DEVYSER
RESULTS FOR LIFE

Devyser CFTR

Art. No.: 8-A103

For Research Use Only

Handbook

TABLE OF CONTENTS

TABLE OF CONTENTS **2**

1. INTRODUCTION TO DEVYSER CFTR **4**
1.1 Intended use 4
1.2 Background 4
1.3 Assay principle 4
1.4 Assay design 5
2. MATERIALS AND EQUIPMENT **7**
2.1 Kit configurations for Devyser CFTR 7
2.2 Equipment and reagents required but not provided 8
2.2.1 Other required Devyser products 8
2.2.2 General 8
2.2.3 DNA extraction 8
2.2.4 Determination of DNA concentration 8
2.2.5 Reagent preparation and amplification 9
2.2.6 Library purification 9
2.2.7 Sequencing 9
2.3 Software 10
2.4 Downloads 10
2.5 Other resources 11
2.5.1 Devyser Sequence Coverage Calculator 11
3. STORAGE REQUIREMENTS **12**
4. WARNINGS AND PRECAUTIONS **13**
5. PROCEDURAL LIMITATIONS **14**
6. SAMPLE REQUIREMENTS **15**
6.1 Samples 15
6.1.1 DNA extraction from whole blood and dried blood spots 15
6.1.2 Determination of DNA concentration 15
6.1.3 Dilution of DNA from samples 15
6.2 PhiX control 15
6.3 Internal system control 15
7. INSTRUCTIONS FOR USE **16**
7.1 Library generation (PCR1) for Devyser CFTR 8, 24 and 96-test kits 17
7.1.1 CFTR mix preparation for 8 and 24-test 17
7.1.2 CFTR mix preparation 96-test 18
7.1.3 Preparation and addition of DNA 18
7.1.4 Thermal cycling PCR1 19
7.2 Library indexing (PCR2) 20
7.2.1 Library indexing - Devyser CFTR 8 test kit 20
7.2.1.1 PCR1 library dilution 20
7.2.1.2 Index preparation 21
7.2.1.3 Addition of diluted PCR1 libraries to Index strip A1 21
7.2.2 Library indexing - Devyser CFTR 24 test kit 21
7.2.2.1 PCR1 library dilution 22
7.2.2.2 Index preparation 22
7.2.2.3 Addition of diluted PCR1 libraries to Index strip A2 22
7.2.3 Library indexing - Devyser CFTR 96 test kit 23
7.2.3.1 PCR1 library dilution 23
7.2.3.2 Index preparation 23
7.2.3.3 Addition of diluted PCR1 libraries to Index plate A3 24

7.2.4 Thermal cycling PCR2 24
7.3 Pooling and purification of libraries using the Devyser Library Clean kit (8-A204) 25
7.3.1 Preparation of Wash solution 26
7.3.2 Pooling 26
7.3.3 Library Purification 27
7.3.4 Library elution 28
7.3.5 Library quantification 28
7.3.6 Library dilution 28
8. SEQUENCING 30
8.1 Number of samples per flow cell 30
8.2 Sample set generation 30
8.3 Index description 30
8.4 Sequencing using MiSeq 31
8.4.1 Denaturation of the purified library pool 31
8.4.2 Sequencing 31
8.5 Sequencing using MiniSeq 32
8.5.1 Denaturation of the purified library pool 32
8.5.2 Sequencing 32
8.6 Sequencing using iSeq100 32
8.6.1 Preparation of the purified library pool 32
8.6.2 Sequencing 33
9. SEQUENCE DATA ANALYSIS 34
9.1 Supported software, instruments and sequencing modes 34
9.2 Guidelines 34
9.3 Sequence data analysis using the SeqNext module of the SeqPilot software 34
9.4 Sequence data analysis using Sophia DDM® 34
9.5 Sequence data analysis using Amplicon Suite (SmartSeq s.r.l) 34
9.6 Interpretation of results 34
10. SYMBOLS USED ON LABELS 35
11. NOTICE TO PURCHASER 36
12. CONTACT INFORMATION 37
12.1 Legal manufacturer 37
12.2 Technical support 37
13. REFERENCES 38
14. ABBREVIATIONS 39
15. REVISION HISTORY 40

1. INTRODUCTION TO DEVYSER CFTR

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1.1 Intended use

The Devyser CFTR kit enables analysis of sequence variants in the CFTR gene in genomic DNA isolated from human tissue.

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

1.2 Background

Cystic Fibrosis is an inherited disorder affecting children and young adults. Since the discovery of the CFTR gene in 1989[1] more than 2000 mutations and variants in the CFTR gene have been described[2]. Mutations in the CFTR gene may cause respiratory disease. Other CFTR related disorders caused by CFTR dysfunction include congenital bilateral absence of vas deferens (CBAVD), acute recurrent or chronic pancreatitis and disseminated bronchiectasis[3, 4].

1.3 Assay principle

The method employed by the Devyser CFTR kit includes multiplex PCR amplification to create a target amplicon library from each DNA sample (PCR1). The library covers the full target region in an overlapping fashion.

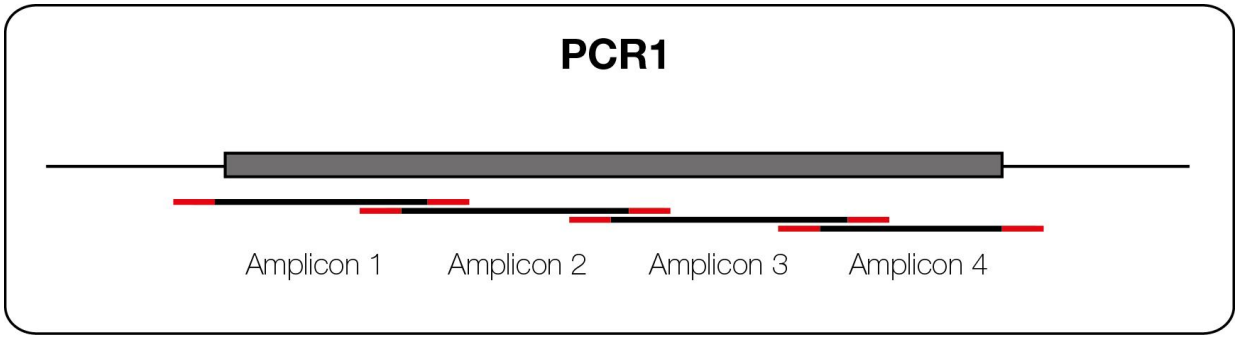

Figure 1. Schematic illustration of PCR1

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 using appropriate software for targeted sequencing.

