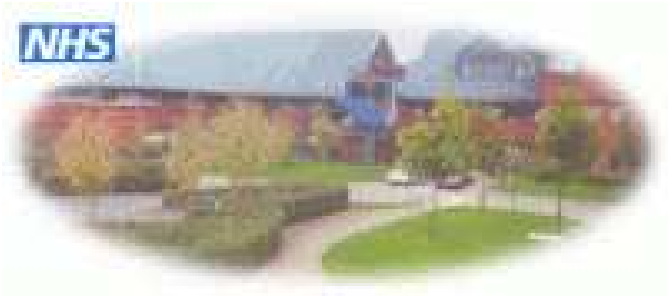
NGRL (Wessex)

DH Department of Health

UK National Genetics Reference Laboratories



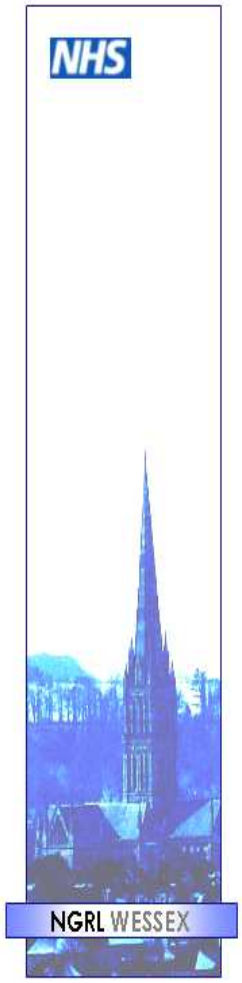
- Established in 2002 by the Department of Health as an initiative by the Department of Health to support the UK NHS genetic lab services
- Two laboratories based in Manchester and Salisbury (Wessex)



- NGRL (Wessex) is integrated with:
    - o Wessex Regional Genetics Laboratory in Salisbury

  and work closely with
    - o Human Genetics Division at the University of Southampton
    - o Wessex Clinical Genetics Service

UK National Genetics Reference Laboratories



- Our work encompasses cytogenetic, molecular cytogenetic and molecular genetic analysis

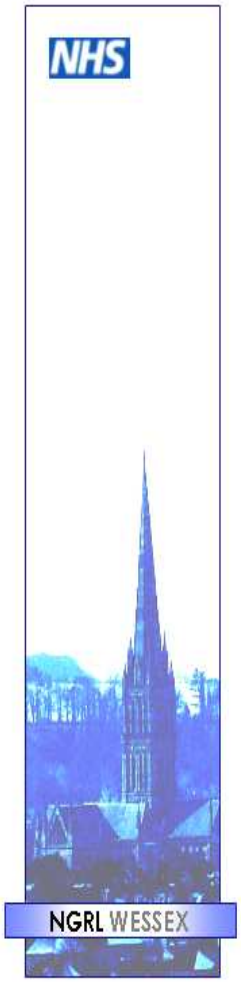
- Principal focus is on development, validation and quality assurance of genetic testing for constitutional and acquired abnormalities

- The specific remit of the laboratories includes:

    - Technology development, assessment and validation
    - Developing new quality management systems
    - Developing reference and control reagents
    - Developing information systems for genetics
    - Providing advice to government and professional bodies

- Our current work programmes are overseen by a steering group

Work programmes 2002 - 2007



Generally reflected genetics labs need to respond to targets set in the Genetics White Paper (2003)

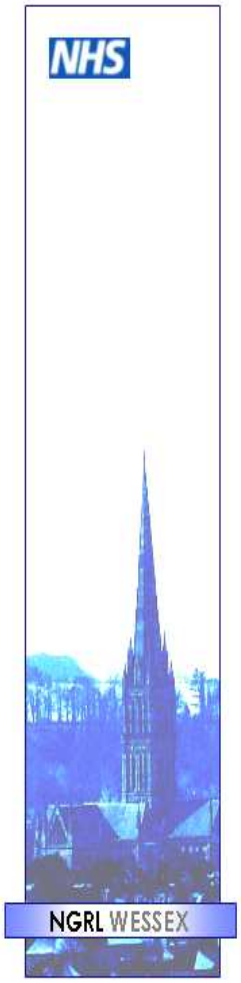
"**Our inheritance, our future: realising the potential of genetics in the NHS"**

Dept of Health invested up to £18 million capital on upgrading NHS genetics laboratory facilities in England.

" As a result of this investment, by 2006 genetic testing times will be cut and the results should be available to the following standards:

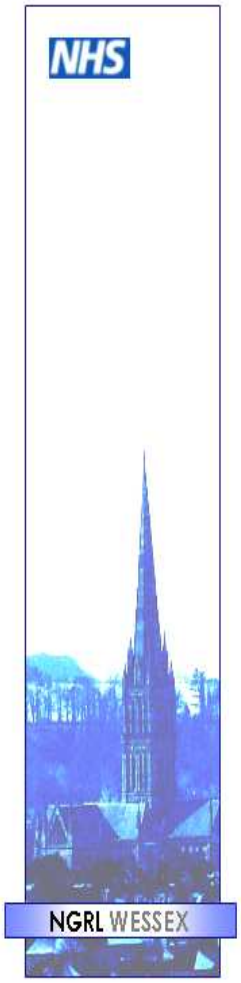
- o within 3 days where the result is urgent (eg. prenatal diagnosis)
- o within 2 weeks where the genetic mutation is already known
- o within 8 weeks for unknown mutations in a large gene"

Work programmes 2002 - 2007



- Reference and Control Materials
- Technology Assessments & White Paper targets
- Genetic diversity and reference ranges
- Developments in cytogenetics and molecular cytogenetics
- Fluorescence in situ hybridisation reference service
- Advice, Training, Education and Horizon Scanning

Control Reagents for Genetic Testing



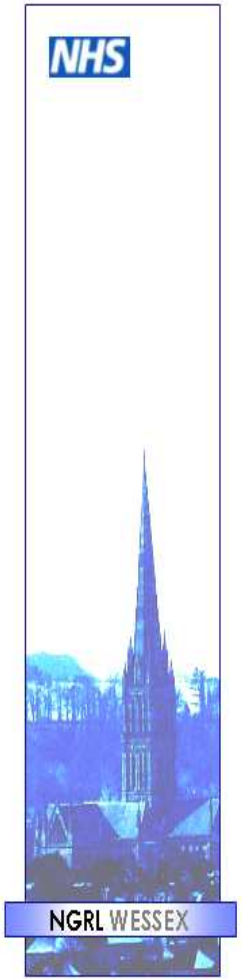
- NHS genetic diagnostic laboratories in the UK (and labs worldwide) perform thousands of mutation detection assays every month using diverse technologies

- Laboratories generally use locally sourced controls as standards to confirm that an assay is working correctly

- Widespread variation in the number and type of controls used in different laboratories

- Can potentially compromise quality assurance

NGRL (Wessex) Reference Material 2002 - 2007



Plasmid based control material for mutation scanning

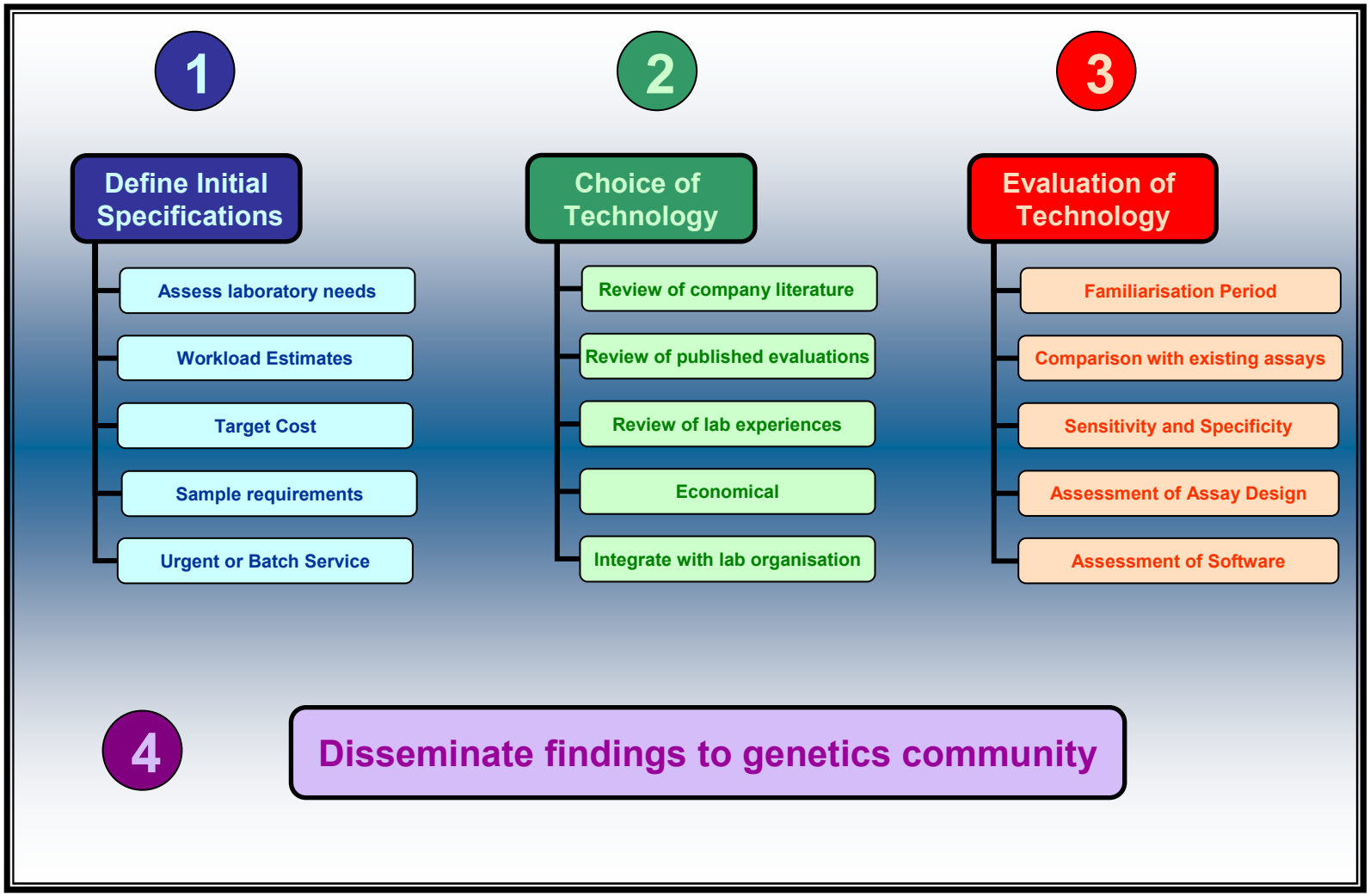
Generated sets of plasmid constructs that harbour defined sequence changes and which can be used as controls for a wide range of mutation assays

