DNA
DIAGNOSTIC

Instruction For Use

HemaVision®-7

Screening test for 7 chromosome translocations and more than 40 breakpoints associated with leukemia

Instruction For Use
Cat No. HV01-7

DNA Diagnostic A/S
www.dna-diagnostic.com
Revision 2021.04.23

IVD CE

HemaVision®-7

Multiplex RT-PCR test

Screens for 7 leukemia causing translocations

t(1;19) (q23;p13) (TCF3-PBX1)

t(4;11) (q21;q23) (KMT2A-AFF1)

t(8;21) (q22;q22) (RUNX1-RUNX1T1)

t(9,22) (q34;q11) (BCR-ABL1)

t(12;21) (p13;q22) (ETV6-RUNX1)

t(15;17) (q24;q21) (PML-RARA)

inv(16) (p13;q22) (CBFB-MYH11)

Instruction For Use for HemaVision®-7
Cat. No. HV01-7
25 tests per kit

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HemaVision®-7

A multiplex RT-PCR screening test for 7 chromosome translocations and more than 40 breakpoints associated with leukemia

User Manual

for

HemaVision®-7, Cat. No. HV01-7

25 tests per kit

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1. PURPOSE OF THE TEST - SCREENING FOR 7 TRANSLOCATIONS (INTENDED USE)

HemaVision®-7 is a CE-marked *in vitro* diagnostic test for qualitative detection of the 7 most frequent leukemia causing chromosomal translocations including more than 40 breakpoints plus associated mRNA splice variants. Furthermore, HemaVision®-7 detects new breakpoints and mRNA splice variants for the 7 translocations. It is a fast one day test. The HemaVision®-7 test has sensitivity (>99%) and specificity (>96%).

Limit of detection is 10^{-8} μg of fusion RNA in a sample of 1 μg total RNA when the RNA quality is good.

This test provides a detailed description of the exon organization of fusion genes originating from chromosome translocations. Use of the test is described by Salto-Tellez et al. (Ref 22).

HemaVision®-7 is a qualitative test intended for use on total RNA samples from human blood or bone marrow for presence of chromosomal translocations associated with leukemia. Tests should be performed and results should be analysed by professionals only. The test is intended for use as an adjunct to evaluation of Leukemia in conjunction with other clinicopathological factors (aid to diagnosis).

The test is for professional use only.

HemaVision®-7 is a qualitative test using total RNA extracted from human whole blood or bone marrow cells as starting material in the test. The test uses reverse transcription of RNA to cDNA followed by multiplex PCR (Polymerase Chain Reaction), agarose gel electrophoresis, and interpretation.

The HemaVision®-7 kit contains primers for 25 cDNA reactions, 25 Master PCR tests, and 15 Split-out PCR tests.

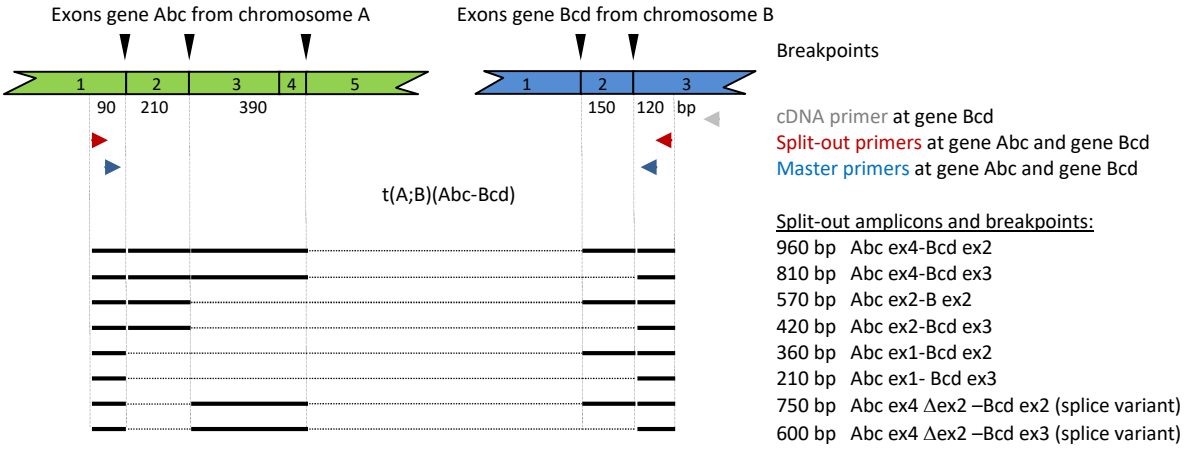


Figure 1 illustrates how HemaVision® kit HV01-7 identifies chromosomes, fusion genes and exons at the breakpoint among seven chromosome translocations causing leukemia. Only breakpoints for fusion genes maintaining the original translational reading frame is presented.

2. PRINCIPLES OF TEST

RNA is template for synthesis of cDNA in a reaction using Reverse Transcriptase (RT) and specific cDNA primers. The cDNA is template for two multiplex Master PCR amplifications using a hot start Taq DNA Polymerase and specific PCR primers. Many of the fusion genes have several breakpoints. Therefore, the PCR primers are designed to bind at positions enabling screening for all these breakpoints as illustrated in figure 1. If a Master PCR reaction is positive for a translocation specific amplicon the cDNA is also used as template for Split-out PCR reactions. PCR products are visualized by agarose gel electrophoresis. A Reaction Control amplicon of 983 bp is also detected in all reactions from the constitutively expressed Biotinidase (BTD) gene. This is a control for the quality of RNA and functionality of the test. The workflow of the test and an example of test results are shown in figure 2.

