Applications of Next Generation Sequencing for Newborn Screening

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Improving testing targeting treatment

Sheffield Children's NHS Foundation Trust

Climb™ Children Living with Inherited Metabolic Diseases

Expanded Newborn Screening

CLAHRC for South Yorkshire

NHS National Institute for Health Research

medipex® healthcare innovation hub

ThermoFisher SCIENTIFIC

Sheffield Diagnostic Genetics Service

life technologies™

Public Health England

The University Of Sheffield.

wellcome

Department of Health

Health Innovation Challenge Fund

Overview of Presentation

- Challenges and advantages of Next Generation Sequencing (NGS) for NBS
- Project aims, progress made and future work
  - 1) Genotype-phenotype database development
  - 2) Laboratory optimisation of NGS for NBS

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Challenges of NGS for NBS

- Hot topic – concerns regarding whole genome or whole exome sequencing for NBS
- Medical, psychological, ethical and economic reasons against
- Need clear best practise implementation guidelines
- Should focus on targeted gene analysis only?

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The potential role of NGS for NBS

- Primary test for current doisoders
- Primary test for disorders where there is no suitable biochemical test available
- Adjunct test to biochemical testing – use in combination to confirm metabolic results
- Obtain a clearer picture of disease severity, expected onset (early or late) and treatment regimen

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Aim 1: database development

- Development of a genotype-phenotype database
- Important clinical resource
- Enhance disease understanding

- For 6 inherited metabolic diseases:

  MSUD, HCU, IVA, GA1, PKU & MCADD

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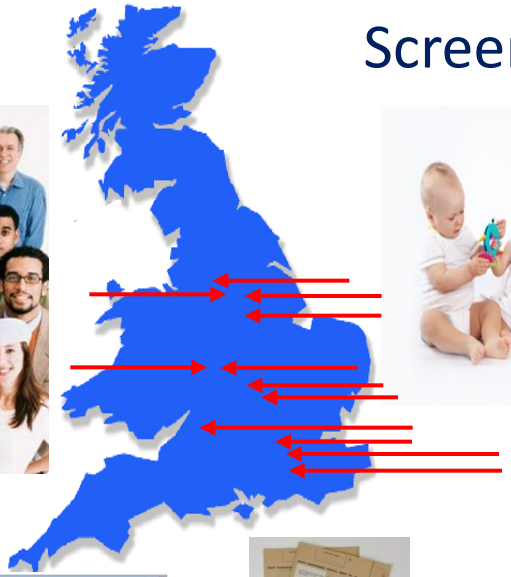
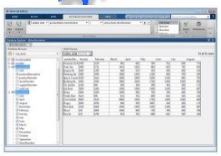
Healthy controls

