

Invivoscribe RUO Portfolio for Clonality testing – Gel and ABI

Assay Reagents, Workflow and
Sample Interpretation

Gel Detection

- B- and T-cell clonality assays
- Translocation assays

ABI Fluorescence Detection

- B- and T-cell clonality assays
- Translocation assays

Invivoscribe developed or based on EuroClonality/BIOMED-2
Concerted Action BMH4-CT98-3936



Gel and Capillary Assays

RUO Assays

B-Cell Assays

IGH + IGK B-Cell Clonality Assays
IGH Gene Rearrangement Assays
IGH Gene Clonality Assays
IGK Gene Clonality Assays
IGL Gene Clonality Assays

T-Cell Assays

TCRB + TCRG T-Cell Clonality Assays
TCRB Gene Clonality Assays
T-Cell Receptor Gamma Gene Rearrangement Assay 2.0
T-Cell Receptor Gamma Gene Rearrangement Assays
TCRG Gene Clonality Assays
TCRD Gene Clonality Assays

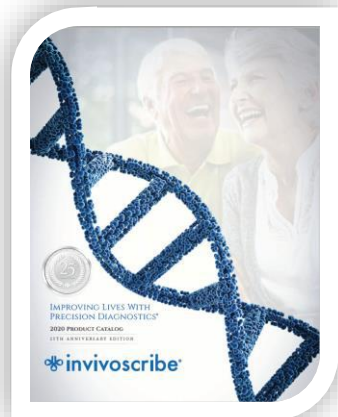
Translocation Assays

BCL1/JH Translocation Assay
BCL2/JH t(14;18) Translocation Assay
BCL2/JH Translocation Assay
BCR/ABL t(9;22) Translocation Assays
PML/RAR α t(15;17) Translocation Assays

Mutation Assays

IGH Somatic Hypermutation Assays v2.0





IGH Gene Clonality Assay

Reagents

Controls

Controls	Concentration	Units in Assay	Units in Assay MegaKit
IVS-0030 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0019 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0024 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0008 Clonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes
IVS-0000 Polyclonal Control DNA	200 µg/mL	1 x 100 µL tube	5 x 100 µL tubes

Master Mixes

Master Mixes	Target	Units in Assay	Units in Assay MegaKit
IGH Tube A	Framework 1 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube B	Framework 2 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube C	Framework 3 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube D	DH1-6 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
IGH Tube E	DH7 + JH	1 x 1500 µL tube	10 x 1500 µL tubes
Specimen Control Size Ladder	Multiple Genes	1 x 1500 µL tube	10 x 1500 µL tubes

IGH Gene Clonality Assay

- More detailed information



Controls and Standards	IVS Catalog #	Concentration
IVS-0030 Clonal Control DNA	4-088-1750	100 µL @200 µg/mL
IVS-0019 Clonal Control DNA	4-088-1090	100 µL @200 µg/mL
IVS-0024 Clonal Control DNA	4-088-1390	100 µL @200 µg/mL
IVS-0008 Clonal Control DNA	4-088-0430	100 µL @200 µg/mL
IVS-0000 Polyclonal Control DNA	4-092-0010	100 µL @200 µg/mL

Master Mixes for 1-101-0020 and 1-101-0040	IVS Catalog #	Target
IGH Tube A - Unlabeled	2-101-0010	Framework 1 + JH
IGH Tube B - Unlabeled	2-101-0020	Framework 2 + JH
IGH Tube C - Unlabeled	2-101-0030	Framework 3 + JH
IGH Tube D - Unlabeled	2-101-0040	DH1-6 + JH
IGH Tube E - Unlabeled	2-101-0050	DH7 + JH
Specimen Control Size Ladder - Unlabeled	2-096-0020	Multiple Genes