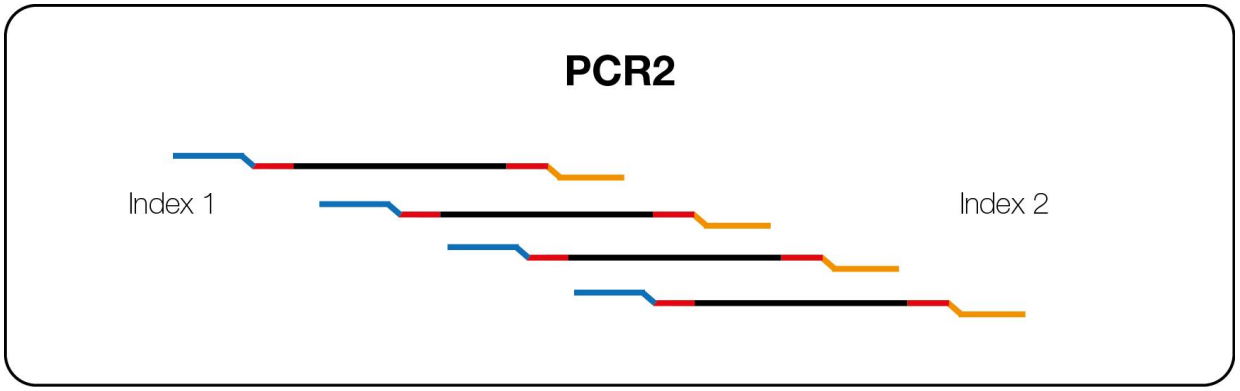

Figure 2. Introduction of adapters including unique index sequences

1.4 Assay design

Devyser CFTR allows detection of single nucleotide variations (SNVs), indels and quantitative detection of exon spanning copy number variations (CNVs), (excluding exon 20), all appearing in the coding regions and adjacent exon-intron boundaries in the CFTR gene (figure 3).

Specifically, the Devyser CFTR kit offers full sequence determination of all coding exons (LRG_ 663t1) and all adjacent exon/intron boundaries, including minimum 20 bp proximal to the 5' end and 10 bp on the 3' end of each exon boundary. In addition, the Devyser CFTR kit also includes targeted detection of the poly-T and TG-repeat region in intron 9 as well as deep intronic mutation detection in introns 7, 11, 12 and 22, as well as part of the promoter region (up to position c.-144). Target specific primers are designed such that primer footprints are covered by an overlapping amplicon to enable detection of primer site SNVs. In addition to quantitative CNV detection, direct detection of 8 common exon spanning CFTR CNVs is enabled through the inclusion of deletion specific primers for the following CFTR CNVs (legacy names):

- CFTRdele1
- CFTRdele2
- CFTRdele2ins182
- CFTRdele2,3
- CFTRdele14b-17b
- CFTRdele17a-18
- CFTRdele22,23
- CFTRdele22-24

CFTR

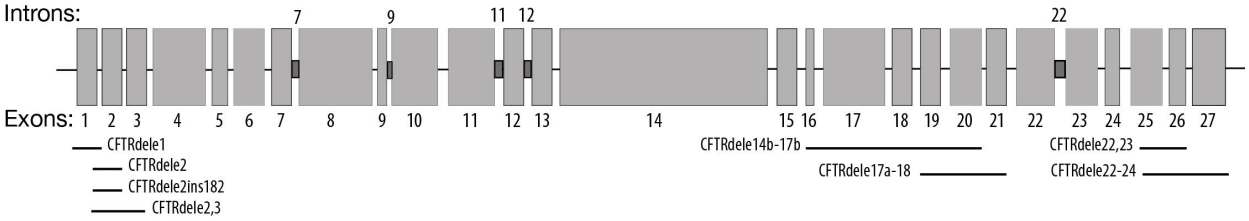

Figure 3. Schematic illustration of CFTR exons, structural deletions and deep intronic regions covered in Devyser CFTR

2. MATERIALS AND EQUIPMENT

2.1 Kit configurations for Devyser CFTR

The Devyser CFTR kit is available in three configurations according to the following tables:

Table 1. Devyser CFTR 8 test configuration (8-A103-8)

Component	Art.No.	Number/kit	Cap color	Storage condition
CFTR mix	4-A246	1	Blue	Below –18°C
Start	4-A243	1	Purple	Below –18°C
Dilution buffer	4-A245	1	White	–25°C to + 8°C
Index mix	4-A247	1	Red	Below –18°C
Index strip A1	-	1	-	Below –18°C
Index buffer	4-A258	1	Green	–25°C to + 8°C
Sealer S	-	1	-	Ambient

Table 2. Devyser CFTR 24 test configuration (8-A103-24)

Component	Art.No.	Number/kit	Cap color	Storage condition
CFTR mix	4-A246	3	Blue	Below –18°C
Start	4-A243	3	Purple	Below –18°C
Dilution buffer	4-A245	3	White	–25°C to + 8°C
Index mix	4-A247	3	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 3. Devyser CFTR 96 test configuration (8-A103-96)

Component	Art.No.	Number/kit	Cap color	Storage condition
CFTR mix, 48 test	4-A286	2	Blue	Below –18°C
Start, 48 test	4-A274	2	Purple	Below –18°C
Dilution buffer, 96 test	4-A275	1	White	–25°C to + 8°C
Index mix, 48 test	4-A276	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	-	2	-	Ambient

2.2 Equipment and reagents required but not provided

2.2.1 Other required Devyser products

- Devyser Library Clean (8-A204)

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 QIAsymphony DSP DNA Midi Kit (Qiagen, cat.#937255) for extraction of DNA from human whole blood
- QIAamp DNA Mini Kit (Qiagen, cat.# 51304/51306) for extraction of DNA from dried blood spots
- If alternative DNA extraction methods and sample materials are used, a thorough evaluation of the performance together with the Devyser CFTR kit should be performed

2.2.4 Determination of DNA concentration

- Determination of DNA concentration according to manufacturer's instructions for use
- Qubit® 2 Fluorometer (cat.# Q32866) or Qubit™ 4 Fluorometer (cat.# Q33226) 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 CFTR 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™, MiniSeq™ or iSeq™ 100
- User-supplied consumables needed for sequencing, according to the Illumina sequencing guide
- Illumina reagent kits according to the following tables:

Table 5. MiSeq reagent kits

Illumina reagent kit	Illumina cat #
Miseq Reagent Nano Kit v2 (300-cycles)	MS-103-1001
Miseq Reagent Nano Kit v2 (500-cycles)	MS-103-1003
Miseq Reagent Micro Kit v2 (300-cycles)	MS-103-1002
Miseq Reagent Kit v2 (300-cycles)	MS-102-2002
Miseq Reagent Kit v2 (500-cycles)	MS-102-2002
Illumina PhiX control v3	FC-110-3001

Table 6. MiniSeq reagent kits

Illumina reagent kit	Illumina cat #
MiniSeq MidOutput Kit (300 cycles)	FC-420-1004
MiniSeq High Output Reagent Kit (300 cycles)	FC-420-1003
NextSeq PhiX Control Kit	FC-110-3002

Table 7. iSeq 100 reagent kits

Illumina reagent kit	Illumina cat #
iSeq 100 i1 Reagent	20021533
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
- Sophia DDM® (Sophia Genetics) pipeline for Devyser CFTR. Contact Devyser or Sophia Genetics to create a Sophia DDM® account
- Amplicon Suite (SmartSeq s.r.l) pipeline for Devyser CFTR. Contact Devyser support at techsupport@devyser.com

2.4 Downloads

Supplementary information and files can be downloaded from the Devyser website: http://devyser.com/ifu using the download code printed on the kit label.

