DEVYSER
RESULTS FOR LIFE

Devyser HBOC

Art. No.: 8-A111-RUO
For Research Use Only

Handbook

TABLE OF CONTENTS

1. INTRODUCTION TO DEVYSER HBOC

Handbook update service

Sign up for the handbook update service to receive notifications via e-mail whenever there is a new version of the handbook available.
Visit www.devyser.com/ifu-subscription to sign up

1.1 Intended use

The Devyser HBOC kit is intended for determination of sequence variants in selected human genes implicated in breast and ovarian cancer. HBOC stands for “Hereditary Breast and Ovarian Cancer”.

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

1.2 Background

Hereditary cancer is caused by genetic mutation(s) inherited from the proband’s parents. Mutations in several different genes have been reported to significantly increase the risk of developing various types of cancer[1]. By knowing the mutation status in genes linked to the development of cancer, a personalized screening plan can be created allowing for earlier detection of cancer and thereby an increased survival rate. The mutation status can in some cases also enable physicians to make informed decisions to help guide treatment.

Devyser HBOC provides information about sequence variants in 12 genes known to significantly increase the risk of developing breast and ovarian cancer[2].

1.3 Assay principle

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

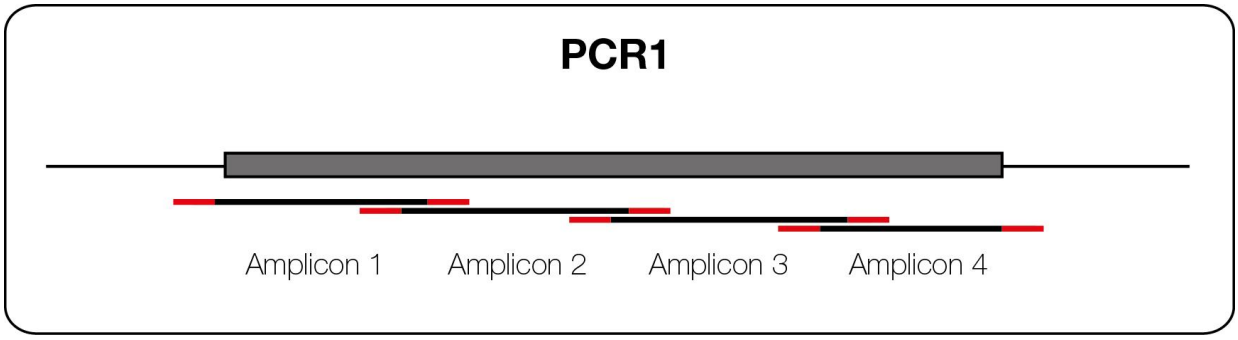


Figure 1. Schematic illustration of PCR1

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

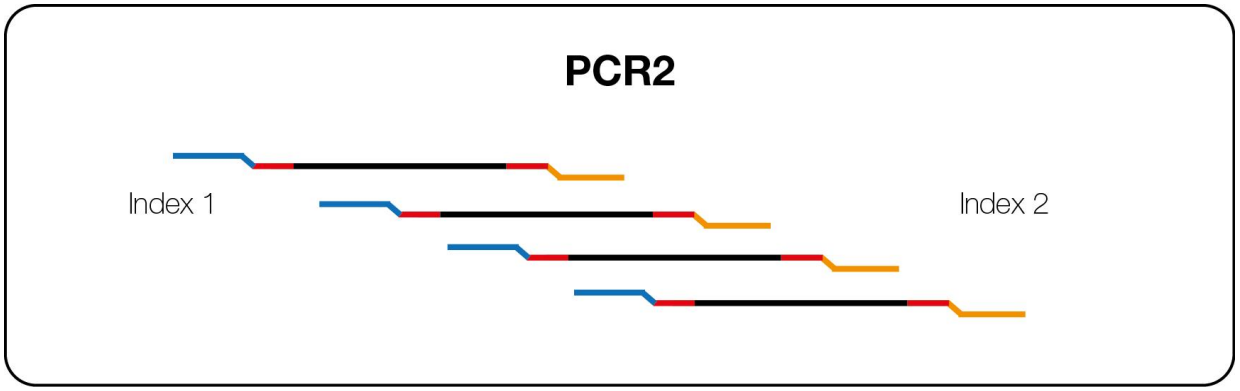


Figure 2. Schematic illustration of PCR2

1.4 Assay design

Devyser HBOC allows detection of SNVs and indels in the coding regions, adjacent exon-intron boundaries and selected introns in the genes listed in table 1. Target specific primers are designed such that most primer footprints are covered by an overlapping amplicon to enable detection of primer site SNVs.