- Familial breast cancer and colorectal cancer (n=193)
- Generic controls for Mutation Scanning (n=52)

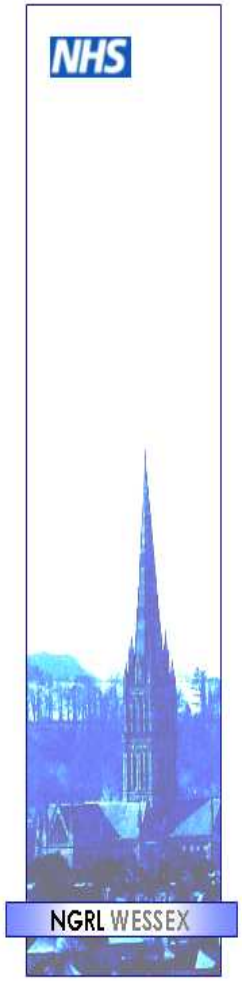
Genomic DNA (lymphoblastoid cell lines)

- Normal controls
- Myotonic dystrophy
- Friedreich ataxia

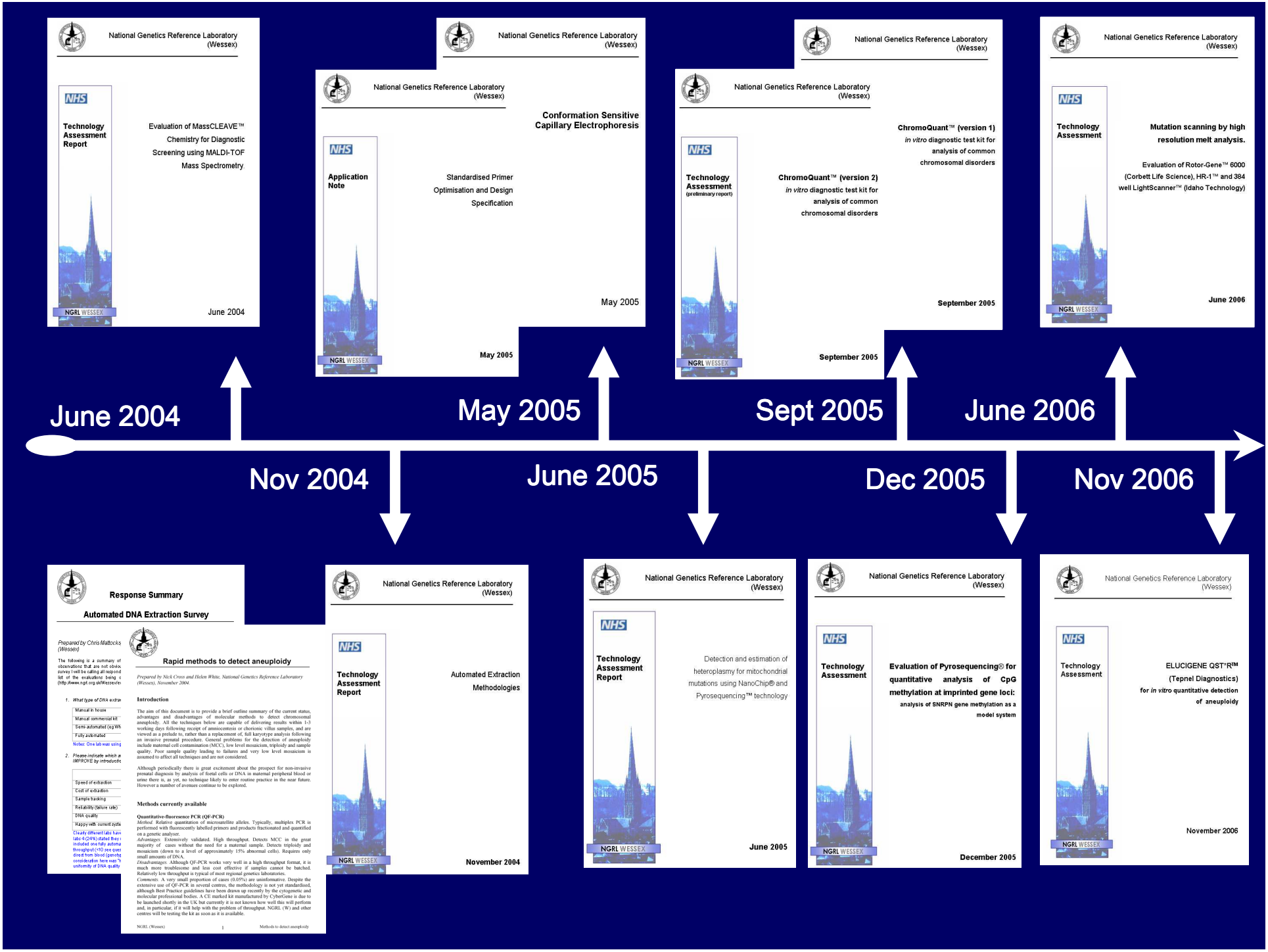
NGRL (Wessex) Health Technology Assessment



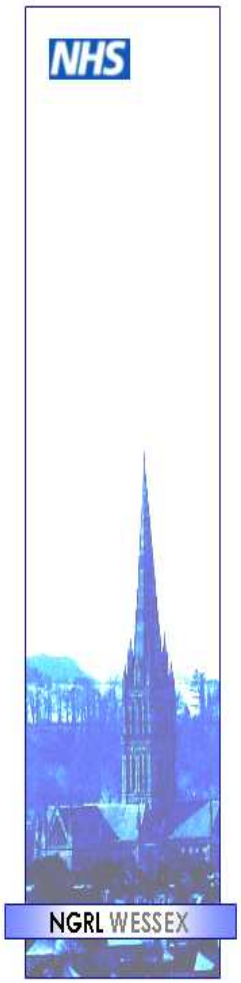
Scope of Technology Assessments 2002 - 2007



- SNP / defined mutation detection
- Allele Quantification / Dosage
- Epigenetic analysis
- Meeting White Paper Targets
    - o Automated DNA extraction platforms
    - o Rapid mutation scanning
    - o Rapid aneuploidy detection


