DEVYSER
RESULTS FOR LIFE

Devyser FH v2

Art. No.: 8-A109-RUO

For Research Use Only

Handbook

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1. INTRODUCTION TO DEVYSER FH v2

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1.1 Intended use

The Devyser FH v2 kit enables analysis of sequence variants in human genes implicated in Familial Hypercholesterolemia (FH) and polymorphisms associated with statin treatment effect (see tables 1-3 for details).

The Devyser FH v2 kit is for research use only, not for use in diagnostic procedures.

1.2 Background

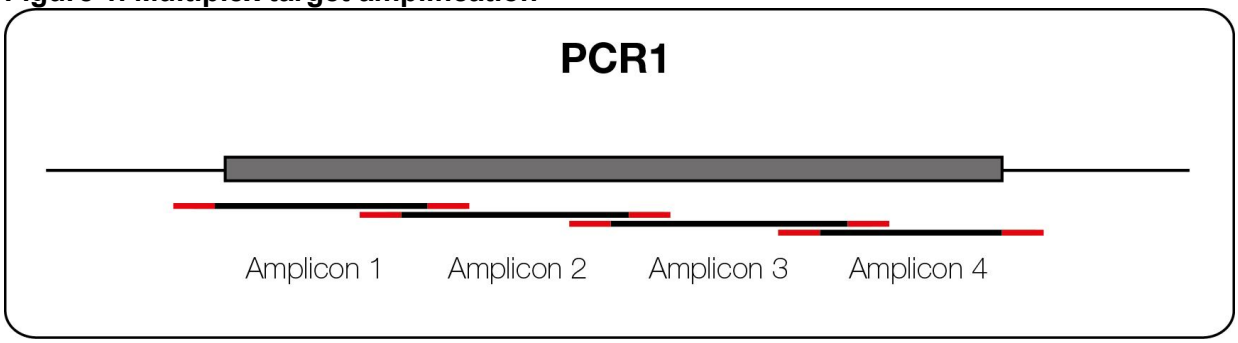
FH is an autosomal dominant hereditary disorder associated with high levels of LDL-cholesterol and early onset cardiovascular disease and death. The clinical diagnosis of FH is based on the presence of high LDL-cholesterol, a family history of hypercholesterolemia, xanthomas or early onset cardiovascular events, presence of tendon xanthomas or arcus senilis on physical examination, as well as early onset cardiovascular disease in the patient.

Sequence variants in several key genes associated with cholesterol metabolism are known to cause FH and the clinical diagnosis can in some cases be confirmed by genetic testing. Genetic testing can also be used when performing “cascade screening” within the family after a proband is diagnosed.

1.3 Assay principle

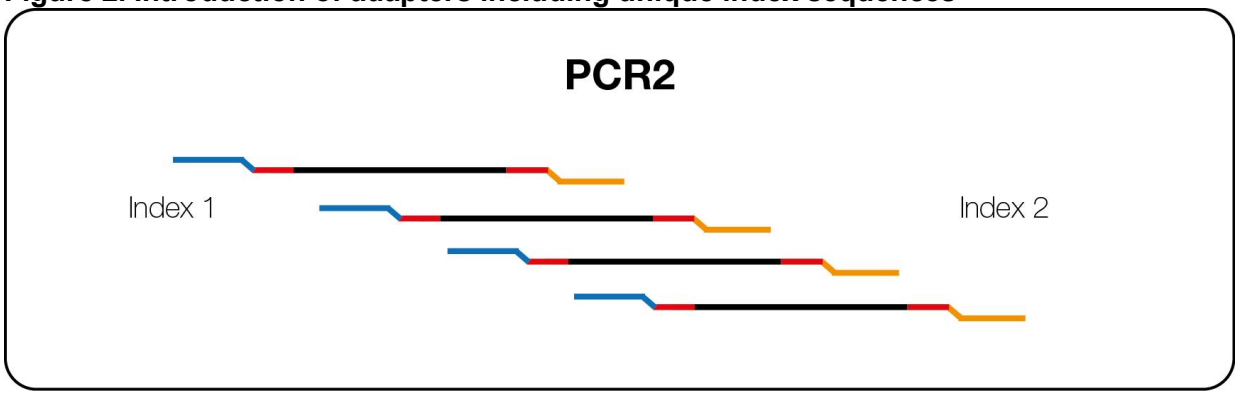
The method employed by the Devyser FH v2 kit includes multiplex PCR amplification to create a target amplicon library from each DNA sample (PCR1). The library covers the full target region in a partly overlapping fashion, as illustrated in figure 1.

Figure 1. Multiplex target amplification



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 using appropriate softwares for targeted sequencing.

Figure 2. Introduction of adapters including unique index sequences



1.4 Assay design

Devyser FH v2 enables target specific library generation for NGS analysis of variants (SNVs and indels), all appearing in the genetic regions outlined in tables 1-3. The design additionally enables CNV analysis to define large, exon spanning deletions and duplications in the LDLR gene.

PCR primers used for the library generation are designed such that primer footprints are normally covered by an overlapping amplicon to enable detection of primer site SNVs.

