Chromosome Translocations

Invivoscribe Offerings

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Improving Lives with Precision Diagnostics®

Translocation Assays

Invivoscribe Portfolio:

- Gel and/or Capillary RUO Assays:
  - *BCL1/JH* Translocation Assay
  - *BCL2/JH* t(14;18) Translocation Assay
  - *BCL2/JH* Translocation Assay
  - *BCR/ABL* t(9;22) Translocation Assay
  - *PML/RARα* t(15;17) Translocation Assay

- Gel CE-IVD IdentiClone Assays:
  - *BCL1/JH* Translocation Assay
  - *BCL2/JH* Translocation Assay


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IdentiClone Assays are *in vitro* diagnostic products. Available outside of North America.

Invivoscribe RUO Translocation Assays

Translocation	Assay Description
BCL1/JH	**Agarose Gel** detection
BCL2/JH	**Agarose Gel** detection
***BCL2/JH* t(14;18)**	**Agarose Gel** detection
***BCR/ABL* t(9;22)**	**Available in Gel and ABI** formats RNA-based qualitative nested PCR assay
***PML/RaRa* t(15;17)**	**Available in Gel and ABI** formats RNA based qualitative nested-PCR assay

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Invivoscribe CE-IVD Translocation Assays

Translocation	Associated Diseases	Assay Description
BCL1/JH	Mantle Cell Lymphoma, Multiple Myeloma other Plasma Cell Neoplasms	**Agarose Gel** detection **CE-IVD** Sensitivity: up to **10^{-4}**
BCL2/JH	Follicular Lymphoma, Diffuse large B-Cell Lymphoma	**Agarose Gel** detection **CE-IVD** Sensitivity: up to **10^{-3}**

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Translocation Assays

BCL1/JH Translocation Assay

BCL1/JH Translocation – CE-IVD

Product Offerings:

- **IdentiClone® *BCL1/JH* Translocation Assays**
- Concordance of 88% positive samples of *BCL1/JH* translocations
- Breakpoints outside of the CCND1/MTC locus will not be identified by this particular test.
- Specificity of 100% and Sensitivity up to **10^{-4}**
- The IdentiClone® *BCL1/JH* Translocation Assay was designed by the EuroClonality group as part of the BIOMED-2 concerted action[1]

Available as **gel detection assay**

[1]Van Dongen, JJM *et al*. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia*. 2003, **17(12)**:2257-2317

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IdentiClone® *BCL1/JH* Translocation Assay

Common sources of genomic DNA:

- 5 mL of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA
- Minimum 5 mm cube of tissue
- 2 µg of genomic DNA
- Formalin-fixed paraffin embedded tissue or slides

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BCL1/JH Translocation - RUO

Product Offerings:

- ***BCL1/JH* Translocation Assay**
- Breakpoints outside of the CCND1/MTC locus will not be identified by this particular test.
- The *BCL1/JH* Translocation Assay was designed by the EuroClonality group as part of the BIOMED-2 concerted action[1]

Available as **gel detection assay**

[1]Van Dongen, JJM *et al*. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia*. 2003, **17(12)**:2257-2317

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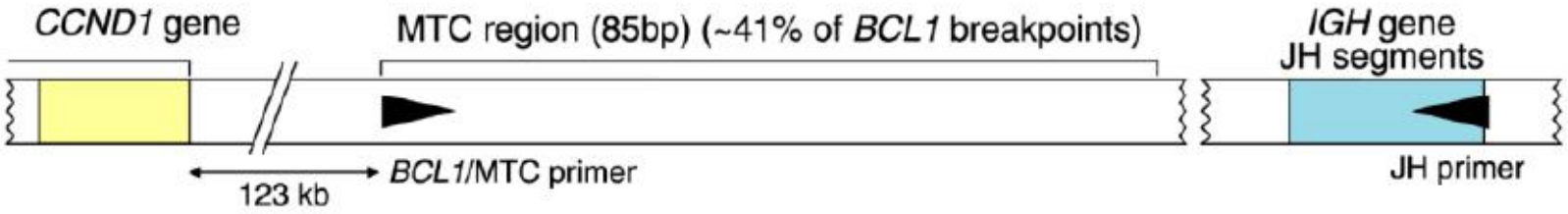
BCL1/JH Assays - Kit Contents

