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Health Innovation Challenge Fund

Bristol Genetics Laboratory

The Application of Next Generation Sequencing to the Analysis of Minimal Residual Disease in Childhood Acute Lymphoblastic Leukaemia

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Bristol Genetics Laboratory WEGMC

MRD Detection

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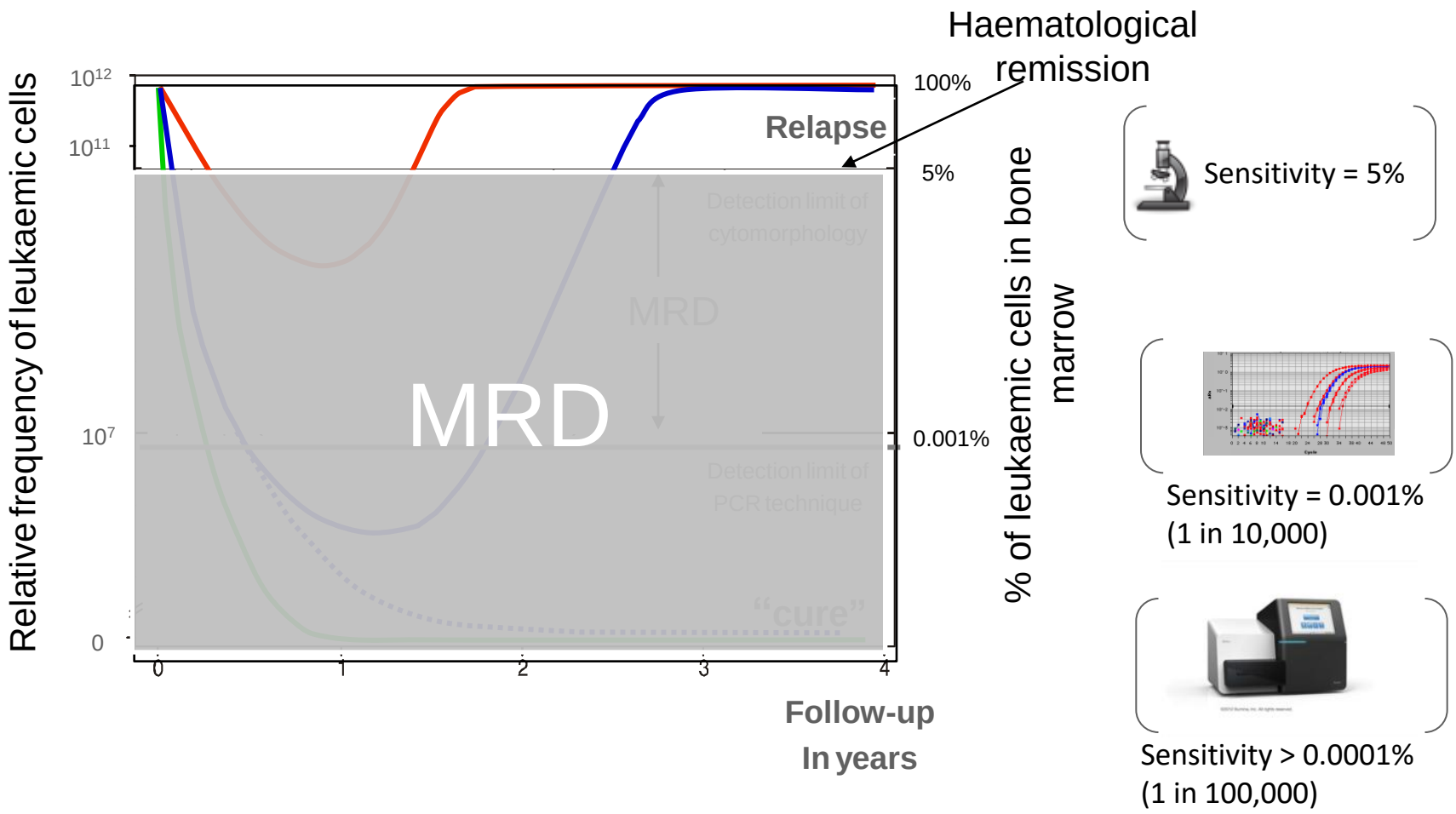
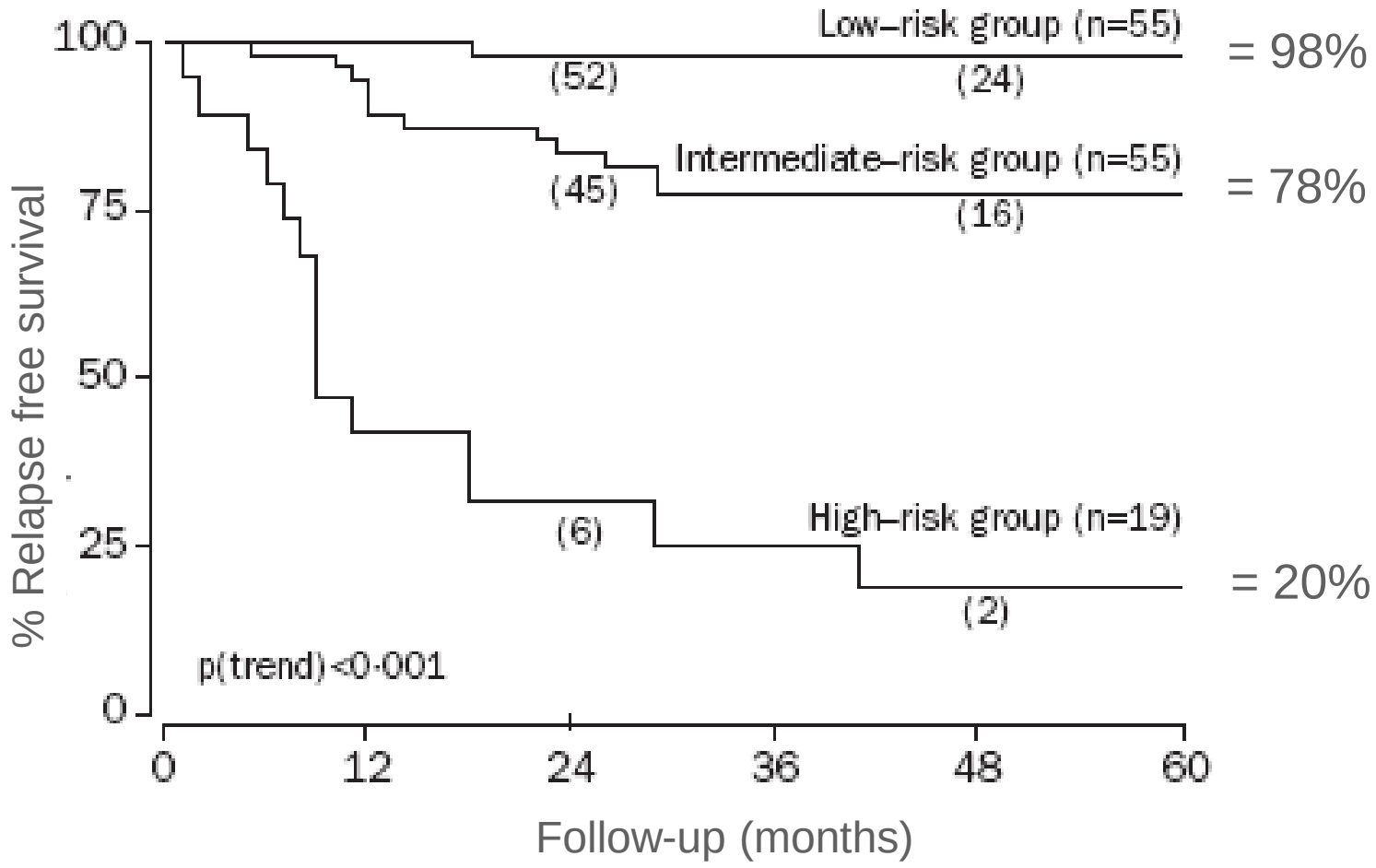