The Devyser HBOC kit consists of two multiplex target amplification reactions per sample (HBOC Mix A and HBOC Mix B), which are pooled before PCR2. Handling and pooling of two mixtures introduces a risk of sample mix-up and hence, the Devyser HBOC kit contains twelve ID markers (control SNVs) according to Eurogentest Guidelines[3].

Table 1. Investigated gene panel

Gene	Transcript for annotation (RefSeq)	LRG ID	Included in HBOC Mix
ATM	NM_000051.3	LRG_135t1	A
BARD1	NM_000465.4	LRG_297t1	B
BRIP1	NM_032043.2	LRG_300t1	B
CDH1	NM_004360.3	LRG_301t1	B
CHEK2	NM_007194.3	LRG_302t1	A
NBN	NM_002485.4	LRG_158t1	B
PALB2	NM_024675.3	LRG_308t1	A
PTEN*	NM_000314.4	LRG_311t1	A
RAD51C	NM_058216.1	LRG_314t1	B
RAD51D	NM_002878.3	LRG_516t1	B
STK11**	NM_000455.4	LRG_319t1	B
TP53	NM_000546.5	LRG_321t1	A

*The confidence of the variant calling may be lower in positions hg19, chr10:89720620-89720648 in PTEN exon 8.

**Six bases in exon 3 of STK11 (hg19, chr19:1,219,407-1,219,412) are not sequenced, however no pathogenic variants are reported in ClinVar at publication of this handbook.

2. MATERIALS AND EQUIPMENT

2.1 Kit configurations for Devyser HBOC

The Devyser HBOC kit for Illumina® sequencing is available in two configurations according to Tables 2 and 3.

Table 2. Devyser HBOC 24 test kit (8-A111-24-RUO)

Component	Art.No.	Number/kit	Cap color	Storage condition
HBOC Mix A	4-A311	1	Blue	-18°C to -28°C
HBOC Mix B	4-A312	1	Blue	-18°C to -28°C
Start 2 S	4-A310	2	Purple	-18°C to -28°C
Dilution buffer	4-A245	3	White	-28°C to +8°C
Index mix S	4-A302	1	Red	-18°C to -28°C
Index strip A2	-	1	-	-18°C to -28°C
Index buffer	4-A258	3	Green	-28°C to +8°C
Sealer S	-	1	-	Ambient

Table 3. Devyser HBOC 48 test kit (8-A111-48-RUO)

Component	Art.No.	Number/kit	Cap color	Storage condition
HBOC Mix A	4-A311	2	Blue	-18°C to -28°C
HBOC Mix B	4-A312	2	Blue	-18°C to -28°C
Start 2 S	4-A310	4	Purple	-18°C to -28°C
Dilution buffer, 96 test	4-A275	1	-	-28°C to +8°C
Index mix S	4-A302	2	Red	-18°C to -28°C
Index plate A3	-	1	-	-18°C to -28°C
Index buffer, 96 test	4-A277	1	-	-28°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 4

Table 4. Devyser Library Clean (8-A204)

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

2.2.2 General

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

2.2.3 DNA extraction

- DNA extraction reagents according to manufacturer's instructions for use
- QIAamp DNA Blood Mini Kit (Qiagen, cat.# 51104/51106) and QIAamp DNA Mini Kit (Qiagen, cat.#51304/51306) or other similar solid phase technologies for extraction of genomic DNA from human whole blood

2.2.4 Determination of DNA concentration

- Determination of DNA concentration according to manufacturer's instructions for use
- Qubit™ 4 Fluorometer (Thermo Fisher Scientific, cat.# Q33226) and required consumables
- Qubit dsDNA 1X 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 alternative thermal cycler is used 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

- Deyvser 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 Illumina's sequencing guide
- Illumina reagent kits (table 5)

Table 5. Illumina 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

- Amplicon Suite (SmartSeq s.r.l) pipeline for Devyser HBOC.