- Robust assay, which targets the Major Translocation Cluster **(MTC)** Region
- **BIOMED-2 design**, 33/330 reactions, 4 tubes (pos./neg. controls+MM+SCSL)

BCL1 MTC primer + JH primer



EuroClonality

MM = Master Mix
SCSL = Specimen Control Size Ladder

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BCL1/JH Translocation Assay

Gel Electrophoresis Detection:

- 2% Agarose Gel
- **One or more prominent positive** bands within the valid size range should be considered as positive for the detection of *BCL1/JH* translocation

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Translocation Assays

BCL2/JH Translocation Assay

BCL2/JH Translocation - CE-IVD

Product Offerings:

- **IdentiClone® *BCL2/JH* Translocation Assays**
- **2% Agarose** gel detection
- Detects about **88%** of *BCL2/JH* translocations[1]
- CE-IVD Sensitivity: up to **10^{-3}**
- This IdentiClone® *BCL2/JH* Translocation Assay was found to be more sensitive than Southern blot analysis
- The IdentiClone® *BCL2/JH* Translocation Assay was designed by the EuroClonality group as part of the BIOMED-2 concerted action[1]

Available as **gel detection assay**

[1]Van Dongen, JJM *et al*. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia*. 2003, **17(12)**:2257-2317

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IdentiClone® *BCL2/JH* Translocation Assay

Common sources of genomic DNA:

- 5ml of peripheral blood, bone marrow biopsy, or bone marrow aspirate anti-coagulated with heparin or EDTA
- Minimum 5mm cube of tissue
- 2µg of genomic DNA
- Formalin-fixed paraffin embedded tissue or slides

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BCL2/JH Translocation - RUO

Product Offerings:

- ***BCL2/JH* Translocation Assay**
- **2% Agarose** gel detection
- Was optimized using positive and negative control samples with multiplex master mixes
- The *BCL2/JH* Translocation Assay was designed by the EuroClonality group as part of the BIOMED-2 concerted action[1]

Available as **gel detection assay**

[1]Van Dongen, JJM *et al*. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia*. 2003, **17(12)**:2257-2317

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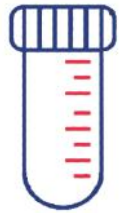
BCL2/JH Assay - Kit Contents

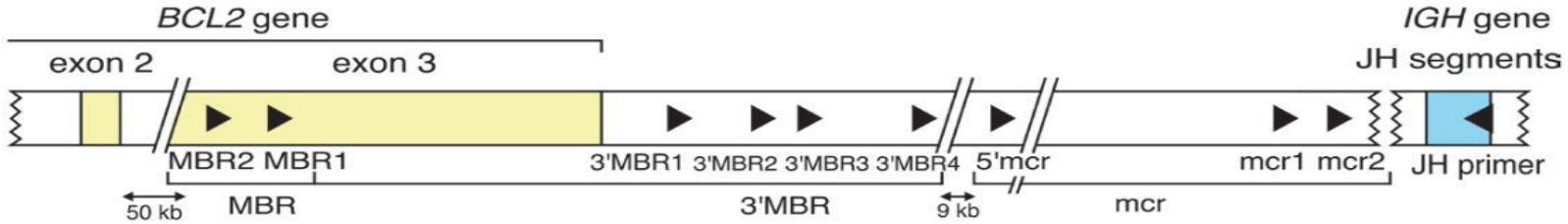
- **BIOMED-2 design**, 8 tubes (3 pos.+1neg controls+ 3 breakpoint MM + SCSL)
- Targets **5´ Mbr** (major breakpoint region), **3´Mbr**, **mcr** (minor cluster region), and JH regions