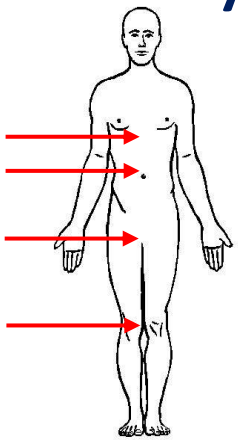


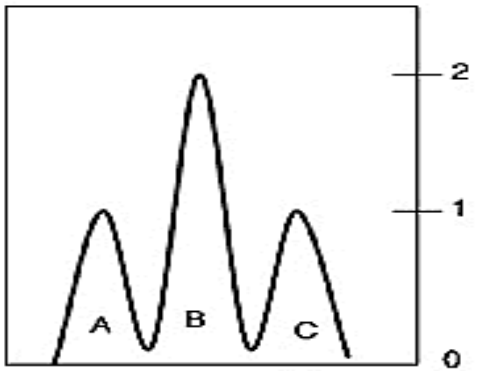
Screen Identified

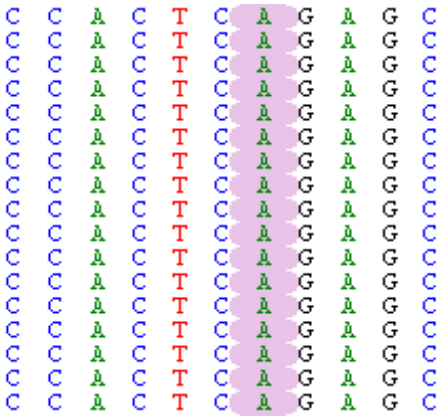
Clinically Identified



Genotype : phenotype database







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Current Data Fields

Participant Information

Birth, Development and Growth

Genetic Sequencing Results

Screen Identified Initial diagnostic visit	Screen Identified Regular visit	Clinically Identified Initial diagnostic visit	Clinically Identified Regular visit
NBS data			
Medication, Diet, other advice; Biochemical tests; Symptoms	Medication, Diet, other advice; Biochemical tests; Symptoms	Medication, Diet, other advice; Biochemical tests; Symptoms	Medication, Diet, other advice; Biochemical tests; Symptoms

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NGS and Reporting

- Sequencing 150 healthy control and 260 patient samples
- 150 healthy controls and 125 patients recruited so far
- Ampliseq Panel Design for six NBS disorders (13 genes)
- Validating specificity and sensitivity of variant calling
- Diagnostic reporting for all patients recruited
- Genetic variant information transferred to project database

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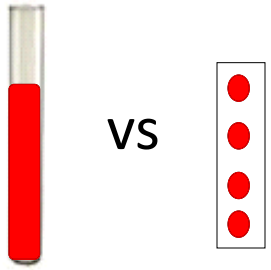
Aim 2: Optimisation of High-Throughput NGS for NBS

- Utilise healthy control individuals' DNA
- Compare DNA extracted from Venous Blood (VB) with DNA extracted from Dried Blood Spots (DBS)
- Aim to obtain same sequence quality from DBS DNA as from VB DNA using NGS



- Use current screened disorders to trial the analysis

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Laboratory Work Overview

DNA extraction: DBS and VB samples

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Assess DNA quality and quantity for NGS

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Design NBS disorder gene panel

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Library to sample preparation for NGS

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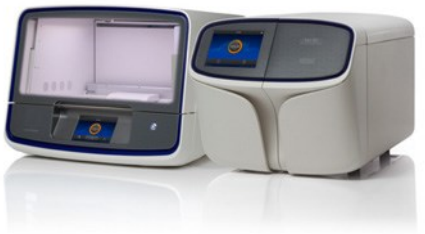
Validation of method for NBS: automation, speed and cost

PGM



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DNA Extraction Results

- A small sample size of healthy control DNA used to optimise extraction methods:
  - 2 variations of VB extraction
  - 10 variations of DBS extraction

- Sufficient quality and quantity of DNA extracted from DBS for use in NGS
  - No significant loss of variant information between VB and DBS DNA
  - Method chosen for DBS extraction that is cost effective and can be automated

