



# Devyser Chimerism

Art. No. 8-A405

For Research Use Only

## Handbook

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## 1. INTRODUCTION

### Handbook update service

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Visit [www.devyser.com/ifu-subscription](http://www.devyser.com/ifu-subscription) to sign up.

#### 1.1 Intended purpose

The Devyser Chimerism kit is a product enabling screening of unique genetic markers in a donor and a recipient prior to a hematopoietic stem cell transplantation (HSCT), as well as quantitative monitoring of chimerism in the recipient following HSCT.

The Devyser Chimerism kit is for research use only, not for use in diagnostic procedures.

#### 1.2 Background

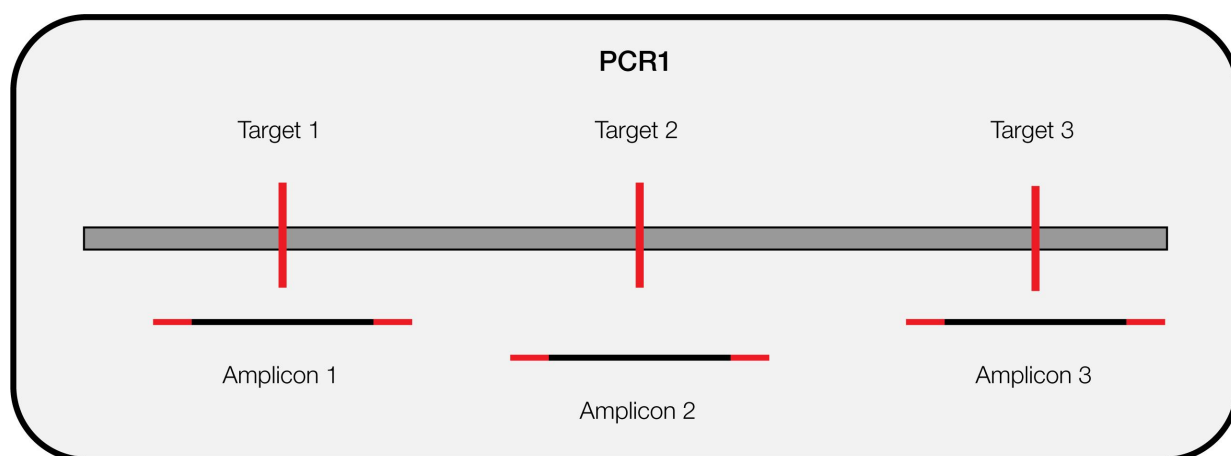
HSCT is the predominant treatment to cure malignant hematological diseases<sup>1</sup>. Monitoring of donor and recipient chimerism can be used to assess engraftment, minimal residual disease, and early detection of relapse after HSCT<sup>2</sup>. Donor- and recipient-derived cells each have unique genetic markers that can be distinguished and measured by analysis.

#### 1.3 Assay principle

The donor's and recipient's unique genetic marker sets can be detected and measured to quantify the ratio of donor's and recipient's cells present in the recipient. Genotype information for the genetic markers used in Devyser Chimerism is required for both donor and recipient for post-transplant monitoring. Determination of the genetic markers (screening) may be performed prior to or at the same time as the analysis of post-transplant samples.

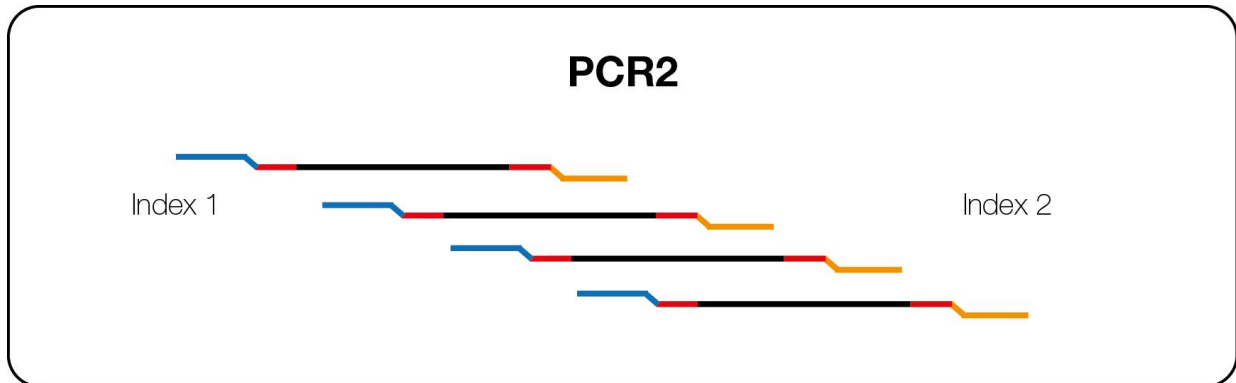
Devyser Chimerism is a targeted, next-generation sequencing (NGS) assay that utilizes differences in genetic markers (short insertions and deletions (indels)) to measure the amount of recipient- and donor-derived DNA present in a patient sample.

The method employed by the Devyser Chimerism kit includes multiplex PCR amplification of human genomic DNA (PCR1) to create a target amplicon library of genetic markers (indels) from each DNA sample as schematically illustrated in Figure 1.



**Figure 1. Schematic illustration of PCR1**

In a second PCR reaction (PCR2), sequencing adapters including unique index sequences are introduced into each amplicon (Figure 2), enabling pooling of up to 96 samples. The sample pool is purified using the Devyser Library Clean (Art. No. 8-A204). The purified sample pool is sequenced using NGS chemistry and the resulting sequences are analyzed as described in Chapter 9.



**Figure 2. Schematic illustration of PCR2**

#### 1.4 Assay design

Devyser Chimerism enables target specific library generation for NGS analysis of 24 genetic markers. See Table 1 for chromosomal marker locations.

**Table 1. Chromosomal marker locations**

<b>Marker</b>	<b>Location</b>
1	1p22.2
2	1q21.3
3	1q41
4	2q36.1
5	4q21.1
6	4q35.1
7	5q13.3
8	6p25.3
9	7p12.3
10	7q21.3
11	8q24.3
12	9p13.1
13	10q25.2
14	10q26.13
15	11p13
16	13q34
17	15q26.2
18	16p13.3
19	16q24.1
20	18p11.23
21	18q11.2
22	20q13.12
23	22q11.21
24	22q11.21

## 2. MATERIALS AND EQUIPMENT

### 2.1 Kit configurations

The Devyser Chimerism kit is available in two configurations according to Tables 2 and 3.

**Table 2. Devyser Chimerism 24 test kit (8-A405-24)**

Component	Art. No.	Number/kit	Cap color	Storage condition
Chim mix	4-A300	1	Blue	Below -18°C
Start M	4-A301	1	Purple	Below -18°C
Dilution buffer	4-A245	3	White	-25°C to +8°C
Index mix S	4-A302	1	Red	Below -18°C
Index buffer	4-A258	3	Green	-25°C to +8°C

**Table 3. Devyser Chimerism 48 test (8-A405-48)**

Component	Art. No.	Number/kit	Cap color	Storage condition
Chim mix	4-A300	2	Blue	Below -18°C
Start M	4-A301	2	Purple	Below -18°C
Dilution buffer, 96 test	4-A275	1	-	-25°C to +8°C
Index mix S	4-A302	2	Red	Below -18°C
Index buffer, 96 test	4-A277	1	-	-25°C to +8°C

### 2.2 Equipment and reagents required but not provided

#### 2.2.1 Other required Devyser products

- Devyser Index Plate LB-A (8-A202) or Devyser Index Plate A (8-A200)\*
- Devyser Library Clean (8-A204), see Table 4

\*Index Plate A has 8 bp dual indexes. Index Plate LB-A has two 10 bp unique index sequences that will reduce index-hopping, reducing background noise compared to Index Plate A

