

# HemaVision<sup>®</sup>-28N

Screening test for 28 chromosome translocations and more than 145 breakpoints associated with leukemia

del1(p32) (STIL-TAL1)	t(9;12) (q34;p13) (ETV6-ABL1)
t(1;11) (p32;q23) (MLL-EPS15)	t(9;22) (q34;q11) (BCR-ABL1)
t(1;11) (q21;q23) (MLL-MLLT11)	t(10;11) (p12;q23) (MLL-MLLT10)
t(1;19) (q23;p13) (TCF3-PBX1)	t(11;17) (q23;q21) (MLL-MLLT6)
t(3;5) (q25;q34) (NPM1-MLF1)	t(11;17) (q23;q21) (ZBTB16-RARA)
t(3;21) (q26;q22) (RUNX1-MECOM)	t(11;19) (q23;p13.1) (MLL-ELL)
t(4;11) (q21;q23) (MLL-AFF1)	t(11;19) (q23;p13.3) (MLL-MLLT1)
t(5;12) (q33;p13) (ETV6-PDGFRB)	t(12;21) (p13;q22) (ETV6-RUNX1)
t(5;17) (q35;q21) (NPM1-RARA)	t(12;22) (p13;q11) (ETV6-MN1)
t(6;9) (p23;q34) (DEK-NUP214)	t(15;17) (q24;q21) (PML-RARA)
t(6;11) (q27;q23) (MLL-MLLT4)	inv(16) (p13;q22) (CBFB-MYH11)
t(8;21) (q22;q22) (RUNX1-RUNX1T1)	t(16;21) (p11;q22) (FUS-ERG)
t(9;9) (q34;q34) (SET-NUP214)	t(17;19) (q22;p13) (TCF3-HLF)
t(9;11) (p22;q23) (MLL-MLLT3)	t(X;11) (q13;q23) (MLL-FOXO4)



**Cat No. HV01-28N**

**USER MANUAL**

# HemaVision<sup>®</sup>-28N

## Multiplex RT-PCR test

### Screens for 28 leukemia causing translocations

#### User Manual

for

HemaVision<sup>®</sup>-28N, Cat. No. HV01-28N

*25 tests per kit*

#### MANUFACTURER:



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## 1. PURPOSE OF THE TEST - SCREENING FOR 28 TRANSLOCATIONS

HemaVision<sup>®</sup>-28N is a CE-marked *in vitro* diagnostic test for 28 leukemia causing chromosomal translocations including more than 145 breakpoints plus associated mRNA splice variants. Furthermore, it detects new breakpoints and mRNA splice variants for the 28 translocations. It is a fast one day test based on the method described by Pallisgard et al. (Ref 58). The HemaVision<sup>®</sup>-28N test has very high sensitivity (>99%) and specificity (>99%) (Ref 59, 60).

Limit of detection is 10<sup>-9</sup> µg of fusion RNA in a sample of 1 µg total RNA when the RNA quality is good.

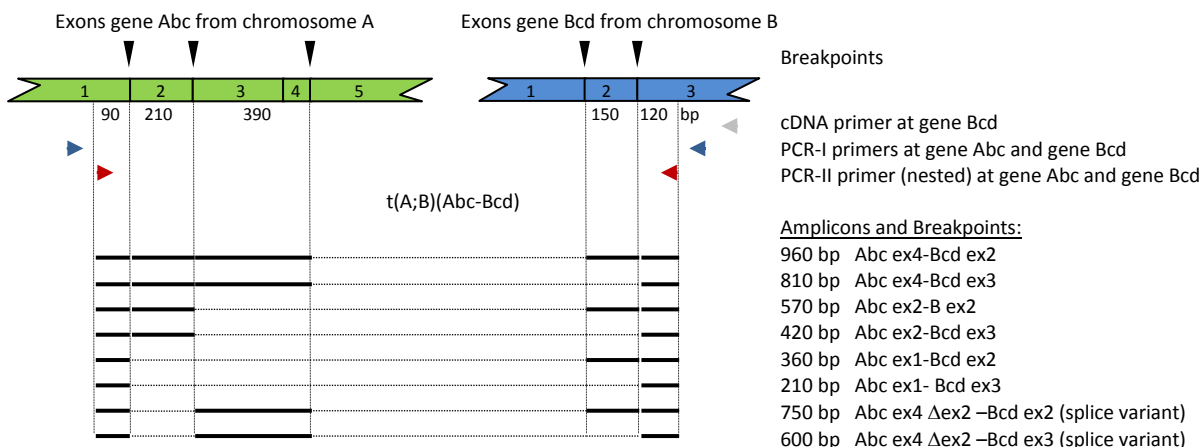
This test brings IVD testing deeper into a detailed description of the exon organization of fusion genes originating from chromosome translocations. This information is important for predicting development of the disease and selection of treatment.

HemaVision<sup>®</sup>-28N 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 nested polymerase chain reactions, agarose gel electrophoresis, and interpretation.

HemaVision<sup>®</sup>-28N identifies chromosomes, genes and exons at the breakpoint in fusion genes. Furthermore, the test identifies mRNA splice variants from fusion genes.

The HemaVision<sup>®</sup>-28N kit contains primers for 25 cDNA reactions, 25 nested PCR master tests, and 15 nested PCR split-out tests.