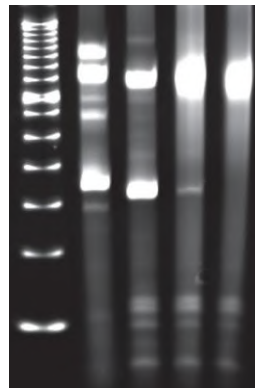
Master Mixes for 1-101-0061 and 1-101-0081	IVS Catalog #	Target
IGH Tube A - 6FAM	2-101-0011	Framework 1 + JH
IGH Tube B - 6FAM	2-101-0101	Framework 2 + JH
IGH Tube C - HEX	2-101-0031	Framework 3 + JH
IGH Tube D - HEX	2-101-0041	DH1-6 + JH
IGH Tube E - 6FAM	2-101-0051	DH7 + JH
Specimen Control Size Ladder - 6FAM	2-096-0021	Multiple Genes

Note: MegaKits contain 10 units of each master mix and 5 units of each Controls and Standards

Clonality Assays Detection Formats

Gel

- 6% non-denaturing PAGE
- Cheaper start-up
- Easier interpretation/ Fewer false positives for some targets (*TRD*, *IGK*, *IGL*)



Capillary Electrophoresis

- Gene Scanning
- ABI (310/3100/3130/3500)*
- High Sensitivity
- High Throughput
- More objective interpretation



*For further information refer to the most current version of the IFU.

Heteroduplex Analysis – Gel Detection

Heat denature DNA amplicons

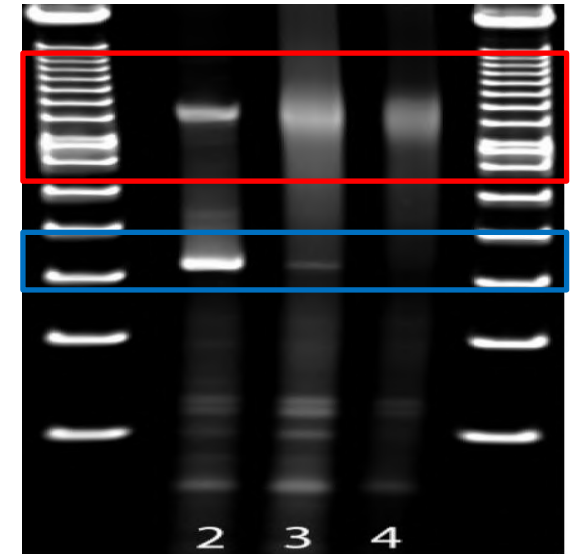
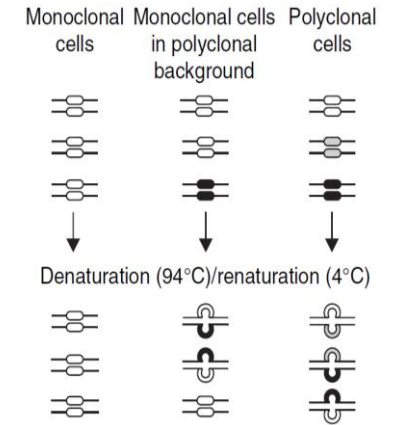
- 94°C for 5 minutes

Snap Chill

- 4 °C for 1 hour

Clonal PCR products re-anneal to each other properly

Polyclonal PCR products' strands bind incorrectly to non-homologous strands forming secondary structures