Table 1. Genetic regions sequenced by Devyser FH v2[1]

Genes	Exons	Promoter region	Minimum exon/intron boundaries sequenced (bp)
LDLR (LRG_274t1)	1-18	236 bp to c.1	-10/+10
APOB	2-29	No	-10/+10
PCSK9 (LRG_275t1)	1-12	439 bp to c.1	-10/+10
LDLRAP1 (LRG_276t1)	1-9	No	-10/+10
APOE	2-3, 4*	No	-10/+10
STAP1	1-9	No	-10/+10

* The following part of APOE exon 4 is sequenced; c.387-529

Table 2. Direct detection of exon spanning CNV[6]

Gene	Mutation	Also known as
LDLR	Exon16-18 deletion	Helsinki

Table 3. Genetic markers sequenced by Devyser FH v2[2-5]

Marker	cDNA position	Gene location	Implication
rs629301	c.*1635T>G	CELSR2	FH risk
rs1564348	c.1599-688T>C	SLC22A1	FH risk
rs1800562	c.845G>A	HFE	FH risk
rs2479409	c.-861A>G	PCSK9	FH risk
rs3757354	c.-2147C>T	MYLIP	FH risk
rs4299376	c.166-718T>G	ABCG8	FH risk
rs6511720	c.67+2015G>T	LDLR	FH risk
rs8017377	c.2932G>A	NYNRIN	FH risk
rs11220462	c.-61+18215G>A	ST3GAL4	FH risk
rs1367117	c.293C>T	APOB	FH risk
rs429358	c.388T>C	APOE	Statin therapy + FH risk
rs7412	c.526C>T	APOE	Statin therapy + FH risk
rs646776	c.*1859T>C	CELSR2	Statin therapy
rs4149056	c.521T>C	SLCO1B1	Statin therapy
rs3798220	c.5673T>C	LPA	Statin therapy
rs10455872	c.3947+467A>G	LPA	Statin therapy

2. MATERIALS AND EQUIPMENT

2.1 Kit configurations for Devyser FH v2

The Devyser FH v2 kit is available in two configurations according to tables 4 and 5.

Table 4. Devyser FH v2, 24 test configuration (8-A109-24-RUO)

Component	Art.No.	Number/kit	Cap color	Storage condition
FH mix	4-A293	1	Blue	Below -18°C
Start, 24 test	4-A280	1	Purple	Below -18°C
Dilution buffer	4-A245	3	White	-25°C to +8°C
Index mix 2, 24 test	4-A279	1	Red	Below -18°C
Index strip A2	-	1	-	Below -18°C
Index buffer	4-A258	3	Green	-25°C to +8°C
Sealer S	-	1	-	Ambient

Table 5. Devyser FH v2, 48 test configuration (8-A109-48-RUO)

Component	Art.No.	Number/kit	Cap color	Storage condition
FH mix	4-A293	2	Blue	Below -18°C
Start, 24 test	4-A280	2	Purple	Below -18°C
Dilution buffer, 96 test	4-A275	1	-	-25°C to +8°C
Index mix 2, 24 test	4-A279	2	Red	Below -18°C
Index plate A3	-	1	-	Below -18°C
Index buffer, 96 test	4-A277	1	Green	-25°C to +8°C
Sealer L	-	1	-	Ambient

2.2 Equipment and reagents required but not provided

2.2.1 Other required Devyser products

- Devyser Library Clean (8-A204), see table 6

Table 6. Devyser Library Clean (8-A204)

Component	Art.No.	Number/kit	Cap color	Storage condition
Clean	4-A255	1	Orange	+2 to +8 °C
Wash	4-A256	1	Yellow	+2 to +8 °C
Dilution buffer	4-A245	1	White	+2 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 QIAsymphony DSP DNA Midi Kit (Qiagen, cat.#937255) for extraction of DNA from human whole blood
- If alternative DNA extraction methods and sample materials are used, a thorough evaluation of the performance together with the Devyser FH v2 kit should be performed

2.2.4 Determination of DNA concentration

- Determination of DNA concentration according to manufacturer's instructions for use
- Qubit™ 4 Fluorometer (cat.# Q33226) or Qubit 2.0 Fluorometer (cat.# Q32866) and required consumables (Thermo Fisher Scientific)
- 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 FH v2 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

- Devyser Library Clean (see 2.2.1)
- Magnetic rack for test tubes (DynaMag™-2 Magnet, Thermo Fisher Scientific or equivalent)
- Ethanol (96 %)

2.2.7 Sequencing

- Illumina® MiSeq™
- User-supplied consumables needed for sequencing, according to the Illumina sequencing guide
- Illumina reagent kits (table 7)

Table 7. MiSeq reagent kits

Illumina reagent kit	Illumina cat #
Miseq Reagent Nano Kit v2 (300-cycles)	MS-103-1001
Miseq Reagent Micro Kit v2 (300-cycles)	MS-103-1002
Miseq Reagent Kit v2 (300-cycles)	MS-102-2002
Illumina PhiX control v3	FC-110-3001

NOTE All equipment should be tested, calibrated and maintained regularly

2.3 Software

- SeqPilot v4.3.1 software including the SeqNext module (JSI Medical Systems GmbH). If using a different version of the software, please consult the manufacturer
- Amplicon Suite (SmartSeq) pipeline for FH v2. Contact Devyser for more information

Contact Devyser support at techsupport@devyser.com for information

2.4 Downloads

Supplementary information and files can be downloaded from 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	Description
Illumina double index	Index sequence information
MiSeq IEM files	Devyser setting files for sample sheet generation: • Generating a Devyser sample sheet for MiSeq.doc • DEVYSER double Index MiSeq.txt • DevyserGenerateFASTQ.txt • DevyserGenerateFASTQ.jpg
SeqNext guide for Devyser FH v2	SeqNext guide for Devyser FH v2 analysis
Devyser FH v2 SeqNext .sge files	Devyser settings files (.sge files) for FH v2 data analysis with SeqNext

2.5 Other resources

2.5.1 Devyser Sequence Coverage Calculator

To plan the sequencing run with respect to coverage needs, please consult the Devyser Sequence Coverage Calculator at www.devyser.com/calculator. As displayed in the calculator, the expected minimum total number of read pairs per sample is 94 500 and minimal coverage per amplicon is 100.

