



**TarCET<sup>®</sup>**  
IVD kits

# INSTRUCTIONS FOR USE



For Diagnostic Procedure Only  
Proprietary Document: WI-03-100-TT-ENG  
Version 4.0



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## Revision History

Date	Version	Description of Change (Initial Version/Revision)
2020-12-21	0.1	Initial Document Issue
2021-03-03	0.2	Edits for including all TarCET products
2022-04-05	1.0	Edits for CE-IVD Submission
2022-09-12	2.0	Edits for Release
2022-11-01	3.0	Edits to Correct Equipment and Reagent's Code and Changes to Reflect Acquisition by Medcover
2023-04-07	4.0	Clarifications on Workflow Steps and Rebranding

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## Symbols



Reagents/Sample



Manufacturer



Keep dry



Telephone number



Consult instructions  
for use (IFU)



Email address



Temperature



Material consists of polypropylene  
and can be recycled with plastic (PMD)



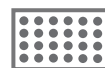
1.5mL tube



Box number



Expiration date



96-well plate



Contains sufficient  
for N tests



10mL tube



20mL tube



30mL tube



Item number



Lot number



GHS07 health  
hazard



Keep away  
from sunlight

## Trademarks and Disclaimers

Use of the TarCET IVD Kit (the **Product**) signifies the agreement of any user of the Product with the following terms:

1. The Product may be used solely in accordance with the instructions for use (the IFU) TarCET IVD workflow and only with components described in the **IFU**.
2. Medicover Genetics Ltd with registration number HE 418406 (ex NIPD Genetics Molecular Laboratories Ltd) (the **Manufacturer**) grants no license to use or incorporate the enclosed component of the Product with any component not included within the kit except as described in the IFU.
3. The Product and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. The sale and the use of this Product is conditioned on not using this product outside such prescribed fields of use or any other purpose not expressly authorized by the Manufacturer.
5. This Product may include limited, non-transferable, licenses under the relevant legislation of patents owned by Medicover Biotech Ltd with registration number HE 418372 (ex NIPD Genetics Biotech Ltd) or Medicover Public Co Ltd with registration number HE 275644 (ex NIPD Genetics Public Company Ltd).

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## Abbreviations

ATP	Adenosine Triphosphate
CI	Confidence Interval
CNVs	Copy Number Variants
°C	Degree Celsius
dNTP	Deoxyribonucleotide Triphosphate
FH	Familial Hypercholesterolemia
GLP	Good Laboratory Practice
HPLC	High Performance Liquid Chromatography Grade Water
INDEL	Insertion or Deletion
IVD	In-Vitro Diagnostic
NGS	Next Generation Sequencing
NTC	No (Negative) Template Control
N/A	Not Applicable
PCR	Polymerase Chain Reaction
Pcs	Pieces
PH	Pulmonary Hypertension
Pkg	Package
P/N	Product Number
Qty	Quantity
Rpm	Revolutions per minute
RT	Room Temperature
Rxn	Reaction
SNVs	Single Nucleotide Variants

## Intended Use

This TarCET IVD Kit is a CE marked, Next Generation Sequencing (NGS) product intended to be used for identification of genetic variants associated with diseases. The TarCET IVD Kit (CE IVD) assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained.

## Purpose, Scope and Users

The purpose of the document is to provide details regarding the methodology, execution, and validation of the Medicover Genetics TarCET genetic testing kit portfolio.

Genetic disorders affect millions of people of all ages globally, with a range of developmental, physical, and socioeconomic implications, often leading to death. For this reason, there is an urgent need for better understanding the cause and prevalence of genetic conditions.

High-throughput and high-resolution advanced technologies, such as NGS, have helped to make great advances in unraveling the underlying mechanisms of genetic disorders as they are able to interrogate thousands of genomic loci for the identification of potentially deleterious genomic alterations. As a result, genetic disorders can be confirmed or ruled out allowing clinicians to provide an accurate diagnosis and choose the appropriate treatment for each disorder. In the context of reproductive health, genetic testing allows prospective parents to know whether they are carriers of genetic alterations as well as inform them about their fertility status. This can guide parents accordingly based on the risk they have of having a child with a disease and make informed decisions regarding their family planning.

Incorporating years of experience in clinical diagnostics along with its highly validated in-solution hybridization-based technology, Medicover Genetics has developed a range of diagnostic/screening testing kits in the fields of Oncology, Postnatal and Neonatal Genetics. The kit solutions include (visit [www.medicover-genetics.com](http://www.medicover-genetics.com) for complete gene list):

1. TarCET Metabolic Kit
2. TarCET Aortopathy Kit
3. TarCET Arrhythmia Kit
4. TarCET Cardiomyopathy Kit
5. TarCET Congenital Heart Defects Kit
6. TarCET FH, PH and RAS Kit
7. TarCET Cardiac Comprehensive Kit
8. TarCET Hereditary Cancer Kit
9. TarCET Infertility Kit
10. TarCET Neonatal Kit
11. TarCET Carrier Screening Core Kit
12. TarCET Carrier Screening Comprehensive Kit



## Rationale

The integration of genetics in clinical practice is becoming increasingly important as it can aid the diagnosis and inform about the prognosis and clinical management of genetic diseases.

Genetic testing has been traditionally offered based on ethnic background or family history; however, in recent years, professional organizations and professional bodies recommend expanded genetic testing to be offered to the general population as it can provide invaluable information for both the patient and their family members.

Medicover Genetics TarCET NGS-based kits offer a wide range of genetic testing solutions providing sensitive and reliable methods for the detection of genetic changes, allowing clinicians to identify individuals at risk for proper future management of many genetic disorders.

## Quality Control and Validity of Results

Medicover Genetics TarCET NGS-based kits should be used with a no (negative) template control (NTC), water, to monitor library preparation and hybridization steps. The negative control can be included in each run for appropriate Quality Control, results interpretation and for the results to be considered valid.

## Introduction

Genetic disorders can be caused by chromosomal abnormalities, single-gene defects or a combination of genetic mutations in multiple genes with variable prevalence among different racial and ethnic groups, with profound consequences for the patients and their families. As such, understanding the role of genetics in disease and the underlying mechanisms has become a central part in clinical practice.

Most genetic diseases are rare and can be caused by mutations inherited from the parents (i.e. sickle cell disease). Other diseases are caused by acquired mutations later in a person's life that are not inherited but can occur randomly (*de novo*). The ability to identify genetic risks and therefore manage a genetic disease before it becomes severe has unprecedented scientific and clinical value.