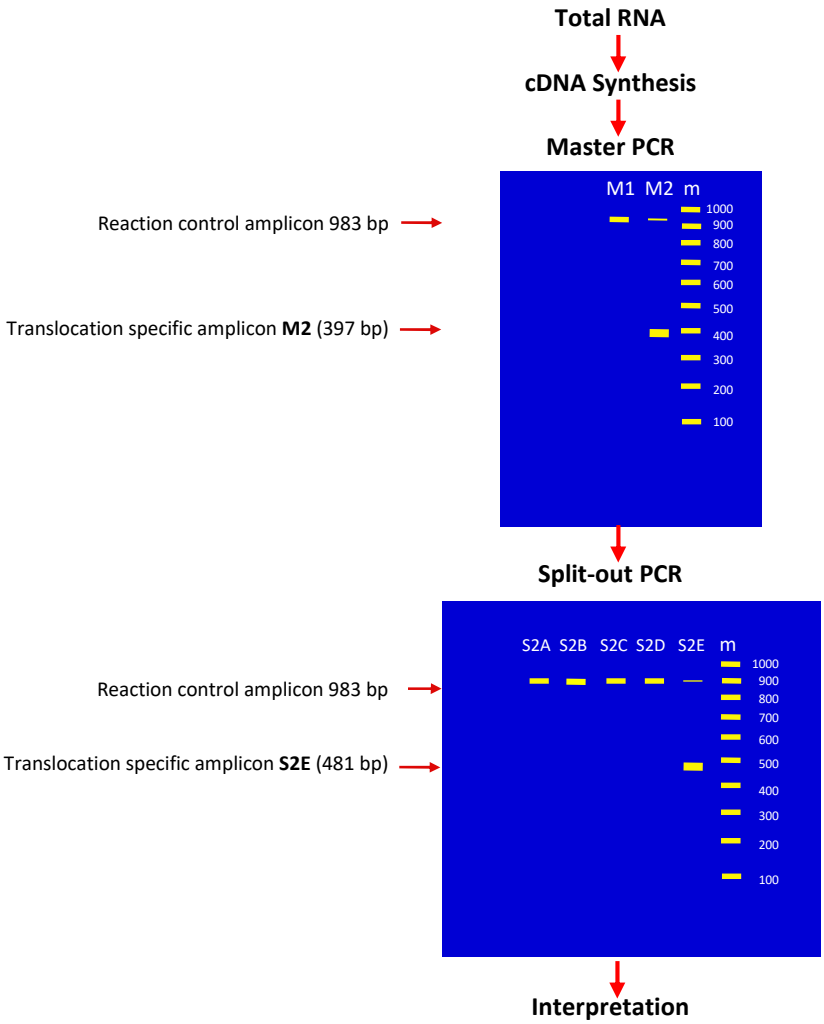


Figure 2. Workflow and results from a test with HemaVision®-7, Cat. No. HV01-7.

Result: 983 bp Reaction Control amplicon present in all lanes. M2 and S2E are positive for amplicon 397 bp and 481 bp respectively.

Conclusion: From the Interpretation Table 7 it can be seen this patient has a translocation at t(9;22)(q34;q11). The breakpoint is in the M-bcr (Major breakpoint cluster region) at position BCRex13-ABL1ex2 (b2a2) generating a P210 (kDa) fusion protein.

RNA Preparation

Total RNA is prepared from whole blood, cell lines, or bone marrow cells with the QIAamp® RNA Blood Mini Kit (Qiagen Cat. No. 52304).

cDNA Synthesis

cDNA is synthesized in a reaction containing the isolated RNA, cDNA Mix (primers) from the HemaVision® kit Cat No. HV01-7 kit and Reverse Transcriptase, 5x Buffer, DTT, and dNTP from the reagent module HemaVision® kit Cat. No. HV06-RMP or Cat No. HV04-RM. Three reagent modules are needed for each HemaVision®-7 kit.

Master PCR

The cDNA is used as a template for 2 multiplex PCR amplification reactions (Master PCR). Both Master PCR reactions use primer mixes from the HemaVision® HV01-7 kit and HemaVision DNA Polymerase, 10xbuffer, and dNTP from HemaVision® kit Cat. No. HV06-RMP. Both Master PCR reaction contains five pairs of translocation specific primers and one pair of reaction control primers specific for the housekeeping gene Biotinidase (BTD). The Master PCR products are analyzed by agarose gel electrophoresis. The 983 bp reaction control band is a positive control for using intact RNA and functionality of the RT-PCR reactions. A translocation specific band show the test is positive for a translocation. The identity of this translocation is established in the Split-out PCR.

Split-out PCR

Each Split-out PCR reactions contain only one pair of translocation specific primers and one pair of reaction control primers. When e.g. master M2 is positive for a translocation specific band continue the test using split-out reactions S2A-S2E. Again, the cDNA is used as template for the Split-out PCR reactions. The split-out PCR amplicons are analyzed by agarose gel electrophoresis. A translocation specific band show the test is positive for a translocation. The identity of this translocation is established by using the Interpretation Table 7.

Interpretation of results

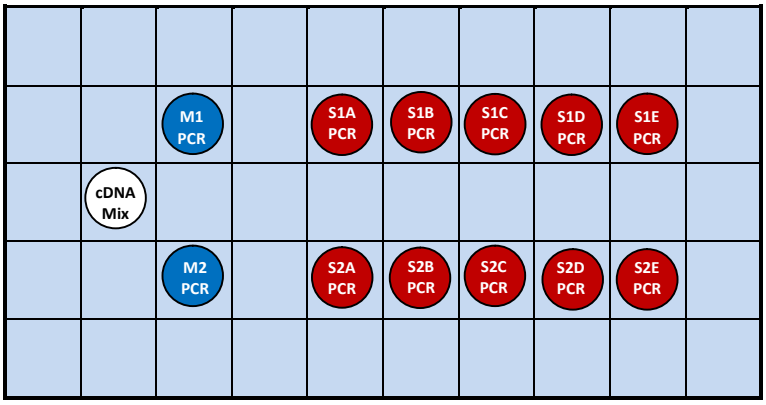
A sample is positive for a translocation when both master and split-out PCR produce a translocation specific band and reaction control amplicons are present. Note, the reaction control band can be weak in reactions with a strong translocation specific band. The breakpoint is identified by the reaction number of the split-out and the molecular size of the translocation specific amplicon using Interpretation Table 7. More than one translocation specific band in both Master and Split-out PCR reactions shows presence of alternative spliced mRNA from the fusion gene.

Figure 2 shows workflow for testing blood or bone marrow samples with HemaVision® test HV01-7. In this example, the test is positive in Master M2 and Split-out S2E with a translocation specific band of 397 bp and 480 bp respectively. The Reaction Control amplicon of 983 bp is present in all master and split-out lanes. From Interpretation Table 7 it can be concluded, the patient has a t(9;22)(q34;q11) translocation with a BCR-ABL1 fusion gene. The breakpoint joins exons BCRex13-ABL1ex2 also named b2a2. This breakpoint belongs to the major breakpoint cluster region (M-bcr) of the BCR gene.

3. KIT COMPONENTS AND STORAGE

The HemaVision® kit Cat. No. HV01-7 contains one box with Primer Mixes (see figure 3) and a User Manual for instruction. The kit is shipped at -20°C or below and the kit must be stored at -20°C by the customer. While performing the test always keep test components on ice (0°C). Each kit contains sufficient primer mixes for 25 cDNA reactions, 25 Master PCR reactions and 15 Split-out PCR reactions.

NOTE: It is essential for functionality of the HemaVision®-7 kit also to obtain and use the reagents provided in HemaVision®-RMP kit, **Cat. No. HV06-RMP** containing: MMLV-Reverse Transcriptase; 5x cDNA buffer; DTT; dNTP, HemaVision DNA Polymerase, 10x PCR buffer.



HemaVision®-7 contains primer mixes:
1 x 200 µL cDNA Mix (white cap)
2 x 155 µL Master PCR (blue caps)
10 x 95 µL Split-out PCR (red caps)

Figure 3 shows content of HemaVision®-7 kit, Cat. No. HV01-7.

4. EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

RNA extraction:
QIAamp® RNA Blood Mini Kit from Qiagen Cat. No. 52304.

Reagent Module:
HemaVision®-RMP kit Cat. No. HV06-RMP contains: MMLV-RT; 5x cDNA buffer; DTT; dNTP, HemaVision DNA Polymerase, 10x PCR buffer. Use three HV06-RMP kits together with each HemaVision®-7.

Instead of using HemaVision®-RMP use HemaVision®-RM Cat. No. HV04-RM containing MMLV-RT; 5x cDNA buffer; DTT; dNTP. HotStarTaq DNA Polymerase 5U/uL is from Qiagen.

Master Mix room – No templates in this room:
Micropipettes, 0.5-10 µL, 20-200 µL,
HemaVision® kit Cat. No. HV06-RMP containing: MMLV-RT; 5x cDNA buffer; DTT; dNTP, HemaVision DNA Polymerase, 10x PCR buffer
Aerosol barrier micropipette tips, 0.5-10 µL, and 20-200 µL
Micro centrifuge
Ice bath
RNase free tubes
Disposable gloves
RNase free water
-20ºC freezer for storage of kits (HemaVision®-7 and HemaVision®-RMP)

cDNA room:
Micropipettes, 0.5-10 µL, 20-200 µL
Aerosol barrier micropipette tips, 0.5-10 µL, and 20-200 µL
Micro centrifuge
Heating block/Water bath
Ice bath
RNase free tubes
Disposable gloves
RNase free water
-80^{o}C freezer for storage of RNA samples

PCR room:
Micropipettes, 0.5-10 µL, 20-200 µL
Aerosol barrier micropipette tips, 0.5-10 µL, and 20-200 µL
Micro centrifuge
Thermal Cycler
Ice bath
PCR tubes (0.1 mL or 0.2 mL) and lids
Disposable gloves

Gel electrophoresis room:
Micropipettes, 0.5-10 µL
Aerosol barrier micropipette tips, 0.5-10 µL
Micro centrifuge
Equipment for agarose gel electrophoresis
Disposable gloves
Molecular size marker (e.g. 100 bp ladder)

5. PRECAUTIONS

General precautions

- The quality and concentration of the RNA sample greatly affects the results of this test. To minimize the risk of degradation by ribonucleases, we strongly recommend purification of total RNA immediately after blood or bone marrow extraction. Optionally, mononuclear cells can be purified prior to RNA extraction using Ficoll Hypaque. Do not freeze Ficoll purified cells without adding a denaturing solution [e.g. containing guanidinium isothiocyanate (GTC)] immediately after isolation and before freezing.
- Use blood from venipuncture collected into a tube containing EDTA. Alternatively, use bone marrow collected into a tube containing EDTA. Do not freeze the blood or bone marrow sample.
- Alternatively, blood samples can be stabilized in PAXgene Blood RNA tubes, Qiagen cat. no. 762165 and bone marrow samples can be stabilized in PAXgene Bone marrow RNA tubes, Qiagen cat. no. 764114.
- Do NOT use Heparin for stabilization of blood samples.
- Always store cell samples and aqueous RNA solutions at -80°C. Even an overnight storage at -20°C may result in RNA degradation.
- When working with RNA always use gloves, as hands are a major source of ribonuclease contamination.
- The integrity and purity of RNA is important for the efficiency of the cDNA synthesis and thus detection of translocations. The quality of RNA can be checked by OD 260/280 nm measurements, agarose gel electrophoresis, or using RIN number from the Agilent Bioanalyzer.

- For more guidance on specimen collection, RNA isolation and storage please refer to "ISO 20186:2019 Molecular in vitro diagnostic examinations – Specifications for pre-examination processes for venous whole blood – Part 1: isolated cellular RNA" and CLSI guidance MM13-A "Collection, Transport, Preparation and Storage of Specimens for Molecular Methods; Approved guideline.
- RT-PCR is a very sensitive technique. Therefore, precautions must be taken to avoid false positive results caused by contamination with RNA, cDNA or PCR products from other samples.
- Dedicate four separate rooms/areas to the following activities:
  - Master mix production – no templates in here
  - cDNA synthesis
  - PCR
  - Gel electrophoresis
- A set of micropipettes, aerosol barrier pipette tips, disposable gloves and clean lab coats should be kept in each of the four rooms. The work must be organized so that mixes and reaction products only moves in the direction from 1-4. NEVER move mixes or reaction products in the opposite direction.
- Laboratory workbenches, pipettes, and lab coats must be cleaned on a regular basis.
- Use of aerosol barrier pipette tips is highly recommended during the entire procedure. It is essential to change gloves very often when handling tubes containing RNA or cDNA.
- For more general guidance on best practice in PCR testing please refer to the CLSI guidance document MM01: Molecular Methods for Clinical Genetics and Oncology Testing, 3rd Edition

Safety

- Read and understand the procedure before starting.
- Normal laboratory aseptic technique should be followed at all times.
- Treat each sample as if it is infectious.
- Wear eye protection and disposable gloves during all steps of the assay.
- The products can be discarded as normal laboratory waste
- Check the safety data sheet for the product on our website for further information or ask for a copy of it.

6. PROCEDURE

Procedural notes

- Store all test components as described in section *3: Kit Components and Storage*.
- Do not mix reagents from different lots.
- Careful pipetting technique is essential for accurate results.
- This protocol is optimized with enzymes and buffers from HemaVision® kit Cat. No. HV06-RMP and HV04-RM + HotStarTaq DNA Polymerase 5U/uL from Qiagen.
- This protocol is optimized for the ABI (Perkin Elmer) GeneAmp 9600/9700 thermal cycler. Use of another thermal cycler may require optimization by the user.
- As a positive control for RNA quality and functionality of each RT-PCR reaction a 983 bp fragment of the housekeeping gene biotinidase must be present in all lanes except in reactions positive for a translocation specific amplicon where it may be weak or missing.
- As a negative control, make the cDNA reaction without RNA template.

RNA preparation

- Due to the inherent instability of RNA use patient samples as fresh as possible. Within 24 hours of sample collection, extract total RNA.

- RNA can be extracted from whole blood with QIAmp RNA blood minikit, Qiagen cat. no. 52304. Typically 5-10 μg total RNA is extracted from 1 x10^7 mononuclear blood cells.
- For blood collected in PAXgene tubes, RNA can be extracted with the PAXgene Blood RNA kit, Qiagen cat. no. 762174.
- For bone marrow collected in PAXgene tubes, RNA can be extracted with PAXgene Bone marrow RNA kit, Qiagen cat. no. 764133.
- Measure the RNA concentration by reading the optical density at 260 nm. An absorbance of 1 unit at 260 nm corresponds to 40 μg of RNA per mL. Adjust the concentration of RNA to 0.1 μg/μL with RNase free H_2O.
- Make 20 μL (0.1 μg/μL) RNA aliquots in RNase free tubes. Store RNA aliquots at –80°C or use RNA immediately for cDNA synthesis.