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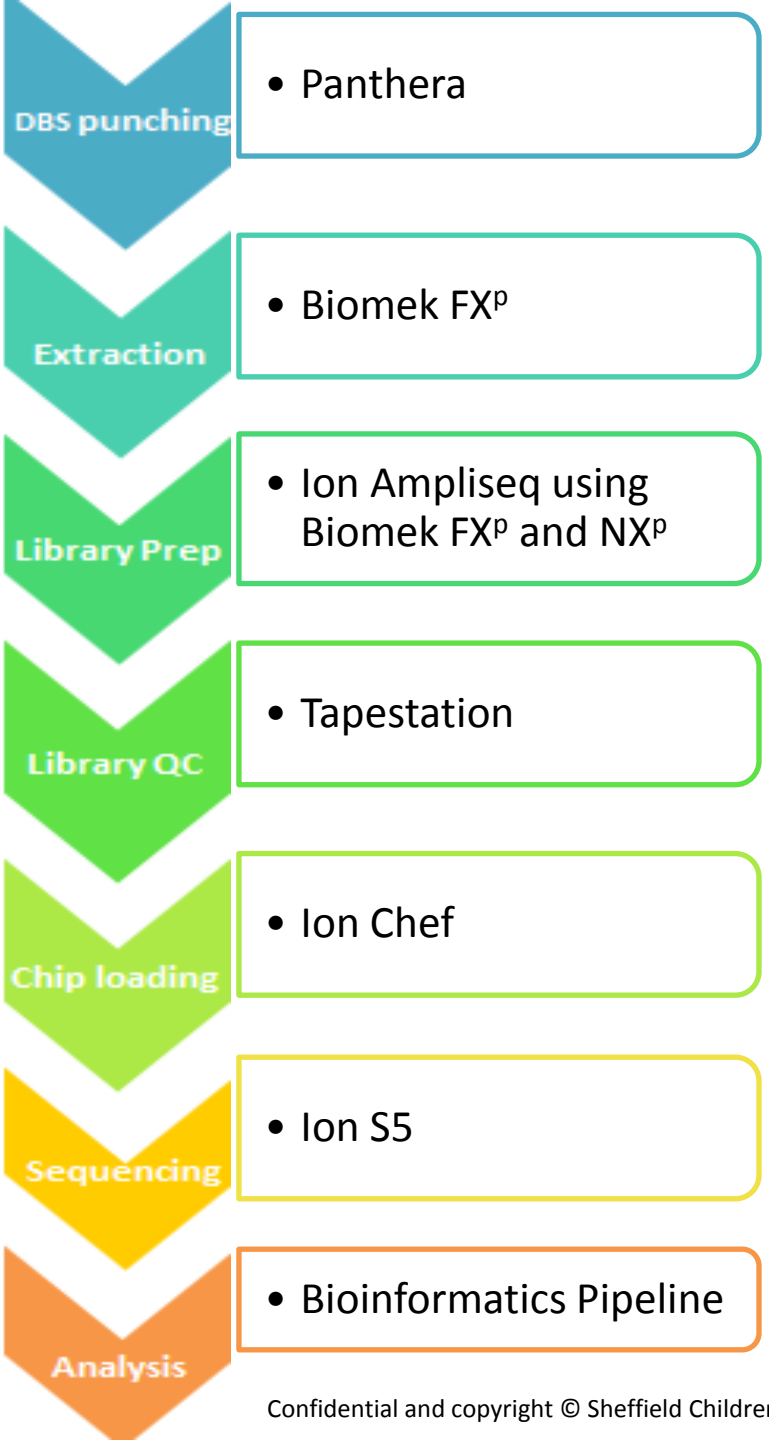

Automated Workflow

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Booking on and Punching

- Booking on of guthrie cards at Sheffield NBS Facility
- Use Panthera DBS puncher with 6 mm punch head
- Punching time: 60 minutes per 96-well barcoded plate
- SDGS will book on 96-well plates containing DBS samples

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DNA Extraction

- Biomek FX^{p} robot program developed
- 3x 96-well plates in 90 minutes
- Sequencing quality of DBS is similar to VB samples