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 6 for details.

Table 6. 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
Devyser HBOC BED files	BED files detailing amplicon positions according to Hg19/GRCh37

Download update service

Sign up for the download update service to receive notifications via e-mail whenever there is a new version of the downloads available.
Visit www.devyser.com/ifu-subscription to sign up

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 table 7.

Table 7. Minimal recommended number of read pairs per sample and amplicon coverage

Detection mode	Read pairs per sample*	Minimal coverage per amplicon**
Germline	167 692	100

*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 HBOC kit at -18°C to -28°C or the individual kit components as specified on the label (see also tables 2 and 3 in 2.1)
- Store the components of the Devyser Library Clean kit at +2°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 (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 IFU
- Deviations from the IFU 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 pool with other kits
- 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 ir moved between areas
- Gloves should be changed between activities

5. PROCEDURAL LIMITATIONS

- The Devyser HBOC 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 HBOC
- Results obtained with the Devyser HBOC kit can only be directly applied to the tissue or specific sample material tested
- Rare primer site sequence variants may affect the function of individual PCR primers used in the Devyser HBOC kit and may result in reduced or no amplification of the affected amplicon.
- The following parameters might affect the quality of the results:
    - Quality and concentration of the DNA
    - Deviations from the protocol
    - The number of samples carrying CNVs
    - The number of samples carrying identical CNVs
    - Sequencing depth
    - Bioinformatic pipeline

6. SAMPLE REQUIREMENTS

DNA concentration, integrity and purity are important parameters for successful testing using the Devyser HBOC 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 HBOC 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 instructions for use (see 2.2.3)

6.1.2 Determination of DNA concentration

- High quality DNA is important for accurate and reproducible determination of DNA concentration
- All DNA concentrations referred to in this handbook were determined using the Qubit Fluorometer and the Qubit dsDNA 1X 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 HBOC kit

6.1.3 Dilution of DNA

- Adjust the concentration of extracted DNA to 2 ng/µL (see 7.1.2) using the Dilution Buffer provided with the kit (see 7.1.2).

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

6.2 PhiX control

Include PhiX control v3 library DNA (see table 5) 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 relevant pre-characterized gene sequence variants (in-house developed or externally sourced) are suitable as system controls.

7. INSTRUCTIONS FOR USE

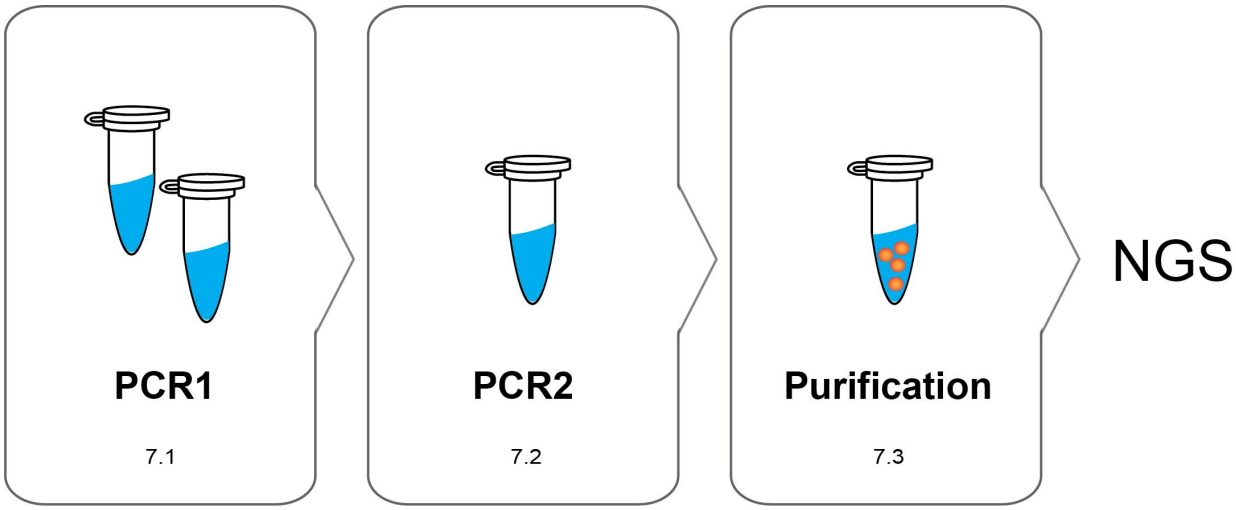


Figure 3. Schematic overview of the Devyser HBOC library preparation procedure (7.1 - 7.3).