Genetic testing is a great medical tool for inherited disease screening. Identification of genetic changes in patients with severe clinical phenotypes can guide physicians to provide suitable therapy and clinical management of the disease, while reducing the psychological distress of the patients and their families. Furthermore, the ability to identify clinically significant variants in patients before the appearance of symptoms allows clinicians to be proactive in order to reduce the clinical severity and promote the quality of life. TarCET NGS-based kits utilize Medicovert Genetics powerful and highly validated in-solution hybridization-based technology to provide genetic information in the field of Oncology, Postnatal and Neonatal Genetics. The tests consist of two components: the TarCET IVD Kit and a visualization software (SIRIUS) which is the data analysis engine.

## Product Description

TarCET NGS-based kits include reagents for universal library preparation for DNA sequencing on Illumina platforms, and reagents for in-solution hybridization for multigene panels for metabolic diseases, cardiac diseases, hereditary cancer, infertility, neonatal health and carrier screening purposes. The kits are used with unique indices allowing combination of multiple libraries onto a single sequencing run. Each kit includes enough reagents for 16 library preparation and in-solution hybridization reactions.

**TarCET Metabolic Kit** (Cat # ET101-00-2016) covers the major classes of inherited metabolic disorders as well as hyperinsulinemic hypoglycemia, which is the most common cause of severe and persistent hypoglycemia in the neonatal period. It is addressed to patients with common symptoms of a metabolic disease such as developmental delay and lethargy, patients with a spectrum of overlapping symptoms that vary in age of onset and severity, undiagnosed adult patients with symptoms related to neurological conditions that failed to improve with routine therapies, and individuals with a positive family history of a metabolic disease.

**TarCET Cardiac Kits** are suitable for the detection of multiple genetic conditions with complex phenotypes, including features of both channelopathy and cardiomyopathy, and for the investigation of sudden cardiac death as these products include large classes of genetic disorders that result in a range of phenotypes. The kits cover:

- Aortopathies (TarCET Aortopathy Kit, Cat # ET103-00-2016)
- Arrhythmias (TarCET Arrhythmia Kit, Cat # ET104-00-2016)
- Cardiomyopathies (TarCET Cardiomyopathy Kit, Cat # ET105-00-2016)
- Congenital Heart Defects (TarCET Congenital Heart Defects Kit, Cat # ET106-00-2016)
- Familial Hypercholesterolemia (FH)/Pulmonary Hypertension (PH)/RASopathy (RAS) (TarCET FH, PH and RAS Kit, Cat # ET107-00-2016)
- Comprehensive cardiology panel (TarCET Cardiac Comprehensive Kit, Cat # ET110-00-2016) covering all the aforementioned groups of diseases

**TarCET Hereditary Cancer Kit** (Cat # ET111-00-2016) provides information for patients with breast, ovarian, endometrial, prostate, colorectal and melanoma cancer and for individuals with a relevant clinical and/or family history suggestive of a hereditary cancer syndrome, as well as individuals included in the guidelines outlined by the National Comprehensive Cancer Network (NCCN). The kit includes an extensive portfolio of cancer-specific gene panels, which can be in silico selected through SIRIUS. It covers cancer predisposing syndromes associated with hereditary cancer. The patients' medical family history, their ethnicity, and any physical findings present that are linked to cancer, should always be taken into consideration.

**TarCET Infertility Kit** (Cat # ET112-00-2016) is used for the detection of genetic variants associated with infertility. The kit includes two panels: female infertility panel and male infertility panel, which can be selected in silico through SIRIUS. It tests for whole, partial and mosaic sex chromosome changes which can cause fertility problems. The female infertility panel screens for sex chromosome numerical abnormalities and disorders related to primary ovarian insufficiency, polycystic ovary syndrome, ovarian hyperstimulation syndrome and hypogonadotropic hypogonadism disorders such as Kallman syndrome. The male infertility panel screens for sex chromosome numerical abnormalities and covers genes related to male sexual development, testicular development and spermatogenesis, including hypogonadotropic hypogonadism, such as Kallmann syndrome.

**TarCET Neonatal Kit** (Cat # ET113-00-2016) is used for the analysis of genes related to metabolic, endocrine and hemoglobin, as well as hearing loss disorders, among others. The kit consists of one panel which targets autosomal recessive, autosomal dominant and X-linked genetic conditions, many with overlapping phenotypes.

**TarCET Carrier Screening Core Kit** (Cat # ET114-00-2016) and **TarCET Carrier Screening Comprehensive Kit** (Cat # ET115-00-2016) can be used in individuals who are potential carriers of genetic diseases. TarCET Carrier Screening Core detects diseases including A-Thalassemia, B-Haemoglobinopathies, Cystic Fibrosis, Dystrophinopathies, Phenylalanine Hydroxylase Deficiency, Fanconi Anemia Group C and Tay-Sachs disease.

For the list of genes included in TarCET Hereditary Cancer, Metabolic, Aortopathy, Arrhythmia, Cardiomyopathy, Congenital Heart Defects, Familial Hypercholesterolemia, Pulmonary Hypertension, RASopathies, Cardiac Comprehensive, Infertility, Neonatal, Carrier Screening Core and Comprehensive Kits please refer to [www.medicover-genetics.com](http://www.medicover-genetics.com)

# General Guidelines

## Transport and Storage Conditions

The reagents are shipped and stored at room temperature (RT), +4°C and -20°C until the expiration date, as stated on the label. All components must be kept away from sunlight and should be protected from humidity.

## Training Requirements

Testing for identifying genetic mutations should be performed in an equipped laboratory with staff trained to carry out the relevant technical procedures according to the Occupational Safety and Health Administration (OSHA) Laboratory standards. Refer to the World Health Organization Interim guidance on laboratory biosafety and the Centers for Disease Control and Prevention (CDC) guidelines for Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with genetic testing.