National Genetics Reference Laboratory (Wessex)
NHS
Technology Assessment Report
Evaluation of MassCLEAVE™ Chemistry for Diagnostic Screening using MALDI-TOF Mass Spectrometry.
June 2004
NGRL WESSEX
National Genetics Reference Laboratory (Wessex)
NHS
Application Note
Conformation Sensitive Capillary Electrophoresis
Standardised Primer Optimisation and Design Specification
May 2005
NGRL WESSEX
May 2005
National Genetics Reference Laboratory (Wessex)
NHS
Technology Assessment (preliminary report)
ChromoQuant™ (version 2)
in vitro diagnostic test kit for analysis of common chromosomal disorders
NGRL WESSEX
September 2005
National Genetics Reference Laboratory (Wessex)
ChromoQuant™ (version 1)
in vitro diagnostic test kit for analysis of common chromosomal disorders
September 2005
National Genetics Reference Laboratory (Wessex)
NHS
Technology Assessment
Mutation scanning by high resolution melt analysis.
Evaluation of Rotor-Gene™ 6000 (Corbett Life Science), HR-1™ and 384 well LightScanner™ (Idaho Technology)
NGRL WESSEX
June 2006
June 2004
May 2005
Sept 2005
June 2006
Nov 2004
June 2005
Dec 2005
Nov 2006
Response Summary
Automated DNA Extraction Survey
Prepared by Chris Mattocks (Wessex)
National Genetics Reference Laboratory (Wessex)
NHS
Technology Assessment Report
Automated Extraction Methodologies
NGRL WESSEX
November 2004
Rapid methods to detect aneuploidy
Prepared by Nick Cross and Helen White, National Genetics Reference Laboratory (Wessex), November 2004.
Introduction
Methods currently available
National Genetics Reference Laboratory (Wessex)
NHS
Technology Assessment Report
Detection and estimation of heteroplasmy for mitochondrial mutations using NanoChip® and Pyrosequencing™ technology
NGRL WESSEX
June 2005
National Genetics Reference Laboratory (Wessex)
NHS
Technology Assessment
Evaluation of Pyrosequencing® for quantitative analysis of CpG methylation at imprinted gene loci:
analysis of SNRPN gene methylation as a model system
NGRL WESSEX
December 2005
National Genetics Reference Laboratory (Wessex)
NHS
Technology Assessment
ELUCIGENE QST*R™ (Tepnel Diagnostics)
for in vitro quantitative detection of aneuploidy
NGRL WESSEX
November 2006

Work programmes 2007 - 2012

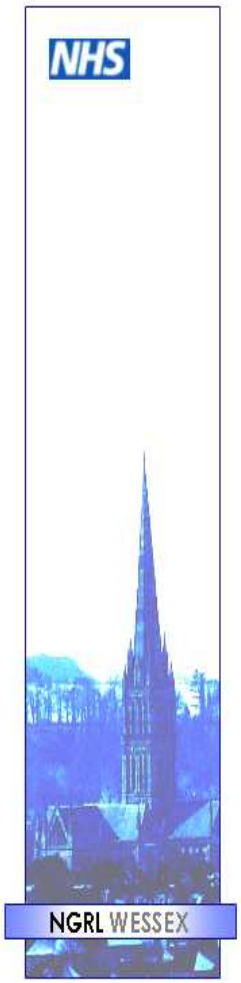


More focused on development of technologies that are likely to have a major impact on genetic testing in the future

- Reference Reagents (Prof Nick Cross & Dr Helen White)
- New generation sequencing (Chris Mattocks)
- Non – invasive prenatal diagnosis (Dr Helen White)
- Array CGH (Dr John Crolla)
- Cytogenetics Resources (Dr John Barber)
- Meeting and Workshops

Reference reagents

Real time Quantitative PCR analysis of the BCR-ABL fusion transcript



- RQPCR is routinely used to quantify levels of BCR-ABL mRNA transcripts from chronic myeloid leukaemia patients

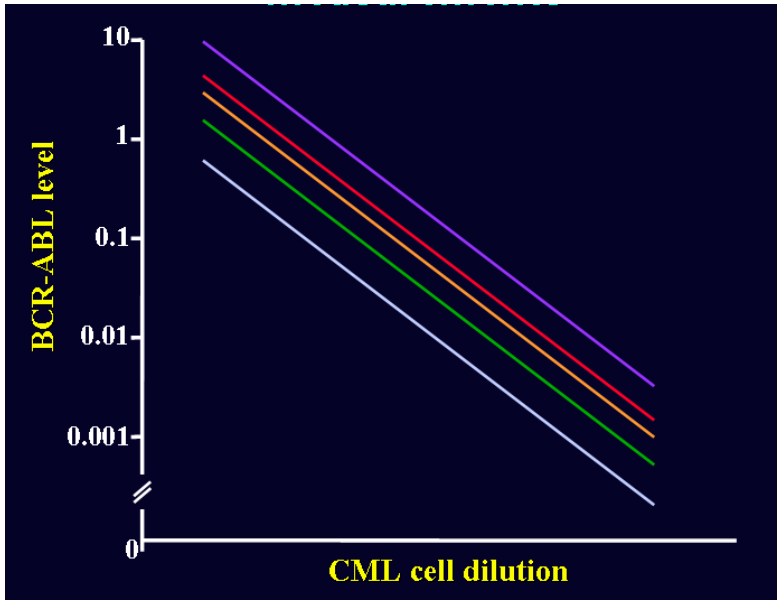
- Accurately determines the response to treatment and is particularly valuable for patients who have achieved complete chromosomal remission.

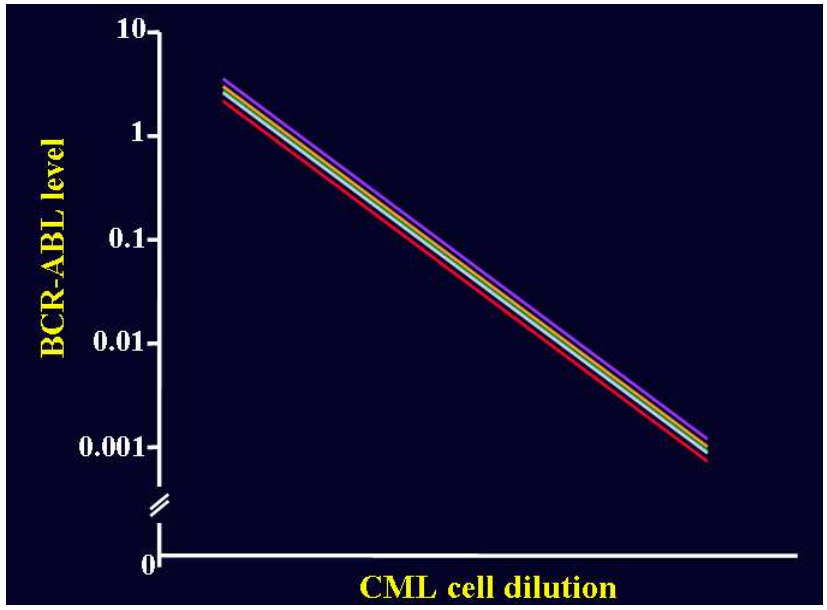
- Despite efforts to establish standardised protocols for BCR-ABL fusion transcript quantitation there is still substantial variation in the way in which RQ-PCR for BCR-ABL is carried out and how results are reported indifferent laboratories worldwide

- An international scale (IS) has recently been established recently that is anchored to two key points defined in the IRIS trial: a common baseline (100% BCR-ABL$_{IS}$) and major molecular response (0.1% BCR-ABL$_{IS}$)

- Definition of the IS currently relies on relating results directly or indirectly to the Adelaide international reference laboratory

- A more robust definition of the IS requires the development of internationally accredited reference reagents





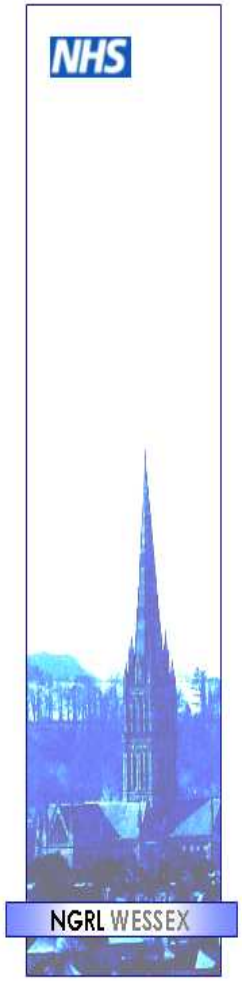
Reference reagents

Real time Quantitative PCR analysis of BCR-ABL

NGRL (Wessex) in collaboration with the National Institute of Biological Standards and Control and Asuragen have developed the following prototype reference materials which were field trialled in 2007.

- Freeze dried cell lines
- Armored RNA constructs

International field trials



- The performance of the freeze dried cells was assessed by 14 labs (7 EU, 4 USA, 3 Asia/Australasia) using 7 different protocols and 9 different RQ-PCR platforms.

- The performance of the aRNA samples was assessed by 29 labs (22 EU, 3 USA, 4 Asia/Australasia) analysing 3 different control genes on 14 different RQ-PCR platforms.

- Both freeze dried cells and aRNAs performed well and appear to be suitable for the development of *BCR-ABL* reference reagents

- Large scale production of the freeze dried cell lines took place in Sept

- Reagents to be used as 'higher order' internationally accredited primary reference materials – field trial due early 2009

- The aRNA constructs will undergo a further round of field trial evaluation with the aim of establishing them as secondary reference reagents.