Master and Split-out tests

Step 1 cDNA Synthesis

1.1 In the Master Mix room prepare *cDNA Synthesis Mix* according to Table 1 using reagents from HemaVision®-RMP reagent module Cat. No. HV06-RMP.

Table 1: *cDNA Synthesis Mix*

Number of samples	1	2
5x MMLV-RT Buffer (μL)	8	16
100 mM DTT (μL)	4	8
10 mM dNTP Mix (μL)	4	8
MMLV-RT (μL)	1	2
Total volume (μL):	17	34

1.2 In the cDNA room add 7 μL *cDNA Mix* (primers) from white capped tube in the HemaVision®-7 kit to one 0.2 mL PCR tube containing 16 μL total RNA (1.6 μg). Mix gently and spin down for 10 seconds.

1.3 In a separate RNase free tube, add 7 μL of *cDNA Mix* (primers) to 16 μL H_2O (negative control).

1.4 Incubate the tubes in a heating block or water bath at 65°C for 5 minutes. Chill tubes on ice and hold on ice.

1.5 Add 17 μL of the *cDNA Synthesis Mix* to the tube with 23 μL RNA+cDNA Mix and the negative control tube from step 1.4. Mix gently and spin down for 10 seconds.

1.6 Incubate at 37°C for 45 minutes.

1.7 Incubate at 95°C for 5 min to inactivate the MMLV-RT enzyme.

1.8 Chill and hold the cDNA tube on ice (0°C, do not freeze) for a maximum of three days before use in Master PCR and Split-out PCR.

Step 2 Master PCR

To achieve maximal sensitivity and specificity Master and Split-out reactions do not contain identical translocation specific primers.

2.1 In the Master Mix room prepare the *Master Mix PCR* for samples and negative control according to Table 2 using HemaVision® reagent module Cat. No. HV06-RMP. Mix and spin down for 10 seconds.

Table 2: *Master Mix PCR*

Number of samples		**1**	**2**
Number of PCR reactions	**1**	**2**	**4**
10x PCR buffer (μL)	2.5	5.0	10.0
dNTP mix (μL)	0.5	1.0	2.0
HemaVision DNA Polymerase (μL)	0.4	0.8	1.6
H_2O (μL)	13.6	27.2	54.4
Total volume (μL):	17	34	68.0

2.2 Label 0.2 mL PCR tubes "M1" and "M2".

2.3 Add 17 μL *Master Mix PCR* to each of the PCR tubes.

2.4 From HemaVision®-7 transfer from blue-capped tubes 5 μL *Master Mix M1* PCR primer to PCR tube labeled "M1" and 5 μL *Master Mix M2* PCR primer to PCR tube labeled "M2".

2.5 In the PCR room add 3 μL cDNA (from step 1.8) to each of the PCR tubes from step 2.4. Close the tubes, mix and spin for 10 seconds. Volume per tube 25 μL.

2.6 Transfer the tubes to a thermal cycler and start the PCR amplification using the PCR cycling parameters in Table 3. Notice during the first 15 cycles, the annealing temperature is reduced by 0.2°C per cycle starting at 65°C and ending at 62°C.

Table 3: Master PCR Amplification Parameters

Step	**Time/Temperature**	**Cycles**
1	15 minutes at 95°C	1
2	30 seconds at 95°C 60 seconds at 65°C minus 0.2°C/cycle. (Touch down program to reduce unspecific amplification) 90 seconds at 72°C	15
3	30 seconds at 95°C 30 seconds at 62°C. 90 seconds at 72°C	22
3	Hold at 4°C	1

Step 3 Gel electrophoresis

3.1 Prepare a 1.5 % (w/v) agarose gel at least 10 cm long in 1X TBE buffer. Add ethidium bromide to a final concentration of 0.5 μg/mL.

3.2 In the Gel Electrophoresis room carefully open the Master PCR tubes without contaminating gloves and surroundings with drops containing high copy numbers of amplicon.

3.3 Add 3 μL of 10x loading buffer into each PCR tube. Load approximately 14 μL per slot in the gel. Finally load a molecular size marker to the gel.

3.4 Run the gel in 1X TBE buffer until the Bromophenol blue dye has migrated approximately 3/4 of the gel.

3.5 Examine the gel with UV-light and document result by photography.

Note! As an alternative to the home made agarose gels, use the very fast gel system "FlashGel System" from LONZA.

Step 4 Interpretation of results from Master PCR

- Positive for one (or more) translocation specific band(s). Continue with the corresponding Split-out reactions to identify translocation. As an example, see figure 2.
- Positive for a translocation specific band but it is very weak or a smear. Positive for reaction control band (983 bp) in all lanes. Continue with the relevant Split-out reactions to identify translocation.
- Negative for a translocation specific band and positive for reaction control band (983 bp) in all lanes M1 and M2. The patient is negative for the 7 translocations detectable by HemaVision®-7.
- Negative for a translocation specific band and negative for reaction control band (983 bp) in all lanes M1-M2. The test failed most likely due to 1) poor quality of RNA, or 2) use of thermal cycler with temperature profile (ramp speed) differing from ABI 9600/9700, or 3) incorrect mixing of reactions. Correct and repeat test.
- Negative for a translocation specific band and positive for reaction control band (983 bp) in some of the lanes M1 and M2. The test failed most likely due to 1) use of thermal cycler with temperature profile (ramp speed) differing from ABI 9600/9700, or 2) incorrect mixing of some reactions. Correct and repeat test.
- Negative Control test is positive (band(s) present): Reaction(s) have been contaminated with DNA. Identify contamination and repeat test.

Step 5 Split-out PCR

When a sample is tested positive for a translocation in a Master PCR the translocation and the breakpoint in the fusion gene can be identified by testing the remaining cDNA in a Split-out PCR reaction.

5.1 In the Master Mix room prepare the *Split-out Mix PCR* according to Table 4 using HemaVision® reagent module Cat. No. HV06-RMP. Mix and spin down for 10 seconds.

Table 4: *Split-out Mix PCR*

Number of positive Master PCR		**1**	**2**
Number of Split-out PCR reactions	**1**	**5**	**10**
10x PCR buffer (μL)	2.5	12.5	25
dNTP mix (μL)	0.5	2.5	5
HemaVision DNA Polymerase (μL)	0.4	2.0	4
H_2O (μL)	13.6	68	136
Total volume (μL):	17.0	85.0	170

5.2 For each positive Master PCR reaction label five PCR tubes (e.g. if Master M2 is positive label five Split-out tubes: S2A, S2B,…..S2E).