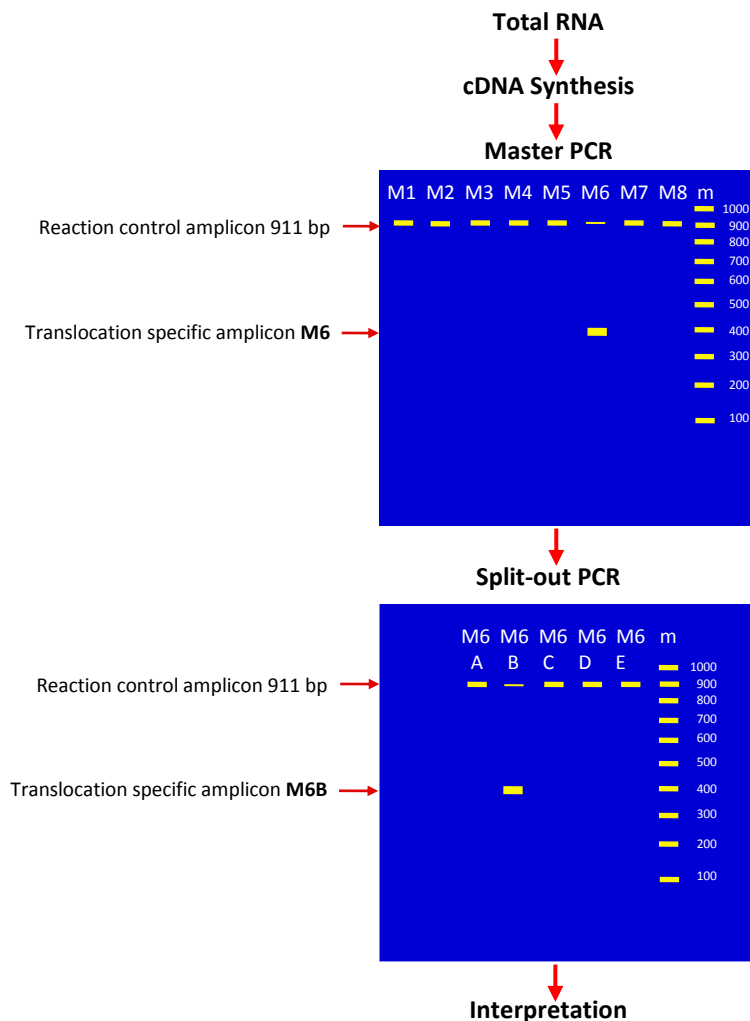
**The test is for professional use only.**



**Figure 1** illustrates how HemaVision<sup>®</sup> kit HV01-28N identifies chromosomes, fusion genes and exons at the breakpoint among 28 chromosome translocations causing leukemia. Only breakpoints for fusion genes maintaining the original translational reading frame are 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 PCR amplification 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. 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® Cat. No. HV01-28N.

Result: M6 and M6B positive, amplicon 397 bp.

Conclusion: From Table 11 it can be seen this patient has a translocation at t(9;22)(q34;q11) with a breakpoint 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 using the cDNA Mix (primers) from the HemaVision® kit Cat No. HV01-28N kit and Reverse Transcriptase, 5x Buffer, DTT, and dNTP, DTT from the reagent module HemaVision® kit Cat. No. HV06-RMP.

## Master PCR

The cDNA is used as a template for eight multiplex PCR amplification reactions (Master PCR-I) subsequently followed by eight nested PCR reactions (Master PCR-II). All reactions use primer mixes from the HemaVision® HV01-28N kit and HemaVision DNA Polymerase, 10xbuffer, and dNTP from HemaVision® kit Cat. No. HV06-RMP. Each Master PCR reaction contains four, five or six pairs of translocation specific primers and one pair of reaction control primers specific for the housekeeping gene *beta-actin*. The Master PCR-II products are analyzed by agarose gel electrophoresis. The 911 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 M6 is positive for a translocation specific band continue the test using split-out reactions M6A-M6E. Again, cDNA is used as template for the split-out PCR-I subsequently followed by nested split-out PCR-II. The nested split-out PCR is analyzed by agarose gel electrophoresis.

## Interpretation of results

A sample is positive for a translocation when both master and split-out PCR produce bands with identical molecular size. The breakpoint is identified by the reaction number of the split-out and the molecular size of the translocation specific amplicon using Interpretation Table 11. 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 HV01-28N. In this example, the test is positive in Master M6 and Split-out M6B with a translocation specific band of 397 bp. From Interpretation Table 11 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 b2-a2. 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. HV01-28N contains two boxes with Primer Mixes (see figure 3) and a User Manual for instruction. The kit is shipped at -20°C or below and both boxes 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 material for 25 Master tests and 15 Split-out tests.

		M1 PCR-I	M2 PCR-I	M3 PCR-I	M4 PCR-I	M5 PCR-I	M6 PCR-I	M7 PCR-I	M8 PCR-I
	cDNA Mix								
		M1 PCR-II	M2 PCR-II	M3 PCR-II	M4 PCR-II	M5 PCR-II	M6 PCR-II	M7 PCR-II	M8 PCR-II

BOX 1:

cDNA and Master PCR primer mixes:

1x 200 µL cDNA Mix (white cap)

8x 155 µL Master PCR-I (blue caps)

8x 155 µL Master PCR-II (red caps)

M1A PCR-I	M1B PCR-I	M1C PCR-I	M1D PCR-I		M1A PCR-II	M1B PCR-II	M1C PCR-II	M1D PCR-II	
M2A PCR-I	M2B PCR-I	M2C PCR-I	M2D PCR-I	M2E PCR-I	M2A PCR-II	M2B PCR-II	M2C PCR-II	M2D PCR-II	M2E PCR-II
M3A PCR-I	M3B PCR-I	M3C PCR-I	M3D PCR-I		M3A PCR-II	M3B PCR-II	M3C PCR-II	M3D PCR-II	
M4A PCR-I	M4B PCR-I	M4C PCR-I	M4D PCR-I		M4A PCR-II	M4B PCR-II	M4C PCR-II	M4D PCR-II	
M5A PCR-I	M5B PCR-I	M5C PCR-I	M5D PCR-I	M5E PCR-I	M5A PCR-II	M5B PCR-II	M5C PCR-II	M5D PCR-II	M5E PCR-II
M6A PCR-I	M6B PCR-I	M6C PCR-I	M6D PCR-I	M6E PCR-I	M6A PCR-II	M6B PCR-II	M6C PCR-II	M6D PCR-II	M6E PCR-II
M7A PCR-I	M7B PCR-I	M7C PCR-I	M7D PCR-I		M7A PCR-II	M7B PCR-II	M7C PCR-II	M7D PCR-II	
M8A PCR-I	M8B PCR-I	M8C PCR-I	M8D PCR-I	M8E PCR-I	M8A PCR-II	M8B PCR-II	M8C PCR-II	M8D PCR-II	M8E PCR-II
				M8F PCR-I					M8F PCR-II

BOX 2:

Split-out PCR primer mixes:

37 x 95 µL Split-out PCR-I (blue caps)

37 x 95 µL Split-out PCR-II (red caps)

Figure 3 shows content of HemaVision® kit Cat. No. HV01-28N.