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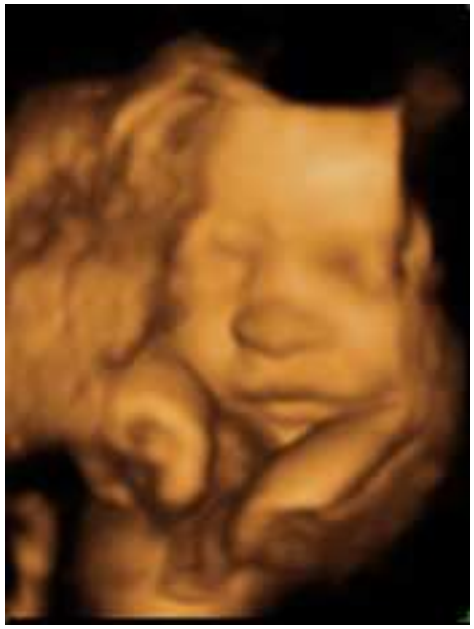
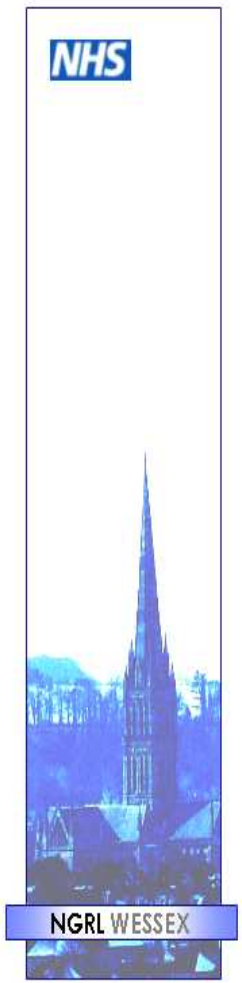
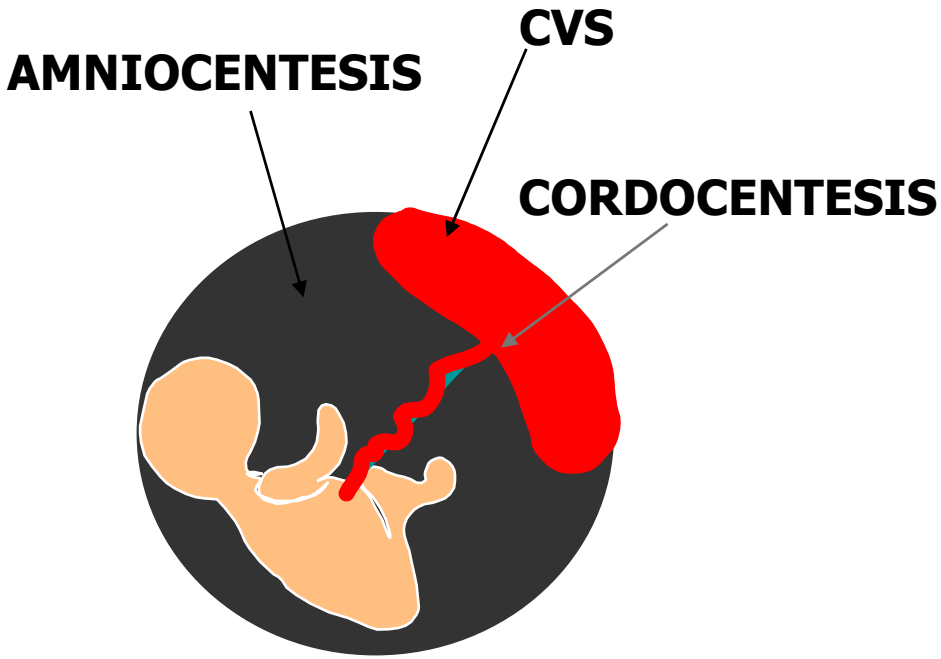


Non invasive pre natal diagnosis of Down Synrome (trisomy 21)

Current prenatal diagnosis requires invasive procedures

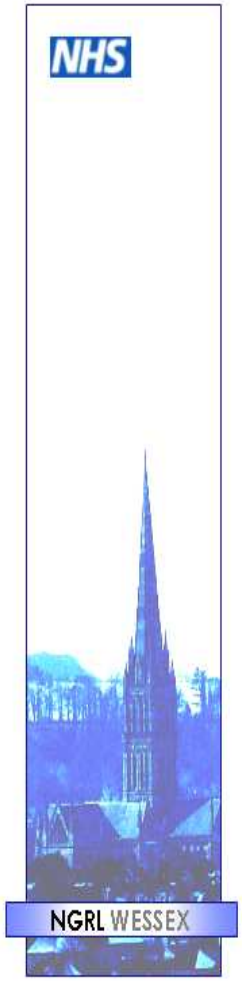




1% risk of miscarriage

Not possible before 11 weeks' gestation

Down syndrome screening in the UK



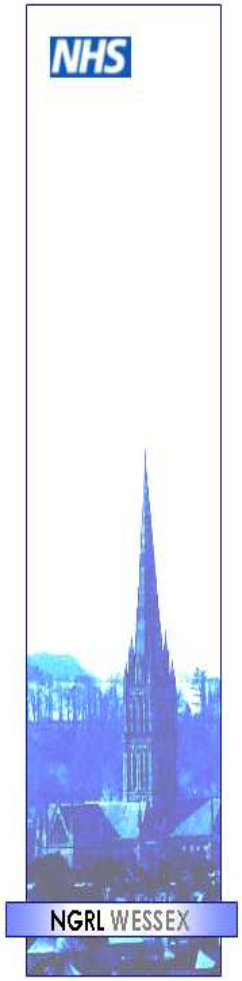
- The invasive approach to obtaining fetal tissue and DNA is currently the gold standard for prenatal diagnosis

- Many women are reluctant to undergo invasive testing, either due to the small but significant risk to the pregnancy, or because they would not terminate the pregnancy irrespective of the results.

- Of the approximately 700,000 pregnant women per year in the UK, around 30,000 amniocentesis tests and 8,000 CVS tests were conducted in the period 2002/03 (Human Genetics Commission, 2004), potentially causing around 460 miscarriages.

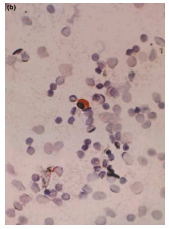
- A reliable and convenient method for non-invasive prenatal diagnosis (NIPD) has therefore long been sought to reduce this risk of miscarriage and allow earlier diagnosis.

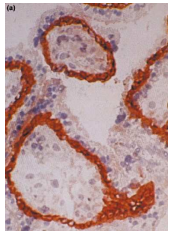
Other sources of fetal tissue for non-invasive prenatal diagnosis



Fetal cells in maternal circulation

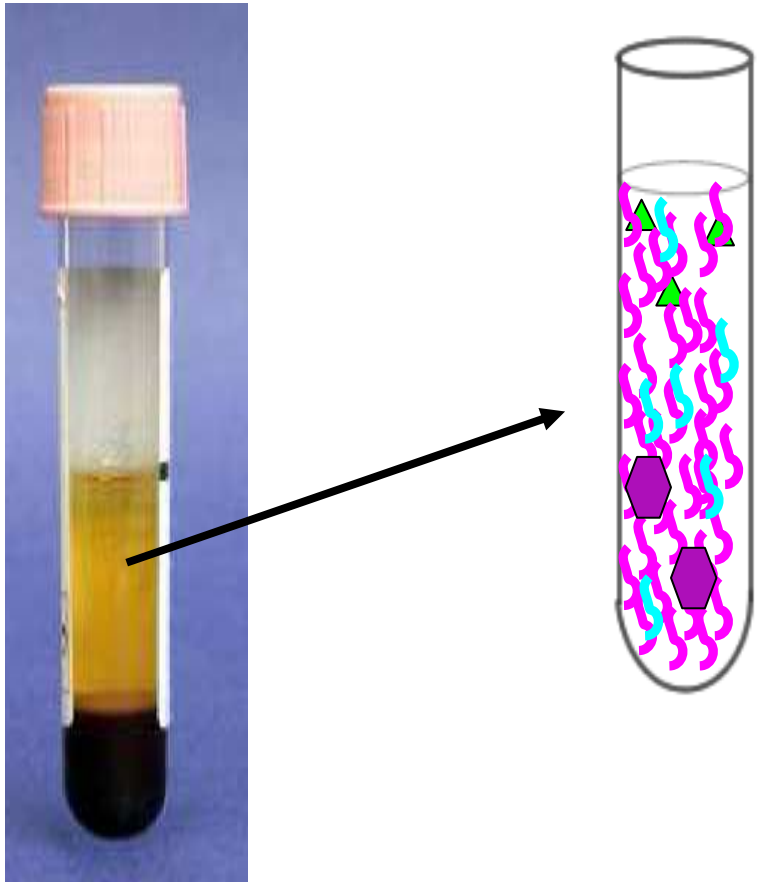
erythroblasts
trophoblastic cells
leucocytes





Difficult to isolate and persist for years after pregnancy

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

Cell free maternal RNA

Cell free fetal RNA

Detectable from 5 weeks' gestation and cleared from circulation within 30 minutes of 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’