5.3 Aliquot 17 μL *Split-out Mix PCR* to each of the PCR tubes.

5.4 To each of these tubes add 5 μL Split-out PCR primers from red-capped tubes (e.g. to a PCR tube labeled S2A add 5 μL S2A PCR primer mix).

5.5 In the PCR room add 3 μL cDNA (from step 1.8) to each of the PCR tubes from step 5.4. Close the tubes, mix and spin for 10 seconds. Volume per tube 25 μL.

5.6 Transfer the tubes to a thermal cycler and start the PCR amplification using the PCR cycling parameters in Table 5. Notice during the first 15 cycles, the annealing temperature is reduced by 0.2°C per cycle starting at 65°C and ending at 62°C to reduce unspecific amplification.

Table 5: Split-out PCR Amplification Parameters

Step	Time/Temperature	Cycles
1	15 minutes at 95°C	1
2	30 seconds at 95°C 60 seconds at 65°C minus 0.2°C/cycle. (Touch down program) 90 seconds at 72°C	15
3	30 seconds at 95°C 30 seconds at 62°C. 90 seconds at 72°C	22
3	Hold at 4°C	1

Step 6 Gel electrophoresis

6.1 Prepare a 1.5 % (w/v) agarose gel at least 10 cm long in 1X TBE buffer. Add ethidium bromide to a final concentration of 0.5 μg/mL.

6.2 In the Gel Electrophoresis room carefully open the Split-out PCR tubes without contaminating gloves and surroundings with drops containing high copy numbers of amplicon.

6.3 Add 3 μL of 10x loading buffer into each PCR tube. Load approximately 14 μL per slot in the gel. Finally load a molecular size marker.

6.4 Run the gel in 1X TBE buffer until the Bromophenol blue dye has migrated approximately 3/4 of the gel.

6.5 Examine the gel with UV-light and document result by photography.

Note! As an alternative to the home made agarose gels, use the very fast gel system "FlashGel System" from LONZA.

7. INTERPRETATION

- The HemaVision®-7 kit tests for 7 chromosomal translocations or chromosomal rearrangements associated with leukemia. It involves more than 40 fusion-gene mRNA variants due to heterogeneity of the breakpoints and/or alternative splicing. After agarose gel electrophoresis do interpretation as follows:
- Look for a positive Biotinidase (BTD) reaction control band (983 bp) in all split-out PCR reactions. The reaction control band can be weak or missing in the lane containing a strong translocation specific band.
- Look for a translocation specific band. The translocation specific Split-out PCR band has a slightly larger molecular size as the translocation specific band in the corresponding Master PCR reaction. Identify the translocation with Table 7.

As an example: M2 and S2E contain a translocation specific amplicon of 397 bp and 481 bp respectively. Table 7 show the sample has a t(9;22)(q34;q11) translocation with a fusion gene BCR-ABL1 having its breakpoint at exons b2a2.

- When two Master PCR and two Split-out PCR reactions contain translocation specific bands it can be caused by two translocations. However, it can also be caused by amplification of two overlapping regions and/or splice-variants from only one translocation.

- E.g. the BCR gene in the t(9;22)(BCR-ABL1) translocation can have leukemia associated breakpoints in introns after exon 1, exon 6, exon 12, exon 13, exon 14, exon 19, and exon 20 resulting in functional fusion proteins with an ABL1 breakpoint at exon 2 or 3. M1 and S1E contain upper PCR primers binding to BCR exon 1. M2 and S2E contain upper PCR primers binding to BCR exon 12. When the breakpoint is located somewhere after BCR exon 12, M2/S2E will be positive. However, sometimes also M1/SE1 is positive together with M2/S2E. In this situation M1/S1E generates an amplicon 1.3 kbp larger than M2/S2E (when no alternative splicing is present). The translocation must be interpreted as a positive S2E, see Table 6. This Table shows translocations with two positive Masters/Split-out reactions.

- The Split-out PCR is negative for the "translocation specific" band seen in the corresponding Master PCR reaction. All the Split-out reactions contain the reaction control band (983 bp). Then the test must be interpreted as negative for a translocation present in Table 7. When some or all Split-out reactions also are negative for the reaction control band the Split-out test failed and it must be repeated.

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Table 6: Translocations with two positive Master and Split-out reactions

Positive in Split-out..	..and also positive in Split-out	Interpret as translocation from Split-out	Translocation
S2D	S1C	**S2D**	inv(16)
S2E	S1E	**S2E**	t(9;22)
S1D	S2B	**S1D**	t(15;17)

Table 7: Interpretation table

PCR	TRANSLOCATION	GENES	BREAKPOINT	MASTER	SPLIT-OUT	REFERENCE
M1/S1A	t(1;19)(q23;p13)	TCF3(19p13.3) PBX1(PRL)(1q23.3)	TCF3 ex16 – PBX1 ex3	376 bp	524 bp	1; 2; 3
M1/S1B	t(12;21)(p13;q22)	ETV6(12p13) RUNX1(21q22.3)	ETV6 ex5 – RUNX1 ex3 ETV6 ex5 – RUNX1 ex4 ETV6 ex6 – RUNX1 ex5 ETV6 ex5 – RUNX1 ex3Δex4	460 bp 421 bp 310 bp 206 bp	495 bp 456 bp 355 bp 241 bp	4; 5; 6; 7
M1/S1C	inv(16)(p13;q22)	CBFB (16q22.1) MYH11 (16p13.11)	CBFB ex4 – MYH11 ex 34 (F) CBFB ex5 – MYH11 ex 34 (A) CBFB ex4 – MYH11 ex 33 CBFB ex5 – MYH11 ex 33 (B) CBFB ex4 – MYH11 ex 32 CBFB ex5 – MYH11 ex 32	213 bp 309 bp 426 bp 522 bp 675 bp 771 bp	415 bp 511 bp 628 bp 724 bp 877 bp 973 bp	8
M1/S1D	t(15;17)(q24;q21)	PML(15q24) RARA(17q21)	PML ex6 - RARA ex3 (L, bcr1) PMLδex6 –RARA ex3 (V, bcr2)	353 bp 100-350 bp	455 bp 202-452 bp	9; 10; 11
M1/S1E	t(9;22)(q34;q11)	BCR(22q11) ABL1(9q34.1)	<u>m-bcr, P190</u> BCR ex1 – ABL1 ex3 (e1a3) BCR ex1 – ABL1 ex2 (e1a2) BCR ex6 – ABL1 ex3 (e6a3) BCR ex6 – ABL1 ex2 (e6a2)	 146 bp 320 bp 788 bp 962 bp	 241 bp 415 bp 883 bp 1057 bp	12; 13; 14