Table 8. Downloads

Download	Description
Illumina double index	Index sequence information
MiSeq IEM files	Devyser setting files for MiSeq sample sheet generation using IEM: • Generating a Devyser sample sheet for MiSeq®.doc • DEVYSER double Index MiSeq®.txt • DevyserGenerateFASTQ.txt • DevyserGenerateFASTQ.jpg
LRM files	Devyser setting files for MiSeq, MiniSeq or iSeq 100 sample sheet generation using LRM: • Generating a Devyser sample sheet with Illumina LRM.doc • Devyser .tsv files
SeqNext guide for Devyser CFTR	SeqNext guide for Devyser CFTR analysis
Devyser CFTR SeqNext sge files	Devyser settings files (.sge files) for CFTR data analysis with SeqNext
Devyser CFTR BED files	BED file detailing amplicon positions according to Hg19/GRCh37 and Hg38/GRCh38

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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 Sequencing Coverage Calculator at www.devyser.com/calculator.

As displayed in the calculator, the expected total number of read pairs for each sample and minimal coverage per amplicon is according to the following table:

Table 9. Minimal recommended number of read pairs per sample and amplicon coverage

Detection mode	Read pairs per sample*	Minimal coverage per amplicon**
Germline	40 000	100
Germline and CNV	80 000	200

*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 CFTR kit in a freezer below –18°C (–28°C to –18°C) or the individual kit components as specified on the label (see 2.1)
- Store the components of the Devyser Library Clean kit at +2°C 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 (see section 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 to use 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

5. PROCEDURAL LIMITATIONS

- This product is intended for research use only. Not for use in diagnostic procedures.
- Sequence variants present in other genes than the CFTR gene will not be detected using Devyser CFTR
- Results obtained with the Devyser CFTR 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 CFTR kit and may result in reduced or no amplification of the affected amplicon
- CFTR exon 20 is not included in the CNV analysis due to varying amplicon coverage
- Accurate detection of homopolymers ≥10 bp is not reliable
- The following parameters might affect the overall performance and in particular the CNV analysis
    - Quality, sample type and concentration of the sample 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
- It is recommended that only samples that have been processed and sequenced together and with the same kit lot should be analyzed together for CNV analysis
- It is recommended that only samples of the same sample type should be analyzed together for CNV analysis
- Running several family members or samples with the same deletions in the same run can affect CNV analysis and should be avoided
- The bioinformatic characterization of CNVs is based on different computational strategies. None of the strategies can guarantee correct detection of all CNVs

6. SAMPLE REQUIREMENTS

DNA concentration, integrity and purity are important parameters for successful testing using the Devyser CFTR 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 CFTR kit can be used with human genomic DNA extracted from whole blood and dried blood spots.

6.1.1 DNA extraction from whole blood and dried blood spots

- DNA extraction reagents according to manufacturer's instructions for use (see 2.2.3)
- When using whole blood as starting material: follow the protocol of the extraction kit starting with 200 µL fresh whole blood and elute in 200 µL elution buffer
- When using dried blood spots as starting material: follow the dried blood spot protocol starting with 3 pieces of 3 mm diameter spots and elute in 25 - 50 µL elution buffer

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 a 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 from the Devyser CFTR kit

6.1.3 Dilution of DNA from samples

- For optimal performance it is recommended to adjust the concentration of the extracted DNA to 2 ng/µL (see 7.1.3)

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 ensure that the sequencing pool has the required diversity for high quality sequencing (see 8.3).

6.3 Internal system control

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

7. INSTRUCTIONS FOR USE

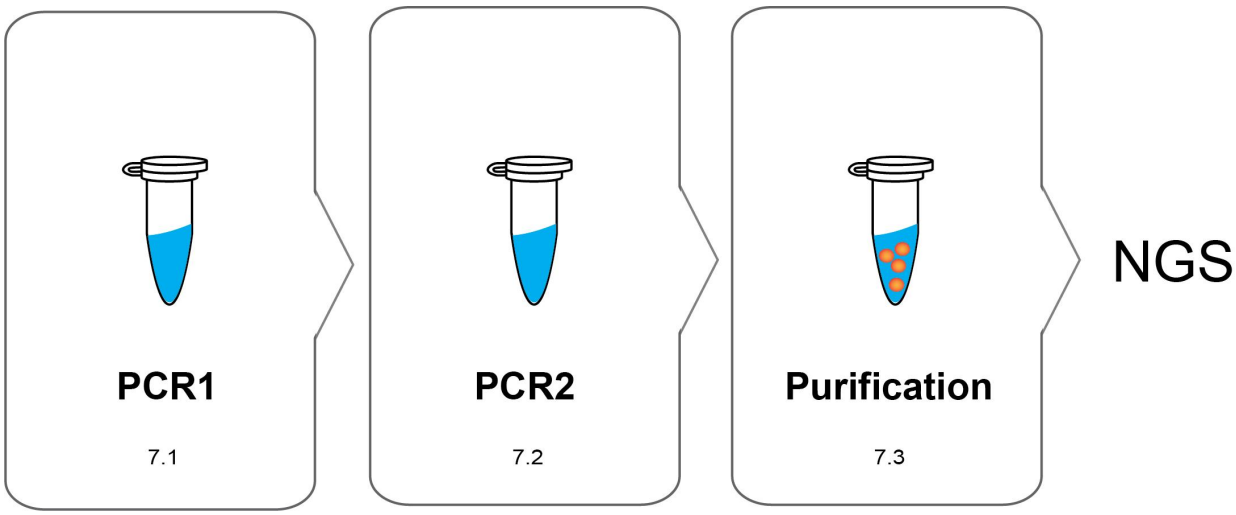


Figure 4. Schematic overview of the Devyser CFTR library preparation procedure

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

PCR1 (7.1)
A CFTR 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.