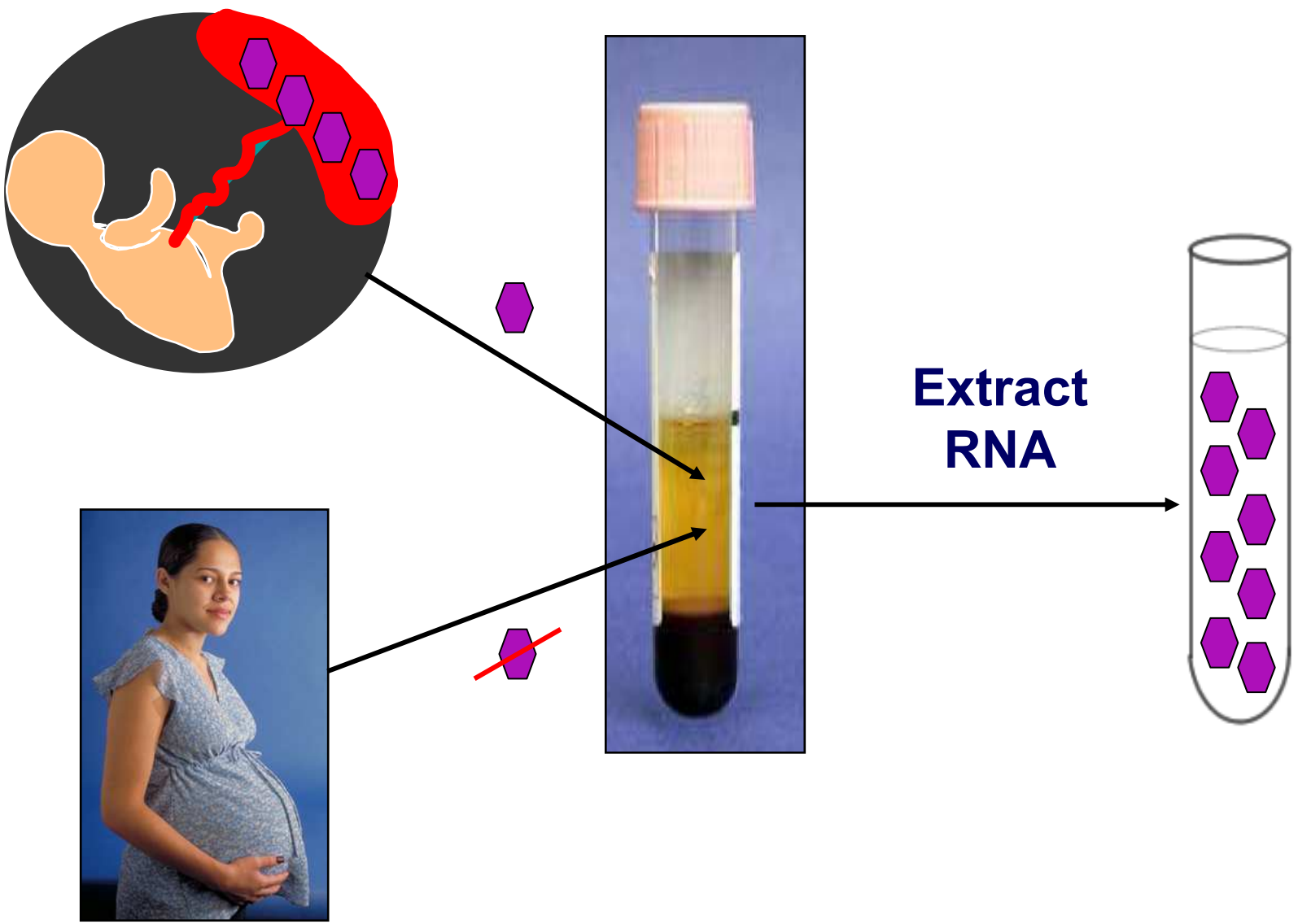
- **Recent major breakthroughs**

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

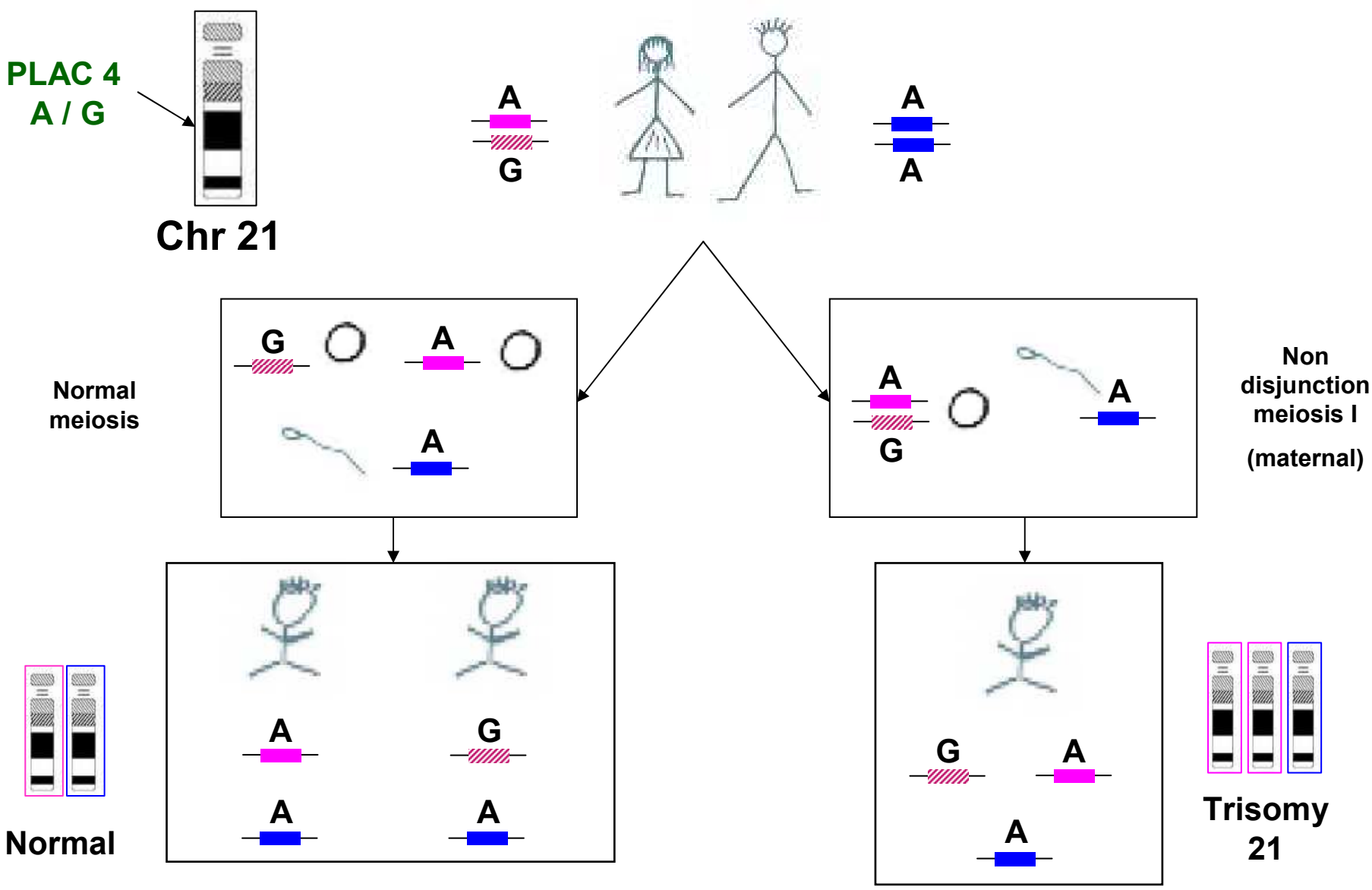
  Digital PCR of cfRNA and cfDNA

  Shotgun sequencing of cfDNA

Quantitative analysis of SNPs in fetal specific mRNA



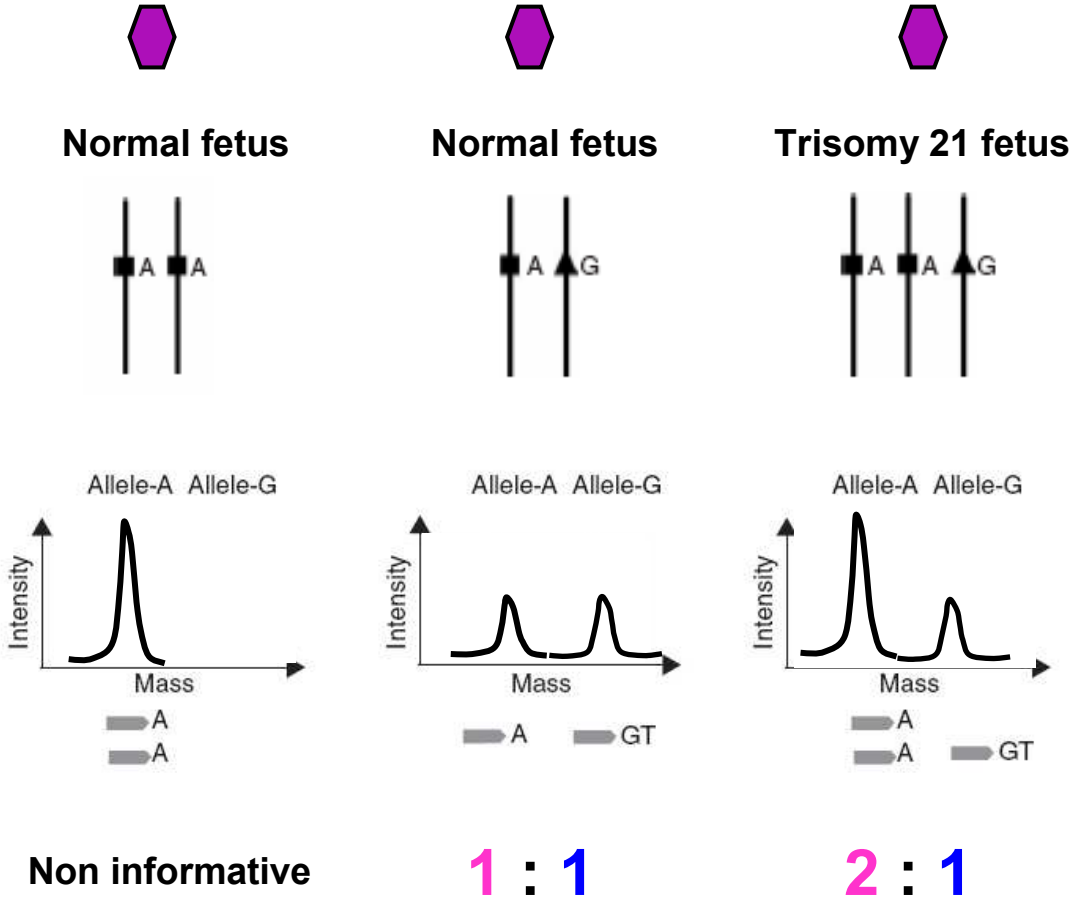
Quantitative analysis of SNPs in fetal specific mRNA



Quantitative analysis of SNPs in fetal specific mRNA

Analysis by MALDI-TOF (mass spectrometry)

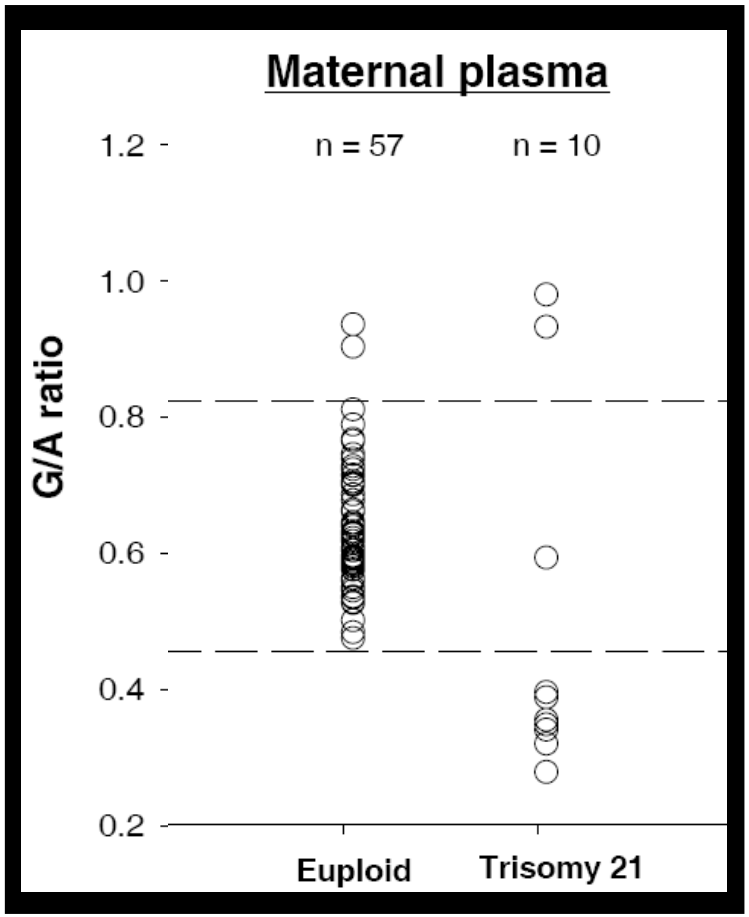
- PLAC4 mRNA ( ) is derived exclusively from fetal chromosome 21
- PLAC4 mRNA expressed in the placenta and is found in the plasma of pregnant women



Lo et al., Nature Med & PNAS, 2007

Quantitative analysis of SNPs in fetal specific mRNA

Analysis by MALDI-TOF (mass spectrometry)

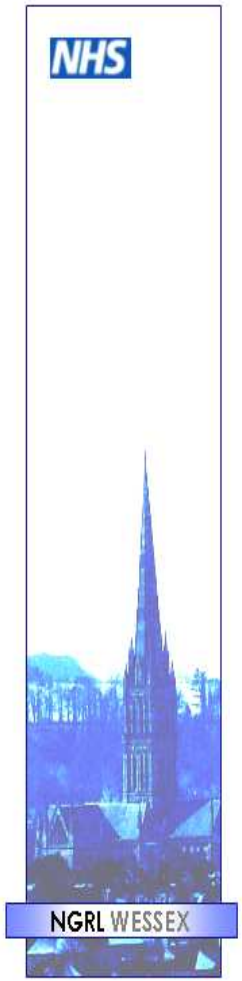


- Correctly diagnosed fetal trisomy 21 in **90%** of +21 cases (n=10)
- Excluded diagnosis of trisomy 21 in **96.5%** of normal controls (n=57)

- Sensitivity: 90%
- Specificity: 96.5%

Lo et al., Nature Med & PNAS, 2007

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:

October 2007

Submission of outline proposal (John Crolla and Helen White: co-applicants)

December 2007

NIHR outline proposal successful. Full submission invited

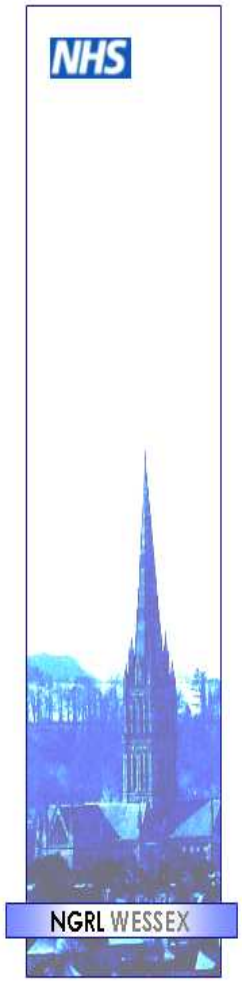
March 2008

Submission of full application (John Crolla and Helen White: co-applicants).

Nov 2008

£2 million awarded

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



(£2 million NIHR Programme Grant – awarded Oct 2008)