PCR	TRANSLOCATION	GENES	BREAKPOINT	MASTER	SPLIT-OUT	REFERENCE
M2/S2A	t(8;21)(q22;q22)	RUNX1(21q22.3) RUNX1T1(8q22)	RUNX1 ex6 -RUNX1T1 ex2 RUNX1 ex7 -RUNX1T1 ex2 RUNX1 ex8 -RUNX1T1 ex2	197 bp 389 bp 551 bp	407 bp 599 bp 761 bp	15; 16; 17
M2/S2B	t(15;17)(q24;q21)	PML(15q24) RARA(17q21)	PML ex3 - RARA ex3 (S, bcr3)	325 bp	490 bp	9; 10; 11
M2/S2C	t(4;11)(q21;q23)	KMT2A(11q23) AFF1(4q21.3)	KMT2A ex8 - AFF1 ex7(1546) KMT2A ex8 - AFF1 ex6(1459) KMT2A ex9 - AFF1 ex7(1546) KMT2A ex8 - AFF1 ex5(1414) KMT2A ex9 - AFF1 ex6(1459) KMT2A ex10 - AFF1 ex7(1546) KMT2A ex9 - AFF1 ex5(1414) KMT2A ex10 - AFF1 ex6(1459) KMT2A ex10 - AFF1 ex5(1414)	293 bp 380 bp 425 bp 425 bp 512 bp 539 bp 557 bp 626 bp 671 bp	313 bp 400 bp 445 bp 445 bp 532 bp 559 bp 577 bp 646 bp 691 bp	18; 19; 20; 21
M2/S2D	inv(16)(p13;q22)	CBFB (16q22.1) MYH11(16p13.11)	CBFB ex4 – MYH11 ex 31 CBFB ex5 – MYH11 ex 31 CBFB ex4 – MYH11 ex 30 (G) CBFB ex5 – MYH11 ex 30 (D) CBFB ex4 – MYH11 ex 29 CBFB ex5 – MYH11 ex 29 (E)	165 bp 261 bp 270 bp 366 bp 477 bp 573 bp	216 bp 312 bp 321 bp 417 bp 528 bp 624 bp	8
M2/S2E	t(9;22)(q34;q11)	BCR(22q11) ABL1(9q34)	<u>M-bcr, P210</u> BCR ex12 – ABL1 ex3 (b1a3) BCR ex13 – ABL1 ex3 (b2a3) BCR ex12 – ABL1 ex2 (b1a2) BCR ex14 – ABL1 ex3 (b3a3) BCR ex13 – ABL1 ex2 (b2a2) BCR ex14 – ABL1 ex2 (b3a2) <u>μ-bcr, P230</u> BCR ex19 – ABL1 ex3 (c3a3) BCR ex20 – ABL1 ex3 (c4a3) BCR ex19 – ABL1 ex2 (c3a2) BCR ex20 – ABL1 ex2 (c4a2)	 118 bp 223 bp 292 bp 298 bp 397 bp 472 bp 838 bp 973 bp 1012 bp 1147 bp	 202 bp 307 bp 376 bp 382 bp 481 bp 556 bp 924 bp 1057 bp 1096 bp 1231 bp	12; 13; 14

Table 7 is used for interpretation of results observed from agarose gel electrophoresis of split-out PCR. The table lists translocations, involved genes, exons at breakpoint, and molecular size of Master and Split-out PCR amplicons. Numbering of exons has been updated year 2014 according to GenBank http://www.ncbi.nlm.nih.gov/genbank/. Only breakpoints maintaining the original translational reading frame from the involved genes are presented.

Note: A PCR amplicon with a molecular size not listed in Table 7 can appear as a consequence of amplification across an unpublished breakpoint or splice variant.

Note: The interpretation table has been updated according to the HUGO Gene Nomenclature Committee (HGNC) see section "8. GENE ABBREVATIONS ACCCORDING TO THE HGNC".

8. GENE ABBREVIATIONS ACCORDING TO THE HGNC:

The HUGO Gene Nomenclature Committee (HGNC) approves a *unique* and *meaningful* name for every known human gene (read more at www.genenames.org). Table 8 contains a list of all relevant genes for the HemaVision®-7 kit, with the old abbreviation and the corresponding HGNC abbreviation. The chromosome position for the gene, HGNC ID number for the protein and NCBI ACCESSION number for the DNA sequence encoding the mRNA are also shown. For details go to the NCBI web site (www.ncbi.nlm.nih.gov)

Table 8

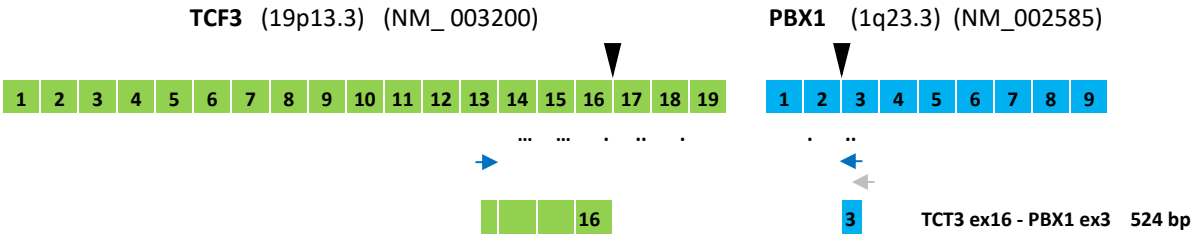
Old Abbreviation	HGNC Abbreviation	Chromosome	HGNC ID	NCBI ACCESSION
ABL	ABL1	9q34.1	76	NM_007313
AF4	AFF1	4q21.3	7135	NM_001166693
AML1	RUNX1	21q22.3	10471	NM_001754
BCR	BCR	22q11	1014	NM_004327
CBFβ	CBFB	16q22.1	1539	NM_022845
E2A	TCF3	19p13.3	11633	NM_003200
ETO	RUNX1T1	8q22	1535	NM_004349
MLL	KMT2A	11q23	7132	NM_001197104
MYH11	MYH11	16p13.11	7569	NM_001040114
PBX1	PBX1	1q23.3	8632	NM_002585
PML	PML	15q24	9113	NM_033238
RARα	RARA	17q21	9864	NM_000964
TEL	ETV6	12p13	3495	NM_001987
BTD	BTD	3p25	1122	NM_000060

9. FIGURES FOR SPLIT-OUT AMPLICONS

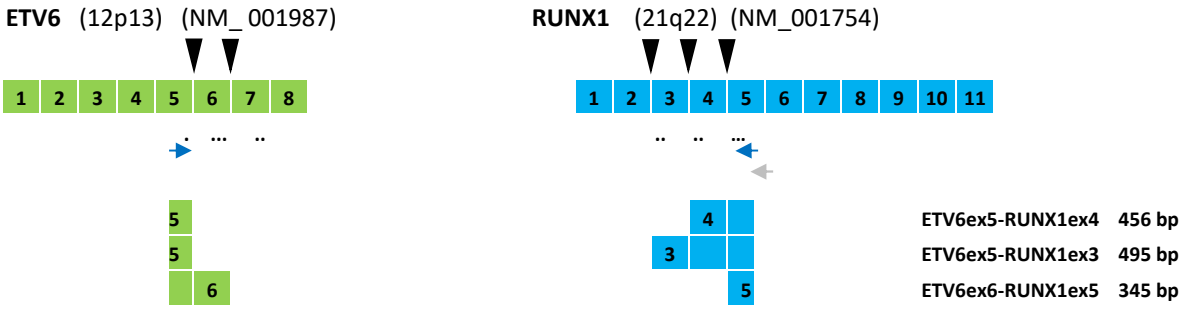
Figures below illustrates principle for the split-out RT-PCR reactions of the HemaVision®-7 kit. The tests identifies chromosomes, fusion genes and exons at the breakpoint. Only intron breakpoints for fusion genes maintaining the original translational reading frame are shown.