Tube A
BCL2 Mbr + *IGH* JH

Tube B
BCL2 3' Mbr + *IGH* JH



Tube C
BCL2 mcr + *IGH* JH



EuroClonality

MM = Master Mix
SCSL = Specimen Control Size Ladder

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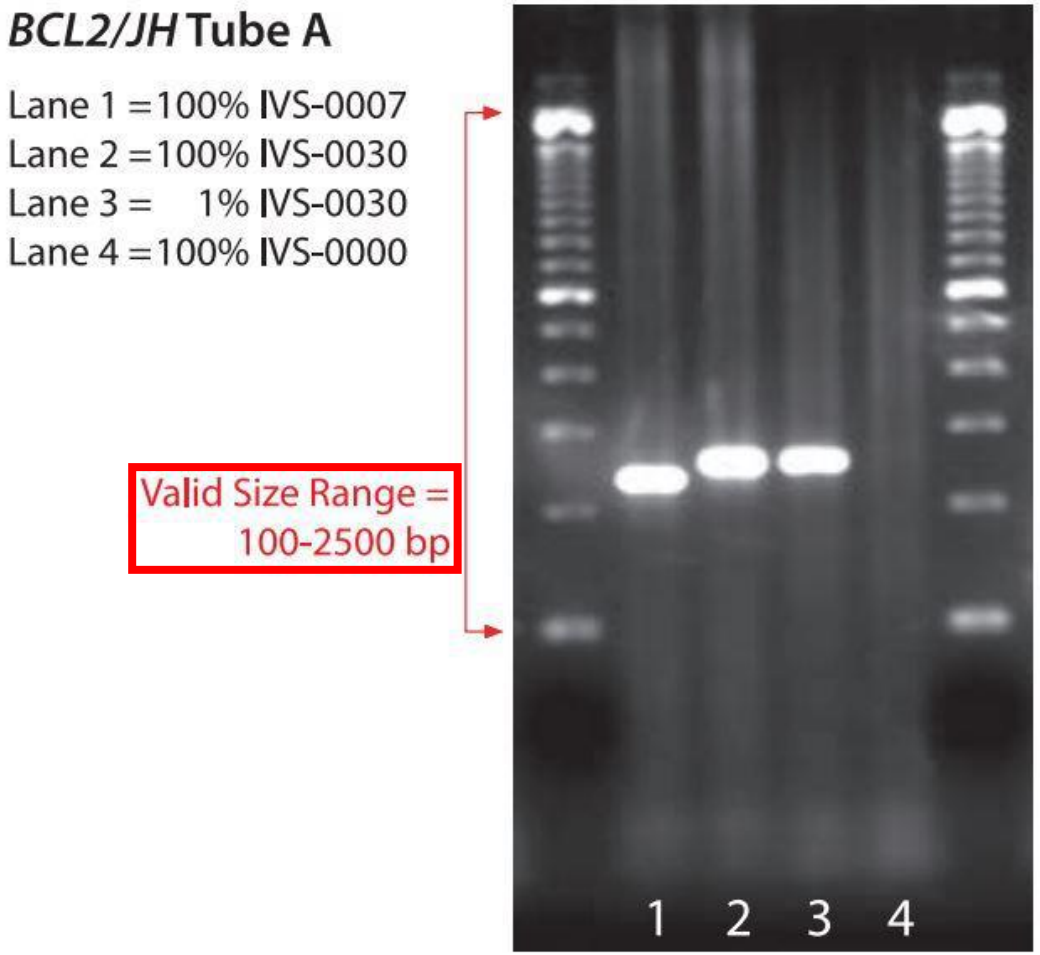
BCL2/JH Translocation Assay

Gel Electrophoresis Detection:

- **2% Agarose Gel**
- **<u>One or more prominent positive bands</u>** within the valid size range should be considered as positive for the detection of *BCL2/JH* t(14;18) translocation



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Translocation Assays

BCL2/JH t(14;18) Translocation Assay

BCL2/JH t(14;18) Translocation Assay

Product Offerings:

- Our ***BCL2/JH* t(14;18) Translocation Assay** identifies *IGH-BCL2* t(14;18)(q32;q21) translocations
- **Agarose Gel** detection
- Can be run either in a standard or nested assay format
- Primers in the *BCL2/JH* master mixes target the joining region of the *IGH* gene and distinct regions of the *BCL2* gene

Specimen Requirements:

- This assay tests genomic DNA as template

Available as **RUO gel detection assay**

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BCL2/JH t(14;18) Assay - Kit Contents