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:

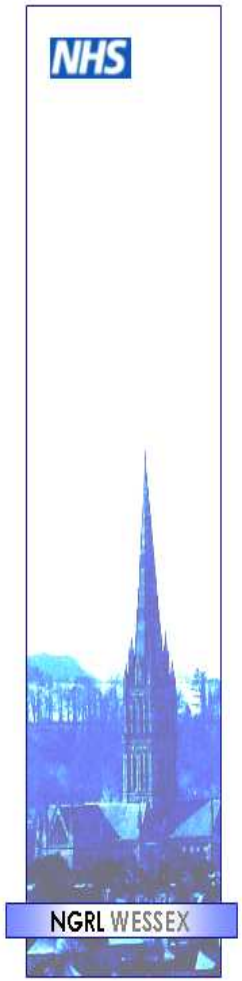
- Fetal sex determination
- Single gene disorders
- Down's syndrome (DS)

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

RAPID: Role of NGRL (Wessex)



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

• Undertake pilot feasibility studies for DS testing

- mass spectrometry (in collaboration with Sequenom)
- digital PCR (in collaboration with Fluidigm)
- targeted new generation sequencing

• Undertake population based feasibility study of NIPD for DS testing

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

• Produce or (co-ordinate production) of prototype reference materials in collaboration with National Institute of Biological Standards and Control and NGRL (M)

• Produce standardised protocols and co-ordinate dissemination in collaboration with Great Ormand Street Hospital and NGRL (M)

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

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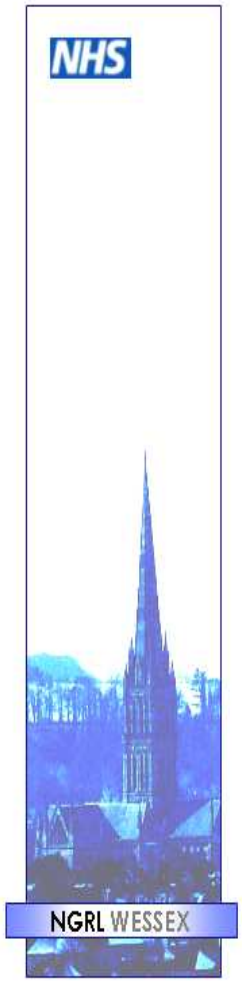
Diagnostic application of next generation sequencing technologies

454 GS FLX
Life Sciences (Roche)

Genetic Analyzer
Solexa (Illumina)

SOLID sequencing
(Applied Biosystems)

Next generation sequencing



- Research is uncovering the genetic causes of multi-factorial diseases where many genes may be implicated

- Implementation of diagnostic tests for such disorders will demand ever increasing capacity to sequence multiple genes for each referral.