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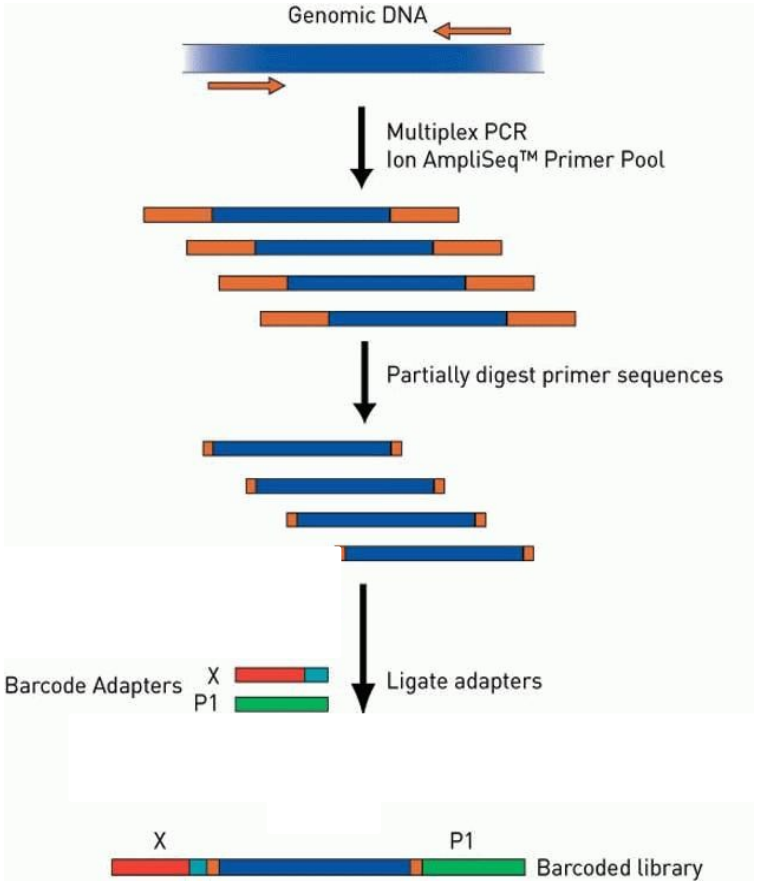
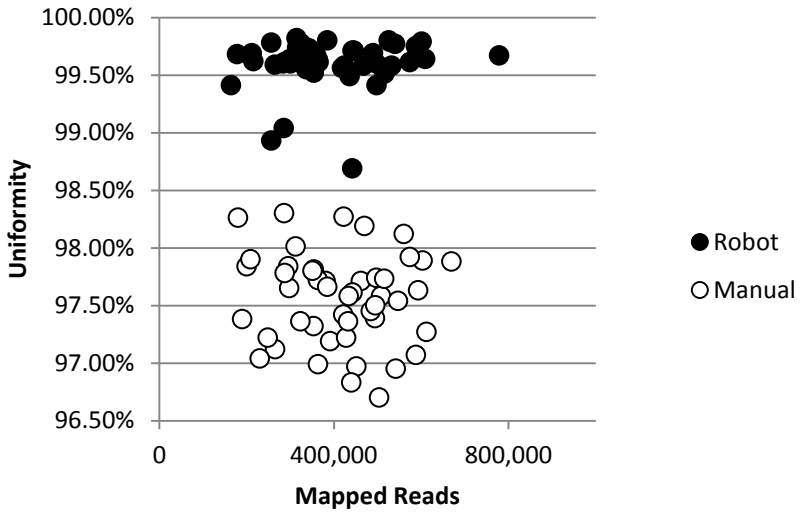
Ampliseq Library Preparation



- Semi-Automated method on the Biomek FX^{p} robot
- Robot outperforms manual library preparation in terms of uniformity of coverage ($p<0.04$)



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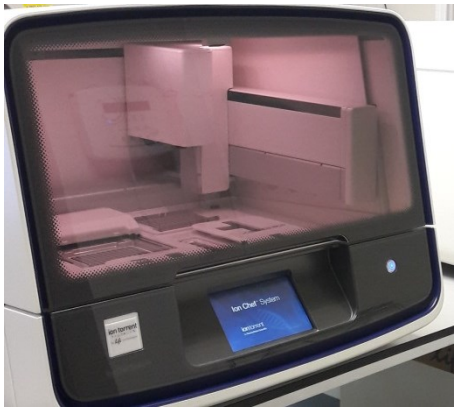
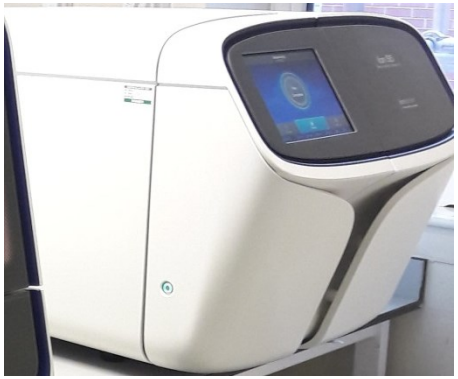
Chip Loading and Sequencing





- Ion Chef: 15 min hands on time; 11h run time (overnight); 2x chips per run
- 192 samples per run using 2x Ion 540 chips
- Ion S5: <15 min hands on time; 2.5h run time per chip
- No significant difference in sequencing coverage or variant calling between DBS and VB samples
- High-throughput bioinformatics analysis pipeline

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Other Laboratory Progress

- Validated 2 NBS panels according to ACGS Guidelines:

  "Best practice guidelines for Targeted Next Generation Sequencing Analysis and Interpretation, 2015"

- Pathogenic variants can be identified using patient sequencing data

- There is no significant effect of the DBS age (1, 7, 30 and 60 days) on sequencing quality and variant calling

- Discussions with Thermo Fisher and Perkin Elmer are helping to drive laboratory developments