NOTE	This kit is available in three test configurations; 8, 24, and 96. Each **Start** and **CFTR mix** tube in the 8 and 24 test configuration is sufficient for 8 reactions, and each **Start** and **CFTR mix** tube in the 96 test configuration is sufficient for 48 reactions . Determine the required number of each component tube before starting

7.1 Library generation (PCR1) for Devyser CFTR 8, 24 and 96-test kits

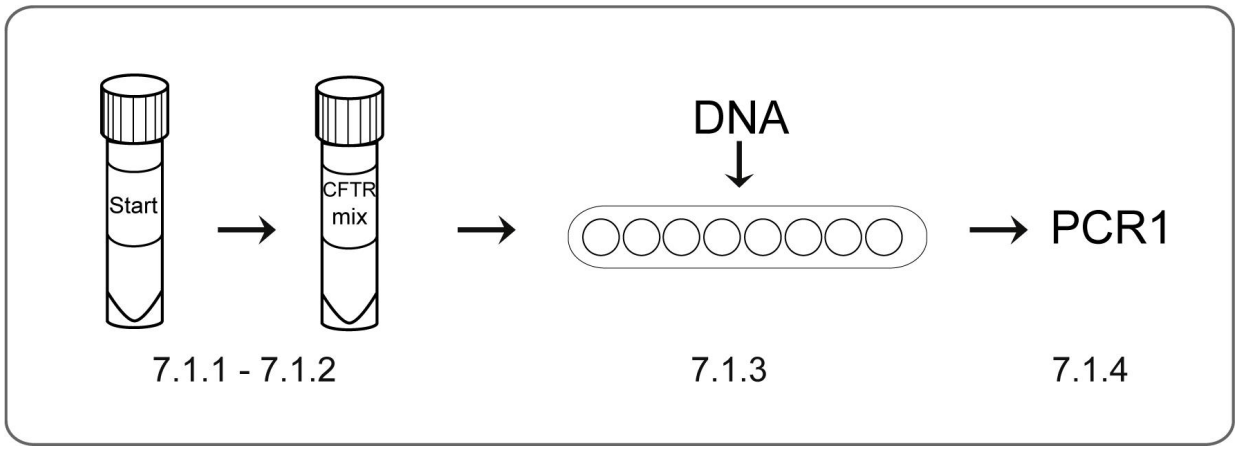


Figure 5. Schematic overview of steps 7.1.1 to 7.1.4

7.1.1 CFTR mix preparation for 8 and 24-test

Required kit components: **Start (4-A243), CFTR mix (4-A246)**

A. Calculate the number of **Start** and **CFTR mix** tubes required. Each tube is sufficient for 8 reactions
B. Ensure that the **Start** and the **CFTR mix** are completely thawed before use
C. Vortex the **Start** tube(s) briefly
D. Briefly centrifuge the **Start** and **CFTR mix** tube(s) to collect the content
E. Add 50 µL of **Start** to the **CFTR mix** tube(s) to obtain an activated **CFTR mix**
F. Vortex the activated **CFTR mix** tube(s) and then centrifuge it briefly to collect the content
G. Dispense 10 µL of the activated **CFTR mix** into separate PCR reaction tubes and cap the tubes
H. Store the dispensed **CFTR mix** at +2°C to +8°C and continue to 7.1.3
I. Any remaining activated **CFTR mix** can be stored in the original tube in a freezer below –18°C for 90 days. Do not aliquot the activated mix.

7.1.2 CFTR mix preparation 96-test

Required kit components: **Start, 48 test (4-A274), CFTR mix, 48 test (4-A286)**

A. Calculate the number of **Start, 48 test** and **CFTR mix, 48 test** tubes required. Each tube is sufficient for 48 reactions
B. Ensure that the **Start, 48 test** and the **CFTR mix, 48 test** are completely thawed before use
C. Vortex the **Start, 48 test** tube(s) briefly
D. Briefly centrifuge the **Start, 48 test** and **CFTR mix, 48 test** tube(s) to collect the content
E. Add 270 µL of Start to the **CFTR mix, 48 test** tube to obtain an activated **CFTR mix, 48 test**
F. Vortex the activated **CFTR mix, 48 test** tube(s) and then centrifuge it briefly to collect the content
G. Dispense 10 µL of the activated **CFTR mix, 48 test** into separate PCR reaction tubes or separate wells in a plate and cap the tubes or seal the plate
H. Store the dispensed **CFTR mix, 48 test** at +2°C to +8°C and continue to 7.1.3
I. Any remaining activated **CFTR mix**, 48 test can be stored in a freezer below –18°C for 90 days. Do not aliquot the activated mix.

7.1.3 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 **CFTR mix** (from 7.1.1 or 7.1.2)
E. Mix by pipetting
F. Cap the tubes or seal the plate and centrifuge briefly to collect the content

7.1.4 Thermal cycling PCR1

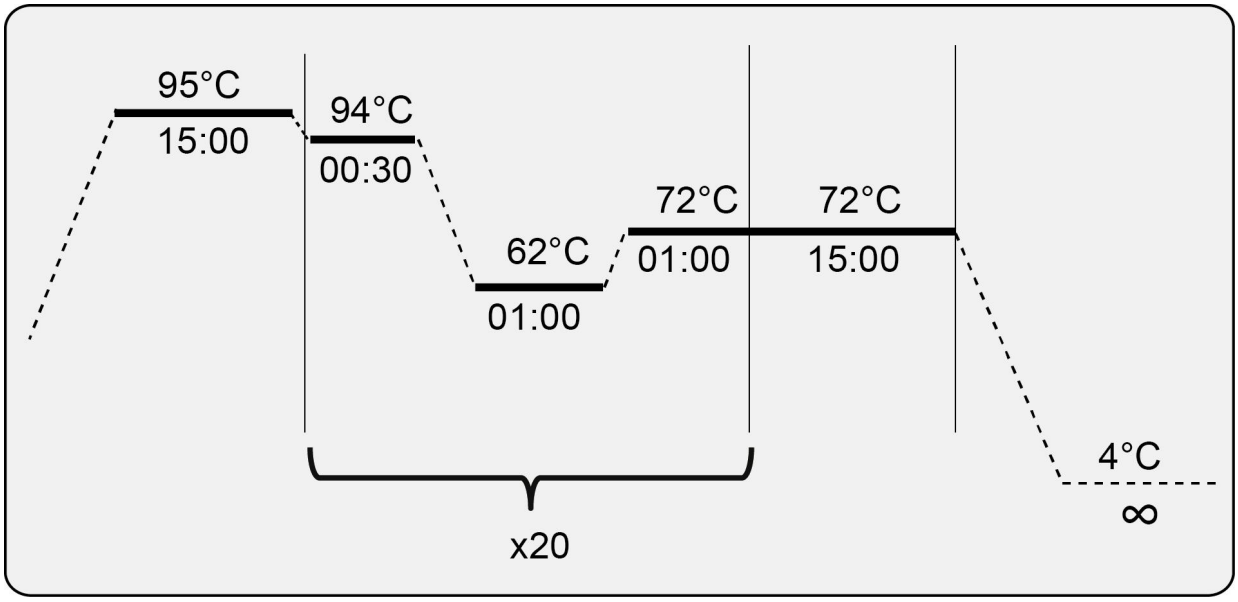


Figure 6. Thermal profile PCR1

A. Program the thermal cycler according to the PCR1 thermal profile shown above
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 to collect the content

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

SUITABLE STOPPING POINT

NOTE	It is of great 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 view, select "9700 Max Mode" and then enter the PCR profile as outlined 7.1.4

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 CFTR 8 test kit proceed to 7.2.1
For the Devyser CFTR 24 test kit proceed to 7.2.2
For the Devyser CFTR 96 test kit proceed to 7.2.3