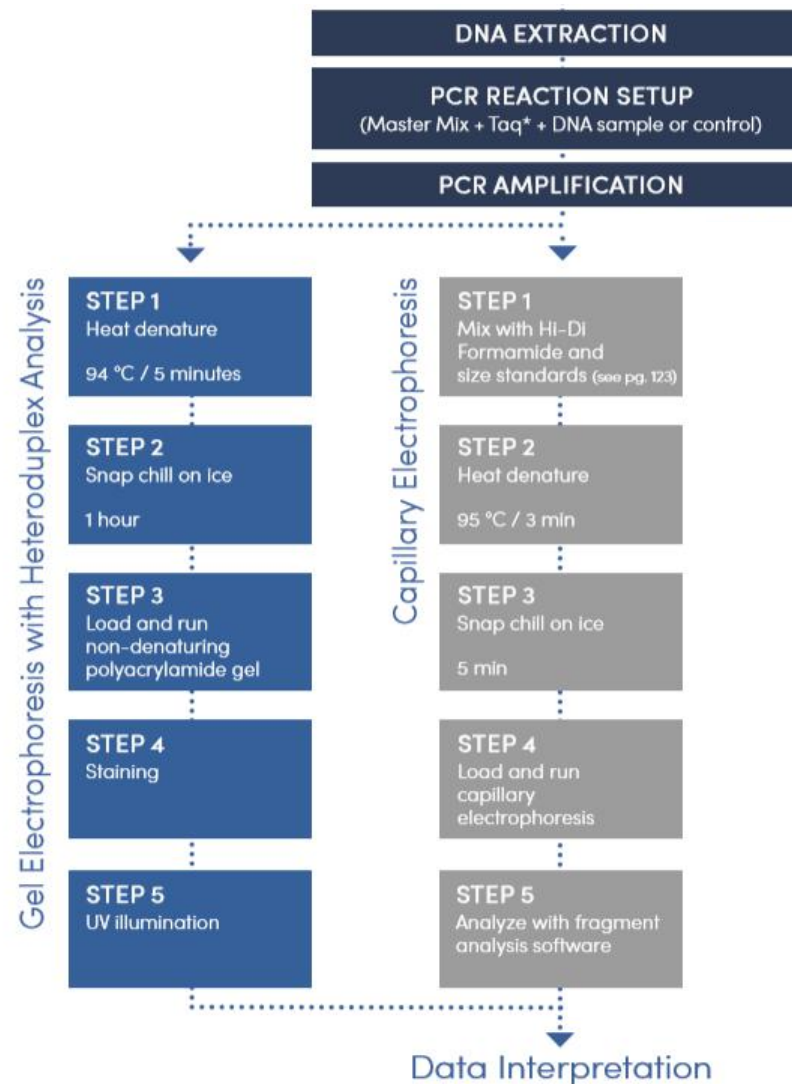


Langerak et al., Expert Opin. Med. Diagn. (2007) 1(4):451-461.