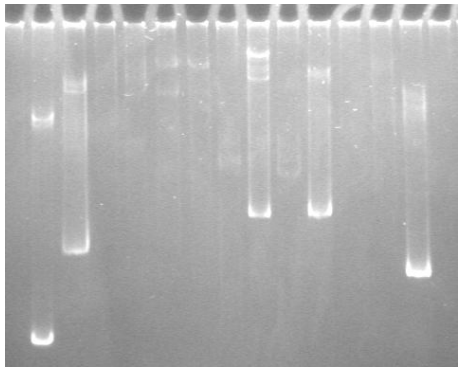
van Dongen *et al*., Lancet 1998

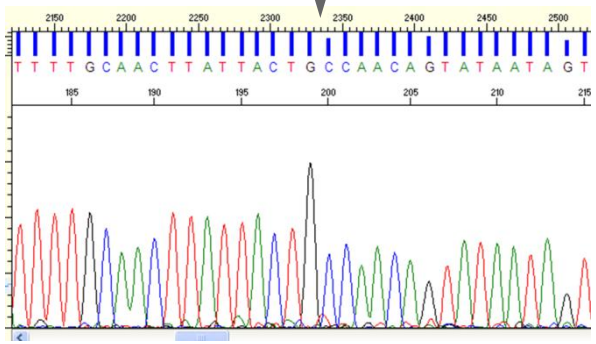


Current Technique

1. Identification of MRD target

- PCR of leukaemic material by IgH, IgK, TcR D, TcR G and TcR B loci
- Heteroduplex analysis
- Purify and sequence clonal PCR product(s)
- Identification of **V**, **D** and **J** segment usage
- Synthesis of 18 - 25 base patient-specific oligonucleotide and optimisation of assays
- ≤ 14 days

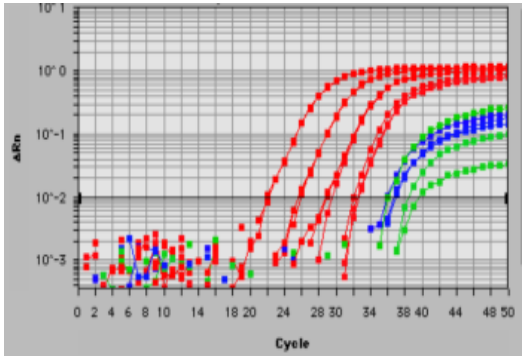


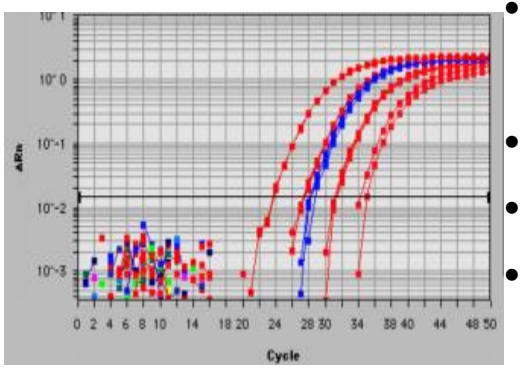


V region ***N region*** ***D region*** ***N region*** ***J region***

...TACTGTGCAATTACGCCTTG**TAGTAGTTACCAGCT**GGAAGC**GCTGAATACTTCCAGC...**

2. Assay Development and Quantitation





- Optimised to a quantitative range 1 in 10,000 cells
- ≤ 7 days assay development
- ≤ 5 days quantitation
- 2 technicians analysing >120 new patients per year

