Comprehensive translocation and clonality detection in lymphoproliferative disorders by next generation sequencing


Published in:
HAEMATOLOGICA

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2016 Ferrata Storti Foundation
This work is made available online in accordance with the publisher’s policies.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.
Comprehensive translocation and clonality detection in lymphoproliferative disorders by next generation sequencing

by Dorte Wren, Brian A. Walker, Monika Bruggemann, Mark A. Catherwood, Christiane Pott, Kostas Stamatopoulos, Anton W. Langerak, and David Gonzalez

Haematologica 2016 [Epub ahead of print]

Citation: Wren D, Walker BA, Bruggemann M, Catherwood MA, Pott C, Stamatopoulos K, Langerak AW, and Gonzalez D. Comprehensive translocation and clonality detection in lymphoproliferative disorders by next generation sequencing. Haematologica. 2016; 101:xxx
doi:10.3324/haematol.2016.155424

Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.
Comprehensive translocation and clonality detection in lymphoproliferative disorders by next generation sequencing

Dörte Wren1*, Brian A. Walker1,2*, Monika Brüggemann3, Mark A. Catherwood4, Christiane Pott3, Kostas Stamatopoulos5, Anton W. Langerak6† and David Gonzalez1,7† (on behalf of the EuroClonality-NGS consortium)

1 The Centre for Molecular Pathology, The Royal Marsden NHS FT, London, UK
2 Myeloma Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA
3 Second Medical Department, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany
4 Department of Haematology, Belfast City Hospital, Belfast, UK
5 Institute of Applied Biosciences, CERTH, Thessaloniki, Greece
6 Department of Immunology, Laboratory for Medical Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands
7 Centre for Cancer Research and Cell Biology, Queen’s University Belfast, Belfast, UK

*These authors contributed equally to this work. †These authors contributed equally to this work and share senior authorship of this article.

Corresponding author: David Gonzalez de Castro, Centre for Cancer Research and Cell Biology, Queen’s University Belfast, 97 Lisburn Road, Belfast, BT9 7BL, Tel: +44 (0)28 9097 2772, Fax: +44(0)28 9097 2776, E-mail: d.gonzalezdecastro@qub.ac.uk

Conflict of interest: The authors declare no conflicts of interest.

Acknowledgements: This work was supported by the EuroClonality-NGS consortium. This work was also supported by the NIHR Biomedical Research Centre at the Royal Marsden NHS Foundation Trust and the Institute of Cancer Research.
Detection and characterization of clonal immunoglobulin (IG)/T-cell receptor (TR) rearrangements and translocations in lymphoproliferative neoplasms provides critical information in the diagnostic pathway and is a valuable tool to address research questions involving B and T cell lymphoproliferative disorders (LPD).\(^1,2\) This includes ascertaining the clonal nature of lymphoid proliferations\(^3,4\), characterization of translocations in lymphomas and leukemias\(^4\), characterization of CDR3 regions for MRD target identification\(^5\) and stereotyping analysis\(^6\), amongst others. Until recently, collecting this information required a combination of different methodologies, such as Gene-scanning/heteroduplex analysis, FISH and Sanger sequencing. The incorporation of next generation sequencing (NGS) in clinical laboratories opens up new possibilities where an integrated NGS approach can provide data on sequence and structural variation in a single assay, including translocations and IG/TR rearrangements, and has been shown to be successful for the characterisation of IG translocations in myeloma and lymphomas.\(^7,8\)