Blue arrow: PCR primers. Grey arrow: cDNA primer. Black arrow: Breakpoints. One, two or three dots indicate translational reading frame at end or beginning of exon.

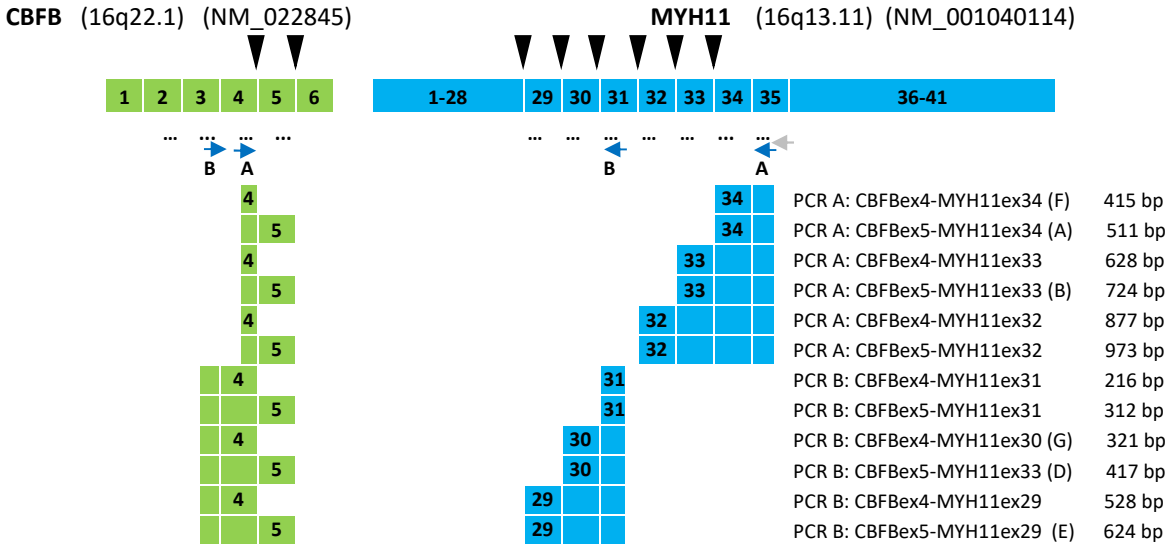
Split-out S1A t(1;19)(TCF3-PBX1)



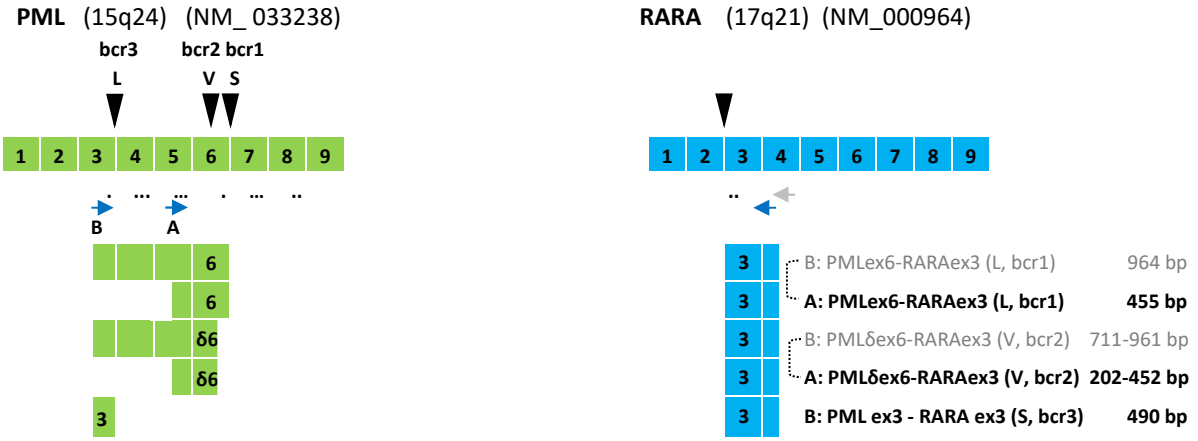
Split-out S1B t(12;21)(ETV6-RUNX1)



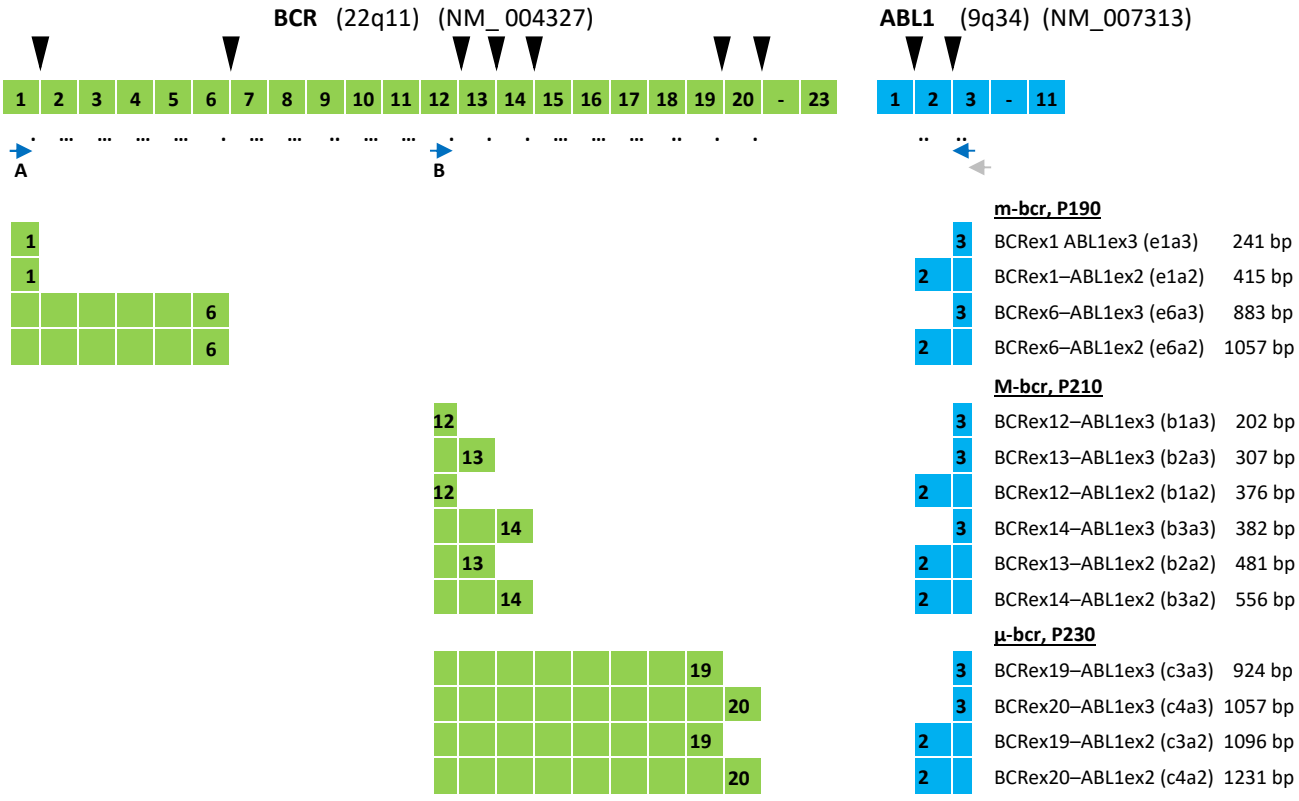
Split-out S1C (primer A) + S2D (primer B) inv(16)(CBFB-MYH11)