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

PCR1 (7.1)
The amplicon library for 12 genes is generated in two multiplex PCR reactions for each sample (HBOC Mix A and HBOC Mix B), which are pooled per sample to obtain a PCR1 library.

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)

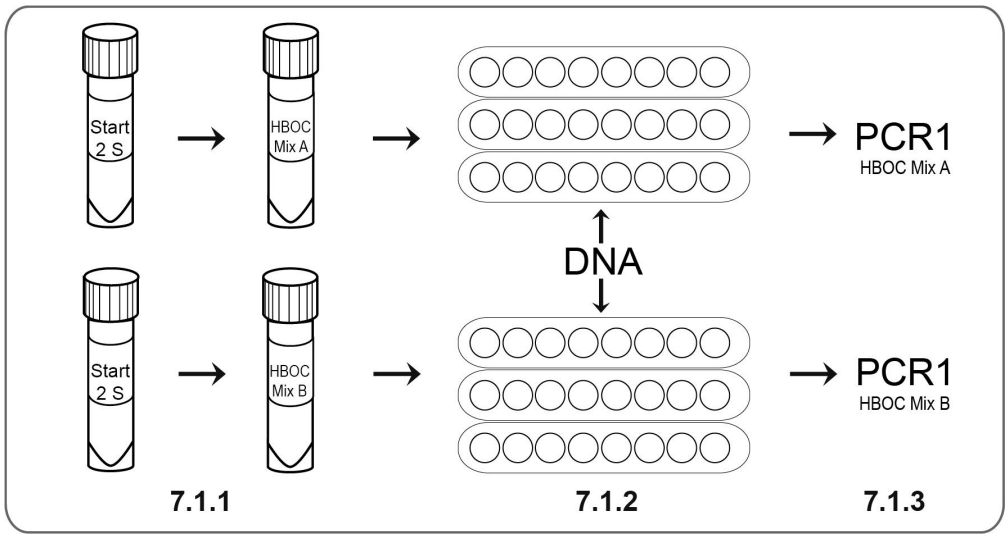


Figure 4. Schematic overview of step 7.1.1 to 7.1.3

7.1.1 HBOC mix preparation

Required kit components:

- 24 test kit: **Start 2 S (4-A310)**, **HBOC Mix A (4-A311)**, **HBOC Mix B (4-A312)**
- 48 test kit: **Start 2 S (4-A310)**, **HBOC Mix A (4-A311)**, **HBOC Mix B (4-A312)**

A. Ensure that the **Start 2 S** and the **HBOC Mix A** and **HBOC Mix B** are completely thawed before use
B. Vortex the **Start 2 S** tubes briefly
C. Briefly centrifuge the **Start 2 S**, **HBOC Mix A** and **HBOC Mix B** tubes to collect the content
D. Add 150 µL of **Start 2 S** to the **HBOC Mix A** and to the **HBOC Mix B** tubes to obtain an activated **HBOC Mix A** and **HBOC Mix B**
E. Vortex the activated **HBOC Mix A** and **HBOC Mix B** tubes and then centrifuge briefly to collect the content
F. Dispense 10 µL of the activated **HBOC Mix A** and **HBOC Mix B** into separate PCR reaction tubes or separate wells. Cap the tubes or seal the plate.
G. Store the dispensed **HBOC Mix A** and **HBOC Mix B** at +2°C to +8°C and continue to 7.1.2. Any remaining activated **HBOC mix** can be stored in a freezer between -18°C and -28°C for 5 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 wells containing activated **HBOC Mix A** and **HBOC Mix B** (from 7.1.1)
E. Mix by pipetting
F. Cap the tubes or seal the plate and centrifuge briefly to collect the content