Limitations of Current Technology

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- Expensive: £3,155 per patient
- Complex and labour intensive:
  - 29 day turnaround time from diagnosis to 1st test result
  - limits analysis of time points such as day 8/15
- Applicability limited to ~ 92% due to:
  - Inadequate sample (5%)
  - No MRD targets (1%)
  - Insensitive MRD targets (1%) – lack of diversity over background
  - Closely related species (1%) – > 2 markers at Ig or TCR loci

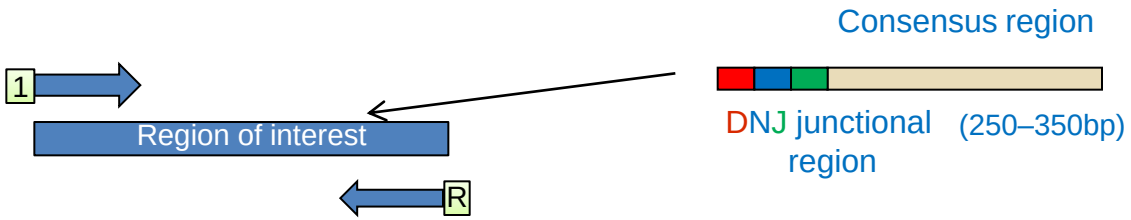
  ➔ *~32 patients each year do not benefit from treatment stratification*
- Limited capacity to learn anything about the leukaemia
  - Inability to study clonal evolution between diagnosis and relapse

Summary of NGS-MRD Methodology

NGS Library Preparation

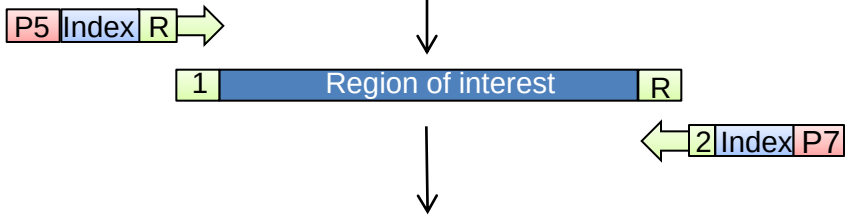
First Stage

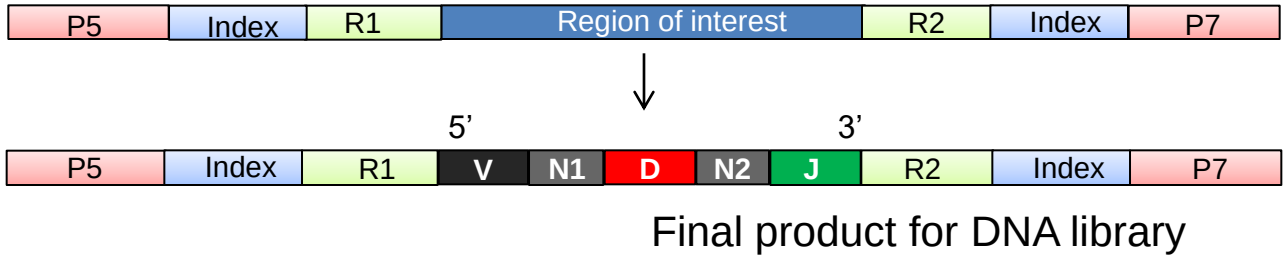
Targeted amplification of Ig and TcR loci