**NOTE:** It is essential for functionality of the multiplex RT-PCR test using HemaVision® kit HV01-28N also to obtain and use the reagents provided in HemaVision® kit **Cat. No. HV06-RMP** containing: MMLV-Reverse Transcriptase; 5x cDNA buffer; DTT; dNTP, HemaVision DNA Polymerase, 10x PCR buffer.

#### 4. EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

##### **RNA extraction:**

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

##### **Reagent Module:**

HemaVision® kit Cat. No. HV06-RMP containing: MMLV-RT; 5x cDNA buffer; DTT; dNTP, HemaVision DNA Polymerase, 10x PCR buffer. Use four HV06-RMP kits together with each HV01-28N kit.

Or as an alternative use: HemaVision® kit Cat. No. HV04-RM containing: MMLV-RT; 5x cDNA buffer; DTT; dNTP and from Qiagen HotStarTaq DNA Polymerase 5 Units/ µL and 10x PCR buffer. Use four HV04-RM kits and 1000 Units HotStarTaq DNA Polymerase together with each HV01-28N kit.

##### **Master Mix room – No templates in this room:**

Micropipettes, 0.5-10 µL, 20-200 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

-20°C freezer for storage of kits (HV01-28N and HV06-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°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

1. The quality of the RNA sample greatly affects the results of this test. To minimize the risk of degradation of RNA by ribonucleases, we strongly recommend lysing the cells in a denaturing solution [e.g. containing guanidinium isothiocyanate (GTC)] immediately after isolation and before freezing. Always store cell samples and aqueous RNA solutions at  $-80^{\circ}\text{C}$ . Even an overnight storage at  $-20^{\circ}\text{C}$  may result in RNA degradation. When working with RNA always use gloves to avoid ribonuclease contamination from hands.
2. 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 to the following activities:

- 1) Master Mix production – No templates in here
- 2) cDNA synthesis
- 3) PCR
- 4) 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 Master Mix room to cDNA room to PCR room to Gel electrophoresis room. NEVER move mixes or reaction products in the opposite direction.

3. Laboratory workbenches, pipettes, and lab coats must be cleaned on a regular basis.
4. 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 DNA. After PCR tubes must be opened with extreme care to avoid spillage of high copy number DNA products.

### 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.



## 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.
- This protocol is optimized for the 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 911 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

- 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 or use samples collected in heparin tubes.
- Prepared mononuclear cells from whole blood or bone marrow by the Ficoll Hypaque method.
- Within 24 hours of collection, extract total RNA with QIAamp® RNA Blood Mini Kit (Qiagen Cat. No. 52304). Typically 5-10 µg total RNA is extracted from 1 x10<sup>7</sup> mononuclear blood cells.
- 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<sub>2</sub>O.
- 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® reagent module Cat. No. HV06-RMP.

Do not add additional volume to compensate for liquid loss during liquating. This has already been factored into the table numbers.

**Table 1: cDNA Synthesis Mix**

Number of samples	1	2
5x MMLV-RT Buffer (µL)	11.0	22.0
100 mM DTT (µL)	5.5	11.0
10 mM dNTP Mix (µL)	5.5	11.0
MMLV-RT (µL)	2.2	4.4
Total volume (µL):	24.2	48.4

- 1.2 In the cDNA room add 8 µL cDNA Mix (primers) from white capped tube in the HemaVision® HV01-28N kit to one tube containing 20 µL total RNA (2 µg). Mix gently and spin down for 10 seconds.
- 1.3 In a separate RNase free tube, add 8 µL of cDNA Mix to 20 µL H<sub>2</sub>O (negative control).
- 1.4 Incubate the tubes in a heating block or water bath at 65°C for 5 minutes. Chill and hold on ice.
- 1.5 Add 22 µL of the cDNA Synthesis Mix to the tube with 28 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 Add 50 µL H<sub>2</sub>O to each cDNA tube.
- 1.8 Incubate at 95°C for 5 min to inactivate the MMLV-RT enzyme.
- 1.9 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-I**

To achieve maximal sensitivity and specificity each sample is tested with both a first and a nested Master PCR reaction.

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

Do not add additional volume to compensate for liquid loss during liquating. This has already been factored (+10 %) into the table numbers.

**Table 2: Master Mix PCR-I**

<b>Number of samples</b>	<b>1</b>	<b>2</b>
<b>Number of PCR reactions</b>	<b>8</b>	<b>16</b>
10x PCR buffer (µL)	22.0	44.0
dNTP mix (µL)	4.4	8.8
HemaVision DNA Polymerase (µL)	3.5	7.0
H <sub>2</sub> O (µL)	102.1	204.2
Total volume (µL):	132.0	264.0

- 2.2 For each sample label the tubes in an 8-microtube strip M1 to M8.
- 2.3 Aliquot 15 µL Master Mix PCR-I to each of the PCR tubes in the 8-microtube strip(s).
- 2.4 To these tubes add 5 µL Master PCR-I primers from blue capped tubes M1-M8 to the corresponding PCR tubes. E.g. to PCR tube labeled M1 add 5 µL M1 PCR-I.
- 2.5 In the PCR room add 5 µL cDNA (from step 1.9) 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.

**Table 3: Master PCR-I Amplification Parameters**

Step	Time/Temperature	Cycles
1	15 minutes at 95°C	1
2	30 seconds at 95°C 30 seconds at 58°C 1 minute 30 seconds at 72°C	25
3	Hold at 4°C	1

**Step 3 Master PCR-II (nested)**

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

Do not add additional volume to compensate for liquid loss during liquating. This has already been factored (+10 %) into the table numbers.