7.1.3 Thermal cycling PCR1

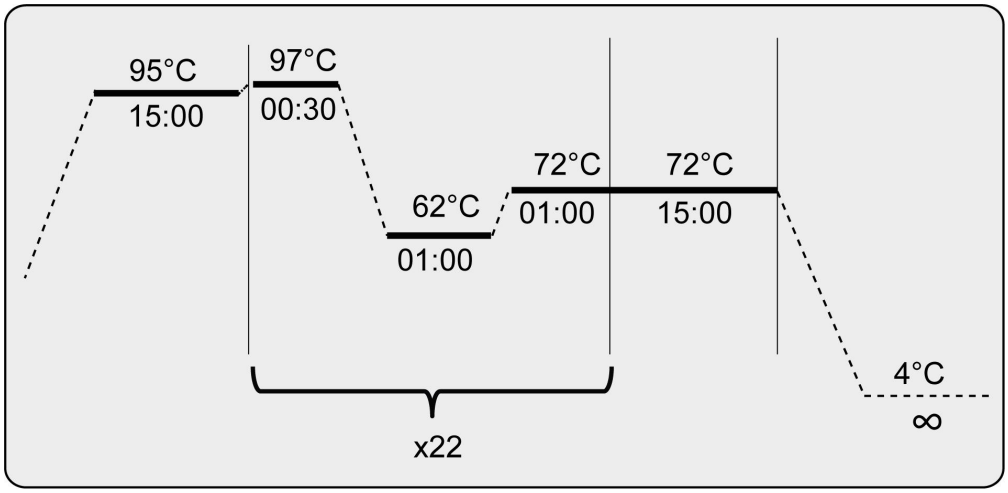


Figure 5. Thermal profile PCR1

A. Program the 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

The PCR1 library can be stored in a freezer between -18°C and -28°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)

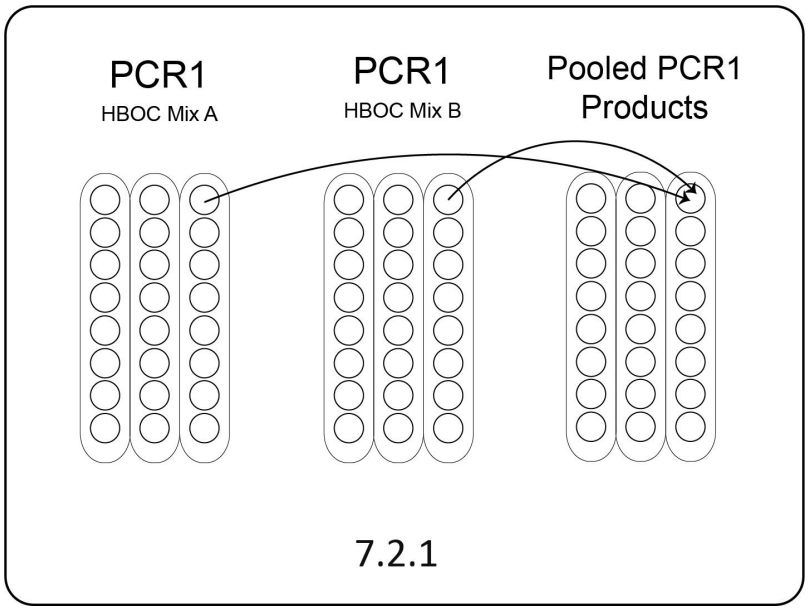


Figure 6. Schematic overview of step 7.2.1

7.2.1 PCR1 library pooling

1. For each DNA sample, pool 10 µL of **HBOC Mix A** library with 10 µL of **HBOC Mix B** library into a new reaction tube or well
2. Mix the pooled PCR1 libraries thoroughly by pipetting