Second Stage

Addition of indices and flow cell adaptors





Final product for DNA library

P5 P7 Bind amplicon to flow cell

R1 R2 Sequencing primers to amplify region of interest

BIOMED-2 primers van Dongen *et al.,* 2003

NGS Workflow

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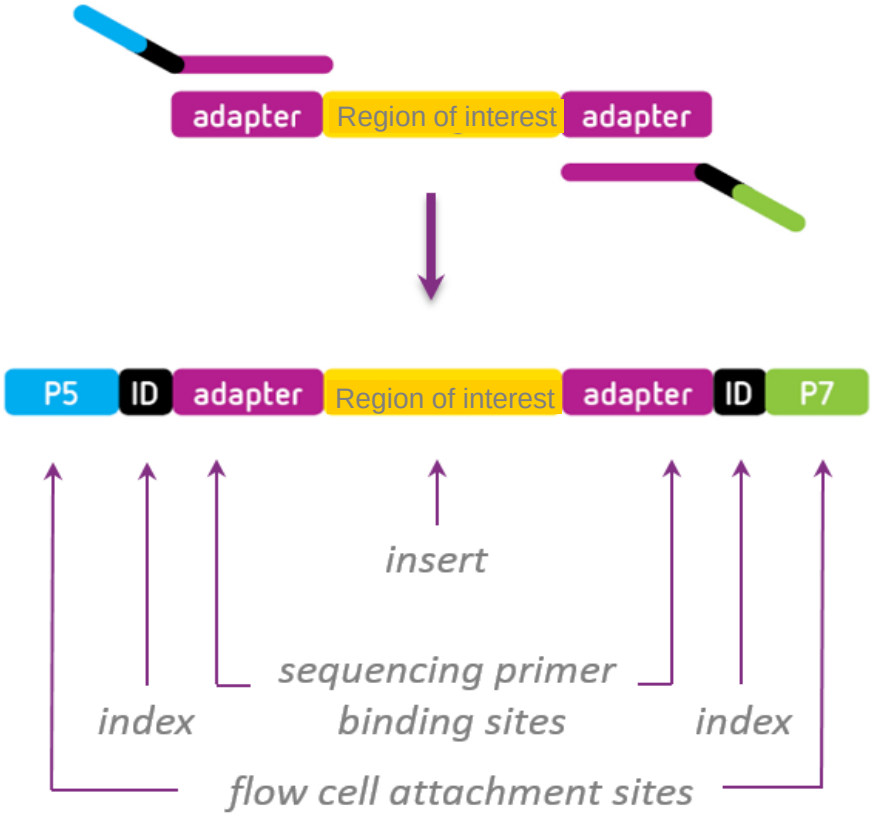
Day 1

1. First stage Ig/TcR PCR
   3 hours

2. AMPure XP clean up
   1 hour

3. Second stage index/adaptor PCR
   2 hours



NGS Workflow

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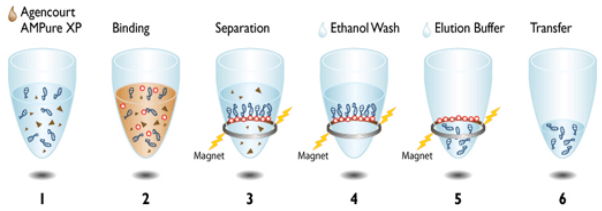
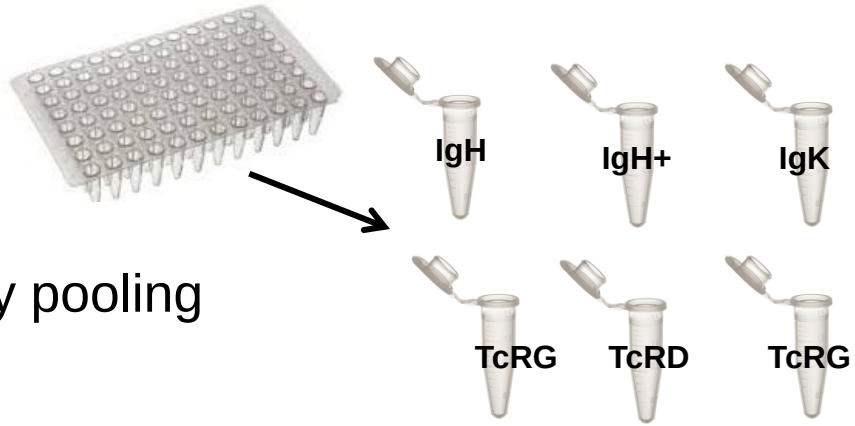
Day 2

1. AMPure XP clean up
   1 hour

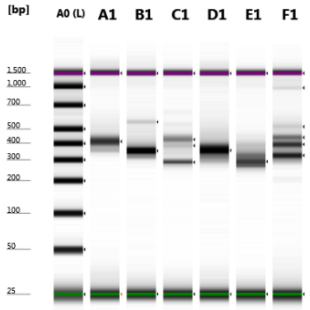


2. Qubit quantification
   1 hour



3. Individual loci library pooling
   1.5 hours

4. TapeStation size measurement
   30 minutes



NGS Workflow

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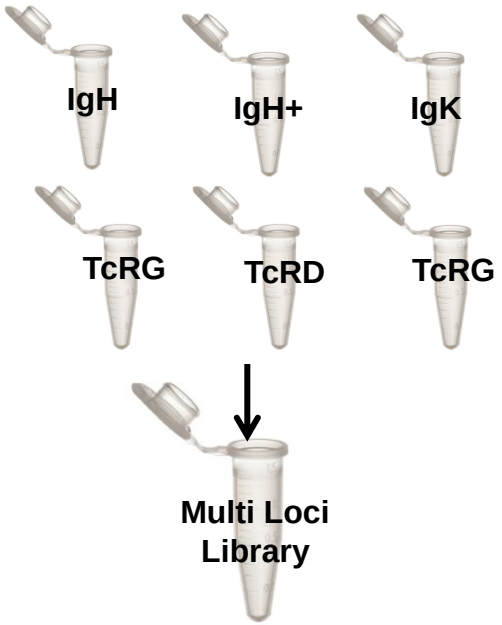
Day 3

1. Final library pooling
   30 minutes

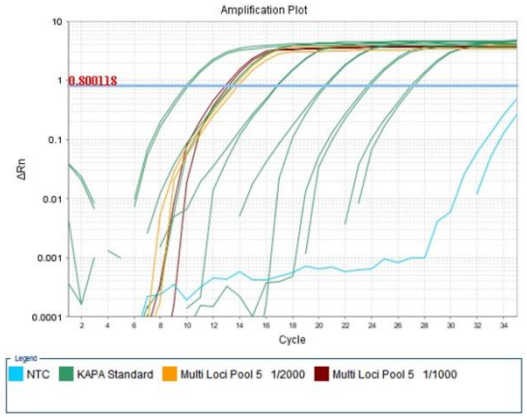
2. KAPA RQ-PCR library quantification
   2.5 hours