After the sequencing run, the total number of read pairs per sample can be found in Illumina Sequencing Analysis Viewer (SAV), BaseSpace or Local Run Manager (LRM). The minimal coverage per amplicon can be found in the analysis software.

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3. STORAGE REQUIREMENTS

- Store the Devyser FH v2 kit in a freezer below -18°C (-28°C to -18°C) or the individual kit components as specified on the label (see also tables 4 and 5 in 2.1)
- Store the components of the Devyser Library Clean kit at +2 to +8 °C
- Do not use components beyond the kit lot 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 specified in this handbook (chapter 7)
- Frozen kit components should be thawed in a refrigerator or at room temperature before use
- Avoid repeated freezing-thawing

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
- Deviations from the handbook will compromise the kit performance
- Modifications of software settings will compromise the kit performance
- Wear powder free disposable gloves, laboratory coat and eye protection when handling samples and kit reagents
- Do not pool reagents with different kit lot numbers or different vials of the same lot
- 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
- Gloves should be changed between activities

5. PROCEDURAL LIMITATIONS

- The Devyser FH v2 kit is for research use only, not for diagnostic procedures
- Sequence variants that may be present in other genes than what is described to be covered in this handbook will not be detected using Devyser FH v2
- Results obtained with the Devyser FH v2 kit can only be directly applied to the tissue or specific sample material tested
- Rare primer site sequence alterations may affect the function of individual PCR primers used in the Devyser FH v2 kit
- It is recommended that only samples that have been processed and sequenced together and with the same kit lot should be analysed together for a CNV analysis
- The bioinformatic characterization of CNVs is based on different computational strategies. None of the strategies can guarantee correct detection of all CNVs
- The following parameters might affect the overall performance and in particular the CNV analysis
    - Quality, sample type and concentration of the DNA
    - Deviations from the protocol
    - The number of control samples used in the analysis
    - The type of control samples used
    - Sequencing depth
    - Bioinformatic pipeline
- The combination of a deletion on one chromosome and a similar sized duplication on the other chromosome may result in false negative CNV results
- Running several family members or samples with the same deletions in the same run can affect CNV analysis and should be avoided

6. SAMPLE REQUIREMENTS

DNA concentration, integrity and purity are important parameters for successful testing using the Devyser FH v2 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 FH v2 kit has been tested using human genomic DNA extracted from whole blood.

6.1.1 DNA extraction from whole blood sample

According to the manufacturer's instruction for use

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 FH v2 kit

6.1.3 Dilution of DNA

- Adjust the concentration of extracted DNA to 2 ng/µL (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

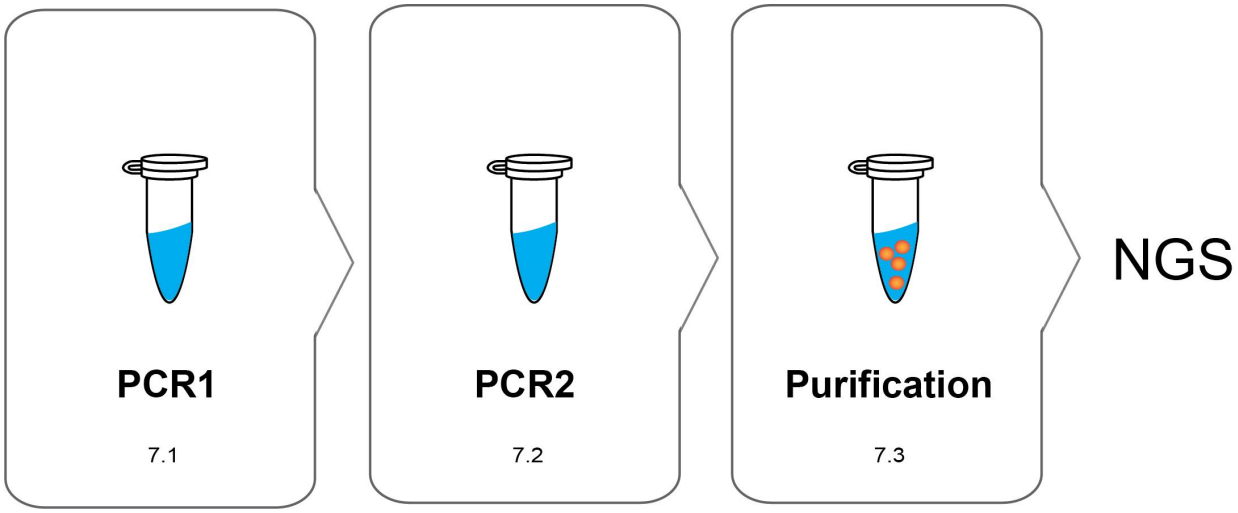
Include PhiX control v3 library DNA (see 2.2.7) in each sequencing run to ensure that the sequencing pool has the required diversity for high quality sequencing (see 8.3).

6.3 Internal system control

We recommend to perform regular internal system control of all equipment and software used in this procedure. Samples with pre-characterized FH associated sequence variants (in-house developed or externally sourced) are suitable as system controls.

7. INSTRUCTIONS FOR USE

Figure 3. Schematic overview of the Devyser FH v2 library preparation procedure (7.1-7.3)



The Devyser FH v2 library preparation procedure consists of the following steps:

PCR1 (7.1)
The amplicon library is generated in one multiplex PCR reaction for each sample.