**Table 4: Master Mix PCR-II**

Number of samples	1	2
Number of PCR reactions	8	16
10x PCR buffer (µL)	22.0	44.0
dNTP mix (µL)	4.4	8.8
HemaVision DNA Polymerase (µL)	3.5	7.0
H <sub>2</sub> O (µL)	137.3	274.6
Total volume (µL):	167.2	334.4

**3.2** For each sample label the tubes in an 8-microtube strip M1 to M8.

**3.3** Aliquot 19 µL Master Mix PCR-II to each of the PCR tubes in the 8-microtube strip(s).

**3.4** To these tubes add 5 µL Master PCR-II primers from red capped tubes M1-M8 to the corresponding PCR tubes. E.g. to PCR tube labeled M1 add 5 µL M1 PCR-II.

**3.5** In the PCR room carefully open without spillage the PCR tubes containing the Master PCR-I reactions. From these tubes transfer 1 µL to the corresponding Master PCR-II tubes from step 3.3. An eight channel pipette can be used for this transfer. Close the tubes, mix and spin for 10 seconds. Volume per tube 25 µL.

**3.6** Transfer the tubes to a thermal cycler and start the PCR amplification using the PCR cycling parameters in Table 5.

**Table 5: Master PCR-II Amplification Parameters**

Step	Time/Temperature	Cycles
1	15 minutes at 95°C	1
2	30 seconds at 95°C 30 seconds at 58°C 1 minute 30 seconds at 72°C	20
3	10 minutes at 72°C	1
4	Hold at 4°C	1

#### **Step 4      Gel electrophoresis**

- 4.1** 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.
- 4.2** In the Gel Electrophoresis room carefully open the PCR tubes with Master PCR-II without contaminating gloves and surroundings with drops containing high copy numbers of amplicon.  
Add 3 µL of 10x loading buffer into each PCR tube. Load approximately 14 µL per slot in the gel (lane 1-8). Finally load a molecular size marker to lane 9.
- 4.3** Run the gel in 1X TBE buffer until the Bromophenol blue dye has migrated approximately 3/4 of the gel.
- 4.4** Examine the gel with UV-light and document result by photography.

#### **Step 5      Interpretation of results from Master PCR-II**

- 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 (911 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 (911 bp) in all lanes M1-M8. The patient is negative for the 28 translocations shown in Table 10.
- Negative for a translocation specific band and negative for reaction control band (911 bp) in all lanes M1-M8. 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, or 3) incorrect mixing of reactions. Correct and repeat test.
- Negative for a translocation specific band and positive for reaction control band (911 bp) in some of the lanes M1-M8. 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 6      Split-out PCR-I**

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

- 6.1** In the Master Mix room prepare the Split-out Mix PCR-I according to Table 6 using HemaVision® reagent module Cat. No. HV06-RMP. Mix and spin down for 10 seconds.  
Do not add additional volume to compensate for liquid loss during liquating. This has already been factored (+10 %) into the table numbers.

**Table 6: *Split-out Mix PCR-I***

Number of positive Master PCR-II	1	1	1
Number of Split-out PCR-I reactions	4	5	6
10x PCR buffer (µL)	11,00	13,75	16,50
dNTP mix (µL)	2,20	2,75	3,30
HemaVision DNA Polymerase (µL)	1,76	2,20	2,64
H <sub>2</sub> O (µL)	51,04	63,80	76,56
Total volume (µL):	66,0	82,5	99,0

- 6.2** Label four, five or six PCR tubes (e.g. if Master M6 is positive label five Split-out tubes: M6A, M6B,.....M6E).
- 6.3** Aliquot 15 µL *Split-out Mix PCR-I* to each of the PCR tubes.
- 6.4** To each of these tubes add 5 µL Split-out PCR-I primers from blue capped tubes (e.g. to the PCR tube labeled M6A add 5 µL M6A PCR-I primer mix).
- 6.5** In the PCR room add 5 µL cDNA (from step 1.9) to each of the PCR tubes from step 6.4. Close the tubes, mix and spin for 10 seconds. Volume per tube 25 µL.
- 6.6** Transfer the tubes to a thermal cycler and start the PCR amplification using the PCR cycling parameters in Table 7.

**Table 7: Split-out PCR-I Amplification Parameters**

Step	Time/Temperature	Cycles
1	15 minutes at 95°C	1
2	30 seconds at 95°C 30 seconds at 58°C 1 minute 30 seconds at 72°C	25
3	Hold at 4°C	1

**Step 7 Split-out PCR-II (nested)**

- 7.1** In the Master Mix room prepare the *Split-out Mix PCR-II* according to Table 8 using HemaVision® reagent module Cat. No. HV06-RMP. Mix and spin down for 10 seconds.

Do not add additional volume to compensate for liquid loss during liquating. This has already been factored (+10 %) into the table numbers.

**Table 8: *Split-out Mix PCR-II***

Number of positive Master PCR-II	1	1	1
Number of Split-out PCR-II reactions	4	5	6
10x PCR buffer (µL)	11,00	13,75	16,50
dNTP mix (µL)	2,20	2,75	3,30
HemaVision Taq DNA Polymerase (µL)	1,76	2,20	2,64
H <sub>2</sub> O (µL)	68,64	85,8	102,96
Total volume (µL):	83,6	104,5	125,4

- 7.2 Label four, five or six PCR tubes (e.g. if Master M6 is positive label five Split-out tubes: M6A, M6B,...M6E).
- 7.3 Aliquot 19 µL Split-out Mix PCR-II to each of the PCR tubes.
- 7.4 To each of these tubes add 5 µL Split-out PCR-II primers from red capped tubes (e.g. to the PCR tube labeled M6A add 5 µL M6A PCR-I primer mix).
- 7.5 In the PCR room carefully open without spillage the PCR tubes containing the Split-out PCR-I reactions. From these tubes transfer 1 µL to the corresponding Split-out PCR-II tubes from step 7.4. Close the tubes, mix and spin for 10 seconds. Volume per tube 25 µL.
- 7.6 Transfer the tubes to a thermal cycler and start the PCR amplification using the PCR cycling parameters in Table 9.