3. MiSeq loading
   1 hour



12 Patients/run

14 Hours Total Technical Time



Bioinformatic Analysis Using Vidjil

Vidjil
High-Throughput Analysis of V(D)J Immune Repertoire

- High-throughput analysis of V,D,J immune repertoires
  - Clonality, repertoire and MRD

- Designed by Bonsai Bioinformatics based in Lille, France

Database

- Easy access and compare results

Analysis pipeline

- Rapid – 100K reads/min
- Customisable settings
- Process all loci (Ig and TcR)

Browser

- Identify and enumerate MRD targets
- Visualisation of clones

Vidjil – Grid View

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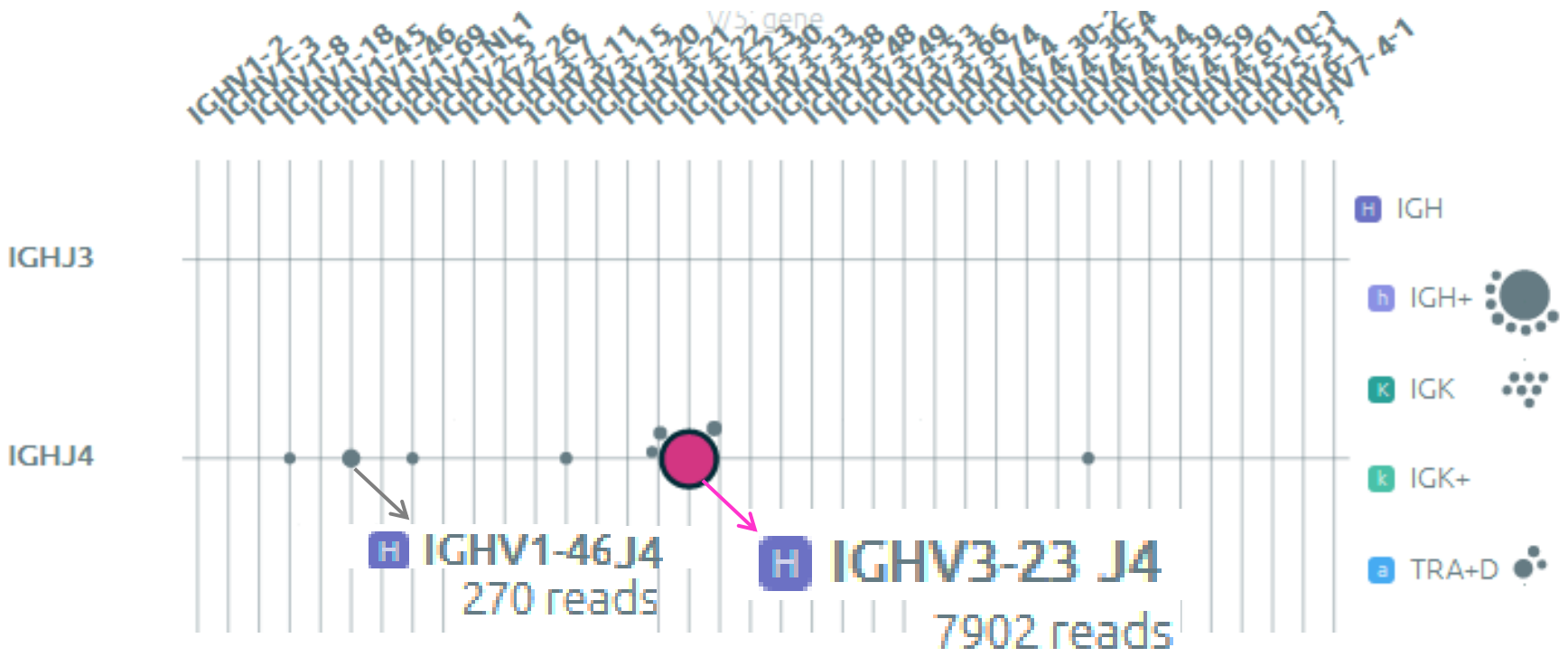

Vidjil – Clone List

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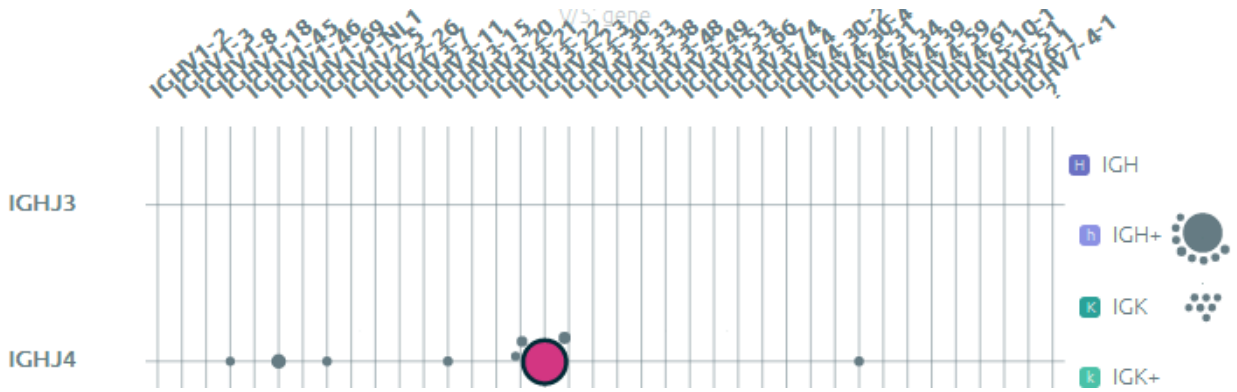