- Over the past two years a new generation of sequencing technologies has become commercially available.

- Very high throughputs have been achieved by massively increasing the density of analyses that can be performed in a single run.

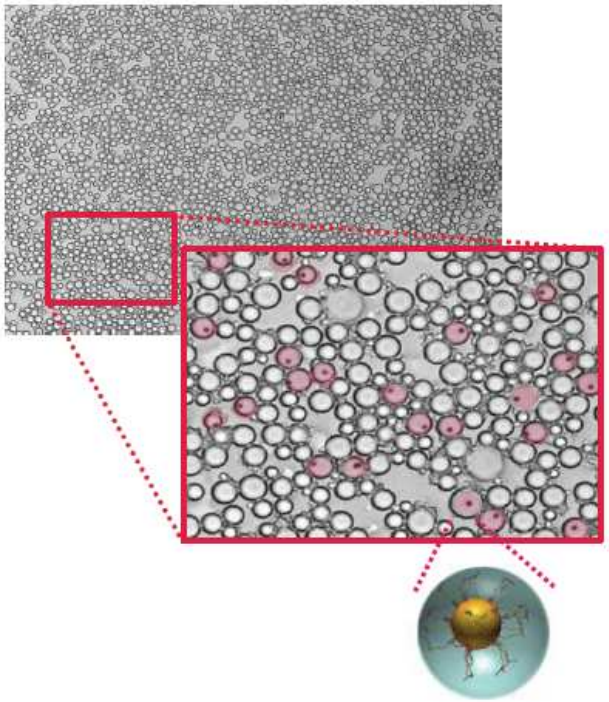
- With conventional Sanger sequencing by capillary electrophoresis up to 384 sequences (more usually 96) can be generated in a single run.

- The new, or “next”, generation sequencing technologies can deliver capacities several orders of magnitude greater than is possible by capillary sequencing.

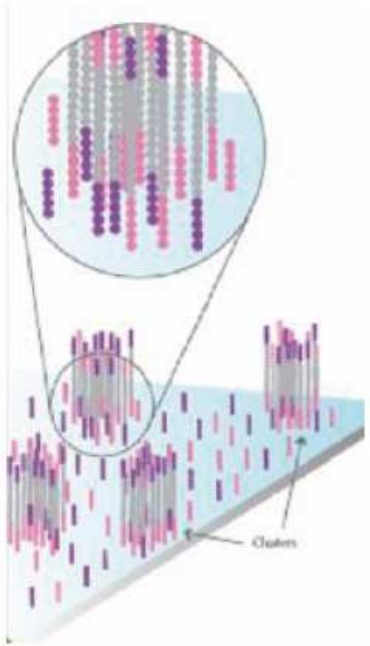
Next generation sequencing

- In situ amplification of single DNA molecule

Emulsion PCR (454 & SOLiD)

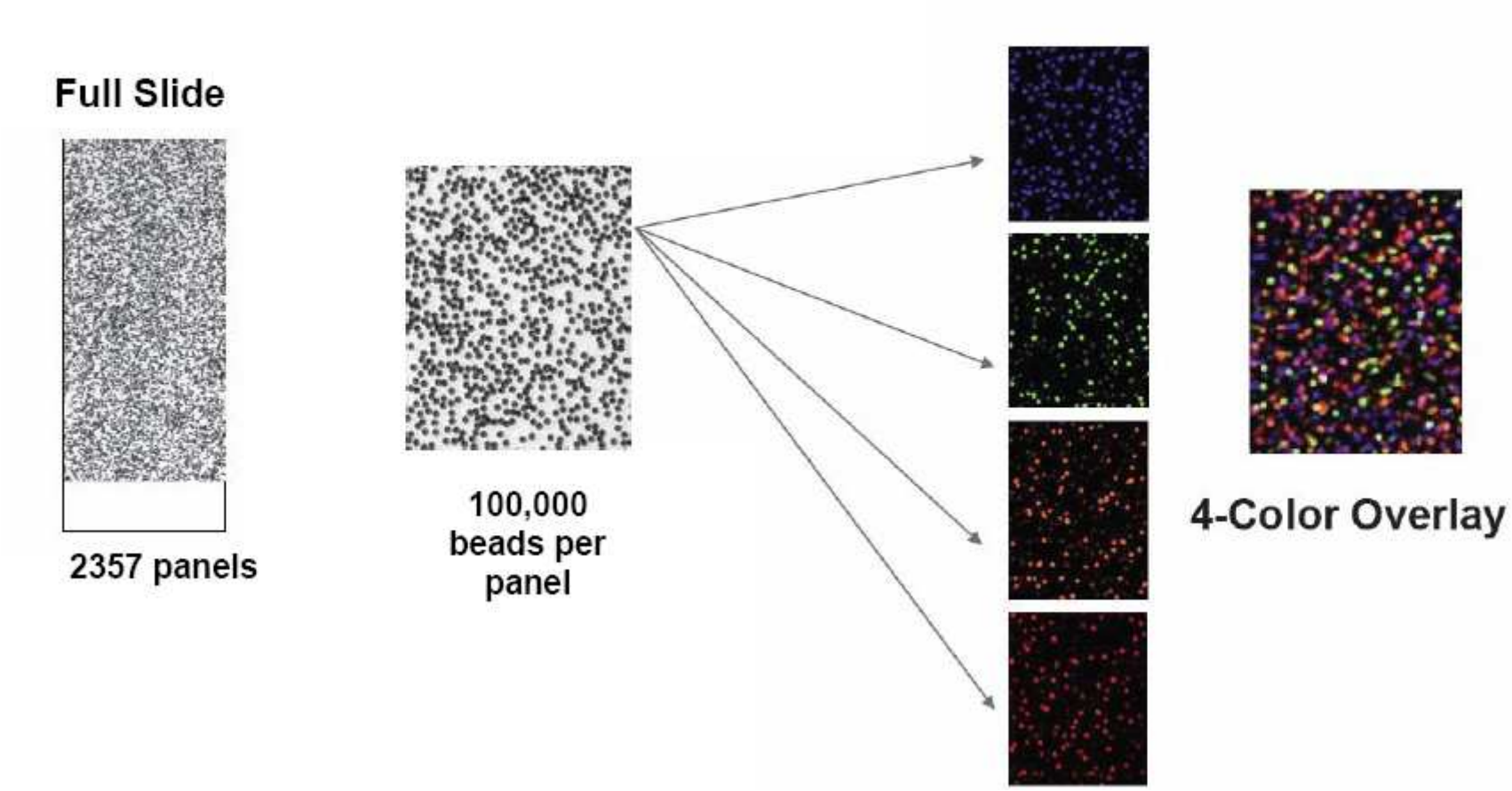


Bridge amplification (Solexa)

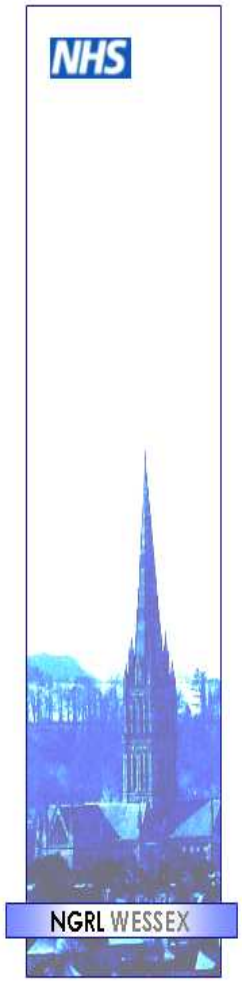


Next generation sequencing

- Massively parallel sequencing



Next generation sequencing



- Application of these technologies has mainly focused on large scale, genome wide, research applications

- But they have potential to profoundly impact on the scope and scale of genetic diagnostics