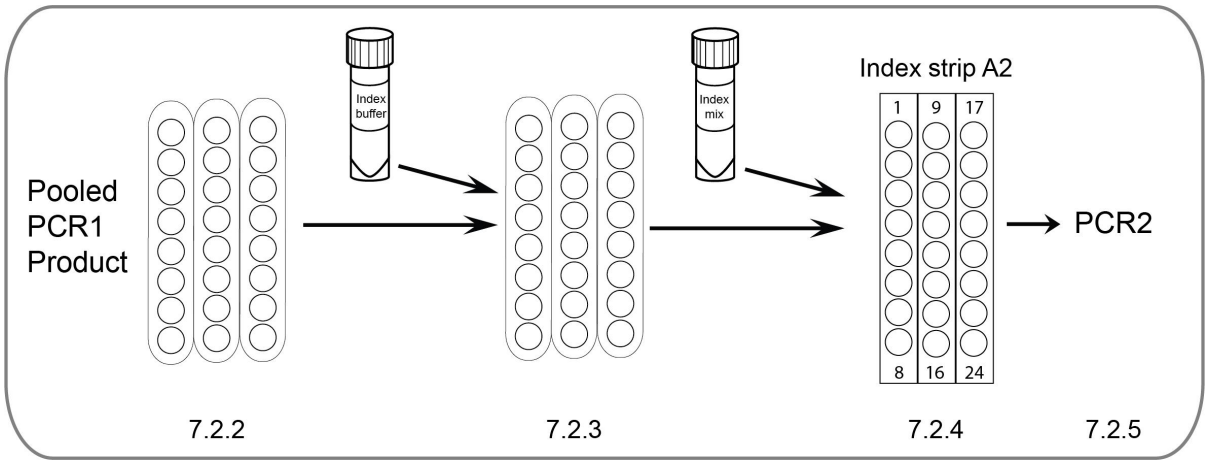


Figure 7. Schematic overview of steps 7.2.2 to 7.2.5

7.2.2 PCR1 library dilution

Required kit components:

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

A. Ensure that the **Index buffer** is completely thawed
B. For each pooled PCR1 product to be diluted, dispense 198 µL **Index buffer** to a new tube
C. Add 2 µL of each pooled PCR1 product 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)

7.2.3 Index preparation

Required kit components:

- 24 test kit: **Index mix S (4-A302)** and 1 **Index strip A2**
- 48 test kit: **Index mix S (4-A302)** and 1 **Index plate A3**

A. Ensure that the **Index mix S** is completely thawed before use
B. Vortex and then briefly centrifuge the **Index mix A** tubes to collect the content
C. Carefully remove the transport seal of **Index strip A2** or **Index plate A3**. **Note! Do not reuse the transport seal**
D. Add 20 µL of **Index mix S** to each well to be used. **Note! Tips must be changed between each individual well**

7.2.4 Addition of diluted PCR1 libraries to Index strip A2 or Index plate A3

Required kit component: **Sealer S** or **Sealer L**

A. Add 5 µL of each diluted PCR1 library (from 7.2.2) to separate wells in **Index strip A2** or **Index plate A3** (prepared in 7.2.3)
B. Mix thoroughly by pipetting to dissolve the colored reagent pellets, using a pipetting volume of at least 15 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** or **Sealer L** to completely cover **Index strip A2** or **Index plate A3**
D. Carefully seal **Index strip A2** or **Index plate A3** and make sure that all wells are covered
E. Centrifuge briefly to collect the content