7.2.1 Library indexing - Devyser CFTR 8 test kit

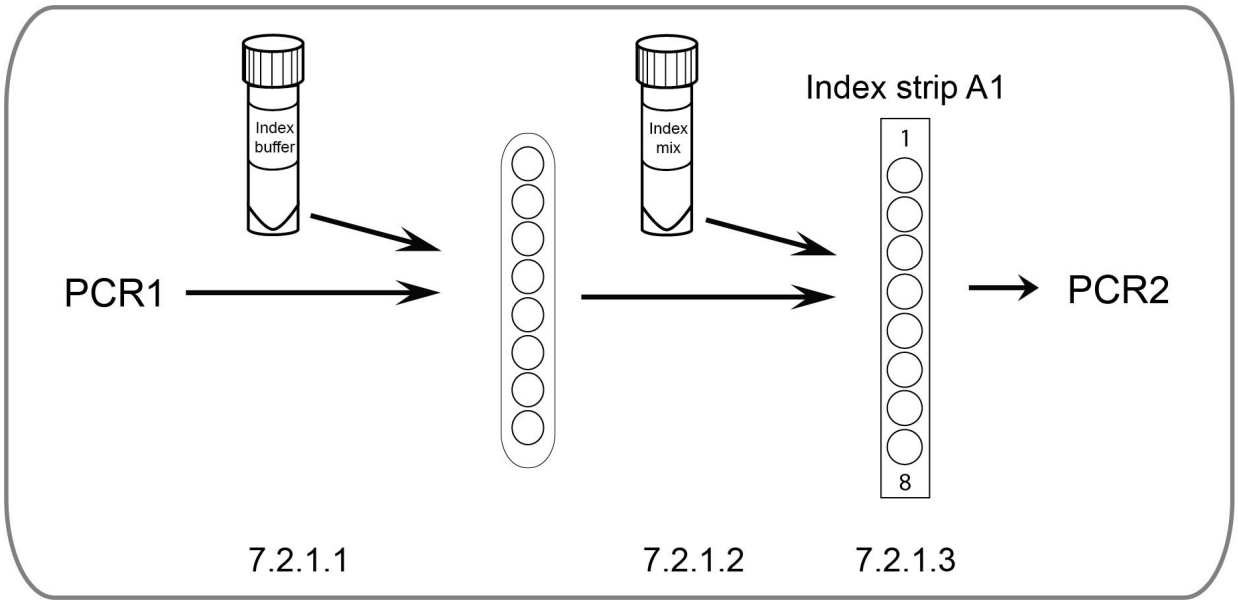


Figure 7. Schematic overview of steps 7.2.1.1 to 7.2.1.3

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 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 (4-A247), Index strip A1**

A. Ensure that the **Index mix** is completely thawed before use
B. Vortex and then briefly centrifuge the **Index mix** tube to collect the content
C. Carefully remove the transport seal of **Index strip A1**. **Note! Do not reuse the transport seal**
D. Add 20 µL of **Index mix** to each of the 8 wells of **Index strip A1**. **Note! Tips must be changed between each individual well**

7.2.1.3 Addition of diluted PCR1 libraries to Index strip A1

Required kit component: **Sealer S**

A. Add 5 µL of each diluted PCR1 library (from 7.2.1.1) to separate tubes in **Index strip A1** (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 A1**
D. Carefully seal **Index strip A1** and make sure that all wells are covered
E. Centrifuge briefly to collect the content
F. Continue to 7.2.4

7.2.2 Library indexing - Devyser CFTR 24 test kit

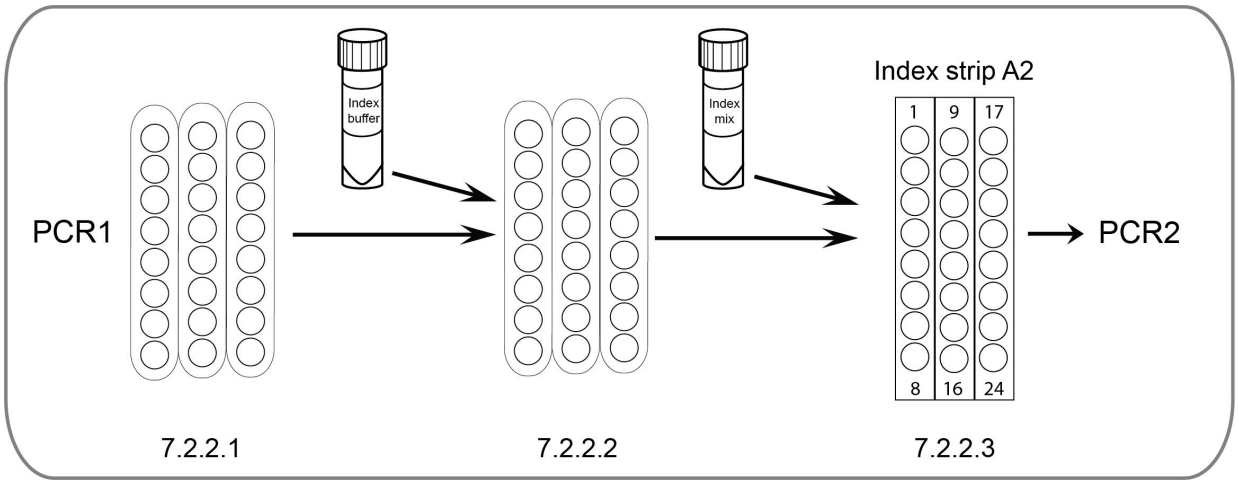


Figure 8. Schematic overview of steps 7.2.2.1 to 7.2.2.3

7.2.2.1 PCR1 library dilution

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

A. Ensure that the **Index buffer** is completely thawed before use
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)

7.2.2.2 Index preparation

Required kit components: **Index mix (4-A247), Index strip A2**

A. Ensure that the **Index mix** is completely thawed before use
B. Vortex and then briefly centrifuge the **Index mix** 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** to each of the wells to be used in **Index strip A2**. **Note! Tips must be changed between each individual well**

7.2.2.3 Addition of diluted PCR1 libraries to Index strip A2

Required kit component: **Sealer S**

A. Add 5 µL of each diluted PCR1 library pool (from 7.2.2.1) to separate wells in **Index strip A2** (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 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 7.2.4