Controls

- Positive
- Negative
- No Template

 Gel
 Capillary

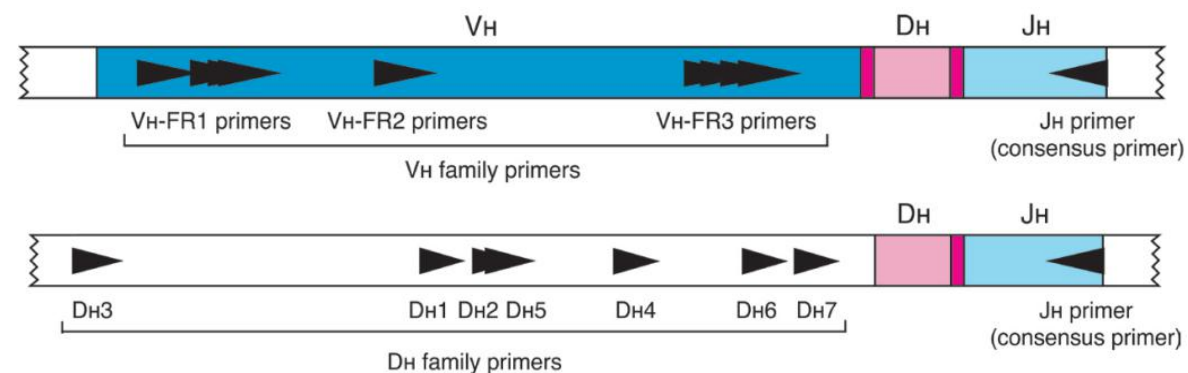


Controls should be run to ensure proper performance of the assay

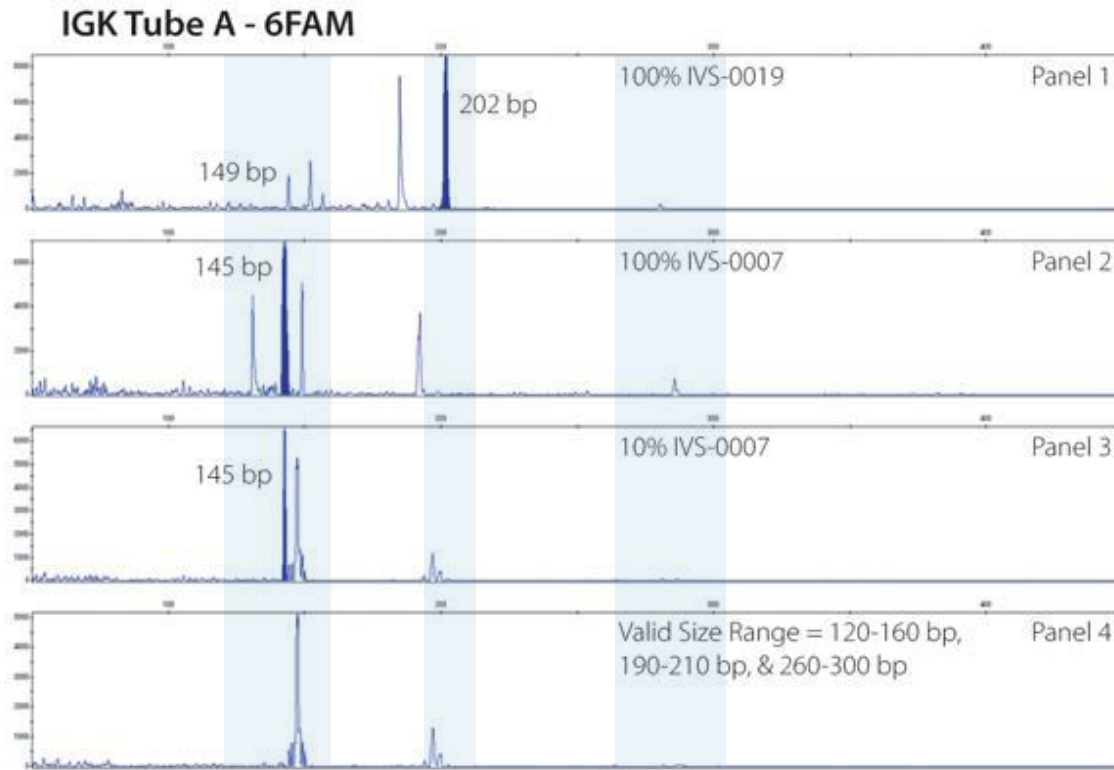
IGH Gene Clonality Assays

Targets

- Framework 1 VH-JH (tube A)
- Framework 2 VH-JH (tube B)
- Framework 3 VH-JH (tube C)
- Incomplete DH-JH (tubes D&E)



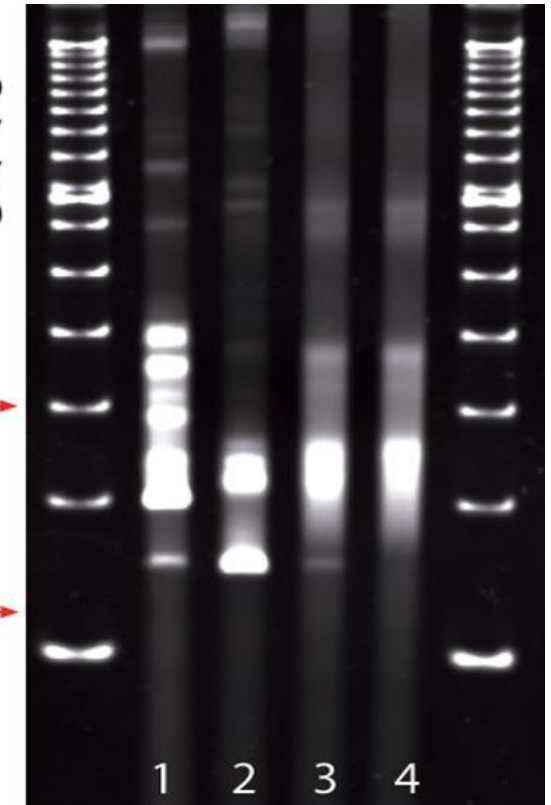
IGK Gene Clonality Assays



IGK Tube A

Lane 1 = 100% IVS-0019
Lane 2 = 100% IVS-0007
Lane 3 = 10% IVS-0007
Lane 4 = 100% IVS-0000

Valid Size Range =
120-160 bp,
190-210 bp,
& 260-300 bp



Multiple Valid Size Ranges Possible!