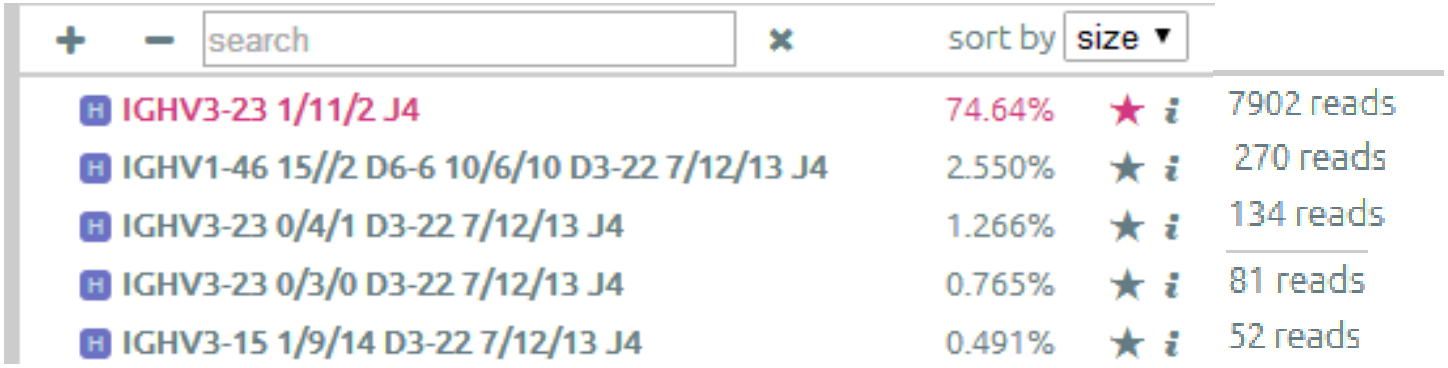

Vidjil – Sequence Alignment

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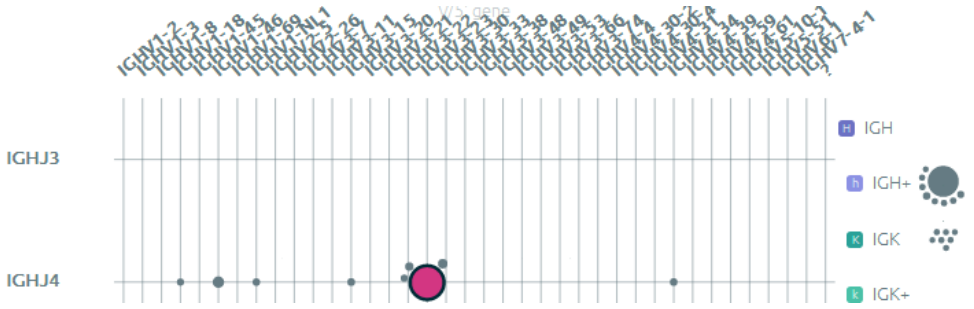
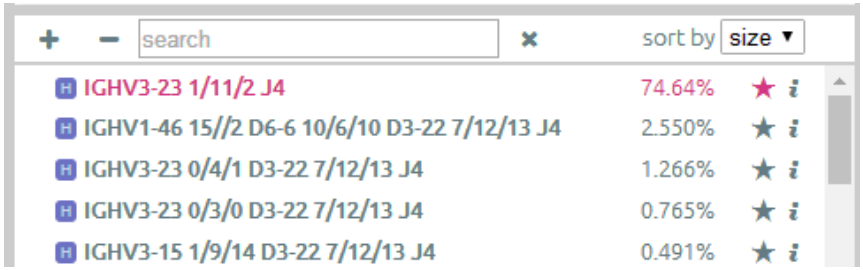
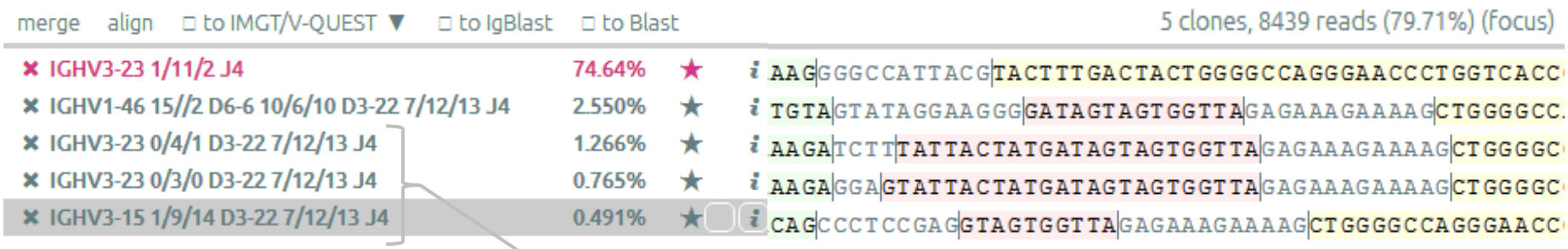
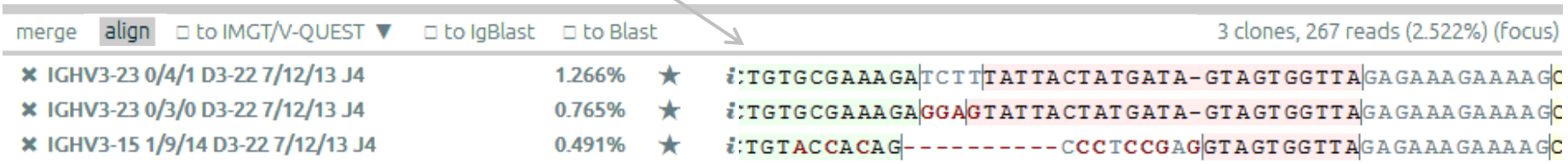
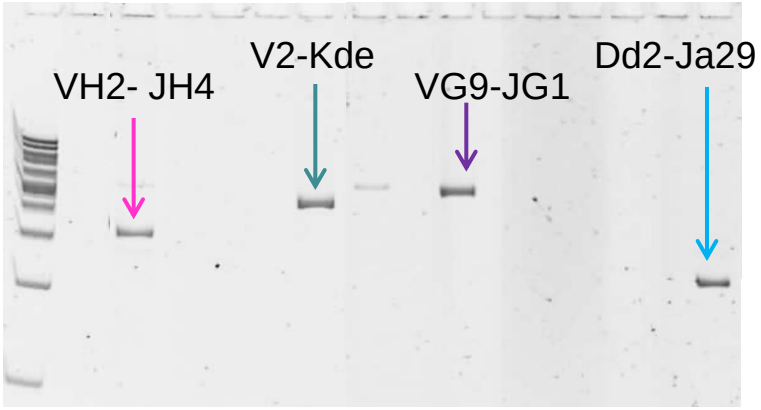