**Table 9: Split-out PCR-II Amplification Parameters**

Step	Time/Temperature	Cycles
1	15 minutes at 95°C	1
2	30 seconds at 95°C 30 seconds at 58°C 1 minute 30 seconds at 72°C	20
3	10 minutes at 72°C	1
4	Hold at 4°C	1

### **Step 8 Gel electrophoresis**

- 8.1 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.
- 8.2 In the Gel Electrophoresis room carefully open the PCR tubes with Master PCR-II without contaminating gloves and surroundings with drops containing high copy numbers of amplicon. 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.
- 8.3 Run the gel in 1X TBE buffer until the Bromophenol blue dye has migrated approximately 3/4 of the gel.
- 8.4 Examine the gel with UV-light and document result by photography.

## **7. INTERPRETATION**

- Positive for the reaction control band (911 bp) in all (four or five or six) split-out PCR-II reactions. The reaction control band can be weak or missing in the lane containing a strong translocation specific band. The translocation specific band has the same molecular size as the translocation specific band in the corresponding Master PCR-II reaction. Identify the translocation with Table 11.  
  
As an example: M6 and M6B both contain a 397 bp amplicon. Table 11 show the sample has a t(9;22)(q34;q11) translocation with a fusion gene BCR-ABL1 having its breakpoint at exons b2-a2.
- When two Master PCR-II and two Split-out PCR-II reactions contain translocation specific bands it can be caused by two translocations. But it can also be caused by amplification of two overlapping regions from only one translocation.

- E.g. the BCR gene in the t(9;22)(BCR-ABL1) translocation can have breakpoints 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. M6 and M6B contain upper PCR primers binding to BCR exon 12. M8 and M8F contain upper PCR primers binding to BCR exon 1. When the breakpoint is located somewhere after BCR exon 12, M6 and M6B will be positive. But sometimes also M8/M8F is positive together with M6/M6B. In this situation M8/M8F generates an amplicon 1351 bp larger than M6/M6B (when no alternative splicing is present). The translocation must be interpreted as a positive M6B, see Table 10. This Table shows translocations with two or three positive Masters/Split-out reactions.

**Table 10: Translocations with two or three positive Master and Split-out reactions**

Positive in Split-out..	..and also positive in Split-out	Interpret translocation from Split-out	Translocation
M1D	M2D & M5B	<b>M1D</b>	t(10;11)
M2D	M5B	<b>M2D</b>	t(10;11)
M2C	M5C	<b>M2C</b>	t(11;19)
M4B	M8B	<b>M4B</b>	t(3;21)
M5D	M2E	<b>M5D</b>	t(9;11)
M6A	M7C	<b>M6A</b>	inv(16)
M6B	M8F	<b>M6B</b>	t(9;22)
M7D	M4B & M8B	<b>M7D</b>	t(3;21)
M8C	M4D	<b>M8C</b>	t(15;17)

- The Split-out PCR-II is negative for the “translocation specific” band seen in the corresponding Master PCR-II reaction. When all the Split-out reactions contain the reaction control band (911 bp) the test must be interpreted as negative for a translocation present in Table 11. 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.
- The HemaVision® kit Cat. No. HV01-28N tests for 28 translocations or chromosomal rearrangements with more than eighty fusion-gene mRNA variants due to heterogeneity of the breakpoints and/or alternative splicing. As a consequence, the number of translocation positive PCR fragments is large. See reference 58.

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- Table 11** is used for interpretation of results observed from agarose gel electrophoresis of split-out PCR-II. The table lists translocations, involved genes, exons at breakpoint, and molecular size of Master and Split-out PCR-II amplicons. Numbering of exons has been updated year 2011 according to GenBank <http://www.ncbi.nlm.nih.gov/genbank/>. Only breakpoints maintaining the original reading frame from the involved genes are presented.
- Note:** A PCR-II amplicon with a molecular size not listed in Table 11 can appear as a consequence of amplification across an unpublished breakpoint or splice variant. Further characterization of this/these amplicons by DNA sequencing can be done free of charge by DNA Diagnostic A/S. Purify the amplicon and send it to DNA Diagnostic A/S.
- Note:** The interpretation table has been updated according to the HUGO Gene Nomenclature Committee (HGNC). For a list of the changes made see section “8. GENE ABBREVIATIONS ACCORDING TO THE HGNC”.

**Table 11: Interpretation table**

<b>M1 and M1A-D</b>				
<b>PCR</b>	<b>TRANSLOCATION</b>	<b>GENES</b>	<b>BREAKPOINTS AND PCR FRAGMENTS</b>	<b>REFERENCE</b>
<b>M1A</b>	t(X;11)(q13;q23)	MLL(11q23) FOXO4(Xq13.1)	MLL ex8 - FOXO4 ex2: 244 bp MLL ex9 - FOXO4 ex2: 376 bp MLL ex10 - FOXO4 ex2: 490 bp	1
<b>M1B</b>	t(6;11)(q27;q23)	MLL(11q23) MLLT4(6q27)	MLL ex8 - MLLT4 ex2: 309 bp MLL ex9 - MLLT4 ex2: 441 bp MLL ex10 - MLLT4 ex2: 555 bp	2; 3
<b>M1C</b>	t(11;19)(q23;p13.1)	MLL(11q23) ELL(19p13.1)	MLL ex8 – ELL ex3: 283 bp MLL ex8 – ELL ex2: 331 bp MLL ex9 – ELL ex3: 415 bp MLL ex9 – ELL ex2: 463 bp MLL ex9 – ELL ex2 ins120: 583 bp MLL ex10 – ELL ex2 ins120: 697 bp	4; 5; 6
<b>M1D</b>	t(10;11)(p12;q23)	MLL(11q23) MLLT10(10p12)	MLL ex8 - MLLT10 ex6: 268 bp MLL ex7 - MLLT10 ex5: 304 bp MLL ex8 - MLLT10 ex4: 433 bp MLL ex7 - MLLT10 ex3: 439 bp	9; 10