**Table 4. Devyser Library Clean (8-A204)**

Component	Art. No.	Number/kit	Cap color	Storage condition
Clean	4-A255	1	Orange	+2°C to +8°C
Wash	4-A256	1	Yellow	+2°C to +8°C
Dilution buffer	4-A245	1	White	+2°C to +8°C

#### 2.2.2 General

- Micropipettes with aerosol barrier tips or dispenser with displacement tips dedicated for pre-PCR
- Micropipettes with aerosol barrier tips or dispenser with displacement tips dedicated for post-PCR
- Disposable powder-free protective gloves
- Reaction tubes

### 2.2.3 DNA extraction

- DNA extraction reagents according to manufacturer's instructions for use
- QIAamp DNA Blood Mini Kit (Qiagen, cat.# 51104/51106) and QIAamp DNA Mini Kit (Qiagen, cat.#51304/51306) or other similar solid phase technologies for extraction of genomic DNA from human whole blood and bone marrow
- If alternative DNA extraction kits and sample materials are used, a thorough evaluation of the performance together with the Devyser Chimerism kit should be performed

### 2.2.4 Determination of DNA concentration

- Qubit Fluorometer (Thermo Fisher Scientific) and required consumables
- Qubit 1X dsDNA HS Assay Kit (Thermo Fisher Scientific, cat.# Q33230/Q33231)

### 2.2.5 Reagent preparation and amplification

- Veriti Thermal Cycler with MicroAmp 96-Well Tray/Retainer Set (Thermo Fisher Scientific)
- If an alternative thermal cycler is used, a thorough evaluation of its performance together with the Devyser Chimerism kit should be performed. It is of high importance that the following ramp rates are applied: heating 1,6 °C/s, cooling 1,6 °C/s
- Consumables for the thermal cycler

### 2.2.6 Library purification

- Magnetic rack for test tubes (DynaMag-2 Magnet, Thermo Fisher Scientific or equivalent)
- Ethanol (96%)

### 2.2.7 Sequencing

- 5% NaClO (sodium hypochlorite), for template line wash (only applicable for Illumina MiSeq)
- Other user-supplied consumables needed for sequencing, according to Illumina's sequencing guide
- Illumina reagent kits (Tables 5, 6 and 7)

**Table 5. Illumina MiSeq reagent kits**

<b>Illumina reagent kit</b>	<b>Illumina cat #</b>
MiSeq Reagent Nano Kit v2 (300-cycles)	MS-103-1001
MiSeq Reagent Micro Kit v2 (300-cycles)	MS-103-1002
MiSeq Reagent Kit v3 (150-cycles)	MS-102-3001
Illumina PhiX control v3	FC-110-3001



**Table 6. Illumina MiniSeq reagent kits**

Illumina reagent kit	Illumina cat #
MiniSeq Mid Output Kit (300-cycles)	FC-420-1004
MiniSeq High Output Reagent Kit (150-cycles)	FC-420-1002
NextSeq PhiX Control Kit	FC-110-3002

**Table 7. Illumina iSeq 100 reagent kits**

Illumina reagent kit	Illumina cat #
iSeq 100 i1 Reagent v2* (300-cycle)	20031371
Illumina PhiX control v3	FC-110-3001

\*iSeq 100 is not recommended to be used with Index Plate A (8-A200) due to a high background signal (i.e. index-hopping on a patterned flow cell)

**NOTE** All equipment should be tested, calibrated, and maintained regularly.

## 2.3 Downloads

Supplementary information and files can be downloaded from [www.devyser.com/ifu](http://www.devyser.com/ifu) using the download code printed on the kit label. See Table 8 for details.

**Table 8. Download files**

Download file name	For use with	Description
LB-A IEM files	Devyser Index Plate LB-A	Devyser setting files for sample sheet generation: <ul style="list-style-type: none"> <li>Devyser LB-A Sample Sheet IEM.pdf</li> <li>DevyserGenerateFASTQ-LB.txt</li> <li>Devyser Index LB-A MiSeq.txt</li> </ul>
LB-A LRM files	Devyser Index Plate LB-A	Devyser setting files for sample sheet generation: <ul style="list-style-type: none"> <li>Devyser LB-A Sample Sheet LRM.pdf</li> <li>Devyser2x76 LB_A.tsv</li> </ul>
MiSeq IEM files	Devyser Index Plate A	Devyser setting files for sample sheet generation: <ul style="list-style-type: none"> <li>Generating a Devyser sample sheet for MiSeq.pdf</li> <li>DevyserGenerateFASTQ.txt</li> <li>DevyserGenerateFASTQ.jpg</li> <li>Devyser double Index MiSeq.txt</li> </ul>
LRM files	Devyser Index Plate A	Devyser setting files for sample sheet generation: <ul style="list-style-type: none"> <li>Generating a Devyser sample sheet with LRM.pdf</li> <li>Devyser 2x76.tsv</li> </ul>

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## 2.4 Other resources

### 2.4.1 Devyser Sequence Coverage Calculator for Chimerism

To plan the sequencing run with respect to coverage needs, please consult the Devyser Sequence Coverage Calculator for Chimerism at [www.devyser.com/calculator](http://www.devyser.com/calculator).

As displayed in the Devyser Sequence Coverage Calculator, the recommended minimum number of read pairs for each sample and minimal coverage per amplicon is according to Table 9.

**Table 9. Recommended minimal number of read pairs per sample and amplicon coverage**

Type of sample	Read pairs per sample*	Coverage per amplicon**
Screening	6 400	200
Monitoring	320 000	10 000

\*After the sequencing run, this information can be found in Illumina Sequencing Analysis Viewer (SAV), BaseSpace or Local Run Manager (LRM)

\*\*After the sequencing run, this information can be found in the analysis software

### 3. STORAGE REQUIREMENTS

- Store the Devyser Chimerism kit below  $-18^{\circ}\text{C}$  ( $-28^{\circ}\text{C}$  to  $-18^{\circ}\text{C}$ ) or the individual kit components as specified on the label (see also Tables 2 and 3 in 2.1)
- Store the components of the Devyser Library Clean kit at  $+2^{\circ}\text{C}$  to  $+8^{\circ}\text{C}$
- Do not use components beyond the kit expiration date
- If handled, reclosed and stored properly, kit components will remain stable until the expiration date of the kit or according to in-use stability
- Frozen kit components should be thawed in a refrigerator or at room temperature before use
- Avoid repeated freeze-thaw cycles

## 4. WARNINGS AND PRECAUTIONS

- Use of this product should be limited to personnel trained in PCR, NGS techniques and NGS data analysis
- The procedure should be performed according to this handbook
- Wear powder-free disposable gloves, laboratory coat and eye protection when handling samples and kit reagents
- Do not pool reagents with different batch numbers or different vials of the same batch
- Do not use damaged reagent vials
- Frozen components should be completely thawed in a refrigerator or at room temperature before use
- Use, storage and disposal of kit components and samples, should be in accordance with the procedures defined by national biohazard safety guidelines and in accordance with country, federal, state and local regulations
- Avoid microbial contamination of reagents when removing aliquots from reagent vials
- The use of sterile disposable aerosol barrier pipette tips is recommended
- It is recommended using different sets of pipettes for the initial addition of DNA samples and for diluting and handling samples after PCR amplification
- Highly concentrated amplicons produced during PCR amplification must be handled with care to avoid contamination in the laboratory environment
- The workflow in the laboratory should proceed in a unidirectional manner, beginning in the reagent preparation area, moving to the DNA extraction area, then to the amplification area and finally to the sequencing area
- Supplies and equipment should be dedicated to each activity and not used for other activities or moved between areas
- Use different indexes in consecutive runs to avoid carry-over contamination in Illumina sequencing instruments<sup>3</sup>
- For Illumina MiSeq: Perform a “Template line Wash” as described in MiSeq System Guide<sup>4</sup> after each sequencing run with Devyser Chimerism

## 5. PROCEDURAL LIMITATIONS

- Rare primer site sequence alterations may affect the function of individual PCR primers used in the Devyser Chimerism kit and may result in reduced or no amplification of the affected amplicon
- The measurement of chimerism is affected by the amount of input DNA
- Do not use the Devyser Chimerism kit to analyze samples from a transplant recipient who has received a transplant from a monozygotic twin
- Devyser Chimerism may show limitations in performance when samples are analyzed from a recipient who has had a blood transfusion that contains white blood cells within the past 30 days
- Do not use the Devyser Chimerism kit to analyze samples from a transplant recipient who has had HSCT procedure(s) which involve three or more genetically distinct donors
- The following parameters might affect the overall performance:
  - Quality and concentration of the DNA
  - Deviations from the protocol
  - Too low sequencing depth

## 6. SAMPLE REQUIREMENTS

DNA concentration, integrity and purity are important parameters for successful testing using the Devyser Chimerism kit. DNA should be free from contaminating proteins, salts and other PCR inhibitors, e.g. residual ethanol from DNA extraction procedures. Poor quality DNA may result in amplification failure and/or increased background signals.