Within the EuroClonality-NGS consortium, we have designed a capture-based protocol covering the coding V, D and J genes of the IG/TR loci, as well as switch regions in the IGH locus. This design allows the identification of D-J and V-(D)-J rearrangements as well as chromosomal translocations involving IG/TR genes by sequencing through the breakpoint regions in genomic DNA. We piloted this approach using a sample cohort (n=24) consisting of 3 B-cell precursor acute lymphoblastic leukemias (BCP-ALL), 4 Burkitt lymphomas, 8 chronic lymphocytic leukemias (CLL), 2 splenic marginal zone lymphomas (SMZL), 2 diffuse large B cell lymphomas (DLBCL), 2 follicular lymphomas (FL), 2 T-cell acute lymphoblastic leukemias (T-ALL) and 1 T-cell non-Hodgkin lymphoma (T-NHL). Twenty-one cases were known to carry a translocation arising within the IG/TR loci with the remaining three cases being included for their well characterised D-J or V-(D)-J gene
rearrangements. Libraries were constructed from 1 µg of genomic DNA which was fragmented to an average of 200 bp using an E220 Focussed-ultrasonicator (Covaris, Woburn, MA, USA). Fragmented DNA was processed using the TruSeq DNA LT sample preparation kit (Illumina, Cambridge, UK). Libraries were hybridized to a custom-designed EZ SeqCap gene panel (Roche-Nimblegen, Madison, MI, USA) which encompassed 180 kb containing the V, D, J and constant regions of the IG and TR loci as well as the switch regions of the IGH locus. Enriched samples were sequenced on a MiSeq (Illumina) using 75bp or120bp paired-end reads. Reads were aligned to the reference genome (hg19) with translocations and variants called using a previously described bioinformatics pipeline9. The average depth of sequencing in the 21 samples with successful NGS results was 322x. IG/TR gene rearrangements were also determined by PCR and Sanger sequencing using the BIOMED-2 protocol in 14 cases1. For the detailed characterization of D-J/V(D)J gene rearrangements the IMGT V-Quest software was used5. IG/TR translocations had been previously determined by routine FISH, karyotyping and/or Sanger sequencing in the referring laboratories.

In 18 out of 21 samples with a known translocation, identified by either FISH or karyotyping, the breakpoints were identified by the NGS capture panel (Table 1). Out of the 3 samples that failed to produce results, 2 concerned fresh frozen samples from lymphomas with degraded and low-quantity DNA (<500ng total DNA and < 1kb median fragment size by TapeStation analysis) and 1 was due to a technical error due to evaporation during the hybridisation step in a BCP-ALL case.

Out of the 18 samples, 15 (83%) were from patients with B-cell LPD (BCLPD), of which 11 had well-known translocations partners: CRLF2 (2 BCP-ALL), MYC (4 Burkitt), BCL11A (1
CLL), BCL2 (2 CLL and 1 DLBCL) and CCND3 (1 SMZL). In these samples, the exact location of different breakpoints could be delineated from the sequencing reads. In addition, analysis of the reads mapping to the IGH locus showed that in 7 cases (47%) the break involved an IGHJ and/or IGHD gene whereas in 8 cases (53%) the break lay within the switch regions. The NGS capture approach thus provided additional information about the timing and aberrant cellular processes giving rise to the translocation, either occurring at the time of the D-J or V-DJ recombination or during class-switch recombination. Identification of the specific breakpoint was possible in the majority of cases, although due to the high homology between the different switch regions it was not always possible to specifically map reads unequivocally.

In 3 of the 15 BCLPD samples (20%) only karyotyping results were available and the location of the breakpoint on the partner chromosome as defined by NGS was mapped to chromosomes 5q14.2 (SMZL), 1q32.1 (CLL) or 16q22.1 (CLL). However, no known lymphoma-associated genes lay in proximity to these breakpoints highlighting the potential of this approach to identify novel translocation partners and/or mechanisms of disease. Alternatively, these chromosomal alterations may represent “passenger” recombination events, where genomic rearrangements are present in the IGH loci due to a by-product of the processing of double-stranded DNA breaks by the enzymatic machinery, not resulting in an oncogenic translocation.

In the remaining BCLPD sample -a case with DLBCL- NGS analysis was able to detect a translocation between IGH and chromosomal location 6p25. The break occurred downstream of EXOC2 and thus in the vicinity of IRF4, a constellation similar to the activating IRF4 translocations described in germinal-centre derived LPD. FISH analysis of the material
using an IGH break-apart probe (Abbott Molecular, Maidenhead, UK) showed the presence of a translocation with no evidence of a MYC, BCL2 or BCL6 rearrangement, supporting the NGS findings and highlighting the strength of NGS capture approaches to identify novel or uncommon translocation partners and breakpoints in one single analysis.

The three T-cell LPD (TCLPD) included in the cohort consisted of two T-ALL and one T-NHL case. The translocations in each of the cases arose from either the TRD, TRG or the TRB locus emphasising the benefit of the NGS capture panel to be able to interrogate all IG/TR loci in samples from different diseases at the same time. In all three samples the NGS approach identified the same rearrangement as demonstrated previously by karyotyping: t(7;10)(q34;q24) involving TRBJ2-5 and TLX1, inv(14)(q11;q32) involving TRDD3 and BCL11B\textsuperscript{11} and inversion on chromosome 7 involving TRGV8 (7p14.2) and TRBJ2-7 (7q34)\textsuperscript{12}.