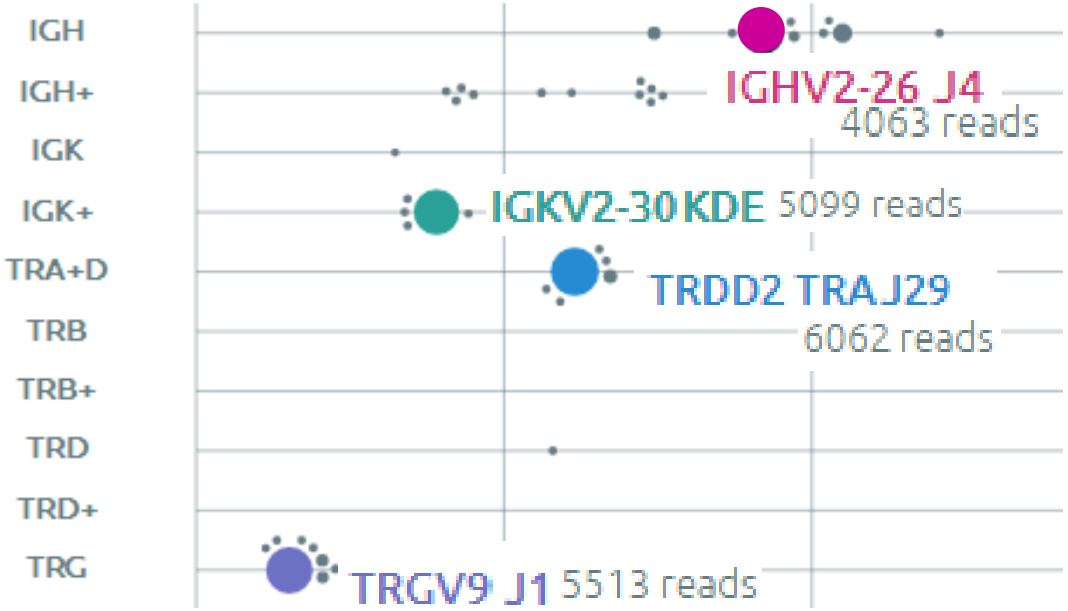


Patient 1



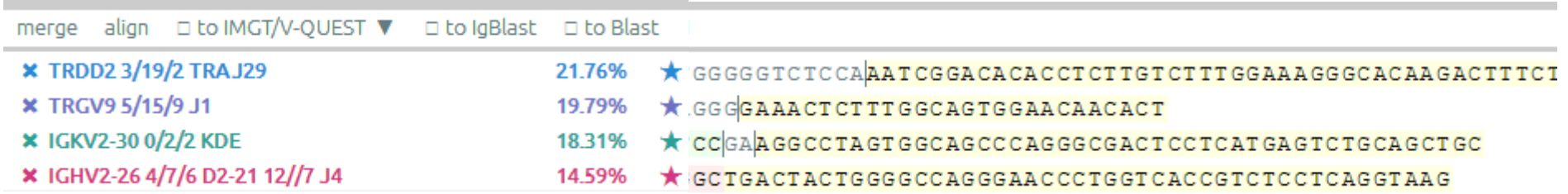
Conventional Analysis

- Four identical IgH, Kappa, Gamma and Delta targets found
- Identical sequences
- Identical junctional analyses