<b>M2 and M2A-E</b>				
<b>PCR</b>	<b>TRANSLOCATION</b>	<b>GENES</b>	<b>BREAKPOINTS AND PCR FRAGMENTS</b>	<b>REFERENCE</b>
<b>M2A</b>	t(1;11)(p32;q23)	MLL(11q23) EPS15 (1p32)	MLL ex8 - EPS15 ex2: 184 bp	7
<b>M2B</b>	t(11;17)(q23;q12-21)	MLL(11q23) MLLT6(17q21)	MLL ex7 - MLLT6 ex11: 304 bp	8
<b>M2C</b>	t(11;19)(q23;p13.3)	MLL(11q23) MLLT1(19p13.3)	MLL ex8 - MLLT1 ex2: 187 bp MLL ex9 - MLLT1 ex2: 319 bp MLL ex10 - MLLT1 ex2: 433 bp MLL ex11 - MLLT1 ex2: 580 bp	30; 32; 34
<b>M2D</b>	t(10;11)(p12;q23)	MLL(11q23) MLLT10(10p12)	MLL ex8 - MLLT10 ex10: 269 bp MLL ex8 - MLLT10 ex9: 365 bp MLL ex9 - MLLT10 ex10: 401 bp MLL ex8 - MLLT10 ex8: 461 bp MLL ex9 - MLLT10 ex9: 497 bp MLL ex9 - MLLT10 ex8: 593 bp	9; 10; 12
<b>M2E</b>	t(9;11)(p22;q23)	MLL(11q23) MLLT3(9p22)	MLL ex8 - MLLT3 ex10: 295 bp MLL ex8 - MLLT3 ex9(B): 367 bp MLL ex9 - MLLT3 ex9(B): 499 bp MLL ex10 - MLLT3 ex9(B): 613bp	3; 30; 35

<b>M3 and M3A-D</b>				
<b>PCR</b>	<b>TRANSLOCATION</b>	<b>GENES</b>	<b>BREAKPOINTS AND PCR FRAGMENTS</b>	<b>REFERENCE</b>
<b>M3A</b>	t(1;19)(q23;p13)	TCF3(19p13.3) PBX1(PRL)(1q23.3)	TCF3 ex16 – PBX ex3 : 376 bp TCF3 ex16 – PBX ex3 (ins 27 bp): 403 bp	13; 14; 15
<b>M3B</b>	t(17;19)(q22;p13)	TCF3(19p13.3) HLF(17q22)	TCF3ex15 – HLF ex4: 207 bp TCF3ex14 – HLF ex3: 269 bp TCF3ex16 – HLF ex3: 552 bp TCF3ex14 – HLF ex2: 605 bp TCF3ex16 – HLF ex2: 888 bp TCF3ex16 – HLF ex4(ins59): 389 bp TCF3ex16 – HLF ex4(ins87): 417 bp	16; 17; 18
<b>M3C</b>	t(12;21)(p13;q22)	ETV6(12p13) RUNX1(21q22.3)	ETV6 ex5 - RUNX1 ex4: 389 bp ETV6 ex5 - RUNX1 ex3: 428 bp	19; 20; 21; 55
<b>M3D</b>	TAL1 <sup>d</sup> 40kbp deletion 1p32	STIL(1p32) TAL1(1p32)	STIL ex1- TAL1 ex1 d1+d2: 184 bp	22; 23



<b>M4 and M4A-D</b>				
<b>PCR</b>	<b>TRANSLOCATION</b>	<b>GENES</b>	<b>BREAKPOINTS AND PCR FRAGMENTS</b>	<b>REFERENCE</b>
<b>M4A</b>	t(8;21)(q22;q22)	RUNX1(21q22.3) RUNX1T1(8q22)	RUNX1 ex6 - RUNX1T1 ex2: 197 bp RUNX1 ex7 - RUNX1T1 ex2: 389 bp RUNX1 ex8 - RUNX1T1 ex2: 551 bp	24; 25; 26
<b>M4B</b>	t(3;21)(q26;q22)	RUNX1(21q22.3) MDS1-EVI1(3q26)	RUNX1 ex6 – MDS1-EVI1 ex2: 291 bp RUNX1 ex7 – MDS1-EVI1 ex2: 483 bp RUNX1 ex8 – MDS1-EVI1 ex2: 645 bp	25; 27; 28
<b>M4C</b>	t(16;21)(p11;q22)	FUS(16p11.2) ERG(21q22.3)	FUS ex7 – ERG ex13: 315 bp FUS ex8 - ERGex11: 453 bp	29; 57
<b>M4D</b>	t(15;17)(q22;q21)	PML(15q22) RARA(17q21)	PML ex3 - RARA ex3 (S, bcr3): 325 bp	50; 51; 52