TCRG Gene Assays (2 options)

Gel & Capillary

TCRG Gene Clonality Assays

Design: BIOMED-2

2 tubes Master Mixes

T-Cell Receptor Gamma Gene Rearrangement Assay 2.0

Design: Invivoscribe

1 tube Master Mix



Just 1 Master Mix

Easier setup and simplified interpretation

Benefits

Smaller amplicon sizes

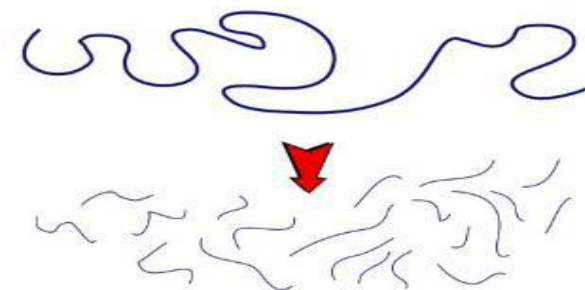
Designed by Invivoscribe

Common sources of DNA:

- Formalin-fixed paraffin-embedded (FFPE), fresh/frozen tissue, peripheral blood, bone marrow.

Common issues:

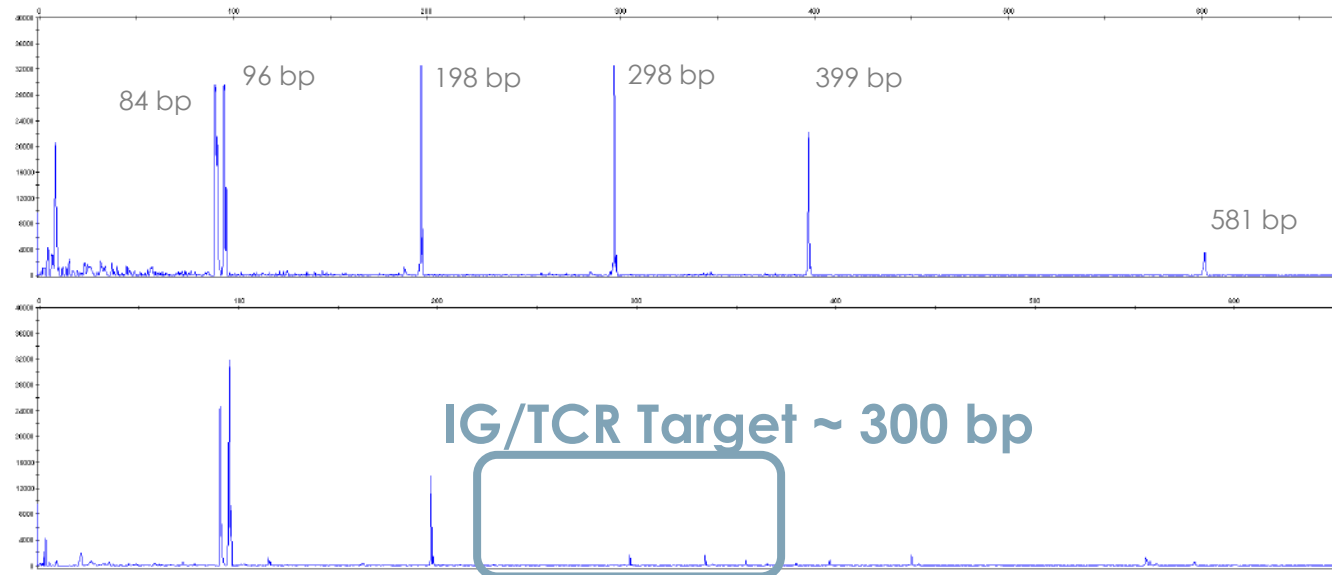
- DNA integrity
- PCR inhibitors



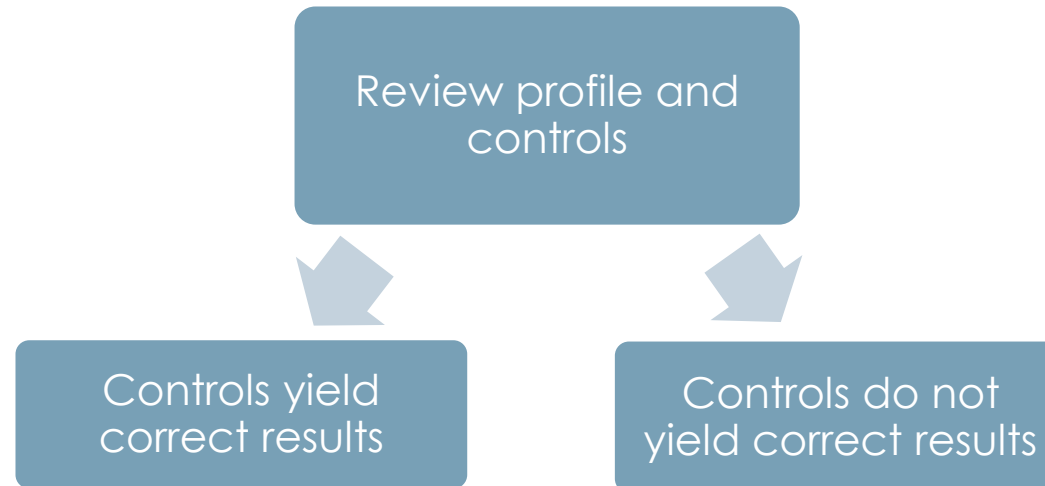
Specimen Control Size Ladder (SCSL)

Tests quality & quantity by amplifying housekeeping genes

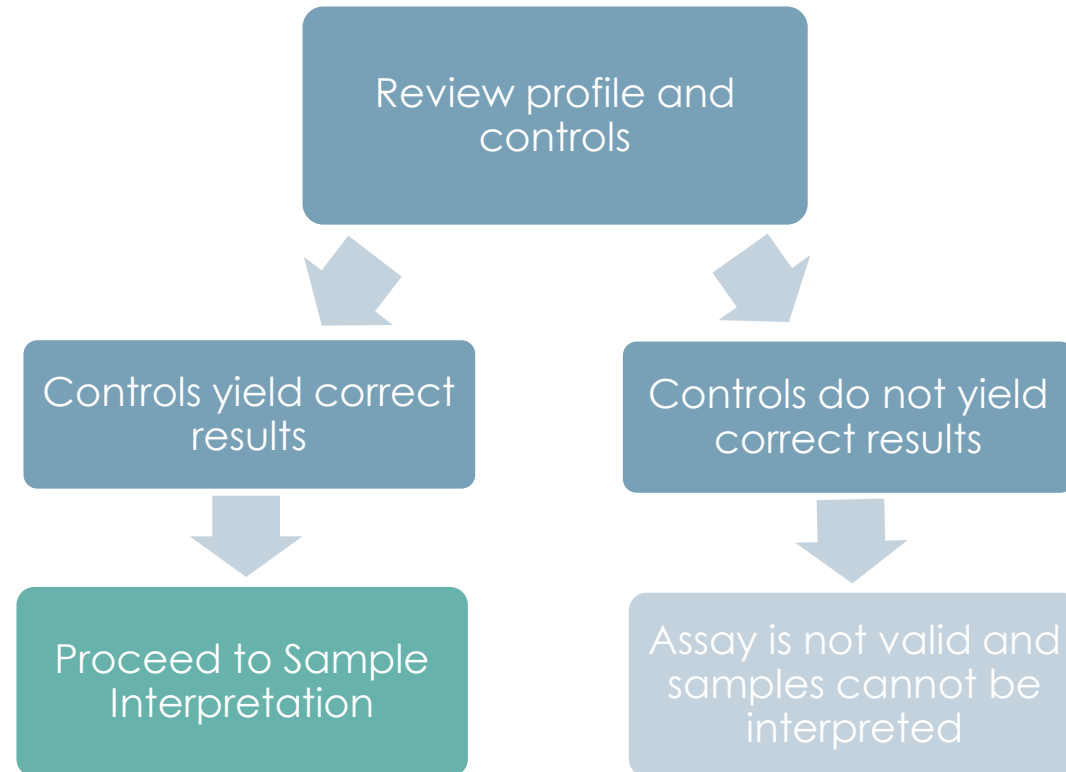
- Are the **fragments** of DNA sufficient for primer binding & amplification?
- Are there **PCR inhibitors** present?
- Is there **enough DNA** for amplification?