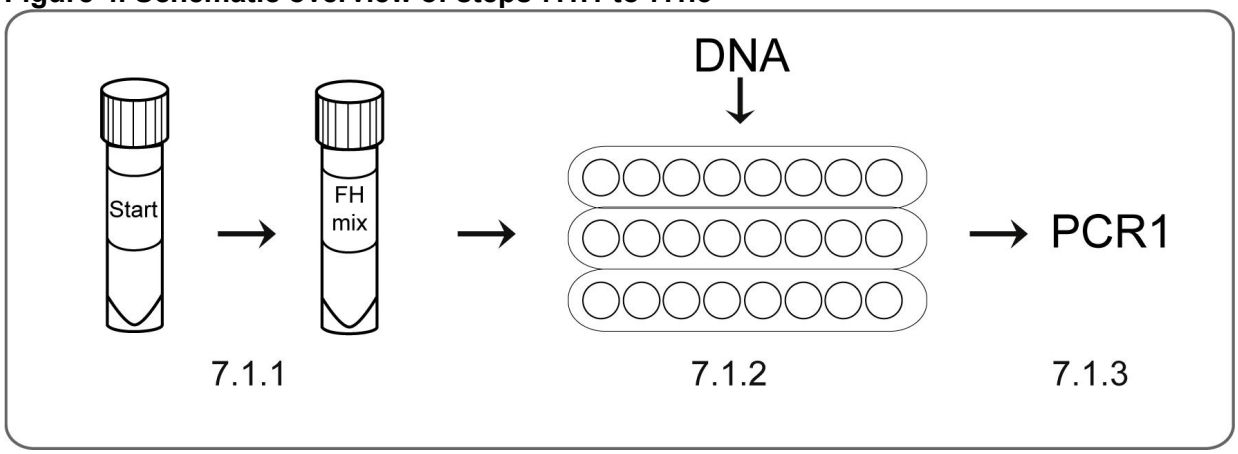
PCR2 (7.2)
Index addition to the PCR1 library is performed in PCR2 to allow pooling of multiple sample libraries for sequencing.

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.

Each step (7.1 - 7.3) is followed by a suitable stopping point where the procedure can be paused and restarted within 30 days.

7.1 Library generation (PCR1) for Devyser FH v2 24 and 48-test kits

Figure 4. Schematic overview of steps 7.1.1 to 7.1.3



7.1.1 FH mix preparation for 24 and 48-test

Determine the number of **Start, 24 test** and **FH mix** tubes required. Each tube is sufficient for 24 reactions.

Required kit components: **Start, 24 test (4-A280), FH mix (4-A293)**

A. Ensure that the **Start, 24 test** and the **FH mix** are completely thawed before use
B. Vortex the **Start, 24 test** tube(s) briefly
C. Briefly centrifuge the **Start, 24 test** and **FH mix** tube(s) to collect the content
D. Add 150 µL of **Start, 24 test** to the **FH mix** tube(s) to obtain an activated **FH mix**
E. Vortex the activated **FH mix** tube(s) and then centrifuge it briefly to collect the content
F. Dispense 10 µL of the activated **FH mix** into separate PCR reaction tubes or separate wells in a plate. Cap the tubes or seal the plate.
G. Store the dispensed **FH mix** at +2 to +8 °C and continue to 7.1.2
H. Any remaining activated **FH mix** can be stored in a freezer below -18°C for 6 weeks. Do not aliquot the activated mix.

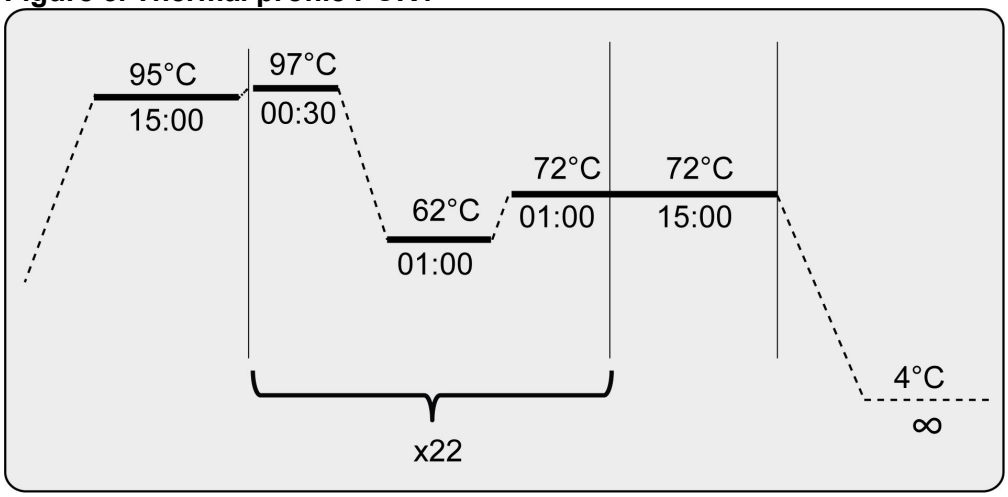
7.1.2 Preparation and addition of DNA

Required kit component: **Dilution buffer (4-A245)** or **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 2 ng/µL using the provided **Dilution buffer**
D. Add 5 µL of diluted DNA from each sample to the separate PCR reaction tubes or the separate wells in the plate containing activated **FH mix** (from 7.1.1)
E. Mix by pipetting
F. Cap the tubes or seal the plate and centrifuge briefly to collect the content
G. Continue to 7.1.3