### 6.1 Samples

The Devyser Chimerism kit has been evaluated using human genomic DNA extracted from whole- blood, bone marrow and cell populations purified from whole-blood and bone marrow.

#### 6.1.1 DNA extraction

- According to manufacturer's instructions for use (see 2.2.3)

#### 6.1.2 Determination of DNA concentration

- High quality DNA is important for accurate and reproducible determination of DNA concentration
- All DNA concentrations referred to in this handbook were determined using the Qubit Fluorometer and the Qubit 1X dsDNA HS Assay Kit
- The DNA concentration determined for a DNA sample may differ between Qubit systems and between the Qubit system and other techniques. It is important to verify that the technique used for determination of DNA concentration correlates to the actual results obtained with the Devyser Chimerism kit

#### 6.1.3 Dilution of DNA

- Adjust the concentration of extracted DNA to 6 ng/μL using the Dilution buffer provided with the kit (see 7.1.2)

#### **NOTE**

The use of high-quality DNA with carefully determined concentration enables direct pooling of equal volumes from each sample library prior to purification and quantification of the library pool (see 7.3).

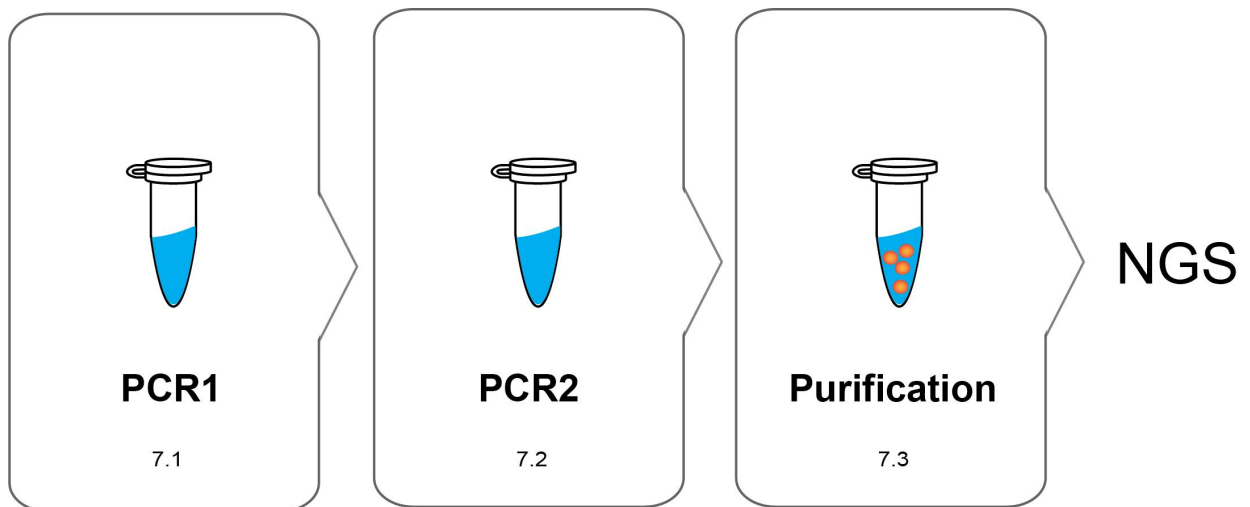
### 6.2 PhiX control

Include PhiX control (see 2.2.7) in each sequencing run to create quality parameters for the sequencing run.

### 6.3 Internal system control

We recommend performing regular internal system control of all equipment and software used in this procedure. Samples with pre-defined mixed chimerism levels (in-house developed or externally sourced) are suitable as system controls.

## 7. INSTRUCTIONS FOR USE



**Figure 3. Schematic overview of the Devyser Chimerism library preparation procedure (7.1 - 7.3)**

The Devyser Chimerism library preparation procedure consists of the following steps:

### **PCR1 (7.1)**

The amplicon library is generated in one multiplex PCR reaction for each sample.

### **PCR2 (7.2)**

Index addition to the PCR1 library is performed in PCR2 to allow pooling of multiple sample libraries for sequencing.

### **Purification (7.3)**

Unique sample libraries generated in PCR2 are pooled and purified in a single tube. The purified library pool is analyzed by NGS.

## 7.1 Library generation (PCR1)

### NOTE

Use the Devyser Sequence Coverage Calculator for Chimerism to determine the number of samples to be tested and for planning the sequencing run (see 2.4.1).

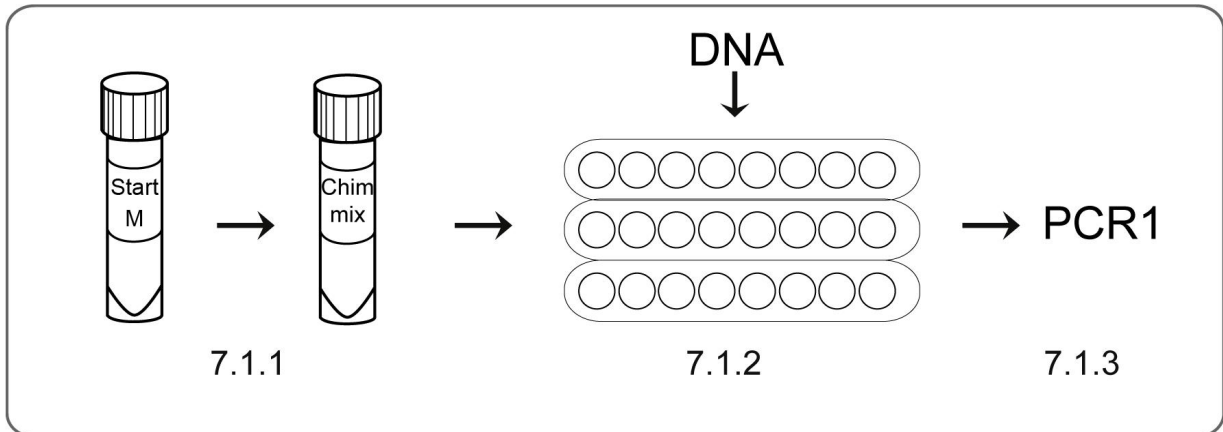


Figure 4. Schematic overview 7.1.1 to 7.1.3

### 7.1.1 Chim mix activation

Required kit components: 24/48 test kit: **Start M (4-A301)**, **Chim mix (4-A300)**

Determine the number of **Start M** and **Chim mix** tubes required. Each tube is sufficient for 24 reactions.