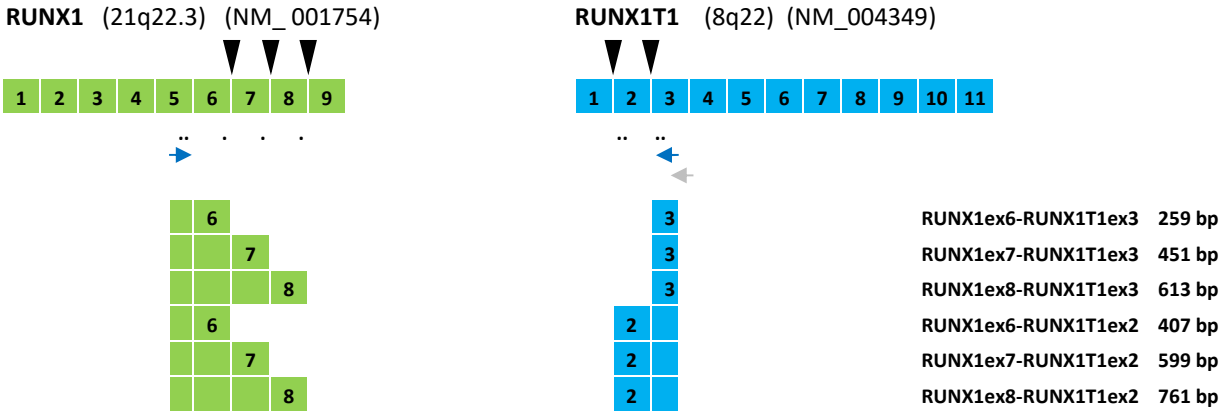
Split-out S1D (primer A) + S2B (primer B) t(15;17)(PML-RARA)



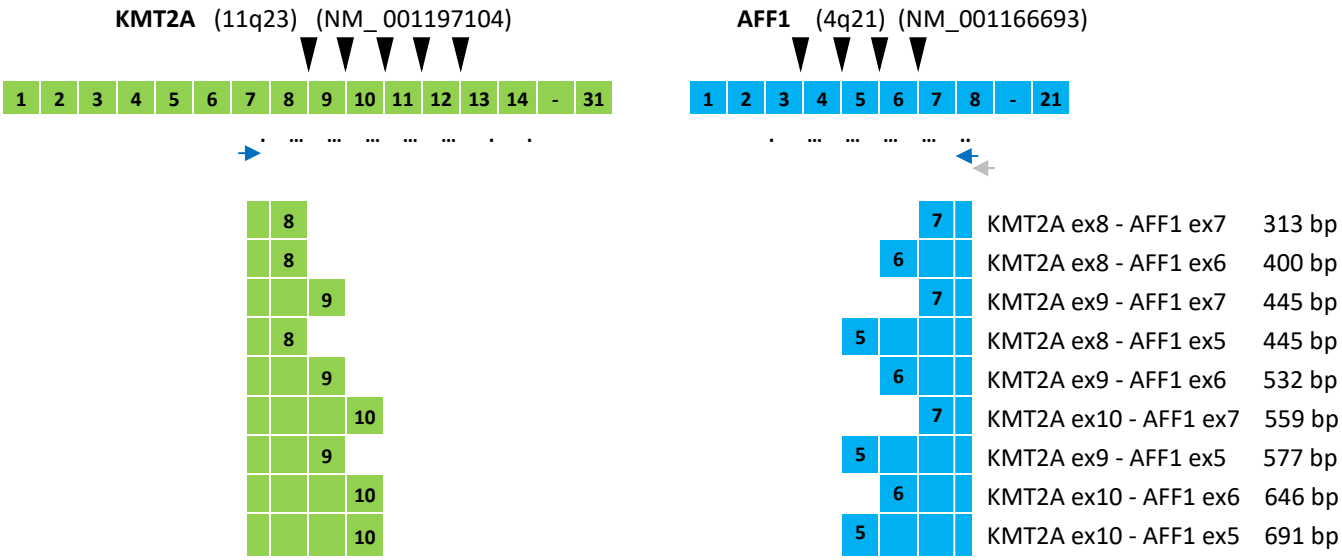
Split-out S1E (primer A) + S2E (primer B) t(9;22)(BCR-ABL1)



Split-out S2A t(8;21)(RUNX-RUNX1T1)



Split-out S2C t(4;11)(KMT2A-AFF1)



Symbols used on tubes and boxes

CE	"Conformité Européenne" ("European Conformity")	IVD	In vitro Diagnostic Medical Device		Consult instructions for use
REF	Catalogue Number	LOT	Lot number	CONT	Contents
	Storage temperature		Expiry Date		Manufacturer

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REVISION HISTORY

REVISION NUMBER	SECTION CHANGED	DATE
8	Updated intended use to more precisely describe. Updated 5. Precautions with more details on specimen collection and RNA extraction. Updated 6. procedure with more details on RNA extraction.	2019.09.19
9	Corrected mistakes in precautions where qPCR was mentioned and it should be PCR	2020.09.15
10	Changed from User Manual to Instruction For Use	2021.04.23

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