7.1.3 Thermal cycling PCR1

Figure 5. Thermal profile PCR1



A. Program the thermal cycler according to the PCR1 thermal profile in figure 5
B. Set the ramp rates to heating 1,6 ˚C/s and cooling 1,6 ˚C/s
C. Set the reaction volume to 15 µL
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 if necessary, to collect the content and continue to 7.2

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

SUITABLE STOPPING POINT

NOTE	It is of high importance that the following ramp rates are applied: heating 1,6 ˚C/s, cooling 1,6 ˚C/s

NOTE	To program the correct ramp rate for the Veriti Thermal Cycler: In the "Tools Menu" select "Convert a Method". In the next select "9700 Max Mode" and then enter the PCR profile as outlined in section 7.1.3

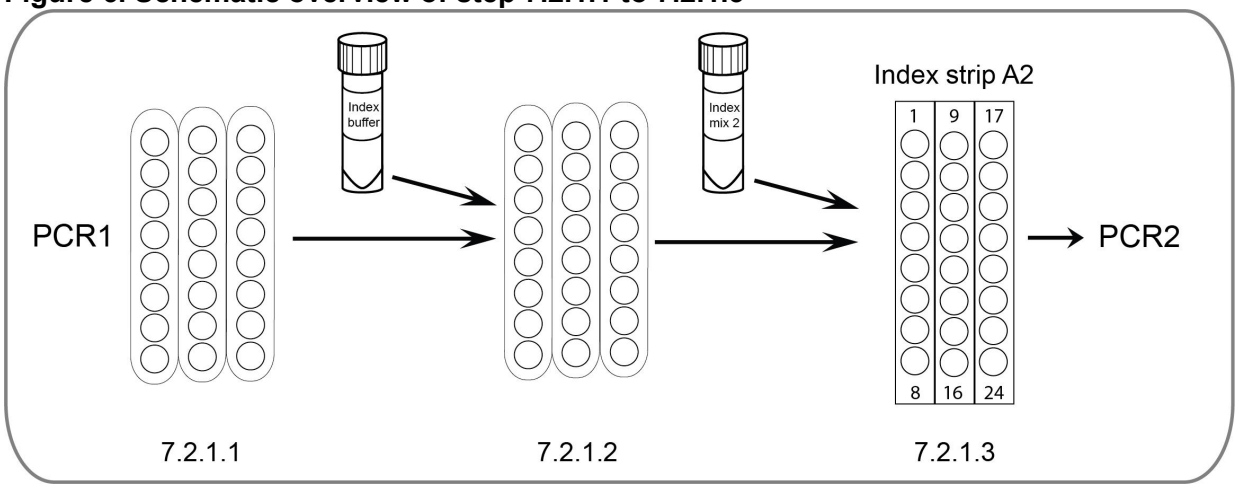
NOTE	If using tubes/strips in a Veriti Thermal Cycler they should first be placed in the MicroAmp 96-Well Tray/Retainer Set for Veriti Systems

7.2 Library indexing (PCR2)

For the Devyser FH v2 24 test kit proceed to 7.2.1
For the Devyser FH v2 48 test kit proceed to 7.2.2

7.2.1 Library indexing - Devyser FH v2 24 test kit (8-A109-24-RUO)

Figure 6. Schematic overview of step 7.2.1.1 to 7.2.1.3



7.2.1.1 PCR1 library dilution

Required kit component: **Index buffer (4-A258)**

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

7.2.1.2 Index preparation

Required kit components: **Index mix 2, 24 test (4-A279), Index strip A2**

A. Ensure that the **Index mix 2, 24 test** is completely thawed before use
B. Vortex and then briefly centrifuge the **Index mix 2, 24 test** tube to collect the content
C. Carefully remove the transport seal of **Index strip A2**. **Note! Do not reuse the transport seal**
D. Add 20 µL of **Index mix 2, 24 test** to each of the 24 wells of **Index strip A2**. **Note! Tips must be changed between each individual well**

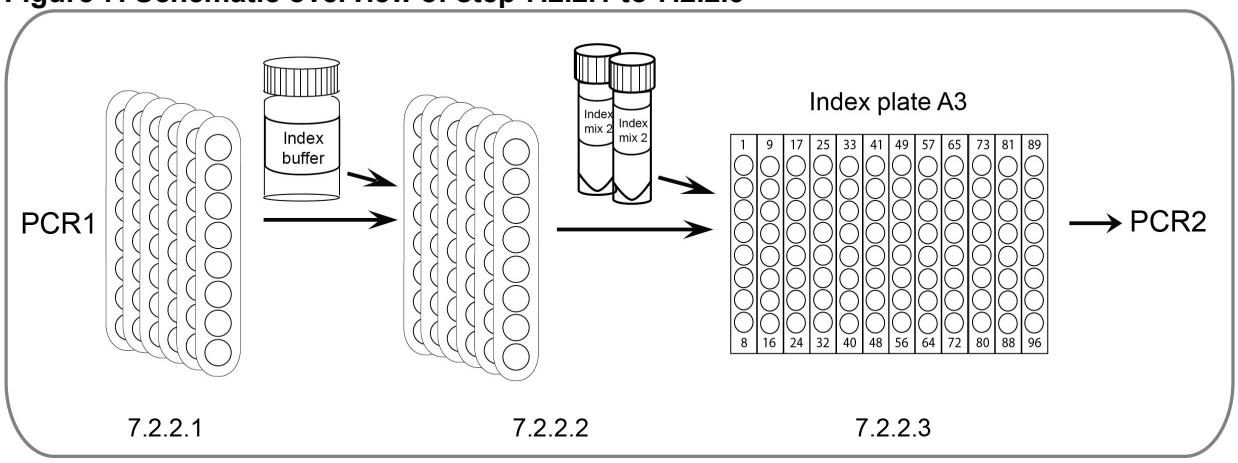
7.2.1.3 Addition of diluted PCR1 libraries to Index strip A2