- Ensure that the **Start M** and the **Chim mix** are completely thawed before use
- Vortex the **Start M** tube(s) briefly
- Briefly centrifuge the **Start M** and **Chim mix** tube(s) to collect the content
- Add 270  $\mu\text{L}$  of **Start M** to the **Chim mix** tube(s) to obtain an activated **Chim mix**
- Vortex the activated **Chim mix** tube(s) and then centrifuge it briefly to collect the content
- Dispense 20  $\mu\text{L}$  of the activated **Chim mix** into separate PCR reaction tubes or separate wells in a PCR plate. Cap the tubes or seal the plate
- Store the dispensed **Chim mix** at +2 °C to +8 °C and continue to 7.1.2
- Any remaining activated **Chim mix** can be stored in a freezer below -18 °C for 90 days. Do not aliquot the activated mix

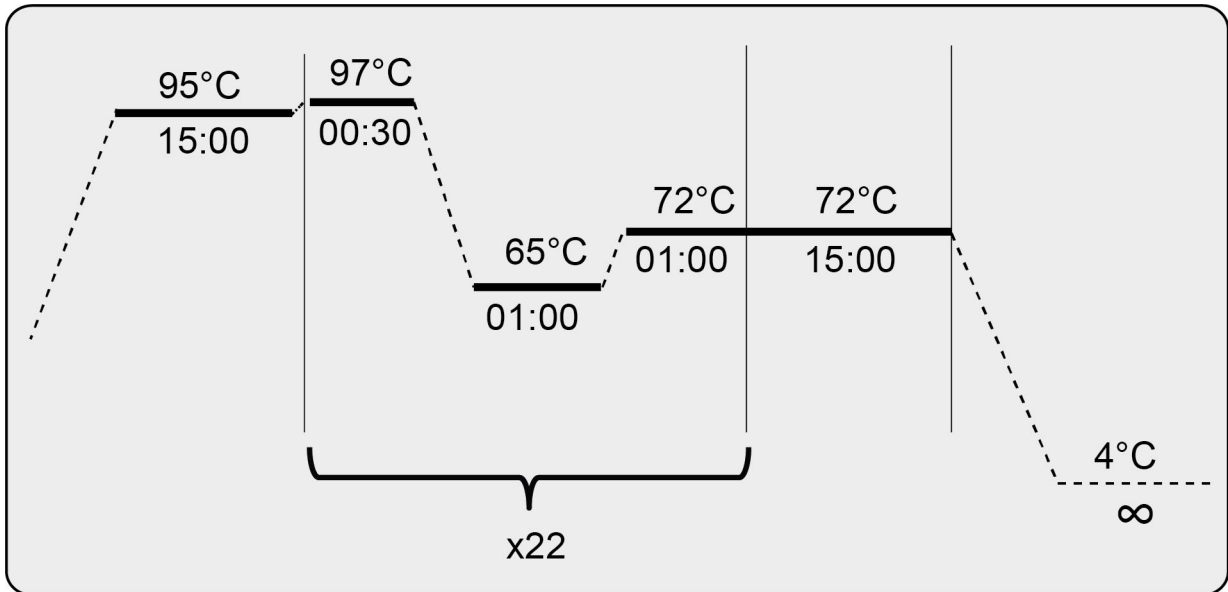
### 7.1.2 Preparation and addition of DNA

Required kit components: 24 test kit: **Dilution buffer (4-A245)**, 48 test kit: **Dilution buffer, 96 test (4-A275)**



- A. Determine the DNA concentration of each DNA sample (see 6.1.2)
- B. Ensure that the **Dilution buffer** is completely thawed before use
- C. Dilute the DNA samples to a final concentration of 6 ng/μL using the provided Dilution buffer
- D. Add 10 μL of diluted DNA from each sample to the separate PCR reaction tubes or the separate wells in the PCR plate containing activated **Chim mix** (from 7.1.1)
- E. Mix by pipetting
- F. Cap the tubes or seal the plate and centrifuge briefly to collect the content

### 7.1.3 Thermal cycling PCR1



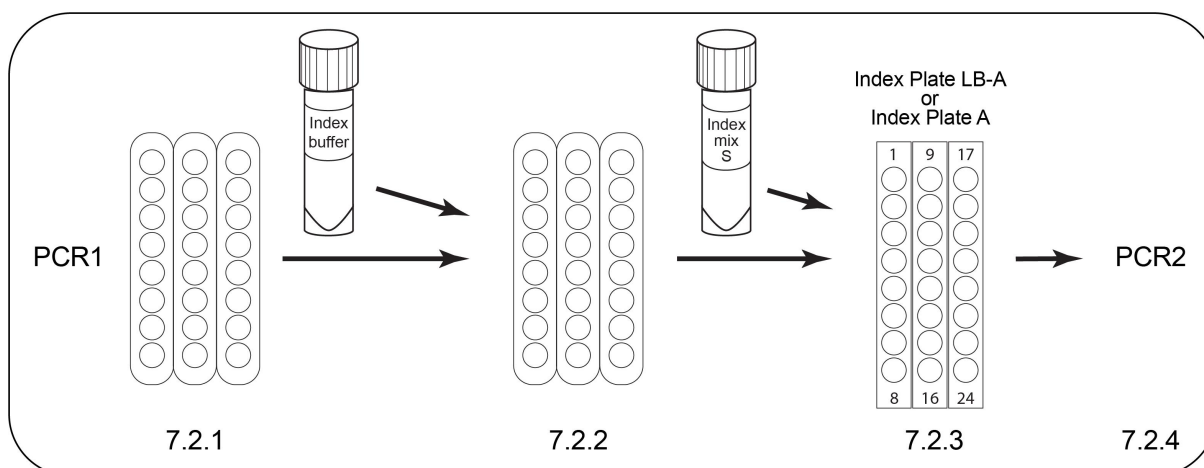
**Figure 5. Thermal profile PCR1**

- A. Program the Veriti Thermal Cycler:  
In the "Tools Menu" select "Convert a Method". In the next step select "9700 MAX Mode" and then enter the PCR profile as outlined in Figure 5. **Make sure that the following ramp rates are applied: heating 1,6 °C/s, cooling 1,6 °C/s**
- B. Set the reaction volume to 30 μL
- C. If using tubes/strips in a Veriti Thermal Cycler they should first be placed in the MicroAmp 96-Well Tray/Retainer Set for Veriti Systems
- D. Place the tubes or the plate in the thermal cycler
- E. Start the amplification (duration approximately 1 hr 45 min)
- F. Following amplification, centrifuge briefly to collect the content

The PCR1 library can be stored in a freezer below -18°C for 30 days.

SUITABLE STOPPING POINT

## 7.2 Library indexing (PCR2)



**Figure 6. Schematic overview 7.2.1 to 7.2.4**

### 7.2.1 PCR1 library dilution

Required kit components: 24 test kit: **Index buffer (4-A258)**, 48 test kit: **Index buffer, 96 test (4-A277)**

- Ensure that the **Index buffer** is completely thawed
- For each PCR1 library to be diluted, dispense 198  $\mu$ L **Index buffer** to a new tube
- Add 2  $\mu$ L of each PCR1 library to the separate dilution tubes containing 198  $\mu$ L **Index buffer**. Make sure no liquid remains in the tip by pipetting repeatedly in the **Index buffer**
- Mix the diluted PCR1 libraries thoroughly by pipetting (using a pipetting volume of at least 100  $\mu$ L)

### 7.2.2 Index preparation

#### NOTE

To avoid potential carry-over, it is strongly recommended to select indexes that have not been used in the previous sequencing run.