## Precautions and Recommendations

- The procedures in this handbook must be followed as described. Any deviations may result in assay failure or cause erroneous results and interpretation.
- Read the entire test procedure before beginning. Take note of stopping points and organize your workflow accordingly.
- Good Laboratory Practice (GLP) is required to ensure the performance of the kit, with care required to prevent contamination of the kit components.
- Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and assay reagents. Wash hands thoroughly after handling specimens and assay reagents.
- Proper laboratory practices and good laboratory hygiene is required to prevent Polymerase Chain Reaction (PCR) products from contaminating reagents, instrumentation, and genomic DNA samples. PCR contamination may cause inaccurate and unreliable results.
- To prevent contamination, ensure that pre-amplification and post-amplification areas have dedicated equipment (e.g., pipettes, pipette tips, vortex, and centrifuge).
- Avoid cross contamination by changing pipette tips between samples and between dispensing reagents. Do not vortex the plates. Using aerosol-resistant tips reduces the risk of amplicon carry-over and sample-to-sample cross-contamination.
- To create master mixes, multiply by the number of samples and add a 10% overfill to compensate for pipetting loss. Add the reagents to the master mix in the specified order.
- Prior to thermal cycling, always seal PCR plates with a pierceable foil heat seal, unless otherwise stated.

- Always prepare fresh 80% ethanol for wash steps. Ethanol can absorb water from the air, impacting results.
- Always equilibrate the Purification Beads (2K1) at RT for at least 30 minutes before use.
- Vortex the Capture Beads and Purification Beads thoroughly until the beads are resuspended. To prevent beads' resettling, do not centrifuge before pipetting.
- When washing the beads:
  - The appropriate magnetic stand for the plate/tube should be used.
  - Do not remove the plate/tube from the magnetic stand before the specified time.
  - Do not disturb the plate/tube while on the magnetic stand, and do not agitate the bead pellet.
  - Dispense back into the plate, on the magnetic stand, in case that beads get aspirated into pipette tips, and wait about 2 minutes until the solution is clear.
  - If there is liquid on the side of the plate/tube, briefly centrifuge.
  - Ensure that all ethanol is removed from the bottom of the wells during wash steps. Residual ethanol may impact results.
  - Adhere to the specified drying time following the magnetic stand step to ensure complete evaporation. Residual ethanol can impact the performance of subsequent reactions.
- When using the Hybridization Buffer (2X) (3L1), pipette slowly in order to minimize foaming.
- Adhere to the specified incubation times. Improper incubation can affect library representation and cluster density/occupancy.
- Thaw reagents on ice prior to use and keep them on ice at all times.
- Do not use reagents past the expiration date. After the expiration date, the quality guarantee is no longer valid.
- Do not mix reagents from different kits and/or lots and/or another supplier.
- Handle all specimens as if infectious using GLP and the OSHA Laboratory standard (29 CFR 1910.1450).
- Dispose of waste in compliance with local, state, and federal regulations.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces with at least 70% (v/v) ethanol.
- Please consult the safety data sheet (SDS) before using this kit, which is available on request.
- Consult each instrument's reference manual for additional warnings, precautions, procedures, and data analysis.

## Kit Contents

### TarCET IVD Kit Reagent boxes

Name	Number of Tests	P/N	Box -20°C	Box +4°C	Box RT
TarCET Metabolic Kit	16	ET101-00-2016	ET101-00-1001	ET101-00-1002	ET101-00-1003
TarCET Aortopathy Kit	16	ET103-00-2016	ET103-00-1001	ET103-00-1002	ET103-00-1003
TarCET Arrhythmia Kit	16	ET104-00-2016	ET104-00-1001	ET104-00-1002	ET104-00-1003
TarCET Cardiomyopathy Kit	16	ET105-00-2016	ET105-00-1001	ET105-00-1002	ET105-00-1003
TarCET Congenital Heart Defects Kit	16	ET106-00-2016	ET106-00-1001	ET106-00-1002	ET106-00-1003
TarCET FH, PH and RAS Kit	16	ET107-00-2016	ET107-00-1001	ET107-00-1002	ET107-00-1003
TarCET Cardiac Comprehensive Kit	16	ET110-00-2016	ET110-00-1001	ET110-00-1002	ET110-00-1003
TarCET Hereditary Cancer Kit	16	ET111-00-2016	ET111-00-1001	ET111-00-1002	ET111-00-1003
TarCET Infertility Kit	16	ET112-00-2016	ET112-00-1001	ET112-00-1002	ET112-00-1003
TarCET Neonatal Kit	16	ET113-00-2016	ET113-00-1001	ET113-00-1002	ET113-00-1003
TarCET Carrier Screening Core Kit	16	ET114-00-2016	ET114-00-1001	ET114-00-1002	ET114-00-1003
TarCET Carrier Screening Comprehensive Kit	16	ET115-00-2016	ET115-00-1001	ET115-00-1002	ET115-00-1003

### TarCET IVD Kit Reagents (Box 1)

Name	ID in Box	Container	Volume (μL)	Qty	Storage	P/N	Box #
ATP	1R1	1.5 mL	128	1	-20°C	ET100-01-01R1	ET1XX-00-1001
dNTP	1R2	1.5 mL	14	1	-20°C	ET100-01-01R2	ET1XX-00-1001
End-repair Buffer	1R3	1.5 mL	68	1	-20°C	ET100-01-01R3	ET1XX-00-1001
End-repair Enzyme 1	1R4	1.5 mL	8	1	-20°C	ET100-01-01R4	ET1XX-00-1001
End-repair Enzyme 2	1R5	1.5 mL	8	1	-20°C	ET100-01-01R5	ET1XX-00-1001
Ligation Enzyme	1G1	1.5 mL	16	1	-20°C	ET100-01-01G1	ET1XX-00-1001
Adapters	1G2	1.5 mL	28	1	-20°C	ET100-01-01G2	ET1XX-00-1001
Adapter Fill-in Buffer	1B1	1.5 mL	4	1	-20°C	ET100-01-01B1	ET1XX-00-1001
Adapter Fill-in Enzyme	1B2	1.5 mL	32	1	-20°C	ET100-01-01B2	ET1XX-00-1001
Indexing Buffer	1W2	1.5 mL	192	1	-20°C	ET100-01-01W2	ET1XX-00-1001
dNTP	1W3	1.5 mL	10	1	-20°C	ET100-01-01W3	ET1XX-00-1001
Indexing Enzyme	1W4	1.5 mL	10	1	-20°C	ET100-01-01W4	ET1XX-00-1001
Blocking Oligos 1	1L1	1.5 mL	48	1	-20°C	ET100-01-01L1	ET1XX-00-1001
Blocking Buffer	1L3	1.5 mL	80	1	-20°C	ET100-01-01L3	ET1XX-00-1001
Blocking Agent 1	1L4	1.5 mL	112	1	-20°C	ET100-01-01L4	ET1XX-00-1001
Blocking Agent 2	1L5	1.5 mL	48	1	-20°C	ET100-01-01L5	ET1XX-00-1001