Required kit component: **Sealer S**

A. Add 5 µL of each diluted PCR1 library (from 7.2.1.1) to separate wells in **Index strip A2** (prepared in 7.2.1.2)
B. Mix thoroughly by pipetting to dissolve the colored reagent pellets, using a pipetting volume of at least 10 µL. **Note! Make sure that the colored reagent pellets are completely dissolved before proceeding to the next step.** Avoid bubbles
C. Cut a piece of **Sealer S** to completely cover **Index strip A2**
D. Carefully seal **Index strip A2** and make sure that all wells are covered
E. Centrifuge briefly to collect the content
F. Continue to 7.2.3

7.2.2 Library indexing - Devyser FH v2 48 test kit (8-A109-48-RUO)

Figure 7. Schematic overview of step 7.2.2.1 to 7.2.2.3



7.2.2.1 PCR1 library dilution

Required kit component: **Index buffer, 96 test (4-A277)**

A. Ensure that the **Index buffer, 96 test** is completely thawed before use
B. For each PCR1 library to be diluted, dispense 198 µL **Index buffer, 96 test** to a new tube
C. Add 2 µL of each PCR1 library to the separate dilution tubes containing 198 µL **Index buffer, 96 test**. Make sure no liquid remains in the tip by pipetting repeatedly in the **Index buffer, 96 test**
D. Mix the diluted PCR1 libraries thoroughly by pipetting (using a pipetting volume of at least 100 µL)

7.2.2.2 Index preparation

Required kit components: **Index mix 2, 24 test (4-A279), Index plate A3**

A. Ensure that the **Index mix 2, 24 test** is completely thawed before use
B. Vortex and then briefly centrifuge the **Index mix 2, 24 test** tube(s) to collect the content
C. Carefully remove the transport seal of **Index plate A3**. **Note! Do not reuse the transport seal**
D. Add 20 µL of **Index mix 2, 24 test** to each of the wells to be used in **Index plate A3**. **Note! Tips must be changed between each individual well**

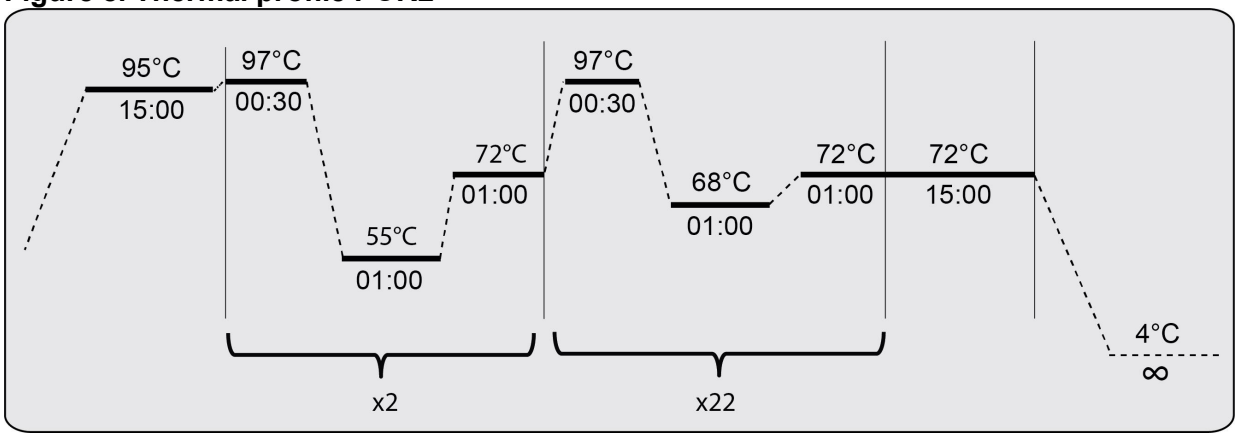
7.2.2.3 Addition of diluted PCR1 libraries to Index plate A3

Required kit component: **Sealer L**

A. Add 5 µL of each diluted PCR1 library pool (from 7.2.2.1) to separate wells in **Index plate A3** (prepared in 7.2.2.2)
B. Mix thoroughly by pipetting to dissolve the colored reagent pellets, using a pipetting volume of at least 10 µL. **Note! Make sure that the colored reagent pellets are completely dissolved before proceeding to the next .** Avoid bubbles
C. Cut a piece of **Sealer L** to completely cover **Index plate A3**
D. Carefully seal **Index plate A3** and make sure that all wells are covered
E. Centrifuge briefly to collect the content

7.2.3 Thermal cycling PCR2

Figure 8. Thermal profile PCR2



A. Program the thermal cycler according to the PCR2 thermal profile in figure 8
B. Set the ramp rates to heating 1,6 ˚C/s and cooling 1,6 ˚C/s
C. Set the reaction volume to 25 µL
D. Place the tubes or the plate in the thermal cycler
E. Start the amplification (duration approximately 1 hr 55 min)
F. If proceeding with sequencing the same day, prepare sequencing reagents (see note in 7.3)
G. Following amplification, centrifuge briefly if necessary, to collect the content and continue to 7.3

PCR2 libraries can be stored in a freezer below -18°C for 30 days.