7.2.3 Library indexing - Devyser CFTR 96 test kit

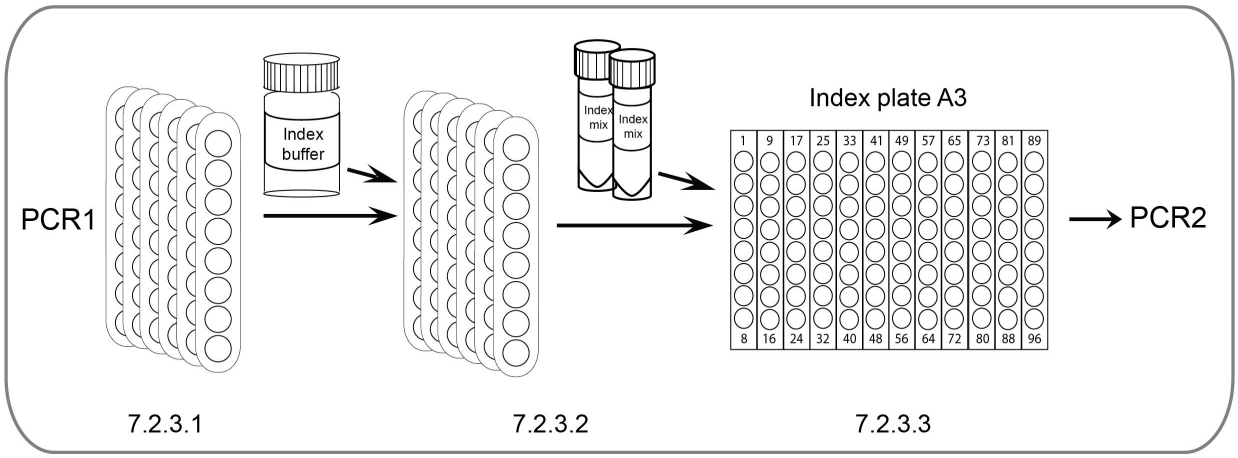


Figure 9. Schematic overview of s 7.2.3.1 to 7.2.3.3

7.2.3.1 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**
D. Mix the diluted PCR1 libraries thoroughly by pipetting (using a pipetting volume of at least 100 µL)

7.2.3.2 Index preparation

Required kit components: **Index mix, 48 test (4-A276), Index plate A3**

A. Calculate the number of **Index mix, 48 test** tubes required. Each tube is sufficient for 48 reactions. Determine the number of indices required and cut the desired wells from the **Index plate A3**. Store the unused wells below –18°C
B. Ensure that the **Index mix, 48 test** is completely thawed before use
C. Vortex and then briefly centrifuge the **Index mix** tube to collect the content
D. Carefully remove the transport seal of **Index plate A3**. **Note! Do not reuse the transport seal**
E. Add 20 µL of **Index mix, 48 test** to each of the wells to be used in **Index plate A3**. **Note! Tips must be changed between each individual well**

7.2.3.3 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 7.2.3.1) to separate tubes in **Index plate A3** (prepared in 7.2.3.2)
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. Use **Sealer L** to completely cover **Index plate A3**, or cut a piece of **Sealer L** to cover wells in use
D. Carefully seal **Index plate A3** and make sure that wells in use are covered
E. Centrifuge briefly to collect the content

7.2.4 Thermal cycling PCR2

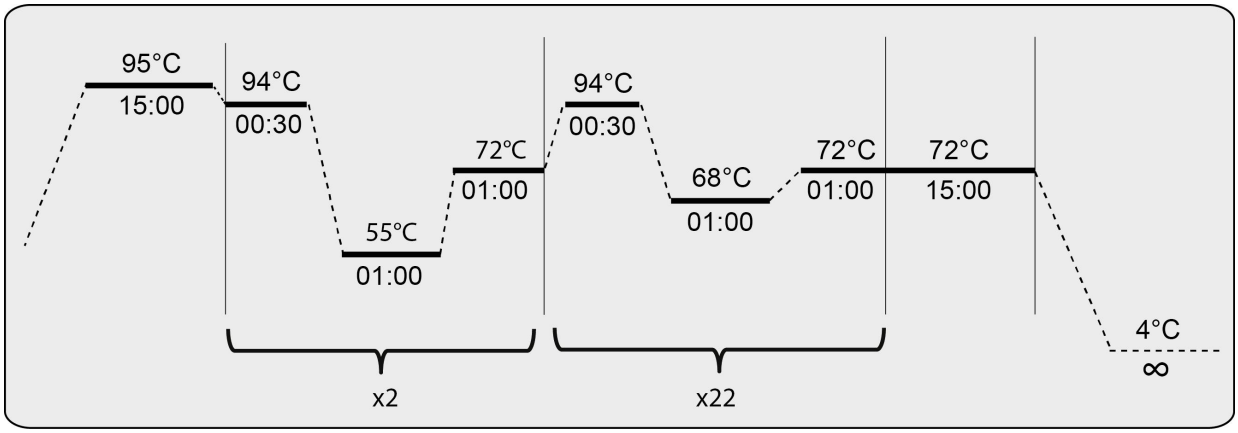


Figure 10. Thermal profile PCR 2

A. Program the thermal cycler according to the PCR2 thermal profile shown above
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 to collect the content

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 view select "9700 Max Mode" and then enter the PCR profile as outlined in 7.2.4

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, MiniSeq or iSeq 100 reagent cartridge well in advance prior to sequencing according to the procedure described in the current "MiSeq, MiniSeq or iSeq 100 System Guide"[7, 16, 15]

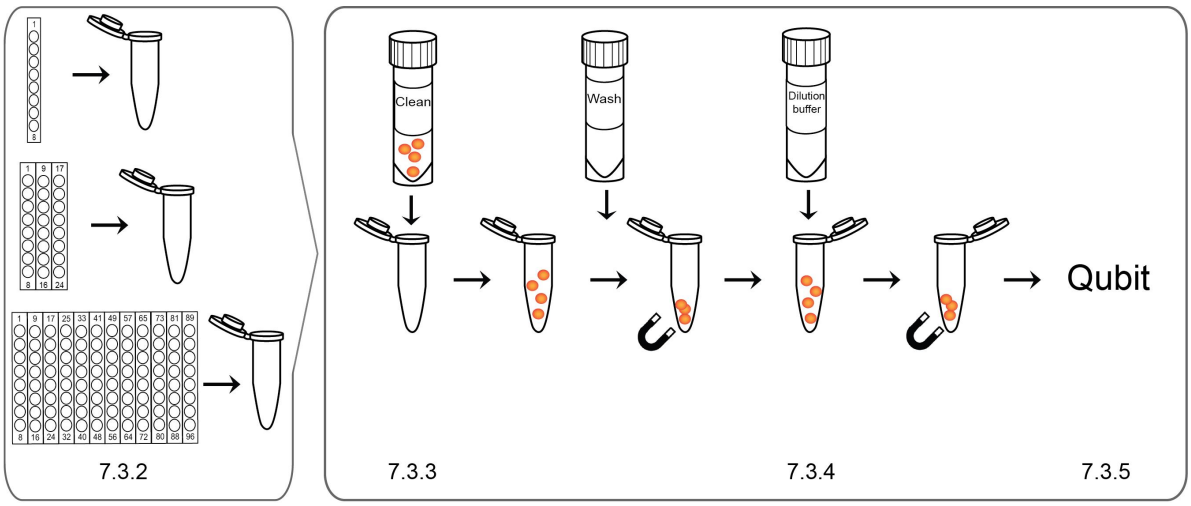


Figure 11. Schematic overview of 7.3.2 to 7.3.5

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°C 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 40 µL for subsequent purification, pool 5 µL from each of the PCR2 libraries (from 7.2.4) into a single tube
B. Mix thoroughly by vortexing and then briefly centrifuge the library pool to collect the content
C. Transfer 40 µL of the library pool into a new tube suitable for placing on a magnetic rack