7.2.5 Thermal cycling PCR2

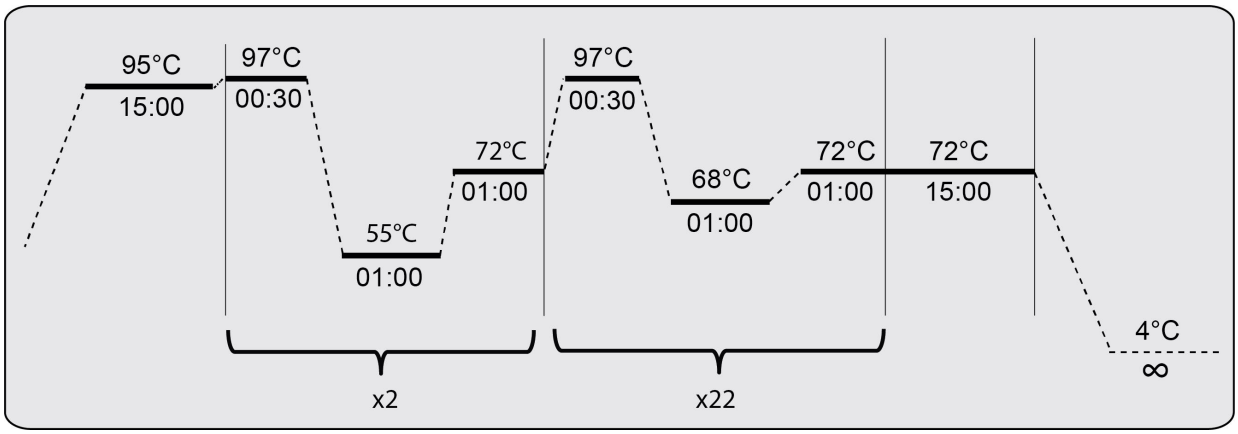


Figure 8. Thermal profile PCR2

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

PCR2 libraries can be stored in a freezer between -18°C and -28°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 7.2.5.

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"[5]

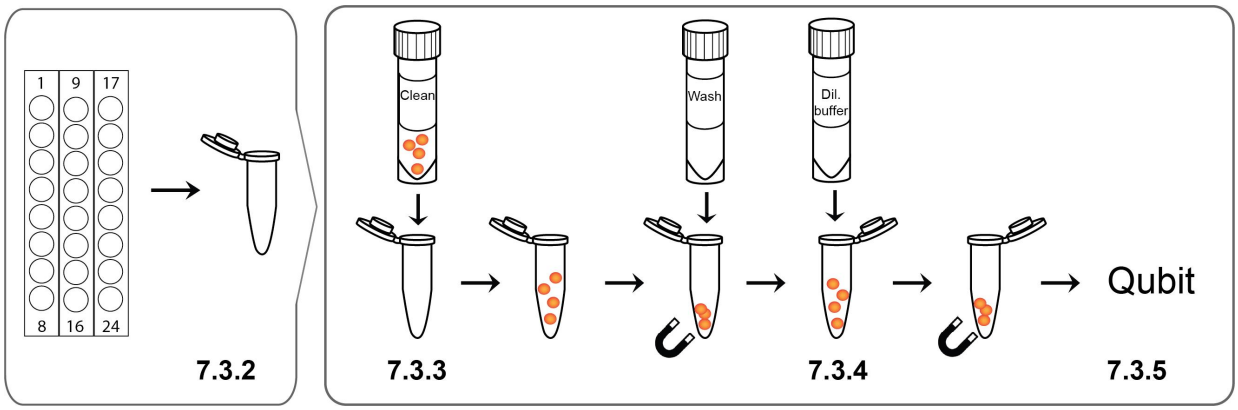


Figure 9. Schematic overview of 7.3.2 to 7.3.5

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°C to +8°C and used within 3 months from day of activation**

7.3.2 Pooling

A. Pool equal volumes, using a pipetting volume of at least 5 µL, from each of the PCR2 libraries (from 7.2.5) into a single tube to obtain a library pool for subsequent purification. Ensure that the volume of the library pool is at least 50 µL
B. Mix thoroughly by vortexing and then briefly centrifuge the library pool to collect the content
C. Transfer 50 µL of the library pool into a new tube suitable for placing on a magnetic rack

NOTE	If using one or two samples, the volume of the library for purification would be less than 50 µL. In this case, use Clean corresponding to 0,6x the volume of the library. For elution, use a volume of the Dilution buffer equal to the volume of the library.

NOTE	It is not recommended to pool Devyser HBOC libraries with libraries prepared using Devyser kits or kits from other vendors.

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 30 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 lustre to lusterless, approximately 3-5 minutes, while remaining on the magnetic rack. **Important! See note below**
L. Remove the tube from the magnetic rack and continue to 7.3.4

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

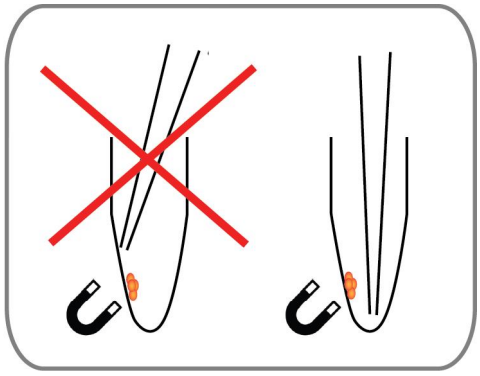


Figure 10. Bead pellet

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 50 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 dsDNA 1X HS Assay Kit** (see 2.2.4)