In all 14 samples with well-characterized V-(D)-J and/or D-J IG gene rearrangements by PCR and Sanger sequencing, NGS was able to detect the same IGH and IGK/IGL gene rearrangements (Table 2), including 4 CLL cases with hypermutated VDJ rearrangements (90% to 96% homology). All cases included in the study were diagnostic material with tumour infiltration >60% and in the remaining six samples clonal V-(D)-J rearrangement(s) were also identified by NGS, consistent with the clonal nature of the disorder; however, PCR and Sanger sequencing data were not available for these six cases for comparison due to insufficient DNA. This version of the EuroClonality-NGS panel did not include probes for the intron RSS or KDE sequence, explaining why the intronRSS-KDE rearrangement found by Sanger sequencing in a Burkitt lymphoma case was not detected by the NGS approach. In two CLL cases, a total of three IGK locus gene rearrangements were detected by NGS in
In each case, raising the possibility of more than one clonal population being present, as
previously described in chronic BCLPD\textsuperscript{13}. Additionally, aberrant clonal rearrangements were
seen that were not detected with conventional PCR based approaches (e.g. IGKV to IGK
intron), which warrant further analysis. In the three TCLPD cases, Sanger sequencing
identified TRDV1-TRDJ1 (T-ALL with inv(14)), a TRBV5-TRBJ1-6 (T-ALL with t(7;10))
and a TRBV5-1-TRBJ2-5 (T-NHL) rearrangements, all of which were also identified in the
NGS analysis. In addition, NGS reads demonstrated functional rearrangements in TRG in
both T-ALL cases (TRGV11-TRGJ1 and TRGV2-TRGJ1/J2), and a non-functional TRDV1-
TRDJ1 rearrangement in one of the T-ALL. No confirmatory Sanger sequencing analysis
was feasible for these latter rearrangements due to insufficient DNA.

In summary, this pilot study demonstrates the ability of the EuroClonality-NGS capture
approach to simultaneously detect IG/TR translocations and V-(D)-J rearrangements in
diagnostic clinical specimens from a range of malignant LPD, including cases with
hypermuted VDJ rearrangements. By using capture probes against the V, D and J gene
regions of the TR and IG loci (with additional switch regions for IGH), clonal
rearrangements and chromosomal translocations arising from these loci can be detected and
at the same time the genomic breakpoint sequence involved in the rearrangements and
translocations can be identified without the need for additional tests. Other technologies such
as target locus amplification (TLA) have also recently demonstrated the ability to detect
structural variants and translocations in cancer\textsuperscript{14}. An important advantage of these approaches
lies in the fact that no prior knowledge of the translocation partner is needed and therefore,
novel or rare chromosomal rearrangements can also be identified by this method, improving
the diagnostic value. Sequencing of the V-(D)-J gene rearrangements in any of the IG/TR
loci can be used not only to assess clonality and enable a more in-depth analysis of clonal
relationships and clonal evolution, but also to identify targets for minimal residual disease (MRD) monitoring and analyse the IG/TR repertoire of diverse lymphoid populations. Additional information, for example the somatic hypermutation status of the IGHV-IGHD-IGHJ gene rearrangements, relevant for prognosis in CLL can also be obtained. A new version of this EuroClonality-NGS panel is being designed to include common non-IG/TR translocations as well as genes relevant for diagnosis and prognosis in LPDs and a clinical multi-centre validation study is now underway within the EuroClonality-NGS consortium.
References