Name	ID in Box	Container	Volume (μL)	Qty	Storage	P/N	Box #
Post-capture Primer 1	1Y1	1.5 mL	72	1	-20°C	ET100-01-01Y1	ET1XX-00-1001
Post-capture Buffer	1Y2	1.5 mL	480	1	-20°C	ET100-01-01Y2	ET1XX-00-1001
dNTP	1Y3	1.5 mL	24	1	-20°C	ET100-01-01Y3	ET1XX-00-1001
Post-capture Enzyme	1Y4	1.5 mL	24	1	-20°C	ET100-01-01Y4	ET1XX-00-1001
Post-capture Primer 2	1Y5	1.5 mL	72	1	-20°C	ET100-01-01Y5	ET1XX-00-1001
Target Capture Mix Metabolic	1L6	1.5 mL	192	1	-20°C	ET101-01-01L6	ET101-00-1001
Target Capture Mix Aortopathy	1L6	1.5 mL	192	1	-20°C	ET103-01-01L6	ET103-00-1001
Target Capture Mix Arrhythmia	1L6	1.5 mL	192	1	-20°C	ET104-01-01L6	ET104-00-1001
Target Capture Mix Cardiomyopathy	1L6	1.5 mL	192	1	-20°C	ET105-01-01L6	ET105-00-1001
Target Capture Mix Congenital Heart Defects	1L6	1.5 mL	192	1	-20°C	ET106-01-01L6	ET106-00-1001
Target Capture Mix FH, PH and RAS	1L6	1.5 mL	192	1	-20°C	ET107-01-01L6	ET107-00-1001
Target Capture Mix Cardiac Comprehensive	1L6	1.5 mL	192	1	-20°C	ET110-01-01L6	ET110-01-1001
Target Capture Mix Hereditary Cancer	1L6	1.5 mL	192	1	-20°C	ET111-01-01L6	ET111-01-1001
Target Capture Mix Infertility	1L6	1.5 mL	192	1	-20°C	ET112-01-01L6	ET112-01-1001
Target Capture Mix Neonatal	1L6	1.5 mL	192	1	-20°C	ET113-01-01L6	ET113-01-1001
Target Capture Mix Carrier Screening Core	1L6	1.5 mL	192	1	-20°C	ET114-01-01L6	ET114-01-1001
Target Capture Mix Carrier Screening Comprehensive	1L6	1.5 mL	192	1	-20°C	ET115-01-01L6	ET115-01-1001

### TarCET IVD Kit Reagents (Box 2)

Name	ID in Box	Container	Volume (μL)	Qty	Storage	P/N	Box #
Purification Beads	2K1	10 mL	6048	1	+4°C	ET100-02-02K1	ET1XX-00-1002
Capture Beads	2L1	1.5 mL	224	1	+4°C	ET100-02-02L1	ET1XX-00-1002

### TarCET IVD Kit Reagents (Box 3)

Name	ID in Box	Container	Volume (μL)	Qty	Storage	P/N	Box #
Hybridization Buffer	3L1	1.5 mL	480	1	RT	ET100-03-03L1	ET1XX-00-1003
Wash Buffer 1 (2X)	3C1	30 mL	23392	1	RT	ET100-03-03C1	ET1XX-00-1003
Wash Buffer 2	3Y1	20 mL	6400	1	RT	ET100-03-03Y1	ET1XX-00-1003
Wash Buffer 3	3C2	20 mL	12528	1	RT	ET100-03-03C2	ET1XX-00-1003

If any of the boxes (TarCET IVD Kit Boxes 1, 2, 3) are missing or damaged upon receipt of the TarCET IVD Kit (3 boxes per Kit), contact Medicover Genetics Ltd. directly.

TarCET IVD Kit reagents boxes 1, 2 and 3, are shipped at -20°C, +4°C and RT respectively. If the box has been opened before arrival, contact Medicover Genetics Ltd. directly. The TarCET IVD Kit should be stored at the temperatures indicated on the box labels.

## User-Supplied Consumables and Equipment

### Consumables

Item	Supplier	Catalogue Number
Microseal® 'B' PCR Plate Sealing Film, adhesive, optical*	Bio-Rad	MSB 1001
PCR Plate Heat Seal, foil, pierceable*	Bio-Rad	1814040
Deepwell Plate, 96/500 µL, DNA LoBind, PCR clean* – PL1	Eppendorf	0030 503.147
15 mL, Conical Centrifuge Tubes	General Laboratory Supplier	Varies
0.2 mL PCR Tubes	General Laboratory Supplier	Varies
0.5 mL Safe-Lock Microcentrifuge Tubes	General Laboratory Supplier	Varies
1.5 mL Safe-Lock Microcentrifuge Tubes	General Laboratory Supplier	Varies
5.0 mL Tubes	General Laboratory Supplier	Varies
1.5 mL LoBind DNA Tubes	General Laboratory Supplier	Varies
twin.tec® 96 Well, PCR plate, unskirted, 150 µL*, – PL2	Eppendorf	0030 133.307
twin.tec® 96 Well LoBind PCR plate, semi-skirted*, – PL3	Eppendorf	0030 129.504
50 mL, Conical Centrifuge Tubes	General Laboratory Supplier	Varies
10 µL Pipette Tips	General Laboratory Supplier	Varies
20 µL Pipette Tips	General Laboratory Supplier	Varies
200 µL Pipette Tips	General Laboratory Supplier	Varies
1000 µL Pipette Tips	General Laboratory Supplier	Varies

Item	Supplier	Catalogue Number
10 mL Serological Pipettes	General Laboratory Supplier	Varies
Qubit™ dsDNA HS Assay Kit*	Thermo Fisher Scientific	Q32854
HPLC Water	General Laboratory Supplier	Varies
Ethanol	General Laboratory Supplier	Varies
Loading Tips, 10 Pk TapeStation*	Agilent	5067-5599
D1000 ScreenTape, TapeStation*	Agilent	5067-5582
D1000 Reagents TapeStation*	Agilent	5067-5583
High sensitivity D1000 ScreenTape, TapeStation*	Agilent	5067-5584
High sensitivity D1000 Reagents TapeStation*	Agilent	5067-5585

(\*or equivalent)