Interpretation of Results



Interpretation of Results



Educational Section



Complementary Targets

Easy to combine

- *IGH* and *IGK*
- *TRB* and *TRG*

Advantages of Combining Targets:

- Increased detection of clonal cells in clonality studies
- Time saving

B-Cell Research Targets

	<i>IGH</i> (F1, 2 & 3)	<i>IGK</i> (Vk - Jk & Kde)	<i>IGH+IGK</i>
MCL(%)	100	100	100
B-CLL/SLL(%)	100	100	100
FL(%)	84	84	100
MZL(%)	87	83	97
DLBCL(%)	79	80	96
Total(%)	88	88	98

PAS Evans et al., *Leukemia*. 2006 21:201-206.

T-Cell Research Targets

	<i>TRB</i>	<i>TRG</i>	<i>TRB+TRG</i>
T-PLL(%)	100	94	100
T-LGL(%)	96	96	100
PTCL-U(%)	98	94	100
AITL(%)	89	92	95
ALCL(%)	74	74	79*
Total(%)	91	89	94 (99)*

J.J.M. van Krieken et al. *Leukemia*. 2007 21:201-206.

*Approximately 20–25% of ALCL are known to have no TCR gene rearrangements and are defined as null ALCL. J.J.M. van Krieken et al. *Leukemia*. 2007 21:201-206.

Low Quality & Quantity of DNA Specimen

Specimen Considerations during Research Studies

Duplicate Assessment Highly Preferred Over Multiple Targets

Priority Selection of Targets

- *IGH* FR3
 - *IGK* V-J
 - *TRG*
- } Smaller Amplicons - Potentially More Reliable

Suspect T-Cell Proliferations

- *TRG* tube A + *TRB* tube A
 - Preferable to also test *TRB* tube B

Suspect B-Cell Proliferations

- *IGH* D-J
 - *IGK* Kde
- } Not Prone to Somatic Mutations

Low % of B- or T-Cells (e.g., Skin or Intestinal Lesions)

- Easily Over Interpreted Due to Coincidental Dominant Peaks
- Test Samples in Duplicate

A.W. Langerak et al. *Leukemia* 2012 26: 2159-71.

No Products after Specimen PCR

Troubleshooting Lack of Amplification in Research Specimens

Poor DNA Quality

- Use Specimen Control Size Ladder (SCSL)

Few B- & T-Cells

- Check Sample via alternative method
- Choose Alternative Specimen Sample

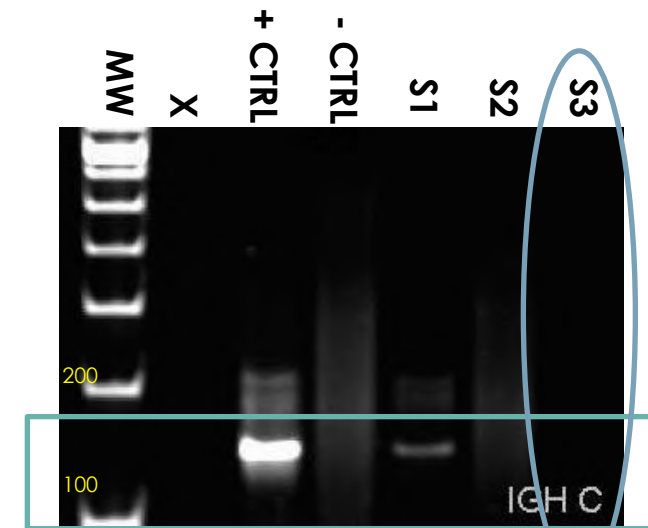
Somatic Hypermutation Affected Primer Binding

- Evaluate other Frameworks or Targets

t(11;14) and t(14;18) Aberrations

- Conduct Complementary Laboratory Tests

What Should Be Considered?