NGS Analysis

- Four major clones
  - IgH, Kappa, Gamma and Delta



Results Summary – Year 1

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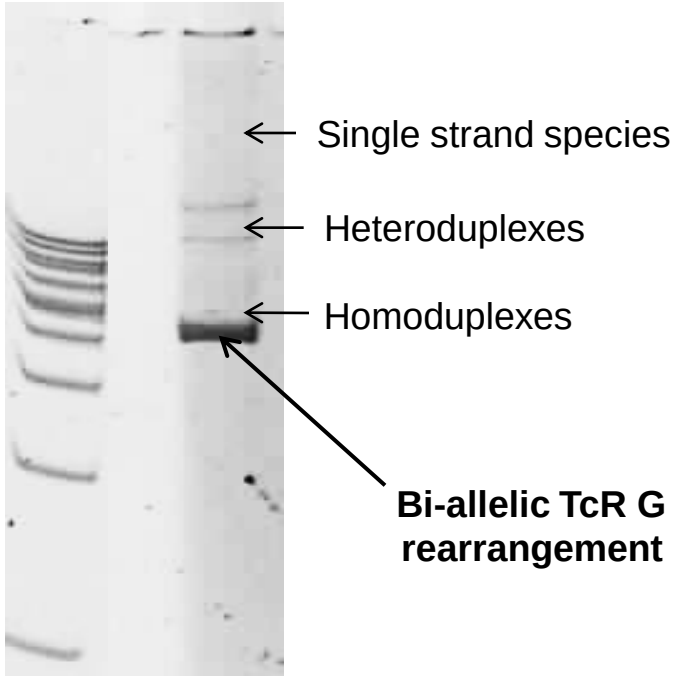
1. Established NGS Methodology for MRD Target Identification
   - Initial singleplex screen
   - Multiplex screen using IgH, IgK, TcRG, TcRD and TcRB

2. Undertaken a Comparison With Conventional Methodology
   - Analysed **63** patients
   - NGS identified **255/256** targets (>99%)
     - TcRG (VG9-JG1) not identified as the NGS primer binding site was deleted during gene rearrangement.

3. Implemented Prospective NGS-MRD Target Identification
   - NGS-MRD now run in parallel with conventional analysis
     - Since January 2016
     - 1 or 2 patients are run each week during assay development

Patient 2

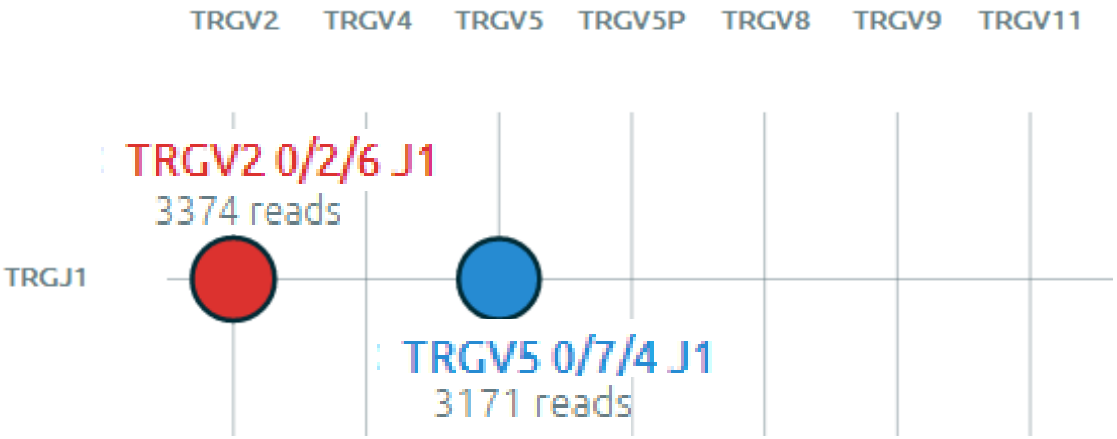


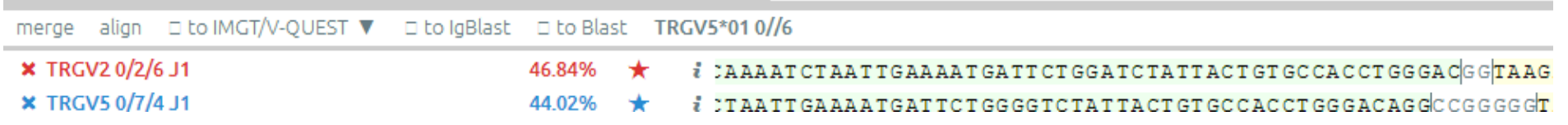
Conventional Analysis

- Bi-allelic TcR-G targets identified but could not be sequenced due to inability to resolve these as separate species
- Therefore two potential MRD markers could not be utilised

NGS Analysis

- NGS successfully sequenced the 2 previously unresolvable TcR-G targets





Future Developments – Year 2

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1. Quantitation
   - Investigate the clinical utility of NGS for MRD-based treatment stratification
   - How best to achieve this?
     - Patient-specific dilution series (as per conventional methodology)
     - Spike-in controls (control cell-lines)
   - Comparison to conventional technology
2. Retrospective Analysis
   - Analysis of a cohort of samples previously quantitated as part of UKALL2011
3. Health Economic Assessment
   - How might this technology be utilised in the UKALL2017 clinical trial?
4. Translation of Research
   - Embedding of NGS-MRD within a service laboratory

Acknowledgements

Bristol Genetics Laboratory

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