## Equipment

Item	Supplier	Catalogue Number
Qubit 4 Fluorometer*	Thermo Fisher	Q33226
Bioruptor® Pico*‡	Diagenode	B01060010
NanoDrop™ 8000 Spectrophotometer*	Thermo Fisher	ND8000LAPTOP
Dry Block Incubator	General Laboratory Supplier	Varies
Shake 'n' Stack™ Hybridization Incubator*	Thermo Fisher	6242

Item	Supplier	Catalogue Number
PX1™ PCR Plate Sealer*	Bio-Rad	1814000
2 µL Pipettes	General Laboratory Supplier	Varies
10 µL Pipettes	General Laboratory Supplier	Varies
20 µL Pipettes	General Laboratory Supplier	Varies
200 µL Pipettes	General Laboratory Supplier	Varies
1000 µL Pipettes	General Laboratory Supplier	Varies
100 mL Single Channel Pipette Controller	General Laboratory Supplier	Varies
Vacuum Concentrator	General Laboratory Supplier	Varies
Thermal Cycler	General Laboratory Supplier	Varies
Microcentrifuge	General Laboratory Supplier	Varies
Vortex Mixer	General Laboratory Supplier	Varies
Rotator SB3*	Stuart	SB3
12-Tube Magnetic Separation Rack*	NEB	S1509S
Magnum FLX Magnet 96 Well plate*	Alpaqua	A000400
4150 TapeStation system*	Agilent	G2992AA

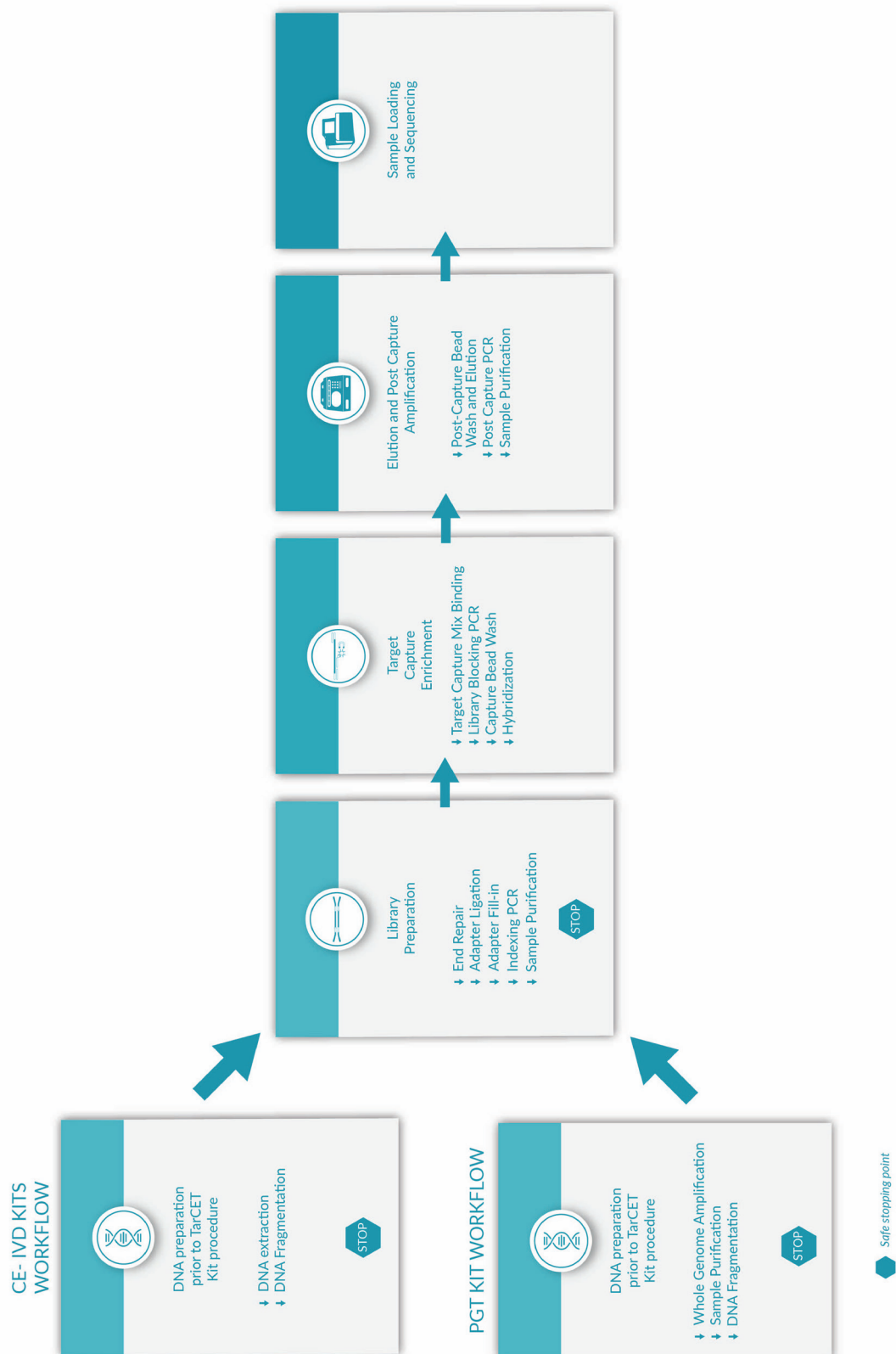
(\*or equivalent) (‡ Bioruptor® Pico, Cat # B01060010, has been discontinued. The new equivalent device is Bioruptor® Pico sonication device, Cat # B01080010)

# Specimen Collection

TarCET NGS Kits were developed and validated on buccal specimens collected using only swabs with a synthetic tip and an aluminum or plastic shaft, and placed in sterile tube containing desiccant. TarCET NGS Kit has been tested on hDNA Free FLOQSwabs, COPAN Diagnostics, Cat # 50C013D01.

# Test Procedure

## Workflow



## Sample Batching

Prior to starting the test procedure, please refer to the SIRIUS user guide to initiate a new batch of samples on the software with their unique identification numbers and indices used to barcode each sample.

## Recommendation for DNA Extraction

The DNA extraction kit used for the development and validation of TarCET IVD Kit is:

- QIAamp Blood Mini Kit (Qiagen, Cat # 51104) for all TarCET NGS assays.