Required kit: **Devysr Index LB-A (8-A202) or Devysr Index Plate A (8-A200)**

Required kit component: 24/48 test kit: **Index mix S (4-A302)**

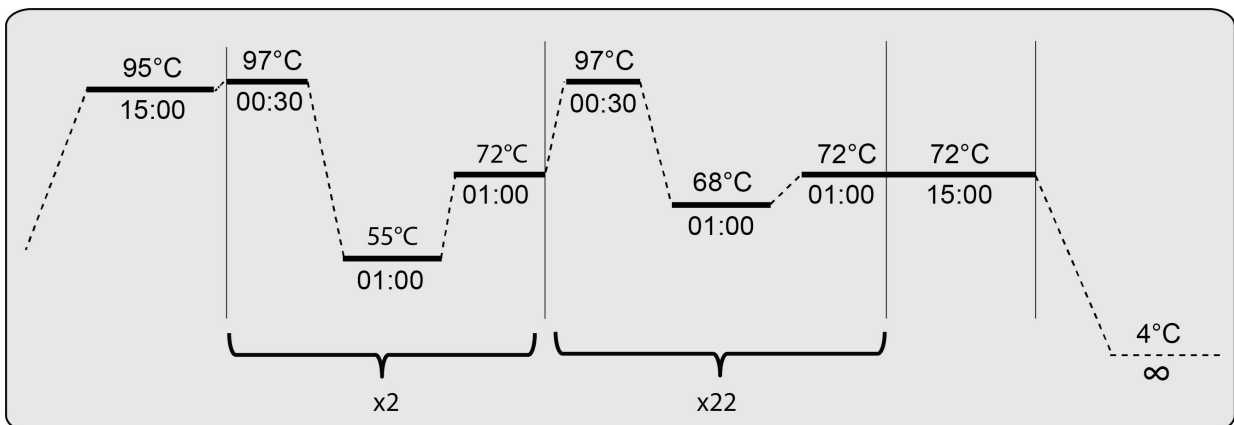
- Determine the number of **Index mix S** tubes required. Each is sufficient for 24 reactions
- Determine the number of indexes required and cut the desired wells from the **Index Plate**. Store the unused wells below  $-18^{\circ}\text{C}$  for a maximum of 24 months
- Ensure that the **Index mix S** is completely thawed before use
- Vortex and then briefly centrifuge the **Index mix S** tube to collect the content
- Carefully remove the transport seal from the wells selected in step A. **Note! Do not reuse the transport seal**
- Add 20  $\mu$ L of **Index mix S** to each well. **Note! Tips must be changed between each individual well**

### 7.2.3 Addition of diluted PCR1 libraries to Index Plate LB-A or Index Plate A

Required kit: 24/48 test kit: **Sealer L**

- Add 5  $\mu\text{L}$  of each diluted PCR1 library (from 7.2.1) to separate wells in the **Index Plate** (prepared in 7.2.2)
- Mix thoroughly by pipetting to dissolve the colored reagent pellets, using a pipetting volume of at least 10  $\mu\text{L}$ . **Note! Make sure that the colored reagent pellets are completely dissolved before proceeding to the next step.** Avoid bubbles
- Use **Sealer L** to completely cover the **Index Plate**, or cut a piece of **Sealer L** to cover wells in use
- Carefully seal the **Index Plate** and make sure that all wells are covered
- Centrifuge briefly to collect the content

### 7.2.4 Thermal cycling PCR2



**Figure 7. Thermal profile PCR2**

- Program the Veriti Thermal Cycler:  
In the "Tools Menu" select "Convert a Method". In the next step select "9700 Max Mode" and then enter the PCR profile as outlined in Figure 7. **Make sure that the following ramp rates are applied: heating 1,6 °C/s and cooling 1,6 °C/s**
- Set the reaction volume to 25  $\mu\text{L}$
- If using tubes/strips in a Veriti Thermal Cycler they should first be placed in the MicroAmp 96-Well Tray/Retainer Set for Veriti Systems
- Place the tubes or the plate in the thermal cycler
- Start the amplification (duration approximately 1 hr 55 min)
- If proceeding with sequencing the same day, prepare sequencing reagents (see note in 7.3)
- Following amplification, centrifuge briefly to collect the content

PCR2 libraries can be stored in a freezer below  $-18^{\circ}\text{C}$  for 30 days.

SUITABLE STOPPING POINT

### 7.3 Pooling and purification of libraries using the Devyser Library Clean kit (8-A204)

#### NOTE

Defrost the Illumina reagent cartridge well in advance prior to sequencing according to the procedure described in the current version of the MiSeq, MiniSeq or iSeq 100 System Guide<sup>4,8,9</sup>.

#### NOTE

The library pool should consist of libraries from samples processed together with the same Devyser library kit. Screening and monitoring samples should be pooled separately.

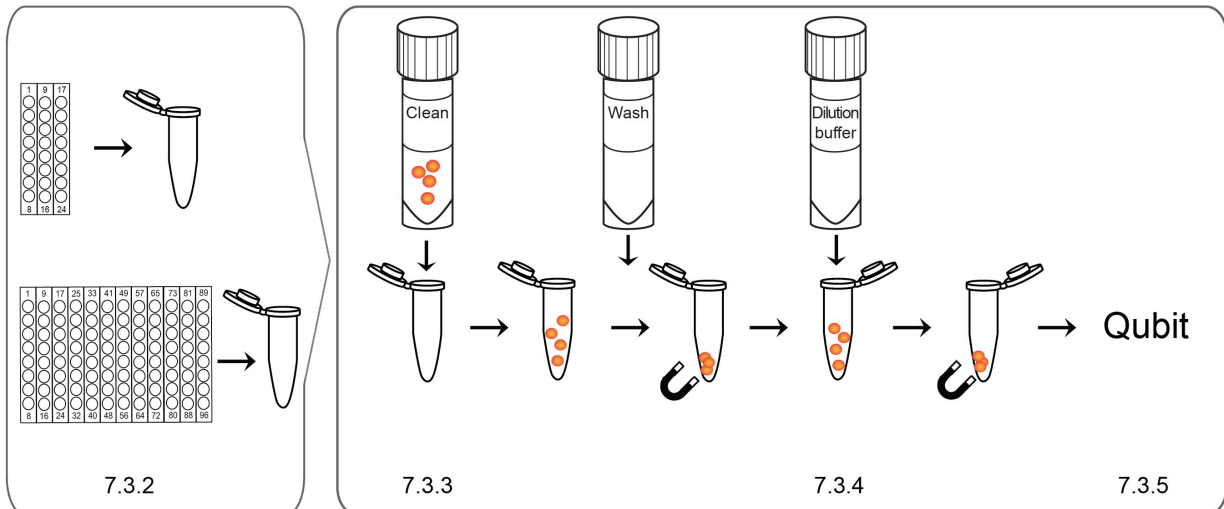


Figure 8. Schematic overview of 7.3.2 to 7.3.5

#### 7.3.1 Preparation of Wash solution

Required kit: **Devyser Library Clean (8-A204)**

Required kit component: **Wash (4-A256)**

- Prepare the **Wash** solution by adding 1500  $\mu\text{L}$  of 96% ethanol to the **Wash** tube
- Mix thoroughly by vortexing
- Tick the box on the **Wash** tube label to indicate that ethanol was added
- Note! The Wash solution should be stored at +2°C to +8°C and used within 3 months from day of preparation**

#### 7.3.2 Pooling

- To obtain a library pool volume of at least 80  $\mu\text{L}$  for subsequent purification, pool at least 5  $\mu\text{L}$  from each of the PCR2 libraries (from 7.2.4) into a single tube
- Mix thoroughly by vortexing and then briefly centrifuge the library pool to collect the content
- Transfer 80  $\mu\text{L}$  of the library pool into a new tube suitable for placing on a magnetic rack

#### NOTE

If less than 16 libraries are pooled, add equal volumes of each PCR2 library to obtain a library pool volume of at least 80  $\mu\text{L}$ . If the total pooled volume is less than 80  $\mu\text{L}$ , use equal volumes of the pooled PCR2 libraries and **Clean**. For elution, use half the library pool volume of **Dilution buffer** (see 7.3.4). However, do not use less than 25  $\mu\text{L}$  of **Dilution buffer** for elution.