NOTE	If less than 8 libraries are pooled, add equal volumes of each PCR2 library to obtain a library pool volume of 40 µL. If the total pooled volume is less than 40 µL, use equal volumes of the pooled PCR2 libraries and **Clean** (see 7.3.3). For elution, use equal volume of **Dilution buffer** as the library pool volume (see 7.3.4). However, do not use less than 25 µL of **Dilution buffer** for elution

NOTE	If highly divergent samples are processed together (e.g. dried blood spots and blood samples or samples processed with different Devyser library kits), only samples of the same category should be pooled prior to library purification

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 40 µL re-suspended **Clean** to the library pool from 7.3.2 and mix by pipetting. See note in 7.3.2 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 12 below**
G. Add 150 µL of prepared **Wash** solution (from 7.3.1)7.3.1to 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 12 below**
K. Leave the lid open until all remaining Wash solution has evaporated and the bead pellet has change 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

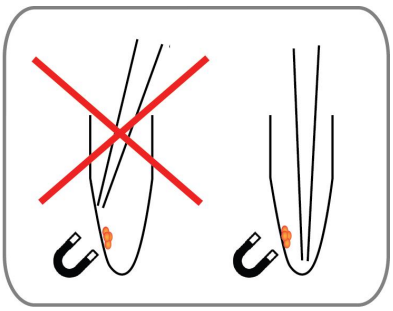


Figure 12. Bead pellet

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 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 µL Qubit 1X dsDNA HS working solution with 10 µL of Qubit™ standard 1
C. Mix 190 µL Qubit 1X dsDNA HS working solution with 10 µL of Qubit™ standard 2
D. Mix 190 µL Qubit 1X 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. Use the **Dilution buffer** to dilute the purified library pool from 7.3.4 to the following concentrations:
   - Illumina MiSeq v2 kits: 0,33 - 0,41 ng/µL
   - Illumina MiniSeq: 0,22 ng/µL
   -Illumina iSeq 100: 0,22 ng/µL
B. Measure the concentration (ng/µL) of the diluted library pool to confirm the concentration by repeating the final two steps in 7.3.5
C. Proceed to sequencing according to section 8

NOTE	The concentration of the purified library pool can be adjusted to ensure that the Illumina specifications for the reagent kit are met.

NOTE	The purified and diluted library pool is not recommended to be stored for long term or to be re-used. For potential re-runs it is instead recommended to repeat the pooling and purification step of this protocol from point 7.3.2, to ensure that the library holds the final concentration range defined in 7.3.6.

SUITABLE STOPPING POINT

8. SEQUENCING

Devyser CFTR can be used with the Illumina MiSeq, MiniSeq and iSeq 100 NGS systems.

NOTE	Defrost the MiSeq, MiniSeq or iSeq 100 cartridge well in advance prior to sequencing according to the procedure described in the current MiSeq, MiniSeq or iSeq 100 System Guide [7, 16, 15].

8.1 Number of samples per flow cell

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

8.2 Sample set generation

Generate a sample sheet for each run in the Illumina Experiment Manager (IEM) or the Local Run Manager (LRM) software by using the MiSeq IEM files or the LRM files (see 2.4) and the current version of the "Illumina Experiment Manager Software Guide"[5] and the "Local Run Manager Software Guide"[8].

8.3 Index description

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

Table 10. Illumina double index used in Index strip A1

	Index 1-8
	Index1: N701
Index2: N501	1
Index2: N502	2
Index2: N503	3
Index2: N504	4
Index2: N505	5
Index2: N506	6
Index2: N507	7
Index2: N508	8

Table 11. 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 12. 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 Sequencing using MiSeq

8.4.1 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 "MiSeq System Denature and Dilute Libraries Guide"[6]

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

8.4.2 Sequencing

A. Prepare the sequencing run according to the current version of the "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

8.5 Sequencing using MiniSeq

8.5.1 Denaturation of the purified library pool

A. Prepare RSB Buffer, Hybridization Buffer, a fresh dilution of 0.1 N NaOK and 1.8 pM denaturated PhiX according to the current version of the "MiniSeq System Denature and Dilute Libraries Guide System"[9]

B. Combine 3,3 µL Dilution buffer with 1,7 µL and vortex

C. Add 5 µL 0.1 N NaOH

D. Briefly vortex, centrifuge and incubate for 5 minutes at room temperature

E. Add 5 µL 200 mMTris-HCl, pH 7.0

F. Briefly vortex and centrifuge

G. Add 985 µL Hybridization Buffer

H. Briefly vortex and centrifuge

I. Combine 180 µL from step H with 320 µL Hybridization buffer

J. Briefly vortex and centrifuge

K. Add 3.3 µL of 1.8 pM denaturated PhiX to the total volume from previous step. The added PhiX will represent approximately 1% of the total number of reads from the sequencing run

L. Repeatedly invert and then vortex the tube to mix and briefly centrifuge to collect the content

8.5.2 Sequencing

A. Prepare the sequencing run according to the current version of the "MiniSeq System Guide"[16]

B. For loading the reagent cartridge, transfer 500 µL 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 correct location for analysis

8.6 Sequencing using iSeq100

8.6.1 Preparation of the purified library pool

A. Prepare RSB Buffer and 50 pM PhiX according to the current version of the "iSeq 100 Sequencing System Guide"[15]

B. Combine 5 µL purified library from 7.3.6 with 95 µL RSB.

C. Briefly vortex and centrifuge

D. To obtain a sequencing mix, add 1 µL 50 pM denaturated PhiX. The added PhiX will represent approximately 1% of the total number of reads from the sequencing run

E. Briefly vortex the tube to mix and centrifuge to collect the content

8.6.2 Sequencing

A. Prepare the sequencing run according to the current version of the "iSeq 100 Sequencing System Guide"[15]

B. For loading the reagent cartridge, transfer 20 µL of the sequencing mix 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 correct location for analysis

9. SEQUENCE DATA ANALYSIS

9.1 Supported software, instruments and sequencing modes

Ensure that the software pipeline to be used is compatible with the instrument and sequencing mode according to the following table.

Table 13. Software and sequencing mode

Software	Instrument	Sequencing mode
SeqNext	MiSeq	SR 1x301 cycles
Sophia DDM	MiSeq	SR 1x301 cycles
Amplicon Suite	MiSeq	SR 1x301 cycles and PE 2x151 cycles
	MiniSeq	PE 2x151 cycles
	iSeq	PE 2x151 cycles

9.2 Guidelines

See references[11, 12, 13].

9.3 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 CFTR (see 2.4) for details on downloading settings and performing analysis and data interpretation.

9.4 Sequence data analysis using Sophia DDM®

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

9.5 Sequence data analysis using Amplicon Suite (SmartSeq s.r.l)

Upload the sequencing data files (FASTQ) and start the analysis in Amplicon Suite according to manufacturer's handbook and quick installation and reference guide for Devyser CFTR.