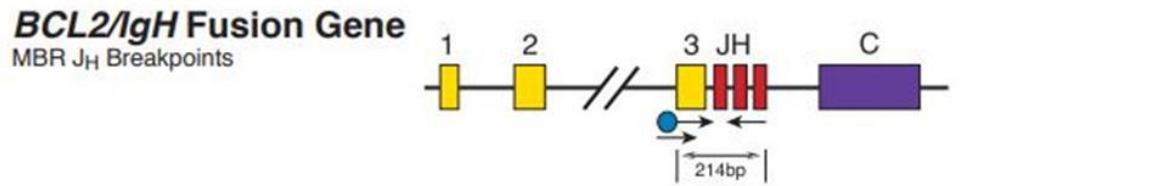
- 8 tubes (3 positive controls + 4 breakpoint MM + amplification control)
- Targets ***BCL2* Mbr** (major breakpoint region)and ***BCL* mcr** (minor cluster region), and JH regions

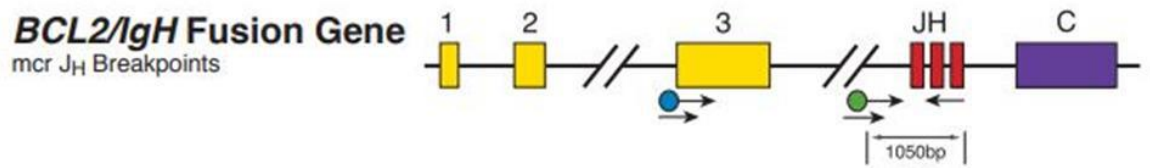
Mix 1a
Outside Mbr primers

Mix 1b
Inside Mbr primers

Mix 2a
Outside mcr primers

Mix 2b
Inside mcr primers





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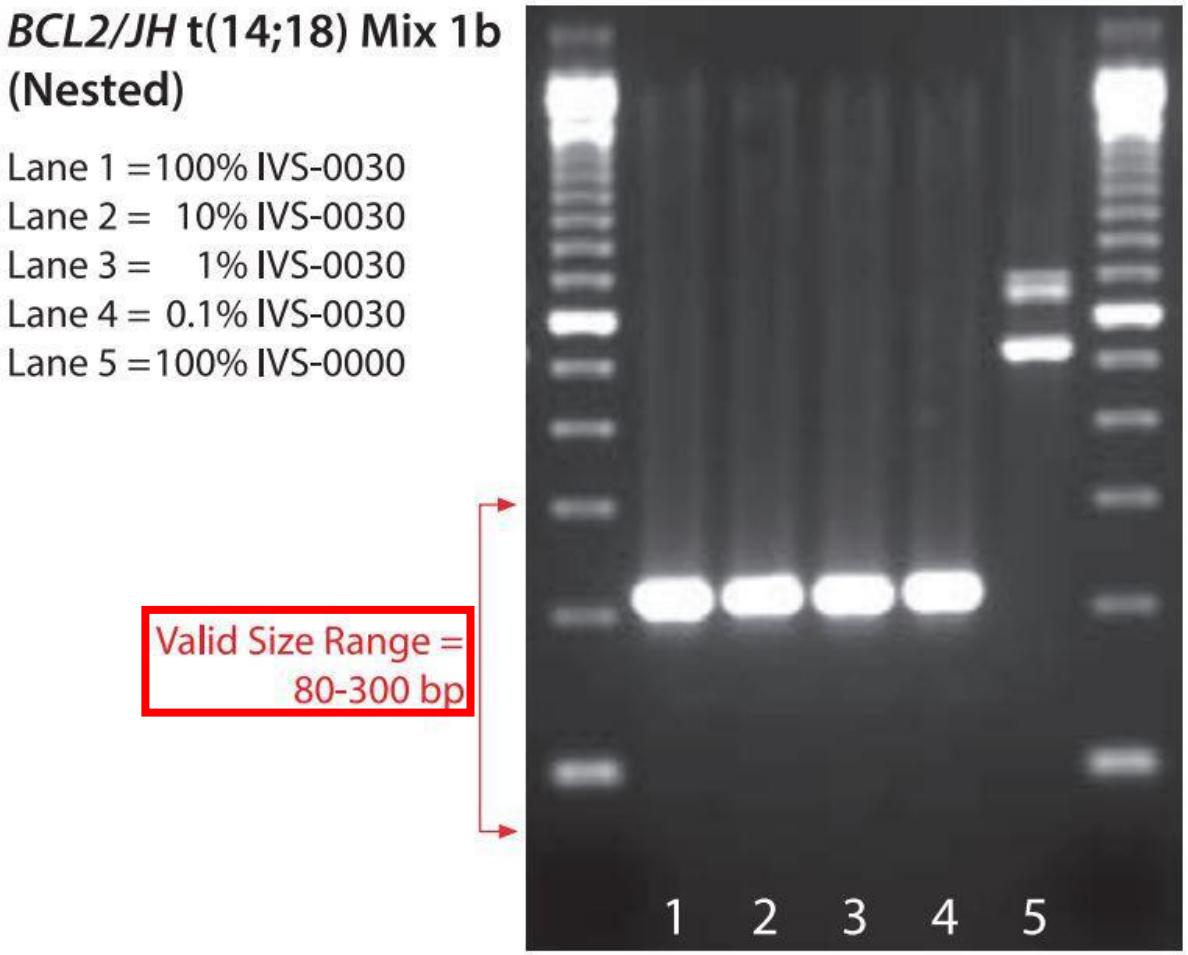
BCL2/JH t(14;18) Translocation Assay