### 7.3.3 Library purification

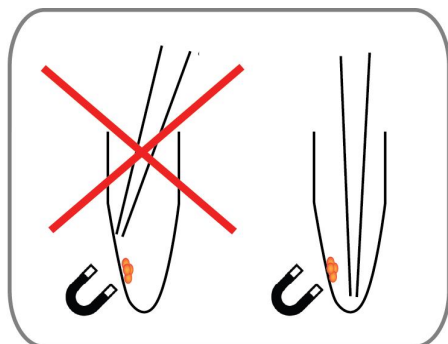
Required kit: **Devysr Library Clean (8-A204)**

Required components: **Clean (4-A255)**, **Wash (4-A256)**

- A. Briefly centrifuge the **Clean** tube to collect the content
- B. Firmly tap the **Clean** tube. Make sure that the bead pellet is re-suspended and that the content is homogenous. If necessary, briefly vortex the tube but avoid extensive vortexing
- C. Add 80  $\mu$ L re-suspended **Clean** to the library pool from 7.3.2 and mix by pipetting. See note for use of alternative volumes of library pool and **Clean**
- D. Incubate the tube at room temperature for 3 minutes
- E. Place the tube onto a magnetic rack until all beads are pelleted and the solution is clear
- F. While keeping the tube on the magnetic rack, carefully remove and discard the solution.  
**Note! It is important to avoid touching the bead pellet during this step (see Figure 9)**
- G. Add 150  $\mu$ L of prepared **Wash** solution (from 7.3.1) to the tube without removing it from the magnetic rack
- H. Slightly lift and rotate the tube two half circles to wash the beads
  - I. Place the tube onto the magnetic rack to pellet the beads
- J. Carefully remove as much **Wash** solution as possible by pipetting from the bottom of the tube. **Note! It is important to avoid touching the bead pellet and the walls of the tube during this step (see Figure 9)**
- K. Leave the lid open until all remaining **Wash** solution has evaporated and the bead pellet has changed from being luster to lusterless, approximately 3-5 minutes, while remaining on the magnetic rack. **Important! See note below**
- L. Remove the tube from the magnetic rack

#### NOTE

It is important that all **Wash** solution has evaporated and that the pellet is dry before continuing. The pellet appearance should change from being luster to lusterless and the color should change slightly to a lighter nuance when dry. If **Wash** solution remains, briefly centrifuge the tube to collect all remaining **Wash** solution, pellet the beads using the magnetic rack, remove the residual **Wash** solution and air dry the pellet again.



**Figure 9. Bead pellet**

### 7.3.4 Library elution

Required kit: **Devyser Library Clean (8-A204)**

Required kit component: **Dilution buffer (4-A245)**

- A. Briefly centrifuge the **Dilution Buffer** tube to collect the content
- B. Add 40  $\mu\text{L}$  **Dilution buffer** to the tube prepared in 7.3.3 and re-suspend the pellet by pipetting and/or tapping the tube. If necessary, collect the liquid by a very brief centrifugation
- C. Place the tube onto the magnetic rack until all beads are pelleted
- D. While keeping the tube on the magnetic rack, transfer the cleared supernatant, containing the purified library pool, to a new tube

### 7.3.5 Library quantification

Required kit: **Qubit 1X dsDNA HS Assay Kit** (see 2.2.4)

Quantify the library as described in the valid user manual for Qubit 1X dsDNA HS Assay Kits for details.

- A. Ensure that all Qubit 1X dsDNA HS solutions are at room temperature
- B. Mix 190  $\mu\text{L}$  Qubit 1X dsDNA HS working solution with 10  $\mu\text{L}$  of Qubit standard 1
- C. Mix 190  $\mu\text{L}$  Qubit 1X dsDNA HS working solution with 10  $\mu\text{L}$  of Qubit standard 2
- D. Mix 190  $\mu\text{L}$  Qubit 1X dsDNA HS working solution with 10  $\mu\text{L}$  of the purified library pool from 7.3.4
- E. Briefly vortex, centrifuge and incubate each tube for 2 minutes at room temperature
- F. Measure the concentration ( $\text{ng}/\mu\text{L}$ ) of the purified library pool on a Qubit Fluorometer

### 7.3.6 Library dilution

Required kit: **Devyser Library Clean (8-A204)**

Required kit component: **Dilution buffer (4-A245)**

- A. Depending on the instrument and reagent kit to be used, dilute the purified library pool from 7.3.4, using **Dilution buffer**, to the following recommended concentrations\* for sequencing:

#### **Index Plate LB-A**

- Illumina MiSeq v2 kits: 0,3 - 0,33  $\text{ng}/\mu\text{L}$
- Illumina MiSeq v3 kit: 0,3 - 0,37  $\text{ng}/\mu\text{L}$
- Illumina MiniSeq: approximately 0,21  $\text{ng}/\mu\text{L}$
- Illumina iSeq 100 v2 kit: approximately 0,23  $\text{ng}/\mu\text{L}$

#### **Index Plate A**

- Illumina MiSeq v2 kits: 0,18 - 0,2  $\text{ng}/\mu\text{L}$
- Illumina MiSeq v3 kit: 0,2 - 0,22  $\text{ng}/\mu\text{L}$
- Illumina MiniSeq: approximately 0,14  $\text{ng}/\mu\text{L}$

- B. Measure the concentration (ng/μL) of the diluted library pool to confirm the concentration by repeating D to F in 7.3.5
- C. Proceed to sequencing according to Chapter 8

\*The recommended concentration of the diluted purified library pool may need to be adjusted to ensure that the Illumina specifications for the reagent kit are met<sup>5,6,7</sup>

**NOTE**

Use the Devyser Sequence Coverage Calculator for Chimerism for information regarding pooling several purified diluted library pools (see 2.4.1).

## 8. SEQUENCING

The Devyser Chimerism can be used with the Illumina MiSeq, MiniSeq and iSeq 100 Systems.

**NOTE** Defrost the MiSeq, MiniSeq or iSeq 100 cartridge well in advance prior to sequencing according to the procedure described in the current version of the MiSeq, MiniSeq or iSeq 100 System Guide <sup>4,8,9</sup>.

### 8.1 FASTQ files for index reads

**NOTE** FASTQ files for iSeq 100 index reads are required for data analysis. FASTQ files for MiSeq and MiniSeq index reads are recommended for data analysis.

FASTQ files for index reads are not generated by default. For MiSeq, follow instructions in the Illumina bulletin "How to generate FASTQ files for index reads in MiSeq Reporter"<sup>10</sup>. For MiniSeq and iSeq 100, follow instructions in the Illumina bulletin "Local Run Manager: How to create FASTQ files for index reads"<sup>11</sup>.

### 8.2 Index layout

The difference between Index Plate LB-A and Index Plate A is that the former contains two unique 10 bp indexes per well and the latter contains two unique 8 bp indexes per well.

The Illumina double indexes introduced during PCR2 are listed in Tables 10 and 11 (see 2.3 for details).

**Table 10. Illumina indexes used in Index Plate LB-A**

	1	2	3	4	5	6	7	8	9	10	11	12
A	LB001	LB009	LB017	LB025	LB033	LB041	LB049	LB057	LB065	LB073	LB081	LB089
B	LB002	LB010	LB018	LB026	LB034	LB042	LB050	LB058	LB066	LB074	LB082	LB090
C	LB003	LB011	LB019	LB027	LB035	LB043	LB051	LB059	LB067	LB075	LB083	LB091
D	LB004	LB012	LB020	LB028	LB036	LB044	LB052	LB060	LB068	LB076	LB084	LB092
E	LB005	LB013	LB021	LB029	LB037	LB045	LB053	LB061	LB069	LB077	LB085	LB093
F	LB006	LB014	LB022	LB030	LB038	LB046	LB054	LB062	LB070	LB078	LB086	LB094
G	LB007	LB015	LB023	LB031	LB039	LB047	LB055	LB063	LB071	LB079	LB087	LB095
H	LB008	LB016	LB024	LB032	LB040	LB048	LB056	LB064	LB072	LB080	LB088	LB096