SUITABLE STOPPING POINT

NOTE	It is of high importance that the following ramp rates are applied: heating 1,6 ˚C/s, cooling 1,6 ˚C/s

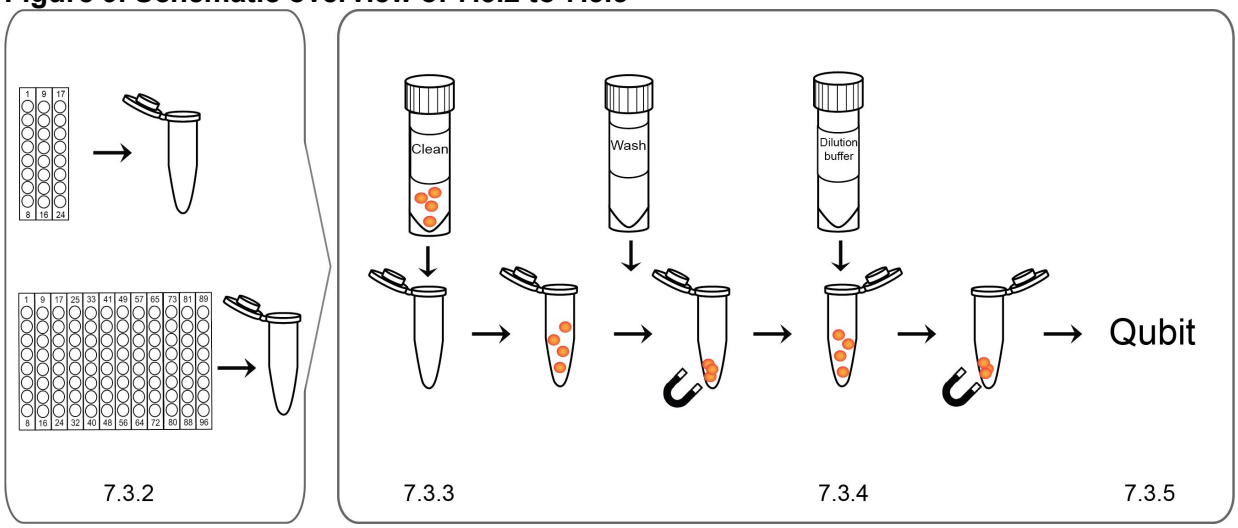
NOTE	To program the correct ramp rate for the Veriti Thermal Cycler: In the "Tools Menu" select "Convert a Method". In the next select "9700 Max Mode" and then enter the PCR profile as outlined in section 7.2.3.

NOTE	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

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

NOTE	Defrost the MiSeq reagent cartridge well in advance prior to sequencing according to the procedure described in the current "MiSeq System Guide"[7]

Figure 9. 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)**

A. Prepare the **Wash** solution by adding 1500 µL of 96 % ethanol to the **Wash** tube
B. Mix thoroughly by vortexing
C. Tick the box on the **Wash** tube label to indicate that ethanol was added
D. **Note! The Wash solution should be stored at +2 to +8 °C and used within 3 months from day of activation**

7.3.2 Pooling

A. To obtain a library pool volume of at least 80 µL for subsequent purification, pool 5 µL from each of the PCR2 libraries (from 7.2.3) into a single tube
B. Mix thoroughly by vortexing and then briefly centrifuge the library pool to collect the content
C. Transfer 80 µL 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 80 µL. If the total pooled volume is less than 80 µL (less than 4 samples), 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 µL of **Dilution buffer** for elution.

NOTE	The library pool should consist of libraries from samples processed together with the same Devyser library kit. If using different Devyser library kits, library pools should be prepared and purified separately.

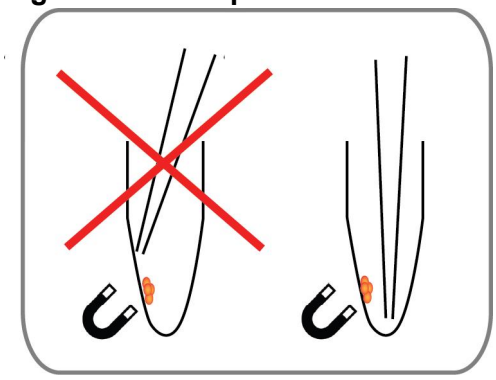
7.3.3 Library Purification

Required kit: **Devyser 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 µL 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 has become 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 10)**
G. Add 150 µL 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 10)**
K. Leave the lid open until all remaining Wash solution has evaporated and the bead pellet has changed from being luster to lustereless, approximately 5-9 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 10. 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** to collect the content
B. Add 40 µL **Dilution buffer** to the tube from 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 current 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 µL Qubit dsDNA HS working solution with 10 µL of Qubit standard 1
C. Mix 190 µL Qubit dsDNA HS working solution with 10 µL of Qubit standard 2
D. Mix 190 µL Qubit dsDNA HS working solution with 10 µL 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 (ng/µL) 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. Dilute the purified library pool from 7.3.4 to a final concentration of 0.25 - 0.30 ng/µL using the **Dilution buffer**
B. Measure the concentration (ng/µL) of the diluted library pool to confirm the concentration by repeating E to F in 7.3.5
C. Proceed to sequencing using the Illumina MiSeq according to chapter 8

NOTE	The concentration of the purified library pool (0.25 - 0.30 ng/µL) can be adjusted to ensure that the Illumina specifications for the reagent kit are met.