IGH Tube C, valid size 100-170bp

A.W. Langerak et al. *Leukemia* 2012 26: 2159-71.

Frequently Asked Support Questions

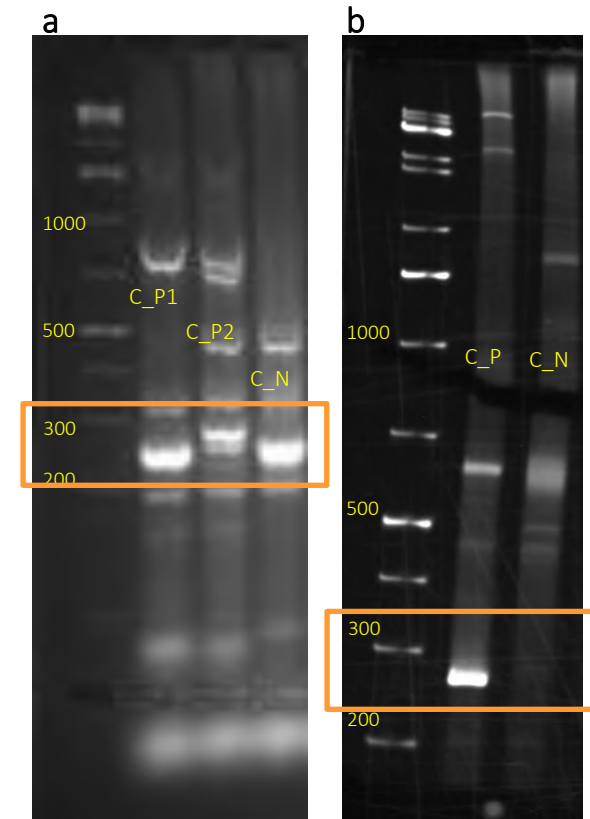
½ Volume Reactions (i.e., 25 µL vs. 50 µL)?

- Conditions in IFU were **optimized for product performance**
- IVS consistently see 25 µL causing less than optimal results & **reduces sampling**



Agarose vs. Polyacrylamide Gels(PAGE)?

- **Agarose Resolution** is not sufficient to discern a monoclonal band from a polyclonal smear



TCRB Tube A. Valid size 240-285 bp
a) No heteroduplex on agarose gel
b) Heteroduplex on polyacrylamide gel

Which ABI instrument can be used for Fluorescence Fragment Detection?*

- ABI 310, 3100, 3130 & 3500

What are the Recommended ABI Calibration Standards?

- DS-30 matrix standards (Dye set D) with ABI 310, 3100, or 3130
- DS-33 matrix standards (Dye Set G5) with ABI 3500

What is the Recommended POP?

- POP-7 if equipment supports fragment analysis & sequencing (IGH SHM)
- POP-4 or POP-7 for fragment analysis only
- POP-6 is not recommended



*Not all assays have been tested on all instruments. For further information refer to the most current version of the IFU.

Master Mix Storage

- Due to high salt concentration, master mixes should be stored at **-85 to -65 °C**
- Labs should **minimize** the number of freeze thaw cycles
- IVS recommends a **maximum of 5 cycles** to avoid performance degradation

DNA/RNA Storage

- DNA controls are best stored at 2 to 8 °C, but can also be stored at -85 to -65 °C
- RNA controls should be stored at -85 to -65 °C

How to Thaw Master Mixes?

- Thaw at **room temperature** for ~45 min, ensure fully thawed
- **Vortex** before use

Take Home Message

- Invivoscribe offers a full range of PCR-based **molecular testing products** for the study of Hematology-Oncology malignancies.
- **PCR-based Clonality Testing** of B- and T- Cell Gene Rearrangements is useful in hematology and oncology research.
- **Testing complimentary targets** results in increased detection of clonal cells in research studies.