**Table 11. Illumina indexes used in Index Plate A**

	<b>Index 1-8</b>	<b>Index 9-16</b>	<b>Index 17-24</b>	<b>Index 25-32</b>	<b>Index 33-40</b>	<b>Index 41-48</b>	<b>Index 49-56</b>	<b>Index 57-64</b>	<b>Index 65-72</b>	<b>Index 73-80</b>	<b>Index 81-88</b>	<b>Index 89-96</b>
	Index1: N701	Index1: N702	Index1: N703	Index1: N704	Index1: N705	Index1: N706	Index1: N707	Index1: N708	Index1: N709	Index1: N710	Index1: N711	Index1: N712
Index2: N501	1	9	17	25	33	41	49	57	65	73	81	89
Index2: N502	2	10	18	26	34	42	50	58	66	74	82	90
Index2: N503	3	11	19	27	35	43	51	59	67	75	83	91
Index2: N504	4	12	20	28	36	44	52	60	68	76	84	92
Index2: N505	5	13	21	29	37	45	53	61	69	77	85	93
Index2: N506	6	14	22	30	38	46	54	62	70	78	86	94
Index2: N507	7	15	23	31	39	47	55	63	71	79	87	95
Index2: N508	8	16	24	32	40	48	56	64	72	80	88	96

### 8.3 Sequencing mode

The Devyser Chimerism libraries should be sequenced in paired end mode:

#### Index Plate LB-A

- MiSeq v2 (300 cycles) and v3 (150 cycles) kits: 2 x 76 bp
- iSeq 100 v2 kit (300 cycles): 2 x 76 bp
- MiniSeq Mid Output Kit (300 cycles): 2 x 76 bp
- MiniSeq High Output Reagent Kit (150 cycles): 2 x 74 bp

#### Index Plate A

- MiSeq v2 (300 cycles) and v3 (150 cycles) kits: 2 x 76 bp
- MiniSeq Mid Output Kit (300 cycles): 2 x 76 bp
- MiniSeq High Output Reagent Kit (150 cycles): 2 x 76 bp

## 8.4 Sequencing using MiSeq

### 8.4.1 Sample sheet generation

Generate a sample sheet for each run in the Illumina Experiment Manager (IEM) software or Local Run Manager (LRM) by using the appropriate Devyser guide (see Table 8) and the Illumina Experiment Manager User Guide<sup>12</sup> or the Illumina Local Run Manager v2 Guide<sup>13</sup>.

### 8.4.2 Denaturation of the purified library pool

- A. Prepare 20 pM PhiX, HT1 and a fresh dilution of 0.2 N NaOH according to the current version of the "Denature and Dilute Libraries Guide"<sup>14</sup>
- B. Combine 5 µL diluted purified library pool from 7.3.6 with 5 µL 0.2 N NaOH
- C. Briefly vortex, centrifuge and incubate for 5 minutes at room temperature
- D. Add 1410 µL prechilled HT1 to dilute the denatured library pool
- E. To obtain a sequencing mix, add 9 µL 20 pM denatured PhiX control DNA to the mixture from D. The added PhiX will represent approximately 1% of the total number of reads from the sequencing run
- F. Repeatedly invert and then vortex the tube to mix and briefly centrifuge to collect the content

### 8.4.3 Sequencing

#### **NOTE**

Make sure FASTQ files for Indexes is activated before starting the sequencing run (see 8.1).

- A. Prepare the sequencing run according to the current version of the "MiSeq System Guide"<sup>4</sup>
- B. Add 600 µL of the sequencing mix (8.4.2 F) to the sample well in the reagent cartridge
- C. Load the flow cell and execute the sequencing run using 2 x 76 bp sequencing mode
- D. After completion of the sequencing run, locate the generated sequencing data files (FASTQ) and move them to the desired location for analysis

## 8.5 Sequencing using MiniSeq

### 8.5.1 Sample sheet generation

Generate a sample sheet for each run in the Local Run Manager (LRM) software by using the appropriate Devyser guide (see Table 8) and the Illumina Local Run Manager v2 Guide<sup>13</sup>.

### 8.5.2 Denaturation of the purified library pool

- A. Prepare 200 mM Tris-HCl, pH 7,0, Hybridization Buffer, a fresh dilution of 0.1 N NaOH and 1.8 pM denatured PhiX according to the current version of the "MiniSeq System Denature and Dilute Libraries Guide System"<sup>15</sup>
- B. Combine 5 µL purified library from 7.3.6 with 5 µL 0.1 N NaOH
- C. Briefly vortex, centrifuge and incubate for 5 minutes at room temperature
- D. Add 5 µL 200 mM Tris-HCl, pH 7.0, briefly vortex and centrifuge
- E. Add 985 µL Hybridization Buffer to the denatured library, briefly vortex and centrifuge
- F. Add 720 µL prechilled Hybridization Buffer to a new tube
- G. Add 180 µL diluted library (from step E) to the Hybridization Buffer (step F) to reach a total volume of 900 µL
- H. To obtain the sequencing mix, add 5 µL of 1,8 pM denatured PhiX to the library. The added PhiX will represent approximately 1% of the total number of reads from the sequencing run
- I. Repeatedly invert and then vortex the tube to mix and briefly centrifuge to collect the content

### 8.5.3 Sequencing

- A. Prepare the sequencing run according to the current version of the "MiniSeq System Guide"<sup>8</sup>
- B. For loading the reagent cartridge, transfer 500 µL of the sequencing mix (8.5.2 I) to the sample well of the reagent cartridge
- C. Load the desired flow cell and execute the sequencing run
- D. After completion of the sequencing run, locate the generated sequencing data files (FASTQ) and move them to the desired location for analysis

## 8.6 Sequencing using iSeq 100

### 8.6.1 Sample sheet generation

Generate a sample sheet for each run in the Local run Manager (LRM) software by using the Devyser Guides "Devyser LB-A Sample Sheet LRM" (see Table 8) and the Illumina Local Run Manager v2 Guide<sup>13</sup>.

### 8.6.2 Preparation of the purified library pool

- A. Prepare 10 mM Tris-HCl, pH 8.5, or RSB buffer and 50 pM PhiX according to the current version of the "iSeq 100 Sequencing System Guide"<sup>9</sup>
- B. Combine 5 µL diluted purified library from 7.3.6 with 95 µL 10 mM Tris-HCl, pH 8.5, or RSB buffer
- C. To obtain a sequencing mix, add 2 µL 50 pM PhiX to the mixture from B. The added PhiX will represent approximately 2% of the total number of reads from the sequencing run
- D. Briefly vortex the tube to mix and centrifuge to collect the content

### 8.6.3 Sequencing

- A. Prepare the sequencing run according to the current version of the "iSeq 100 Sequencing System Guide"<sup>9</sup>
- B. For loading the reagent cartridge, transfer 20 µL of the sequencing mix (8.6.2 D) to the library reservoir
- C. Load the flow cell and execute the sequencing run
- D. After completion of the sequencing run, locate the generated sequencing data files (FASTQ) and move them to the desired location for analysis

## 9. CHIMERISM CALCULATION

Samples to be analyzed using Devyser Chimerism are divided into two categories: screening and monitoring samples.

Screening samples are pre-transplant samples obtained from the recipient and the donor. Screening samples are analyzed to identify informative markers to be used for monitoring chimerism after HSCT.

Monitoring samples are taken from the recipient post-HSCT and are analyzed to determine the amount of recipient and/or donor DNA in the recipient.

For screening samples, each marker should be assigned a genotype accordingly to Table 12. For screening samples, the minimum required coverage per marker should be 200 paired end (PE) reads. A marker is considered reliable and useful if the VAF is:

- Heterozygous: above 40% and below 60%
- Homozygous: above 99% or below 1%

**Table 12. Interpretation of genotype output for a given marker**

Marker output	Interpretation
+/+	Marker is homozygous for the reference sequence according to hg19
-/-	Marker is homozygous for the alternate sequence according to hg19
+/-	Marker is heterozygous for the reference and alternate sequences according to hg19

Each monitoring sample from a recipient should be associated with both screening samples, one coming from the recipient and one from the donor. The informativity of the markers should subsequently be defined according to Table 13.