Gel Electrophoresis Detection:

- **2% Agarose Gel**
- **One or more positive bands** within the valid size range should be considered as positive for the detection of *BCL2/JH* translocation



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Translocation Assays

BCR/ABL t(9;22) Translocation Assays

BCR/ABL t(9;22) Translocation Assays

Product Offerings:

- ***BCR/ABL* t(9;22) Translocation Assay**
- **RNA-based** qualitative **nested PCR** assay
- Molecular detection of **Philadelphia chromosome (Ph1)-positive** cells by nested reverse transcriptase PCR is faster and significantly more sensitive than cytogenetics or other methods
- This assay detects and identifies the variety of p190-, p210-, and p230-type transcripts produced from all known *BCR/ABL* translocations

Specimen Requirements

- This assay tests complementary DNA (cDNA) template

Available as **RUO assay** for **agarose/ polyacrylamide gel** as well as **ABI formats**

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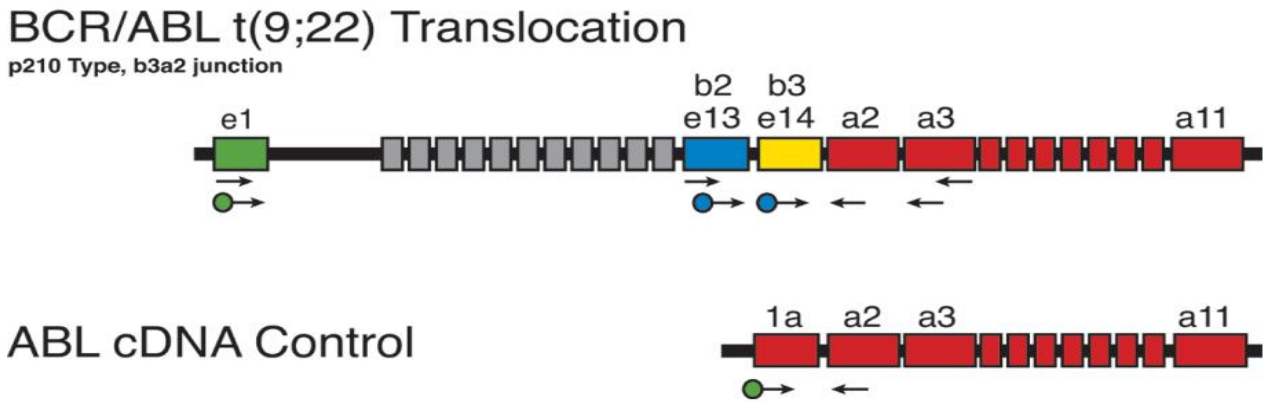
BCR/ABL t(9;22) Translocation Assays