## DNA Fragmentation and Quality Check

1. Quantify the extracted DNA using a High-Sensitivity fluorometric method (Qubit Fluorometer).
2. Fragment the extracted DNA to an average length of 200-250 bp. *For the development and validation of TarCET IVD Kit, fragmentation was performed using the Bioruptor® Pico (Diagenode, Cat # B01060010) according to the manufacturer's instructions.*
3. Quantify the fragmented sample using a High-Sensitivity fluorometric method (Qubit Fluorometer). Ensure that you have 250 ng of DNA in a total of 20 µL prior to proceeding to library preparation. Agilent Tapestation can be used to ensure desired fragment size.

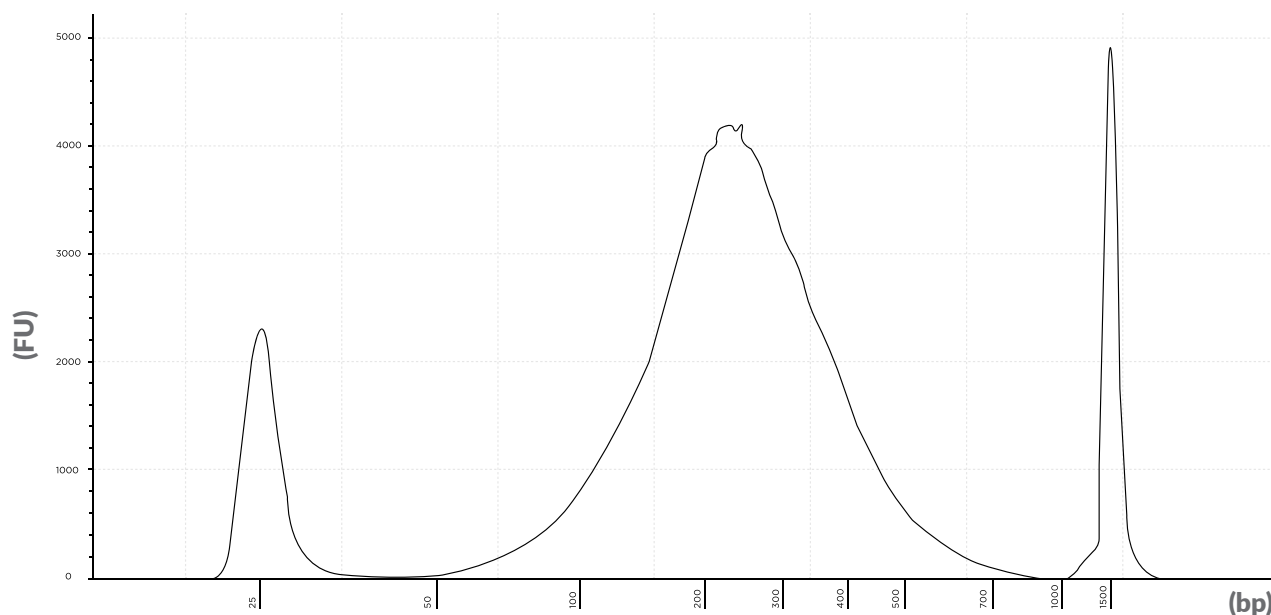


Figure 1: Representative electropherogram of post-sonicated DNA sample.  
Accepted fragment size peak ~ 200 bp - 250 bp.



Safe stop: Fragmented DNA can be stored overnight at -20°C.



## Library Preparation

1. Thaw all Library Preparation reagents on ice and program a thermal cycler as described in Table 2 below.
2. Transfer 20  $\mu\text{L}$  of the extracted DNA (250 ng) per sample to a PCR plate (PL2) or 0.2 mL PCR tubes, then place the samples on ice.

## End Repair

3. Prepare the End Repair reaction master mix according to Table 1. Vortex, centrifuge briefly, and then place the tube on ice.


Reagent Code	Reagent Name	1 rxn ( $\mu\text{L}$ )	Storage
1R1	ATP	8	TarCET Kit Box 1 (-20°C) 
1R2	dNTP	0.84	
1R3	End-repair Buffer	4.2	
1R4	End-repair Enzyme 1	0.5	
1R5	End-repair Enzyme 2	0.5	
N/A	HPLC water	1.26	
Total Master Mix Volume		15.3	

Table 1 End Repair reaction Master Mix

4. Add 15.3  $\mu\text{L}$  of the End Repair reaction mix to each well/tube containing DNA samples to a final volume of 35.3  $\mu\text{L}$  and seal the plate/tubes.
5. Vortex or mix by pipetting and briefly centrifuge the samples.
6. Place the samples in a thermal cycler with the End Repair reaction program as described in Table 2.

## Heated Lid at 99°C

Temperature (°C)	Time
20	30 min
75	20 min
16	$\infty$

Table 2 End Repair reaction conditions

### Adapter Ligation

7. Prepare the Adapter Ligation reaction master mix according to Table 3. Vortex, centrifuge briefly, and then place the tube on ice.


Reagent Code	Reagent	1 rxn (μL)	Storage
1G1	Ligation Enzyme	1	TarCET Kit Box 1 (-20°C) 
1G2	Adapters	1.7	
Total Master Mix Volume		2.7	

Table 3 Adapter Ligation reaction Master Mix

8. Add 2.7 μL of the Adapter Ligation reaction master mix to each well/tube containing the samples to a final volume of 38 μL.
9. Thoroughly vortex to mix well and briefly centrifuge the samples. When using a plate, seal with adhesive film.
10. Place the samples in a thermal cycler with the Adapter Ligation reaction program as described in Table 4.

### Lid Off

Temperature (°C)	Time
25	25 min
4	∞

Table 4 Adapter Ligation reaction conditions

### Adapter Fill-in

11. Prepare the Adapter Fill-in reaction master mix according to Table 5. Vortex, centrifuge briefly, and then place the tube on ice.


Reagent Code	Reagent	1 rxn (μL)	Storage
1B1	Adapter Fill-in Buffer	0.2	TarCET Kit Box 1 (-20°C) 
1B2	Adapter Fill-in Enzyme	2	
N/A	HPLC water	1.8	
Total Master Mix Volume		4	

Table 5 Adaptor Fill-in reaction Master Mix

12. Add 4  $\mu\text{L}$  of the Adapter Fill-in reaction master mix to each well/tube containing the samples to a final volume of 42  $\mu\text{L}$ .
13. Thoroughly vortex to mix well and briefly centrifuge the samples.
14. Place the samples in a thermal cycler with the Adapter Fill-in reaction program as described in Table 6.