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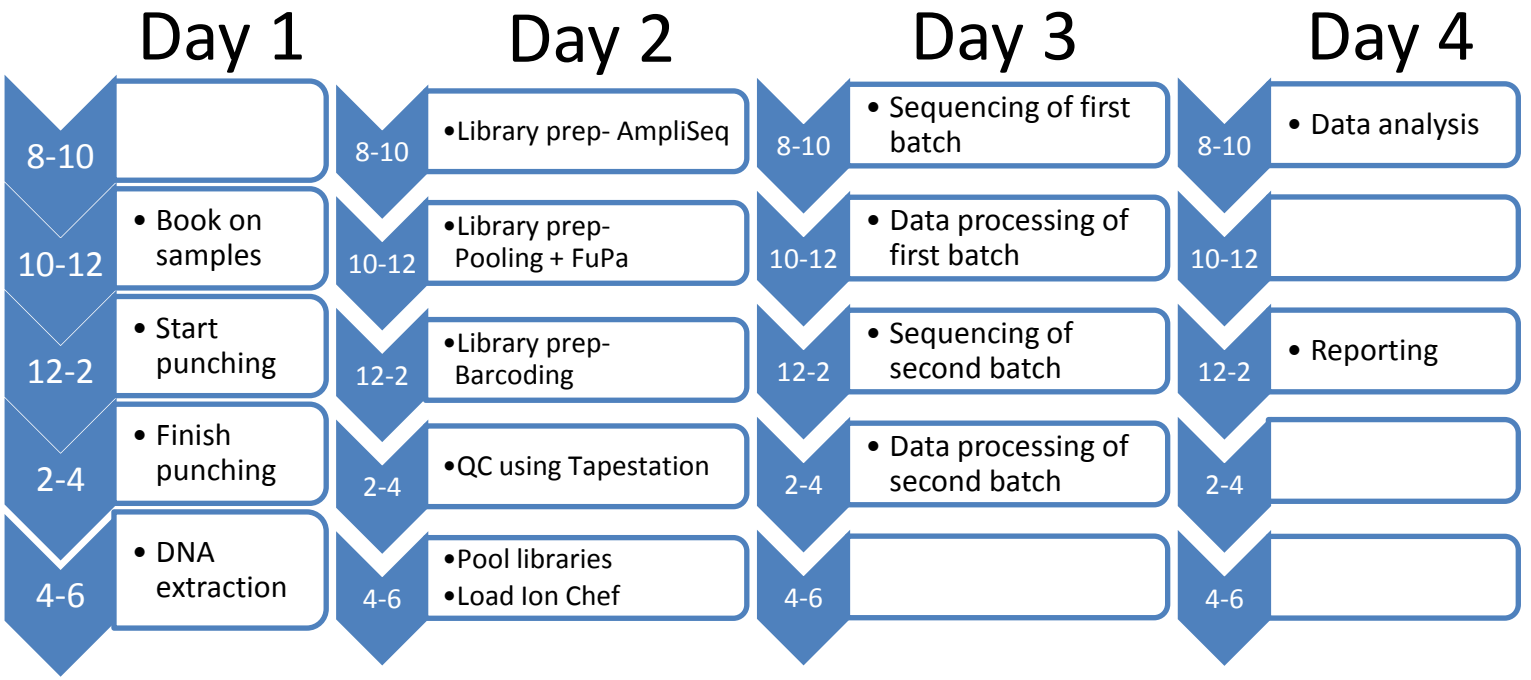
Current Timeline for NBS



- 2 x 96 samples would be punched per day meaning that ~ 1000 samples could be sequenced per week.
- Doubling up on automation equipment would increase sample high-throughput capability.

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On-going work

- Aim1: Database Development
  - Continue sequencing healthy control and patient VB samples
  - Collect patient medical record information into database
  - Perform genotype-phenotype correlative analysis

- Aim 2: Development of high-throughput NGS for NBS:
  - Validate specificity and sensitivity of NGS process
  - Test high-throughput capability and turnaround times of the whole process from booking on to NGS reporting
  - Confirm proof of concept: 1000-2000 samples in one week
  - Perform cost-benefit analysis

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Acknowledgements

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