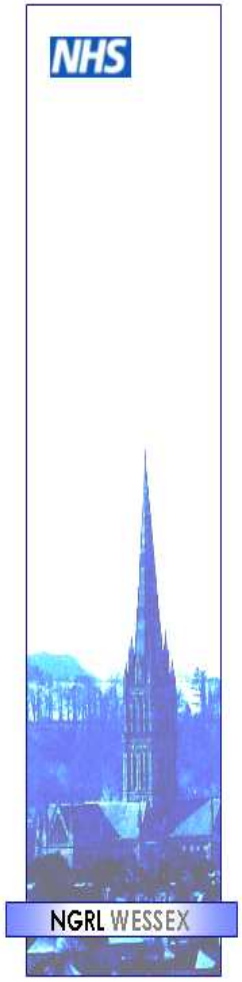
- Significant innovations are required to utilise these technologies in the diagnostic setting:

    - The ability to target regions / gene of clinical interest

    - The ability to ID tag samples to enable multiple tests to run in parallel

    - IT infrastructure and knowledge to enable analysis of the data

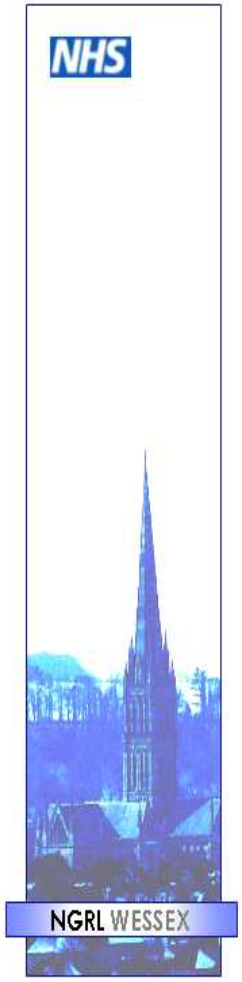
Next generation sequencing



Aims

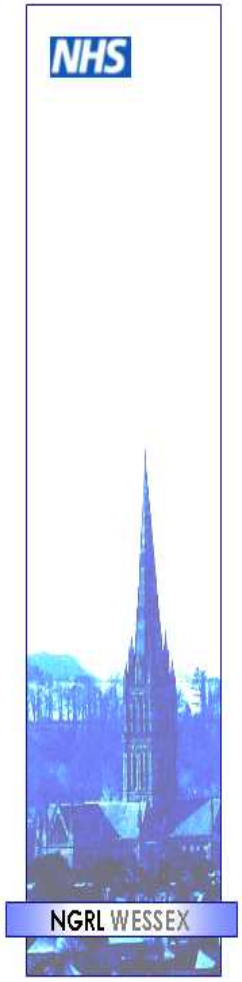
- Co-ordinate efforts of early users within the NHS via an advisory group and the ***CMGS NGS interest group***
- Develop protocols for sample preparation for a range of applications applicable to diagnostic use.
- Ongoing evaluation of the different platforms
- Provide timely feedback to companies
- Monitor development of existing and new technologies

Next generation sequencing: specific projects



- **Three main applications**
  - mutation scanning DNA / RNA
  - quantitative analysis
  - genome architecture
- **The key areas of interest will be:**
  - Enabling analysis of different samples / tests on one instrument run.
  - Rationalising the sample preparation workflow.
  - Interaction with NGRL(M) regarding data analysis and IT issues.

Next Generation Sequencing: In the pipeline

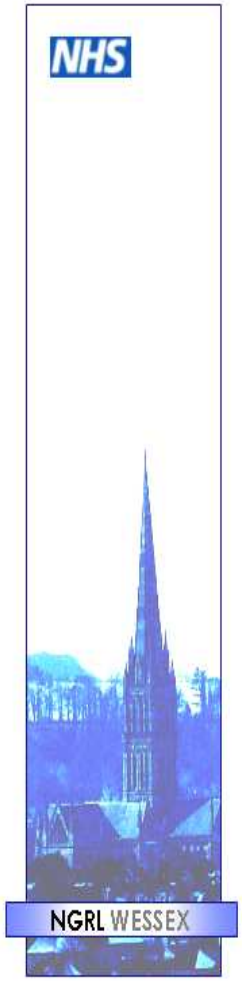


NEAT grant application:

- This application will allow us to expand the scope of our work on NGS technologies – Should the application not be successful we will continue with plans outlined in the proposal but the scale of work will be reduced

- Funding decision expected By 28th November 2008

Johan den Dunnen
Head of Centre
Leiden Genome Technology Centre



Future ahead:

"***Soon*** we will be able to sequence a complete human genome"

but if we can not make sense out of the variants detected, as to whether they are "pathogenic or not", this information is useless and misinterpretation…."

Summary:

"As clinical lab,
do not buy a system yet,
use that of your colleague,
but start saving money,
in 3-5 years you need it…!!"

Array CGH

NHS

NGRL WESSEX

- Aim: To develop a robust, cost effective, validated targeted oligonucleotide array CGH platform to detect common microdeletions and microduplications

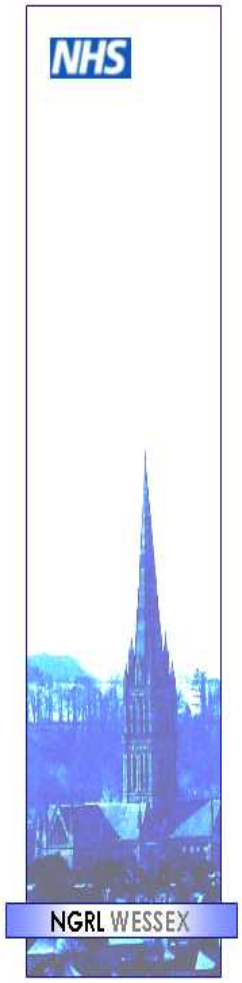
- We have designed and validated a customised 4x44K Agilent array for constitutional aCGH:

  - o designed to provide the maximum genome wide resolution with the probes available

  - o Focuses specifically on the known micro-deletion and duplication syndromes.

- Design currently being upgraded to the new 4x180K format to provide better genome wide coverage

Cytogenetic Resources



Chromosome Anomaly Collection

Catalogue of unbalanced structural chromosome abnormalities without phenotypic effect.

Chromosome Microdeletion / duplication syndrome

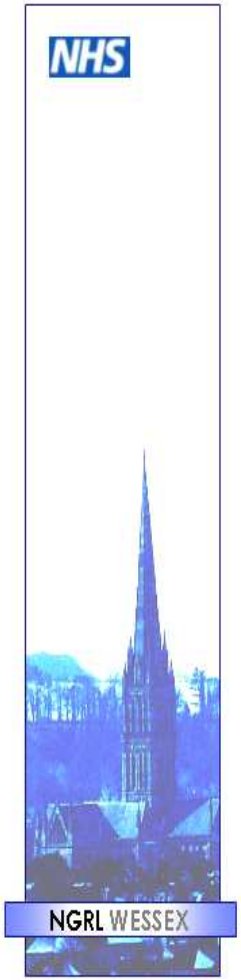
This collection was compiled by searching:

- o OMIM and PubMed databases using “microdeletion syndrome”, “monosomy syndrome” and “microduplication syndrome”
- o Data from current published microarray series
- o Tests currently offered by the Key Locus Service of the NGRL (Wessex)

Subtelomeric collection

Catalogue of sub-telomeric deletions, duplications and rearrangements that may have no phenotypic consequences

Cytogenetic Resources



Probe bank and key locus service

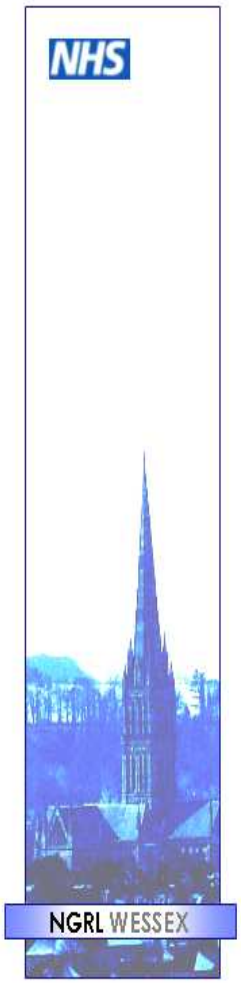
Utilise the molecular cytogenetics resources of the Wessex Regional Genetics Laboratory by obtaining probes from:

- o Human Genome Mapping Project Resources
- o Research Collaborators
- o Web-based FISH probe resources

Within the resources of the NGRL we are able to:

- o Interrogate the www databases to identify locus specific probes
- o Order BACs, PACs, cosmids etc from genome resource centres
- o Prepare the selected probes for FISH
- o Validate the FISH probes
- o Store glycerol stocks
- o Store genomic and labelled DNA from all probes

Meetings and Communication



Meetings

New and Developing technologies meeting: Salisbury, July 2010

BCR-ABL meeting: London, 27th Nov

CMGS NGS Interest group meeting: Leeds, 15th Dec

Conferences:

CMGS Exeter March 2009

BSHG Warwick August 2009

More information:

Website: www.ngrl.org.uk/Wessex

E Bulletin: LISTSERV@LISTSERV.MANCHESTER.AC.UK

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Jan Mitchell