#### Heated Lid at 99°C

Temperature (°C)	Time
65	20 min
80	20 min
4	$\infty$

Table 6 Adapter Fill-in reaction conditions

#### Indexing PCR

15. Prepare the Index PCR reaction master mix according to Table 7. Vortex, centrifuge briefly, and then place the tube on ice.


Reagent Code	Reagent	1 rxn ( $\mu\text{L}$ )	Storage
1W2	Indexing Buffer	12	TarCET Kit Box 1 (-20°C) 
1W3	dNTP	0.6	
1W4	Indexing Enzyme	0.6	
Total Master Mix Volume		13.2	

Table 7 Index PCR reaction Master Mix

16. Transfer 13.2  $\mu\text{L}$  of master mix to the samples and mix well by pipetting up and down.
17. Pierce the foil of the index plate (UltraVerse Index Oligos) and add to each well/tube containing the samples, 4.8  $\mu\text{L}$  of pre-mixed unique index primer set.



**Important note:** Each index pair is unique to a sample and no Index oligo pair should be used twice when running samples in the same run.

18. Place the samples in a thermal cycler and perform the PCR reaction using the following conditions as described in Table 8.

#### Heated Lid at 99°C

Temperature (°C)	Time	Cycles
95	3 min	x1
95	30 sec	x12
60	30 sec	
72	30 sec	
72	3 min	x1
4	∞	

Table 8 Index PCR reaction conditions

#### Sample Purification

19. Equilibrate the Purification Beads (● 2K1, TarCET Kit Box 2) at RT for at least 30 minutes before use. Prepare fresh 80% ethanol in HPLC water.
20. Transfer the PCR amplified libraries (60 µL) in a 500 µL LoBind deep well plate (PL1) or 1.5 mL LoBind tubes.
21. Add 108 µL of well resuspended Purification Beads to each sample and mix well by pipetting >10 times.
22. Incubate for 10 minutes at RT and pipette up and down 10 times, every 2 minutes.
23. Place the plate/tubes on a magnetic stand for 5 minutes or until the solution is clear.
24. Remove and discard the supernatant without disturbing the beads.
25. Wash the beads by adding 200 µL of 80% ethanol to each well/tube, while on magnet, and incubate for 30 seconds. Aspirate out ethanol and discard.
26. Repeat step 25. Remove as much excess ethanol as possible.
27. Incubate the beads on the magnetic stand for 2 minutes to dry.
28. Remove the plate/tubes from the magnetic stand and elute by adding 20 µL HPLC water to each well/tube and mix well by pipetting >10 times, until homogenous. Incubate for 2 minutes at RT.
29. Place the plate/tubes back on the magnetic stand to separate the beads from the solution for 2 minutes, or until the solution is clear. Then carefully transfer the supernatant into a new plate (PL2) or tubes.
30. Quantify the libraries using a nanodrop spectrophotometer.



Safe stop: Libraries can be stored overnight at -20°C.

## Targeted Capture Enrichment

### Preparation of Reagents and Samples

1. Thaw all Capture Preparation reagents on ice, dilute 1.05 mL of Wash Buffer 1 (3C1) (2X) with 1.05 mL of HPLC water for a total volume of diluted Wash Buffer 1 (3C1) of 2.1 mL (1X).
2. Set two thermo blocks at 100°C and 50°C and the oven at 65°C. Pre-heat 1.4 mL of diluted Wash Buffer 1 (3C1) (1X) in the thermo block set at 50°C.
3. Add the appropriate amount of library (Table 9) to a LoBind PCR plate (PL3) and add HPLC water to a total volume of 12 µL. Place the plate on ice, ready to be used in step 10.

Assay	Amount of Library required for capture preparation (µg)
TarCET Metabolic	2
TarCET Aortopathy	1.2
TarCET Arrhythmia	1.2
TarCET Cardiomyopathy	1.2
TarCET Congenital Heart Defects	1.2
TarCET FH/PH/RAS	1.2
TarCET Cardiac Comprehensive	1.2
TarCET Hereditary Cancer	2
TarCET Infertility	1.2
TarCET Neonatal	1.2
TarCET Carrier Screening Core	1.2
TarCET Carrier Screening Comprehensive	1.2

Table 9 Amount of Library required for Target Capture Reaction

### Target Capture Mix Binding

4. Add 12 µL of Target Capture Mix (1L6) and 12 µL of Wash Buffer 1 (3C1) (2X) per library sample to a clean 1.5 mL LoBind tube. Denature by incubating the tube at 100°C for 6 minutes, and then placing it immediately on ice for 10 minutes. Keep on ice, ready to be used in step 7.
5. Transfer 700 µL of diluted Wash Buffer 1 (3C1) (1X) in a new 1.5 mL LoBind tube and add 14 µL of Capture Beads (●2L1) per library sample. Place the tube on a magnetic stand for 2 minutes, or until the solution is clear, then carefully discard the supernatant.
6. Remove the tube from the magnetic stand and resuspend the beads with 700 µL of Wash Buffer 3 (3C2). Then place the tube on the magnetic stand for 2 minutes, or until the solution is clear, and carefully discard the supernatant.
7. Remove the tube from the magnetic stand and resuspend the beads with the denatured Target Capture Mix from step 4.

- Place the Capture Beads-Target Capture Mix on a rotator, for 30 minutes at RT on a 25 cm radius wheel rotating at 18 rpm.

### Library Blocking PCR

- During the 30 minute incubation, prepare the Hybridization master mix according to Table 10. Mix carefully to avoid bubbles, briefly centrifuge the tube, and keep it at RT.



Reagent Code	Reagent	1 rxn (μL)	Storage
1L1	Blocking Oligos 1	3	TarCET Kit Box 1 (-20°C) 
1L3	Blocking Buffer	5	
1L4	Blocking Agent 1	7	
1L5	Blocking Agent 2	3	
3L1	Hybridization Buffer*	30	TarCET Kit Box 3 (RT) 
Total Master Mix Volume		48	

Table 10 Hybridization reaction Master Mix

\*Slowly pipette the Hybridization Buffer to minimize foaming. Do not vortex.

- Add 48 μL of Hybridization Master mix per library sample in the PL3 plate containing the libraries from step 3. Heat seal the plate, mix well, briefly centrifuge the plate and place it in a thermal cycler with the Blocking PCR reaction program as described in Table 11.