<b>M5 and M5A-E</b>				
<b>PCR</b>	<b>TRANSLOCATION</b>	<b>GENES</b>	<b>BREAKPOINTS AND PCR FRAGMENTS</b>	<b>REFERENCE</b>
<b>M5A</b>	t(4;11)(q21;q23)	MLL(11q23) AFF1(4q21.3)	MLL ex8 - AFF1 ex8(1546): 191 bp MLL ex8 - AFF1 ex7(1459): 278 bp MLL ex9 - AFF1 ex8(1546): 323 bp MLL ex8 - AFF1 ex6(1414): 323 bp MLL ex9 - AFF1 ex7(1459): 410 bp MLL ex10 - AFF1 ex8(1546): 437 bp MLL ex9 - AFF1 ex6(1414): 455 bp MLL ex10 - AFF1 ex7(1459): 524 bp MLL ex10 - AFF1 ex6(1414): 569 bp	30; 31; 32; 33
<b>M5B</b>	t(10;11)(p12;q23)	MLL(11q23) MLLT10(10p12)	MLL ex7 - MLLT10 ex17: 200 bp MLL ex8 - MLLT10 ex16: 386 bp MLL ex7 - MLLT10 ex15: 491 bp MLL ex9 - MLLT10 ex16: 518 bp MLL ex7 - MLLT10 ex14: 539 bp MLL ex7 - MLLT10 ex13: 572 bp MLL ex7 - MLLT10 ex12: 617 bp	9; 10; 11
<b>M5C</b>	t(11;19)(q23;p13.3)	MLL(11q23) MLLT1(19p13.3)	MLL ex 8 - MLLT1 ex7(B): 315 bp MLL ex 9 - MLLT1 ex7(B): 447 bp MLL ex 10 - MLLT1 ex7(B): 561 bp	30; 34
<b>M5D</b>	t(9;11)(p22;q23)	MLL(11q23) MLLT3(9p22)	MLL ex 8 - MLLT3 ex6(A): 322 bp MLL ex 9 - MLLT3 ex6(A): 454 bp MLL ex 10 - MLLT3 ex6(A): 568 bp MLL ex 8 - MLLT3 ex5: 1027 bp MLL ex 9 - MLLT3 ex5: 1159 bp MLL ex 10 - MLLT3 ex5: 1273 bp	3; 30; 35
<b>M5E</b>	t(1;11)(q21;q23)	MLL(11q23) MLLT11(1q21)	MLL ex 8 - MLLT11 ex2: 401 bp MLL ex 9 - MLLT11 ex2: 533 bp MLL ex 10 - MLLT11 ex2: 647bp	36

<b>M6 and M6A-E</b>				
<b>PCR</b>	<b>TRANSLOCATION</b>	<b>GENES</b>	<b>BREAKPOINTS AND PCR FRAGMENTS</b>	<b>REFERENCE</b>
<b>M6A</b>	inv(16)(p13;q22)	CBFB (16q22.1) MYH11 (16p13.11)	CBFB ex4 - MYH11 ex30(G): 192 bp CBFB ex4 - MYH11 ex29: 399 bp CBFB ex5 - MYH11 ex30(D): 288 bp CBFB ex5 - MYH11 ex29(E): 495 bp	47
<b>M6B</b>	t(9;22)(q34;q11)	BCR(22q11) ABL1(9q34.1)	<u>M-bcr, P210:</u> BCR ex12 - ABL1 ex3 (b1a3): 118 bp BCR ex13 - ABL1 ex3 (b2a3): 223 bp BCR ex12 - ABL1 ex2 (b1a2): 292 bp BCR ex14 - ABL1 ex3 (b3a3): 298 bp BCR ex13 - ABL1 ex2 (b2a2): 397 bp BCR ex14 - ABL1 ex2 (b3a2): 472 bp <u>μ-bcr, P230:</u> BCR ex19 - ABL1 ex3 (c3a3): 838 bp BCR ex20 - ABL1 ex3 (c4a3): 973 bp BCR ex19 - ABL1 ex2 (c3a2): 1012 bp BCR ex20 - ABL1 ex2 (c4a2): 1147 bp	37; 38; 39
<b>M6C</b>	t(9;12)(q34;p13)	ETV6(12p13) ABL1(9q34.1)	ETV6 ex2 - ABL1 ex3: 121 bp ETV6 ex3 - ABL1 ex3: 286 bp ETV6 ex2 - ABL1 ex2: 295 bp ETV6 ex4 - ABL1 ex3: 421 bp ETV6 ex3 - ABL1 ex2: 460 bp ETV6 ex4 - ABL1 ex2: 595 bp ETV6 ex5 - ABL1 ex3: 967 bp ETV6 ex5 - ABL1 ex2: 1141 bp	40; 41
<b>M6D</b>	t(5;12)(q33;p13)	ETV6(12p13) PDGFRB(5q33)	ETV6 ex4 – PDGFRB ex11: 464 bp ETV6 ex4 – PDGFRB ex9: 800 bp	42
<b>M6E</b>	t(12;22)(p13;q11-12)	ETV6(12p13) MN1(22q12.1)	ETV6 ex2 - MN1 ex2(1): 244 bp ETV6 ex3 - MN1 ex2(2): 409 bp ETV6 ex4 - MN1 ex2: 544 bp	43

<b>M7 and M7A-D</b>				
<b>PCR</b>	<b>TRANSLOCATION</b>	<b>GENES</b>	<b>BREAKPOINTS AND PCR FRAGMENTS</b>	<b>REFERENCE</b>
<b>M7A</b>	t(6;9)(p23;q34)	DEK(6p23) NUP214(9q34)	DEK ex9 - NUP214 ex18: 320 bp DEK ex10 - NUP214 ex18: 449 bp DEK ex9 - NUP214 ex17: 479 bp DEK ex10 - NUP214 ex17: 608 bp DEK ex9 - NUP214 ex16: 629 bp DEK ex9 - NUP214 ex15: 716 bp DEK ex10 - NUP214 ex16: 758 bp DEK ex10 - NUP214 ex15: 845 bp	44
<b>M7B</b>	t(9;9)(q34;q34)	SET(9q34) NUP214(9q34)	SET ex7 - NUP214 ex18: 246 bp SET ex8 - NUP214 ex18: 393 bp SET ex7 - NUP214 ex17: 405 bp SET ex8 - NUP214 ex17: 552 bp SET ex7 - NUP214 ex16: 555 bp SET ex7 - NUP214 ex15: 642 bp SET ex8 - NUP214 ex16: 702 bp SET ex8 - NUP214 ex15: 789 bp	45; 46
<b>M7C</b>	inv(16)(p13;q22)	CBFB (16q22.1) MYH11 (16p13.11)	CBFB ex4 - MYH11 ex34 (F): 174 bp CBFB ex5 - MYH11 ex34 (A): 270 bp CBFB ex4 - MYH11 ex33: 387 bp CBFB ex5 - MYH11 ex33 (B): 483 bp CBFB ex4 - MYH11 ex32: 636 bp CBFB ex5 - MYH11 ex32: 732 bp CBFB ex4 - MYH11 ex31: 789 bp CBFB ex5 - MYH11 ex31: 885 bp	47
<b>M7D</b>	t(3;21)(q26;q22)	RUNX1(21q22.3) RPL22P1 (3q26)	RUNX1 ex6 – RPL22 P1, 22bp from 3'-end ex2: 127 bp RUNX1 ex7 – RPL22 P1, 22bp from 3'-end ex2: 319 bp	25; 27; 28; 56