<table>
<thead>
<tr>
<th>Sample</th>
<th>Diagnosis</th>
<th>Karyotyping or FISH results</th>
<th>der(IG/TR)</th>
<th>NGS Capture results</th>
<th>der(partner chromosome)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Break 1 Location</td>
<td>Break 2 Location</td>
<td></td>
<td>Break 1 Location</td>
</tr>
<tr>
<td>EC20</td>
<td>BCP-ALL</td>
<td>t(X;14)(p22;q32)</td>
<td>IGHJ6</td>
<td>chr14:106329465</td>
<td>CRLF2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IGHD3-9</td>
</tr>
<tr>
<td>EC19</td>
<td>BCP-ALL</td>
<td>t(Y;14)</td>
<td>IGHJ5</td>
<td>chr14:106330070</td>
<td>CRLF2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IGHD6-19</td>
</tr>
<tr>
<td>EC21</td>
<td>Burkitt lymphoma</td>
<td>t(8;14)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IGHA1</td>
</tr>
<tr>
<td>EC23</td>
<td>Burkitt lymphoma</td>
<td>t(8;14)</td>
<td>IGH1 and</td>
<td>ch14: complex</td>
<td>MYC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IGHD2-2</td>
<td></td>
<td>IGHG2/3*</td>
</tr>
<tr>
<td>EC18</td>
<td>Burkitt lymphoma</td>
<td>t(8;14)</td>
<td>IGHJ4</td>
<td>chr14:106330462</td>
<td>MYC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>EC17</td>
<td>Burkitt lymphoma</td>
<td>t(8;14)</td>
<td>IGHA1</td>
<td>chr14:106177015</td>
<td>MYC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IGHA1</td>
</tr>
<tr>
<td>EC30</td>
<td>CLL</td>
<td>t(2;14)(p13;32)</td>
<td>IGHM</td>
<td>chr14:106327487</td>
<td>BCL11A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IGHM</td>
</tr>
<tr>
<td>EC31</td>
<td>CLL</td>
<td>t(14;18)(q32;q21)</td>
<td>IGHJ6</td>
<td>chr14:106329459</td>
<td>BCL2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>EC29</td>
<td>CLL</td>
<td>t(14;18)(q32;q21)</td>
<td>IGHJ5</td>
<td>chr14:10630071</td>
<td>BCL2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IGHD2-15</td>
</tr>
<tr>
<td>EC5</td>
<td>DLBCL</td>
<td>t(14;18)</td>
<td>IGHJ5</td>
<td>chr14:106330072</td>
<td>BCL2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IGHV3-21</td>
</tr>
<tr>
<td>EC34</td>
<td>SMZL</td>
<td>t(6;14)(p21;q32)</td>
<td>IGHA2*</td>
<td>chr14</td>
<td>CCND3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>EC3</td>
<td>DLBCL</td>
<td>IGH break‡</td>
<td>IGHM</td>
<td>chr14:106362706</td>
<td>IRF4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IGHG4*</td>
</tr>
<tr>
<td>EC25</td>
<td>CLL</td>
<td>t(14;16)(q32;q22)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IGHD2-2</td>
</tr>
<tr>
<td>EC24</td>
<td>CLL</td>
<td>t(1;14)(q32;q32)</td>
<td>IGHM or</td>
<td>chr14</td>
<td>IGHM or</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IGHA1*</td>
<td></td>
<td>IGHA1*</td>
</tr>
<tr>
<td>EC33</td>
<td>SMZL</td>
<td>t(5;14)(q13;q32)</td>
<td>IGHM</td>
<td>chr14:106326138</td>
<td>unknown¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IGHM</td>
</tr>
<tr>
<td>EC11</td>
<td>T-ALL</td>
<td>inv14(t14;14)</td>
<td>TRDD3</td>
<td>chr14:22918106</td>
<td>BCL11B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>EC12</td>
<td>T-ALL</td>
<td>t(7;10)</td>
<td>TRBJb2.5</td>
<td>chr7:142494805</td>
<td>TLIX1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ND na</td>
</tr>
<tr>
<td>EC14</td>
<td>T-NHL</td>
<td>inv7</td>
<td>TRGV8</td>
<td>ND</td>
<td>TRBJb2.7</td>
</tr>
</tbody>
</table>