**Table 13. Definition of informative markers**

A marker is informative if:	Recipient	Donor
Recipient and the donor are opposite homozygous for reference and alternate sequences	+/+	-/-
	-/-	+/+
Recipient is heterozygous and donor is homozygous for reference and alternate sequences	+/-	-/-
	+/-	+/+

Chimerism should be calculated for all informative markers and the average chimerism percentage should also be calculated. At least two informative markers are required to reliably calculate chimerism in a sample.

For a given informative marker, chimerism should be calculated as the percentage for the allele belonging to the recipient present in the monitoring sample. In the case of a heterozygous informative marker the percentage should be multiplied by two. Alternatively, chimerism can be calculated as the percentage for the allele belonging to the donor in the monitoring sample.

## 10. TROUBLESHOOTING

Possible results requiring troubleshooting are shown in Table 14.

**Table 14. Troubleshooting**

Observation	Possible cause	Interpretation/action
Calculated chimerism values vary notably between informative markers	Poor DNA quality and/or DNA input concentration is below 6 ng/μL	Repeat extraction of sample and/or repeat dilution of DNA to 6 ng/μL
	Possible sample mix-up	Investigate non-informative markers in monitoring sample for divergent or conflicting VAF (e.g. donor and recipient are +/+ and monitoring sample is -/-)
	Same indexes were used in two consecutive runs without performing template line wash between runs (only applicable for MiSeq)	Repeat sequencing run with other indexes and/or perform template line wash
Coverage is below 10.000 reads/marker	Poor DNA quality and/or DNA input concentration is below 6 ng/μL and/or sub-optimal PCR profile resulting in sub-optimal sequencing depth	A coverage of >10.000 reads is required to determine a low level (<0,1%) of minority fraction chimerism with high precision and sensitivity. For a minority fraction chimerism level of >1%, a coverage of >1.000 reads is sufficient
Coverage is below 1.000 reads/marker		Determine if the low coverage applies to a single marker or if there is a general problem in the sample. If related to a single informative marker: do not use this marker to calculate and interpret chimerism. If related to all informative markers: repeat DNA extraction and/or repeat dilution of DNA to 6 ng/μL
Evaporation of PCR1 and/or PCR2 reaction(s)	The lid of the PCR instrument and/or the closure of the plate does not seal properly	Repeat procedure from suitable step according to this handbook
Library concentration too low after clean-up	Possible error during PCR or library clean-up	Repeat clean-up step. A library QC evaluation is recommended using automated electrophoresis (a smear around 210 bp should be seen). If no library is detected, repeat procedure from suitable step according to this handbook
High cluster density and low passing filter	Loading concentration is too high	Decrease loading concentration to match Illumina specifications output
Low cluster density and insufficient amount of data generated	Loading concentration is too low	Increase loading concentration to match Illumina specifications output

## 11. SYMBOLS USED ON LABELS

**LOT**

Lot or batch number



Expiry date



Number of tests



Store below temperature shown



Temperature limit



Consult instructions for use

**REF**

Catalogue number



Manufacturer

**RUO**

Research Use Only

## 12. NOTICE TO PURCHASER

Results from the Devyser Chimerism kit should not be used for diagnostic purposes. This product is intended for research use only.

Purchase of this product does not provide a license to perform PCR under patents owned by any third party.

MiSeq™, MiniSeq™ and iSeq™ 100 are trademarks of Illumina® Corporation.

Qubit™, DynaMag™, Veriti™ and MicroAmp™ are trademarks of Thermo Fisher Scientific Corporation.

Devyser® is a registered trademark of Devyser Diagnostics AB.



## 13. CONTACT INFORMATION

### 13.1 Legal manufacturer

Devyser AB  
Instrumentvägen 19  
SE-126 53 Hägersten  
SWEDEN

Phone: +46-8-562 15 850  
Homepage: [www.devyser.com](http://www.devyser.com)

### 13.2 Technical support

E-mail: [techsupport@devyser.com](mailto:techsupport@devyser.com)

## 14. REFERENCES

- <sup>1</sup> Gratwohl A, Baldomero H, Passweg J, Frassoni F, Niederwieser D, Schmitz N and Urbano-Ispizua A. "Hematopoietic stem cell transplantation for hematological malignancies in Europe", *Leukemia*. 2003, 17, 941-959.
- <sup>2</sup> Reshef R, Hexner EO, Loren AW, Frey NV, Stadtmayer EA, Luger SM, Mangan JK, Gill SI, Vassilev P, Lafferty KA, Smith J, Van Deerlin VM, Mick R, and Porter DA. "Early donor chimerism levels predict relapse and survival after allogeneic stem-cell transplantation with reduced intensity conditioning", *Biol Blood Marrow Transplant*. 2014 November 1758–1766.
- <sup>3</sup> Reducing Run-to-Run Carryover on the MiSeq Using Dilute Sodium Hypochlorite Solution. Technical note from Illumina, posted on 2018-08-29, 02:38 by Illumina Inc.
- <sup>4</sup> MiSeq System Guide (Document # 15027617)
- <sup>5</sup> <https://www.illumina.com/systems/sequencing-platforms/miseq/specifications.html>. Cited 2020-11-11
- <sup>6</sup> <https://emea.illumina.com/systems/sequencing-platforms/miniseq/specifications.html> Cited 2020-11-11
- <sup>7</sup> <https://emea.illumina.com/systems/sequencing-platforms/iseq/specifications.html> Cited 2020-11-11
- <sup>8</sup> MiniSeq System Guide (Document # 1000000002695)
- <sup>9</sup> iSeq 100 Sequencing System Guide (Document #10000000036024)
- <sup>10</sup> "How to generate FASTQ files for index reads in MiSeq Reporter", Jun 7, 2018. Available at: <https://support.illumina.com/bulletins/2016/06/how-to-create-fastq-files-for-index-reads-in-miseq-reporter.html>, verified 2020-11-25
- <sup>11</sup> "Local Run Manager: How to create FASTQ files for index reads", Mar 26, 2018. Available at: <https://support.illumina.com/bulletins/2018/03/local-run-manager--how-to-create-fastq-files-for-index-reads.html>, verified 2020-11-25
- <sup>12</sup> Illumina Experiment Manager User Guide (Document # 15031335)
- <sup>13</sup> Illumina Local Run Manager v2 Guide (Document #1000000002702)
- <sup>14</sup> MiSeq System Denature and Dilute Libraries Guide (Document # 15039740)
- <sup>15</sup> MiniSeq System Denature and Dilute Libraries Guide (Document # 1000000002697)

## 15. ABBREVIATIONS

<b>Abbreviation</b>	<b>Explanation</b>
bp	base pairs
CV	coefficient of variation
DNA	deoxyribonucleic acid
dsDNA	double stranded DNA
gDNA	genomic DNA
HSCT	hematopoietic stem cell transplantation
Indel	insertion and / or deletion
IEM	Illumina Experiment Manager
LB	low background
LRM	local run manager
MC	mixed chimerism
NGS	next generation sequencing
PCR	polymerase chain reaction
PE	paired end
RUO	research use only
VAF	variant allele frequency

## 16. REVISION HISTORY

### Version 2021-04-15

#### Editorial changes

8.6.2 Corrected instructions in step C to not state that added PhiX should be denatured

### Version 2021-02-22

#### Editorial changes

2.2.1 Removed note specifying that Index Plate A can be used when pooling with other Devyser products

2.3 Corrected Download file names "Index Plate A IEM files" and "Index Plate A LRM files" to "MiSeq IEM files" and "LRM files"

8.3 Added number of cycles for the MiSeq v2, MiSeq v3 and iSeq 100 v2 kits

8.5 Removed RSB Buffer from steps A and D

8.6.2 Corrected Tris-HCl concentration, changing it from 200 mM to 10 mM, and added it to step B

9 Corrected minimum required coverage per marker for screening from 100 paired end reads to 200

10 Corrected the level of minority fraction chimerism that a coverage of >10.000 reads is sufficient for detecting, from <1% to <0.1%

10 Specified the fragment sizes to expect if automated electrophoresis is used for a library QC evaluation

### Version 2021-01-25

#### New