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

A. Ensure that all Qubit 1X dsDNA HS solutions are at room temperature
B. Mix 190 µL Qubit 1X ds DNA HS working solution with 10 µL of Qubit standard 1
C. Mix 190 µL Qubit 1X ds DNA HS working solution with 10 µL of Qubit standard 2
D. Mix 190 µL Qubit 1X ds DNA 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 0.23 - 0.27 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 (0.23 - 0.27 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 between -18°C and -28°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 Samples 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®" available at www.devyser.com and the Illumina document #15031335:"Illumina Experiment Manager Software Guide"[6].

8.3 Index description

The Illumina double indexes introduced during PCR2 are listed in tables 8 and 9. Detailed information about the index combinations and index sequences can be found online (see 2.4 for details).

Table 8. 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 9. 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 "MiSeq System Denature and Dilute Libraries Guide"[7]

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

8.4.1 Illumina Sequencing

A. Prepare the sequencing run according to the current version of "MiSeq System Guide"[5]

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 Sequence data analysis using Amplicon Suite

Data analysis is performed using the Devyser HBOC pipeline in Amplicon Suite.

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

Indications of CNVs are given for all genes listed in table 1 with the exception of STK11.

10. SYMBOLS USED ON LABELS

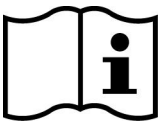
Lot or batch number

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.

The Devyser HBOC kit is for research use only and not for use in diagnostic procedures.

12. CONTACT INFORMATION

12.1 Legal manufacturer

Devyser AB
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

[1] Hereditary Cancer Syndromes - A Primer on Diagnosis and Management: Part 1: Breast-Ovarian Cancer Syndromes, Samadder NJ et al., Mayo Clin Proc., 2019.

[2] NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®), Genetic/Familial High-Risk Assessment: Breast and Ovarian, Version 2.2019, July 30, 2018.

[3] Guidelines for diagnostic next-generation sequencing, Gert Matthijs et al., European Journal of Human Genetics, 2016, https://www.nature.com/articles/ejhg2015226

[4] https://www.illumina.com/systems/sequencing-platforms/miseq/specifications.html. Cited 2019-09-24.

[5]MiSeq System Guide (Document # 15027617)

[6] Illumina Experiment Manager Software Guide (Document # 15031335)

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

14. ABBREVIATIONS

Abbreviation	Explanation
bp	basepairs
CNV	Copy Number Variation
DNA	Deoxyribonucleic acid
HBOC	Hereditary Breast and Ovarian Cancer
IEM	Illumina Experiment Manager
Indel	insertion and / or deletion
NGS	next generation sequencing
PCR	polymerase chain reaction
PE	paired end
RUO	Research use only
SNV	Single Nucleotide Variation

15. REVISION HISTORY

Version 2020-02-13

General: Editorial changes.

Chapter 2
Added section 2.5 and inserted table 7 with recommended number of read pairs per sample and amplicon coverage.

Chapter 4
Added a point to emphasize that HBOC should not be pooled with other kits.

Chapter 7
7.1.1 G. Added the sentence "Do not aliquot the activated mix."
7.3.6 Concentration of the library pool was updated from 0.40 - 0.45 ng/µL to 0.23 - 0.27 ng/µL in the text and the note.

Version 2019-11-06

Chapter 1
1.4, Table 1. Added column specifying genes included in HBOC Mix A and B.

Chapter 7
New library purification protocol:

7.3.2. Library pool volume changed from 40 µL to 50 µL (step A and C). Step A rephrased. Information revised in the two notes below.

7.3.3 Clean volume changed from 40 µL to 30 µL (step C).

7.3.4 Volume of Dilution buffer changed from 40 µL to 50 µL (step C).

7.3.6 Concentration of the library pool changed from 0.24 - 0.3 ng/µL to 0.40 - 0.45 ng/µL.
In note: Recommended cluster density removed due to new cleaning protocol.

Version 2019-09-25
New