- Tests e1a2, e1a3, b2a2, b2a3, b3a2 and b3a3
- Detects control ABL, **p190**, **p210**, **p230** transcripts
- Both p190 and p210 transcripts encode proteins with aberrant **tyrosine kinase activity**



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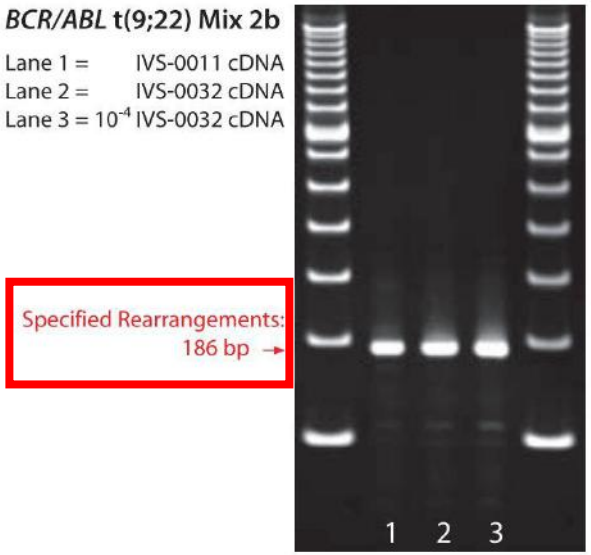
BCR/ABL t(9;22) Translocation Assays

Gel Electrophoresis Detection:

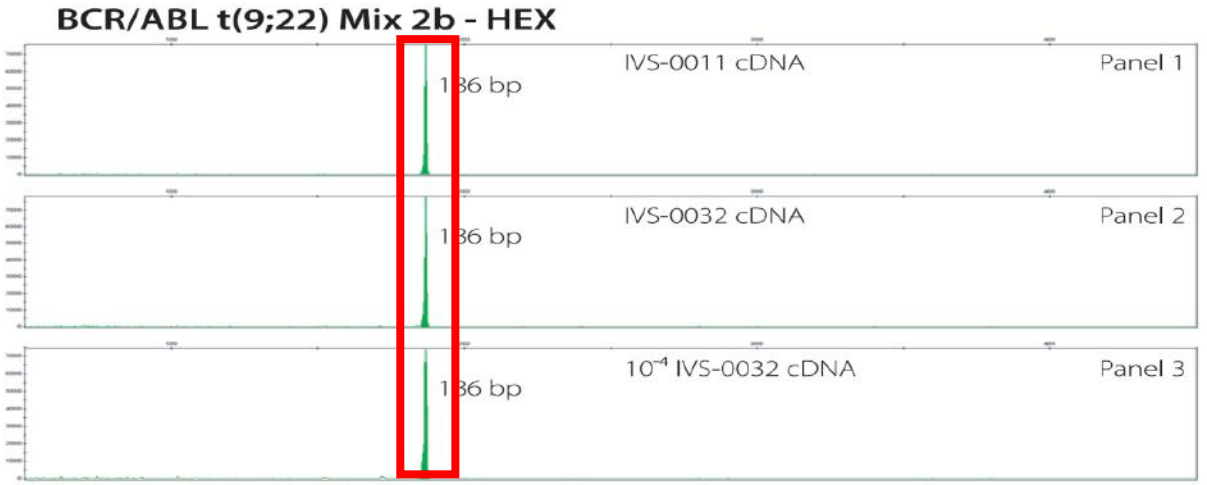
- **2% agarose or 6% polyacrylamide/TBE gel**
- A *BCR-ABL* translocation is indicated if **<u>just one of the 2nd round master mixes</u>** (Mix 2b, Mix 2c, Mix 3b, Mix 3c, or Mix 3d) generates product(s) of the valid size



Amplified products were run on a 2% agarose gel

Capillary Electrophoresis Detection (ABI):

- Panel 1: cDNA synthesized from an alternative 100% control RNA
- Panel 2: cDNA synthesized from the recommended 100% clonal control RNA
- Panel 3: cDNA synthesized from a 10^{-4} dilution of the recommended clonal control RNA