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

SUITABLE STOPPING POINT

8. SEQUENCING USING MiSeq

8.1 Number of samples per flow cell

Calculate the number of samples to be sequenced per flow cell by using the Devyser Sequence Coverage Calculator (see 2.5.1).

8.2 Sample sheet generation

Generate a sample sheet for each run in the Illumina Experiment Manager (IEM) software by using the Devyser Guide "Generating a Devyser Sample Sheet for MiSeq" (see 2.4 for details) and the Illumina document # 15031335: " Illumina Experiment Manager User Guide"[8].

8.3 Index description

The Illumina double index introduced during PCR2 are listed in tables 9 and 10. Detailed information about the index combinations and index sequences can be found online (see 2.4 for details).

Table 9. Illumina double index used in Index strip A2

	Index 1-8	**Index 9-16**	**Index 17-24**
	Index1: N701	Index1: N702	Index1: N703
Index2: N501	1	9	17
Index2: N502	2	10	18
Index2: N503	3	11	19
Index2: N504	4	12	20
Index2: N505	5	13	21
Index2: N506	6	14	22
Index2: N507	7	15	23
Index2: N508	8	16	24

Table 10. Illumina double index used in Index plate A3

	Index 1-8	Index 9-16	Index 17-24	Index 25-32	Index 33-40	Index 41-48	Index 49-56	Index 57-64	Index 65-72	Index 73-80	Index 81-88	Index 89-96
	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

8.4 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 Illumina document # 15039740: "MiSeq System Denature and Dilute Libraries Guide"[9]

B. Combine 5 µL 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 denaturated PhiX control DNA. The added PhiX will represent approximately 1 % of the total number of reads from the sequencing run

F. Repeateadly invert and then vortex the tube to mix and briefly centrifuge to collect the content

8.5 Illumina sequencing

A. Prepare the sequencing run according to the current version of the Illumina document # 15027617: "MiSeq System Guide"[7]

B. For loading the reagent cartridge, transfer 600 µL of the sequencing mix to the sample well in 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 correct location for analysis (see 2.3 and 9)

9. SEQUENCE DATA ANALYSIS

9.1 Supported software and sequencing mode

The Devyser FH v2 libraries can be sequenced in paired-end mode (2 x 151 cycles) (table 11).

Table 11. Software and sequencing mode

Software	Sequencing mode
SeqNext	Paired end 2x151 cycles*
Amplicon Suite	Paired end 2x151 cycles

*For some heterozygous genetic variants, the presented VAF values may be imprecise. See Devyser SeqNext guide for additional information (see 2.4).

9.2 Sequence data analysis using the SeqNext module of the SeqPilot software

Transfer the sequencing data files (FASTQ) to an appropriate location and start the analysis in SeqNext according to manufacturer's instructions for use. Consult the SeqNext guide for Devyser FH v2 (see 2.4) for details on downloading settings and performing analysis and data interpretation.

9.3 Sequence data analysis using Amplicon Suite

Upload the sequencing data files (FASTQ) and start the analysis in Amplicon Suite according to manufacturer's instructions for use.

10. SYMBOLS USED ON LABELS

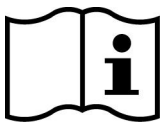
Lot or batch number

Expiry date

Number of tests

Store below temperature shown

Temperature limit



Consult instructions for use

Catalogue number

Manufacturer

Research Use Only

11. NOTICE TO PURCHASER

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

MiSeq is a registered trademark of Illumina Corporation.
Qubit, DynaMag and Veriti are trademarks of Thermo Fischer Scientific Corporation.

12. CONTACT INFORMATION

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13. REFERENCES

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[7] MiSeq System Guide (Document # 15027617)

[8] Illumina Experiment Manager User Guide (Document # 15031335)

[9]MiSeq System Denature and Dilute Libraries Guide (Document # 15039740)

14. ABBREVIATIONS

APOB apolipoprotein B

APOE apolipoprotein E

bp basepairs

CNV copy number variation

FH Familial Hypersholesterolemia

IEM Illumina Experiment Manager

Indel insertion and / or deletion

LDLR low-density lipoprotein receptor

LDLRAP1 low-density lipoprotein receptor adaptor protein 1

NGS next generation sequencing

PCR polymerase chain reaction

PCSK9 proprotein convertase subtilisin/kexin type 9

PE paired end

ROI region of interest

RUO research use only

SNV single nucleotide variation

STAP1 signal-transducing adaptor protein 1

VAF variant allele frequency

15. REVISION HISTORY

Version 2020-01-09

Editorial changes

1.4. Added CNV in LDLR

2.2.1 Other required Devyser products added (moved from 2.2.6)

2.2.4. Removed Qubit dsDNA HS Assay Kit

Table 8. Sge file description revised

2.5. New

Chapter 3. Storage requirements. Specific storage recommendations stated for below -18 °C: -28°C to -18°C.

Chapter 5. Procedural limitations. Point 7: sample type added. Two last points added

7.1.1, H. Added: Do not aliquot the activated mix.

7.3.6. Note added

Chapter 8. Illumina MiSeq Sequencing. Moved calculator text to 2.5.1. Info regarding sequencing modes moved from introduction to chapter 9. 2 x 151 bp changed to 2 x 151 cycles.

8.1. Number of samples per flow cell. New

9.1 Supported software and sequencing mode. New

Chapter 14. List updated (VAF added)

Version 2018-12-13

Editorial changes

Updated Article number and Product name

Table 1: LRG names added and information about covered exons clarified

Section 1.5 regarding Assay procedure was moved to chapter 2

Version 2018-07-06
New