<b>M8 and M8A-F</b>				
<b>PCR</b>	<b>TRANSLOCATION</b>	<b>GENES</b>	<b>BREAKPOINTS AND PCR FRAGMENTS</b>	<b>REFERENCE</b>
<b>M8A</b>	t(11;17)(q23;q21)	ZBTB16(11q23) RARA(17q21)	ZBTB16 ex4(A) - RARA ex3: 285 bp ZBTB16 ex5(B) - RARA ex3: 373 bp	48; 49
<b>M8B</b>	t(3;21)(q26;q22)	RUNX1(21q22.3) EVI1(3q26)	RUNX1 ex6 – MDS1-EVI1 ex5: 91 bp RUNX1 ex7 – MDS1-EVI1 ex5: 283 bp RUNX1 ex8 – MDS1-EVI1 ex5: 445 bp RUNX1 ex6 – MDS1-EVI1 ex2: 667 bp RUNX1 ex7 – MDS1-EVI1 ex2: 859 bp RUNX1 ex8 – MDS1-EVI1 ex2: 1021 bp	25; 27; 28
<b>M8C</b>	t(15;17)(q24;q21)	PML(15q24) RARA(17q21)	PML ex6 - RARA ex3 (L, bcr1): 353 bp PML $\delta$ ex6 –RARA ex3 (V, bcr2): 97-350 bp	50; 51; 52
<b>M8D</b>	t(5;17)(q35;q21)	NPM1(5q35) RARA(17q21)	NPM1 ex4(S) – RARA ex3: 91 bp	53
<b>M8E</b>	t(3;5)(q25.1;q35)	NPM1(5q35) MLF1(3q25.1)	NPM1 ex6 – MLF1 ex4: 276 bp	54
<b>M8F</b>	t(9;22)(q34;q11)	BCR(22q11) ABL1(9q34.1)	<u>m-bcr, P190:</u> BCR ex1 – ABL1 ex3 (e1a3): 146 bp BCR ex1 – ABL1 ex2 (e1a2): 320 bp BCR ex6 – ABL1 ex3 (e6a3): 788 bp BCR ex6 – ABL1 ex2 (e6a2): 962 bp	37; 38; 39

## 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](http://www.genenames.org)). Table 12 contains a list of all relevant genes for the HemaVision® product series, with the old abbreviation and the corresponding HGNC abbreviation. The table also contains the NCBI ([www.ncbi.nlm.nih.gov/nucleotide](http://www.ncbi.nlm.nih.gov/nucleotide)) accession numbers.

**Table 12**

Old Abbreviation	HGNC Abbreviation	Chromosome	HGNC ID	NCBI Accession
ABL	ABL1	9q34.1	HGNC:76	NG_012034.1
AF10	MLLT10	10p12	HGNC:16063	NG_027818.1
AF17	MLLT6	17q21	HGNC:7138	NM_005937.3
AF1p	EPS15	1p32	HGNC:3419	NM_001981.2
AF1q	MLLT11	1q21	HGNC:16997	NM_006818.3
AF4	AFF1	4q21.3	HGNC:7135	NM_001166693.1
AF6	MLLT4	6q27	HGNC:7137	NM_001207008.1
AF9	MLLT3	9p22	HGNC:7136	NM_004529.2
AFX1	FOXO4	Xq13.1	HGNC:7139	NM_005938.3
AML1	RUNX1	21q22.3	HGNC:10471	NG_011402.2
BCR	BCR	22q11	HGNC:1014	NG_009244.1
CAN	NUP214	9q34	HGNC:8064	NG_023371.1
CBFβ	CBFB	16q22.1	HGNC:1539	NG_009281.1
DEK	DEK	6p23	HGNC:2768	NM_003472.3
E2A	TCF3	19p13.3	HGNC:11633	NG_029953.1
ELL	ELL	19p13.1	HGNC:23114	NM_006532.3
ENL	MLLT1	19p13.3	HGNC:7134	NM_005934.3
ERG	ERG	21q22.3	HGNC:3446	NG_029732.1
ETO	RUNX1T1	8q22	HGNC:1535	NG_023272.2
FUS	FUS	16p11.2	HGNC:4010	NG_012889.2
HLF	HLF	17q22	HGNC:4977	NM_002126.4
MDS1-EVI1	MECOM	3q26	HGNC:3498	NG_028279.1
MLF1	MLF1	3q25	HGNC:7125	NG_027720.1
MLL	MLL	11q23	HGNC:7132	NG_027813.1
MN1	MN1	22q12.1	HGNC:7180	NG_023258.1
MYH11	MYH11	16p13.11	HGNC:7569	NG_009299.1
NPM	NPM1	5q35	HGNC:7910	NG_016018.1
PBX1	PBX1	1q23.3	HGNC:8632	NG_028246.1
PDGFRβ	PDGFRB	5q31-q32	HGNC:8804	NG_023367.1
PLZF	ZBTB16	11q23	HGNC:12930	NG_012140.1
PML	PML	15q24	HGNC:9113	NG_029036.1
RARα	RARA	17q21	HGNC:9864	NM_000964.3
SET	SET	9q34	HGNC:10760	NG_030356.1
SIL1	STIL	1p32	HGNC:10879	NG_012126.1
Tal1	TAL1	1p32	HGNC:11556	NM_003189.2
TEL	ETV6	12p13	HGNC:3495	NG_011443.1
TLS	FUS	16p11.2	HGNC:4010	NG_012889.2








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