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Translocation Assays

PML/RARα t(15;17) Translocation Assays

PML/RARa t(15;17) Translocation Assays

Product Offerings:

- ***PML/RARa* t(15;17) Translocation Assays**
- **RNA based** qualitative **nested-PCR** assay
- Detects all three forms of ***PML/RARa*** translocation; **short (S)**, **long (L)** and **variable (V)** forms

Specimen Requirements

- This assay tests complementary DNA (cDNA) template

Available as **RUO assay** for **agarose/ polyacrylamide gel** as well as **ABI formats**

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PML/RARα t(15;17) Assay - Kit Contents

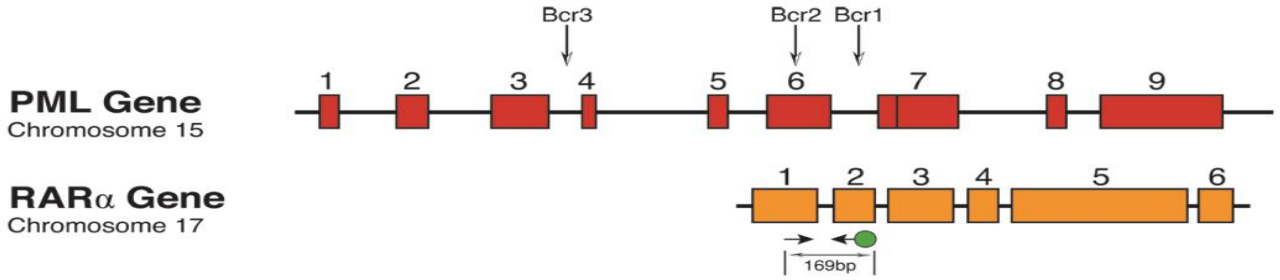
- 6 tubes (2 RNA controls + 4 MM)
- Targets: *RARa*, *PML/RARα*, S- & L-forms and L-forms

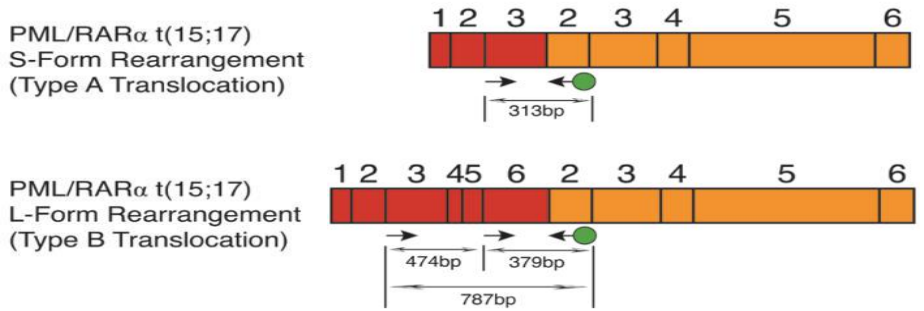
Mix 1
RARa

Mix 2a
PML/RARa

Mix 2b
S- and L-forms

Mix 2c
L-forms





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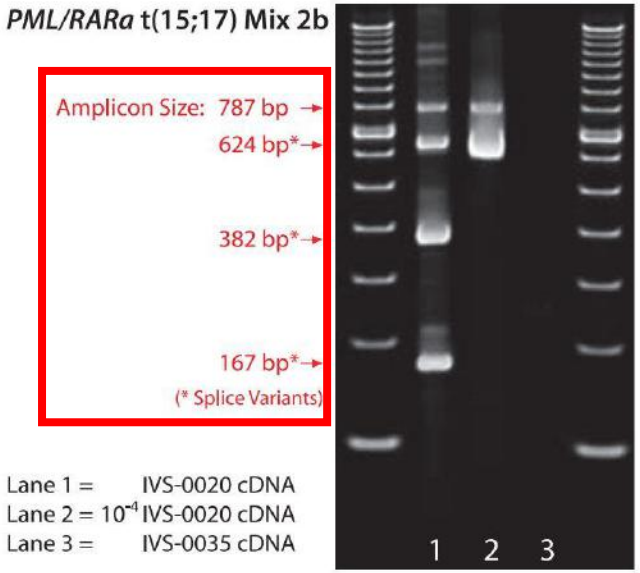
PML/RARα t(15;17) Translocation Assays