The screening list version used in Amplicon Suite is v.CFTR2 Aug. 2018.

9.6 Interpretation of results

An expert should perform technical evaluation of the results and interpretation of detected variants.

10. SYMBOLS USED ON LABELS

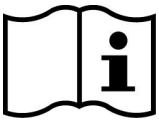
Lot or batch number

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, MiniSeq and iSeq are registered trademarks of Illumina Corporation.

Sophia DDM is a registered trademark of Sophia Genetics.

Qubit, DynaMag and Veriti are trademarks of Thermo Fischer Scientific Corporation.

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

12. CONTACT INFORMATION

12.1 Legal manufacturer

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Instrumentvägen 19
SE-126 53 Hägersten
SWEDEN

Phone: +46-8-562 15 850
Homepage: www.devyser.com

12.2 Technical support

Phone: +46-8-562 15 850
E-mail: techsupport@devyser.com

13. REFERENCES

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4. Southern KW. "Cystic fibrosis and formes frustes of CFTR-related disease". Respiration. 2007;74(3):241-51.
5. Illumina Experiment Manager Software Guide (Document # 15031335)
6. MiSeq System Denature and Dilute Libraries Guide (Document # 15039740)
7. MiSeq® System Guide (Document # 15027617)
8. Local Run Manager Software Guide (Document # 1000000002702)
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15. iSeq 100 Sequencing System Guide (Document # 1000000036024)
16. MiniSeq System Guide (Document # 1000000002695)

14. ABBREVIATIONS

Abbreviation	Explanation
BED	browser extensible data
bp	basepairs
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CBAVD	congenital bilateral absence of vas deferens
CNV	copy number variation
DDM	data driven medicine
DNA	deoxyribonucleic acid
hg19/GRCh37	human genome, version 19
hg38/GRCh38	human genome, version 38
IEM	Illumina Experiment Manager
Indel	insertion and / or deletion
LRM	local run manager
NGS	next generation sequencing
PCR	polymerase chain reaction
PE	paired end
ROI	region of interest
RUO	research use only
SNV	single nucleotide variation
SR	single read
VAF	variant allele frequency

15. REVISION HISTORY

Version 2020-07-01
General: Editorial changes

Chapter 1:
1.4 Assay design information updated

Chapter 2:
2.2.7 Sequencing: added MiniSeq and iSeq information
2.3 Software: added Amplicon Suite
2.4 Downloads: added MiniSeq and iSeq

Chapter 5:
Added bullet regarding same sample type should be analyzed together for CNV analysis

Chapter 7.
7.3 Updated note
7.3.6 Added concentrations for MiniSeq and iSeq 100

Chapter 8:
8.1 updated
Added: Sequencing using MiSeq
Added: Sequencing using MiniSeq
Added: Sequencing using iSeq 100
8.2 updated
8.5 added
8.6 added

Chapter 9.
9.1 updated supported software, instrument and sequencing mode
9.5 updated version of the screening list

Version 2020-01-23
General: Editorial changes

Section 1.5 removed. Information is already available in other sections in the IFU.

2.2.1 Other required Devyser products, 2.2.2 General and 2.2.4 Determination of DNA concentration added

2.2.3. Added QIAamp DNA Mini Kit (Qiagen, cat.# 51304 /51306) for dried blood spots

2.3 Amplicon Suite added

2.4. Table 6. Downloads. Removed "MiSeq sample sheet guide". File included in "MiSeq IEM files". Sge file description revised

2.5.1. Revised. Minimum total number of read pairs per sample and amplicon coverage for germline and CNV added.

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

Chapter 5. Procedural limitations. Added: CFTR exon 20 is not included in the CNV analysis (also in 1.4). in point 5: sample type added. Last point added.

6.1.2 and 7.3.5 Changed from Qubit dsDNA HS Assay Kit to Qubit 1X dsDNA HS Assay Kit

7.1.1 and 7.1.2, step I: Added "Do not aliquot the activated mix"

7.3.2. Last note updated with highly divergent samples (e.g. dried blood spots and blood samples)

7.3.6. Removed "(corresponding to approximately 2 nM)" and changed the concentration from 0.38-0.45 µL to 0.33 - 0.41 ng/µL. Note added.

Chapter 8. Illumina MiSeq Sequencing. Removed calculator text (already included in 2.5.1). Info regarding sequencing modes revised (paired end mode removed) and moved to chapter 9.

8.1. Number of samples per flow cell. New

9.1 Supported software and sequencing mode added

Amplicon coverage (9.5) moved to 2.5.1. 9.5 Sequence data analysis using Amplicon Suite added

Chapter 15 Abbreviations. List updated (VAF added)

Version 2018-12-11

General:
Editorial changes

96 test kit configuration (8-A103-96) added in chapters/sections:
- 2.1, 7.1 and 7.2

Specific storage recommendations added in sections:
- 7.1.1, 7.1.2, 7.1.4, 7.2.4, 7.3.1 and 7.3.6

Other changes

Chapter 2
2.2.1 (previously 2.3.1):
- Reference to extraction reagents and information regarding alternative methods added
- RNase A (Qiagen cat.# : 19101) removed

2.2.2 (previously 2.3.2):
- 96-Well Tray/Retainer Set added
- Information about alternative thermal cyclers added

2.2.3 (previously 2.3.3):

- Devyser Library Clean added (moved from previous section 2.2)
- Corrected cat.# for Qubit fluorometer from Q32851 to Q33216
- Added another kit format for Qubit dsDNA HS Assay: Q32854

2.2.4 Sequencing (previously 2.3.4 Detection):
- individually specified the different Illumina sequencing kits
- Added information about other user-supplied consumables

2.4, table 5 (previously table 4)
- Guide for SeqNext and sample sheet generation added
- Pooling strategies removed (replaced with calculator)

2.5. New. Devyser Sequence Coverage Calculator.

Chapter 5
- CNV information revised

Chapter 7
- Change of lab workflow: Index preparation and Wash preparation moved to the first section
- 7.1.4 and 7.2.4: Use of 96-Well Tray/Retainer Set added
- 7.3.3 Library purification (previously Clean up using the Devyser Library Clean kit): general clarifications and addition of information.
- 7.3.5 Library quantification (previously Preparation of the purified library pool): Qubit kit added. Figure 4 with information removed (replaced with calculator)

Chapter 8
- Compatible sequencing modes added
- Previous 8.1 (Flowcell selection) removed (replaced with calculator)
- Prevous 8.2 (Sample sheet generation) content moved to separate Devyser guide: "Generating a Devyser Sample Sheet for MiSeq®"
- 8.3 and 8.4. New, containing revised information from previous section 8.2.3

Chapter 9
- SeqNext information (previous 9.2) moved to separate Devyser guide: "SeqNext guide for Devyser CFTR"
- 9.5. Coverage information added
- 9.2 Amplicon Suite information added

Version 2017-07-14
New