* Alignment equivocal due to sequence homology.  
† No known lymphoma/leukaemia-associated gene in proximity.  
‡ demonstrated by FISH IGH break-apart probe.  
ND: exact breakpoint could not be determined due to insufficient or lack of aligned reads.
Table 2: IGH and IGK rearrangements detected by Sanger sequencing and the EuroClonality NGS panel.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diagnosis</th>
<th>Results</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Allele 1</th>
<th>Allele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IGHV</td>
<td>IGHJ</td>
<td>IGHD</td>
<td>IGHD</td>
<td>IGJ</td>
</tr>
<tr>
<td>EC21</td>
<td>Burkitt Lymphoma</td>
<td>Sanger Seq.</td>
<td>3-15</td>
<td>4</td>
<td>3-16</td>
<td>3-22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC23</td>
<td>Burkitt Lymphoma</td>
<td>Sanger Seq.</td>
<td>3-23</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>4-23</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>EC18</td>
<td>Burkitt Lymphoma</td>
<td>Sanger Seq.</td>
<td>3-22</td>
<td>4</td>
<td>6-13</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>3-22</td>
<td>4</td>
<td>6-13</td>
<td>-</td>
</tr>
<tr>
<td>EC30</td>
<td>CLL</td>
<td>Sanger Seq.</td>
<td>4-39</td>
<td>4</td>
<td>6-25</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>4-39</td>
<td>4</td>
<td>6-25</td>
<td>4</td>
</tr>
<tr>
<td>EC31</td>
<td>CLL</td>
<td>Sanger Seq.</td>
<td>3-30</td>
<td>4</td>
<td>2-2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>na</td>
<td>4</td>
<td>2-2</td>
<td>4</td>
</tr>
<tr>
<td>EC29</td>
<td>CLL</td>
<td>Sanger Seq.</td>
<td>5-51</td>
<td>4-17</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>5-51</td>
<td>4-17</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>EC25</td>
<td>CLL</td>
<td>Sanger Seq.</td>
<td>4-34</td>
<td>6-18</td>
<td>6</td>
<td>5-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>4-34</td>
<td>6-18</td>
<td>6</td>
<td>5-18</td>
</tr>
<tr>
<td>EC24</td>
<td>CLL</td>
<td>Sanger Seq.</td>
<td>4-61</td>
<td>5</td>
<td>6-19</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>4-61</td>
<td>5</td>
<td>6-19</td>
<td>4</td>
</tr>
<tr>
<td>EC33</td>
<td>SMZL</td>
<td>Sanger Seq.</td>
<td>3-23</td>
<td>3</td>
<td>3-22</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>3-23</td>
<td>3</td>
<td>3-22</td>
<td>3</td>
</tr>
<tr>
<td>EC32</td>
<td>SMZL</td>
<td>Sanger Seq.</td>
<td>2-5</td>
<td>6-19</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>2-5</td>
<td>6-19</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>EC34</td>
<td>SMZL</td>
<td>Sanger Seq.</td>
<td>5-51</td>
<td>4-4</td>
<td>6</td>
<td>5-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>5-51</td>
<td>4-4</td>
<td>6</td>
<td>5-18</td>
</tr>
<tr>
<td>EC27</td>
<td>CLL</td>
<td>Sanger Seq.</td>
<td>4-39</td>
<td>6-13</td>
<td>5</td>
<td>5-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>4-39</td>
<td>6-13</td>
<td>5</td>
<td>5-18</td>
</tr>
<tr>
<td>EC26</td>
<td>CLL</td>
<td>Sanger Seq.</td>
<td>4-39</td>
<td>6-13</td>
<td>5</td>
<td>5-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGS</td>
<td>4-39</td>
<td>6-13</td>
<td>5</td>
<td>5-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>NGS</td>
<td>4-39</td>
<td>6-13</td>
<td>5</td>
<td>-</td>
<td>2-2</td>
</tr>
<tr>
<td>EC28</td>
<td>CLL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NGS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC20</td>
<td>BCP-ALL</td>
<td>Sanger Seq.</td>
<td>4-39</td>
<td>6-13</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NGS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC22</td>
<td>BCP-ALL</td>
<td>Sanger Seq.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NGS</td>
<td>FAIL</td>
<td>FAIL</td>
<td>FAIL</td>
<td>FAIL</td>
<td>FAIL</td>
</tr>
<tr>
<td>EC19</td>
<td>BCP-ALL</td>
<td>Sanger Seq.</td>
<td>3-30</td>
<td>2-2</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NGS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC17</td>
<td>Burkitt Lymphoma</td>
<td>Sanger Seq.</td>
<td>3-72</td>
<td>6-13</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NGS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC3</td>
<td>DLBCL</td>
<td>Sanger Seq.</td>
<td>1-46</td>
<td>6-6</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NGS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC5</td>
<td>DLBCL</td>
<td>Sanger Seq.</td>
<td>3-48</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
</tbody>
</table>

Blank cells: No Sanger sequencing data available. *intronRSS-Kde not detectable by NGS as no probes against this region were included in the original panel design. † More than 2 rearrangements detected.