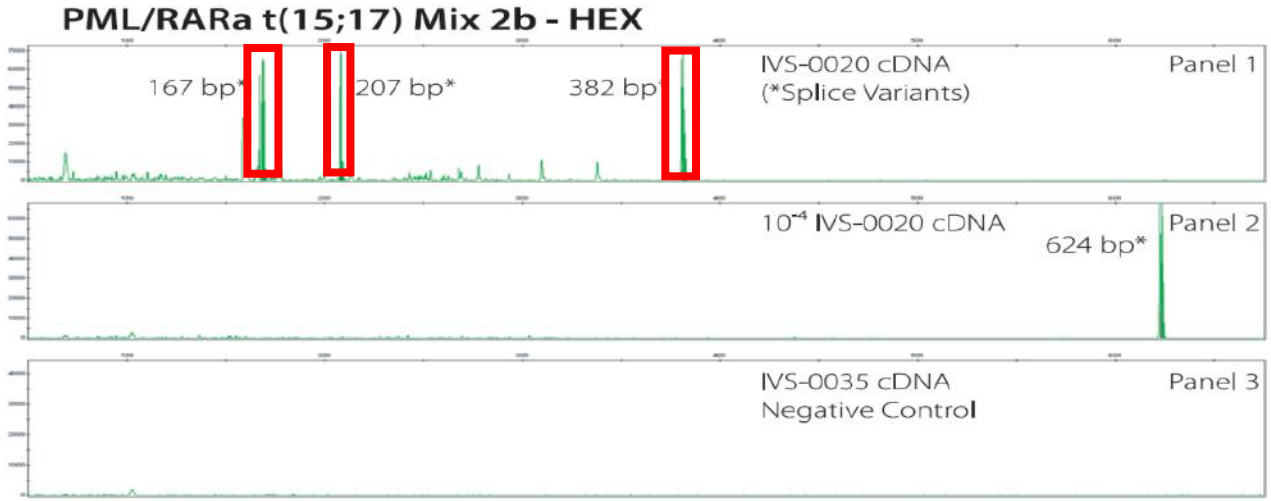
Gel Electrophoresis Detection:

- **2% agarose or 6% Polyacrylamide/TBE Gel**
- **<u>One or more positive bands</u>** within the valid size range should be considered as positive for the detection of *PML/RARa* translocation



Amplified products were run on a 6% PAGE gel

Capillary Electrophoresis Detection (ABI):



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Take Home Message

Translocation Assays

Take Home Message

IVS Translocation Assays Strengths

- Molecular identification of genetic markers
- Available for Gel and/or ABI formats
- Efficient and reliable standardized tests, reagents, and controls
- Extensive quality control and quality assurance
- Cutting-edge tools used for improving the quality of healthcare
- IdentiClone® products were designed by the EuroClonality group as part of the BIOMED-2 concerted action

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Translocation Assays

Quiz

Quiz

Which Invivoscribe translocation assays are available as CE-IVD products?

- *PML/RARa*
- *BCL2/JH t(14;18)*
- *BCR/ABL t(9;22)*
- *BCL1/JH*
- *BCL2/JH*

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Quiz

What are key characteristics of a nested PCR?

- Faster TAT
- A first and a second stage of PCR
- Increased LOD

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Quiz

Which Invivoscribe translocation assays are based on the EuroClonality/BIOMED-2 Concerted Action?

- *PML/RARa* Translocation
- *BCR/ABL* t(9;22)
- IdentiClone *BCL1/JH*
- IdentiClone *BCL2/JH*
- *BCL2/JH* t(14;18)

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Quiz

Which Invivoscribe translocation assays are available for gel and ABI detection?

- *PML/RARa* Translocation
- *BCR/ABL* t(9;22)
- IdentiClone *BCL1/JH*
- IdentiClone *BCL2/JH*
- *BCL2/JH* t(14;18)

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Thank You!

Any questions?

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