### Heated Lid at 99°C

Temperature (°C)	Time
95	3 min
37	30 min
37	∞

Table 11 Blocking PCR reaction conditions

### Capture Bead Wash

11. When the 30 minutes incubation of the Target Capture Mix-Capture Beads mixture is completed (step 8), place the tube on a magnetic stand for 2 minutes, or until the solution is clear, and then carefully discard the supernatant.
12. Remove the tube from the magnetic stand and resuspend the beads with 700  $\mu$ L of diluted Wash Buffer 1 (3C1) (1X) preheated at 50°C.
13. Place the beads on a magnetic stand for 2 minutes, or until the solution is clear, and then carefully discard the supernatant.
14. Repeat steps 12 and 13 for a total of 2 washes.
15. Resuspend the beads in 50  $\mu$ L of Wash Buffer 3 (3C2) per library sample. Mix well by pipetting and briefly centrifuge. Then transfer 50  $\mu$ L of the resuspended beads in a new LoBind plate (PL3), filling a well for every sample being captured.

### Hybridization

16. When step 10 is completed, place the resuspended beads from step 15 on a magnetic stand for 2 minutes, or until the solution is clear. Then carefully discard the supernatant.
17. Remove the plate from the magnetic stand and resuspend the beads with the hybridization mix containing the libraries from step 10.
18. Heat-seal the plate at 170°C for 3 seconds and let it to cool down for 1 minute. Vortex the plate thoroughly.
19. Incubate the plate at 65°C for at least 15 hours in a rotating oven of 10 cm radius at 10 rpm.

### Elution and Post-Capture Amplification

#### Post-Capture Bead Wash and Elution

1. Thaw all Post-capture reagents on ice and dilute 400  $\mu$ L of Wash Buffer 1 (3C1) (2X) with 400  $\mu$ L of HPLC water, for a total volume of diluted Wash Buffer 1 (3C1) of 800  $\mu$ L (1X) per sample. Set a thermal cycler at 60°C with a heated lid set at 65°C.
2. Pre-heat 400  $\mu$ L per sample of Wash Buffer 2 (3Y1) in the pre-heated thermal cycler set at 60°C.
3. Remove the sample plate from the hybridization oven, spin down, unseal and place it on a magnetic stand for 1 minute and carefully remove the supernatant.
4. Wash the beads with 200  $\mu$ L of diluted Wash buffer 1 (3C1) (1X) while on magnet. Incubate for 1 minute, or until the solution is clear, and remove the supernatant. Repeat this step for a total of 3 washes.
5. Place the plate on bench and resuspend the beads with 200  $\mu$ L of preheated Wash Buffer 2 (3Y1).
6. Seal the plate with adhesive seal and incubate at 60°C for 2 minutes in the thermal cycler.
7. Remove the plate from the thermal cycler and unseal.
8. Place the plate on a magnetic stand for 1 minute, or until the solution is clear, to capture the beads, and then carefully remove the supernatant.

9. Remove the plate from the magnetic stand and repeat steps 5-8.
10. Wash the beads with 200  $\mu$ L of diluted Wash Buffer 1 (3C1) (1X) while on magnet. Incubate for 1 minute, or until the solution is clear, and carefully remove the supernatant.
11. Resuspend the beads in 33  $\mu$ L of Wash Buffer 3 (3C2) and transfer the resuspended beads in a clean PCR column or a new LoBind PCR plate (PL3).
12. Heat-seal and incubate at 95°C for 3 minutes in a thermal cycler, then vortex thoroughly for 10 seconds and briefly centrifuge the plate.
13. Place the plate on a magnetic stand for 1 minute, or until the solution is clear, to capture the beads and transfer the supernatant in a new PCR plate (PL2).

### Post-Capture PCR

14. Prepare the Post-capture PCR reaction master mix according to Table 12. **Perform 3 Post-capture PCR reactions per sample.** Vortex, centrifuge briefly, and then place the tube on ice.


Reagent Code	Reagent	1 rxn ( $\mu$ L)	Storage
1Y1	Post-capture Primer 1	1.5	TarCET Kit Box 1 (-20°C) 
1Y2	Post-capture Buffer	10	
1Y3	dNTP	0.5	
1Y4	Post-capture Enzyme	0.5	
1Y5	Post-capture Primer 2	1.5	
N/A	HPLC water	26	
Total Master Mix Volume		40	
Template DNA		10	

Table 12 Post-capture PCR reaction Master Mix

15. Transfer 40  $\mu$ L of master mix to each sample and mix well by pipetting.
16. Place the plate in a thermal cycler with the Post-capture PCR reaction program as described in Table 13.



**Heated Lid at 99°C**

Temperature (°C)	Time	Cycles
95	3 min	x1
95	30 sec	x11
55	30 sec	
72	30 sec	
72	3 min	x1
4	∞	

Table 13 Post-capture PCR conditions

**Post-Capture PCR Sample Purification**

17. Equilibrate the Purification Beads (●2K1, TarCET Kit Box 2) at RT for at least 30 minutes before use. Prepare fresh 80% ethanol in HPLC water.
18. Pool the 3 Post-capture PCR replicates of each sample together, in a 500 µL LoBind deep well plate (PL1) or 1.5 mL LoBind tubes.
19. Add 270 µL of well resuspended Purification beads to each sample and mix well by pipetting >10 times.
20. Incubate for 10 minutes at RT and pipette up and down 10 times, every 2 minutes.
21. Place the plate/tubes on a magnetic stand for 5 minutes or until the solution is clear.
22. Without disturbing the beads, remove and discard the supernatant.
23. Wash the beads by adding 200 µL of freshly prepared 80% ethanol to each well/tube, while on magnet, and incubate for 30 seconds. Aspirate out ethanol and discard.
24. Repeat step 23 for a total of 2 washes. Remove as much excess ethanol as possible.
25. Incubate the beads on the magnetic stand for 2 minutes to dry.
26. Remove the plate/tubes from the magnetic stand and elute by adding 20 µL HPLC water to each well/tube and mix well by pipetting >10 times, until homogenous. Incubate for 2 minutes at RT.
27. Place the plate/tubes on the magnetic stand to separate the beads from the solution for 2 minutes, or until the solution is clear. Then carefully transfer the supernatant into a new PCR plate (PL2) or tubes.

**Post-Capture DNA Quality Check**

28. Quantify the amplified Post-captured sample using a High-Sensitivity fluorometric method (Qubit Fluorometer).
29. Assess the size distribution of the Post-captured sample using Agilent TapeStation.

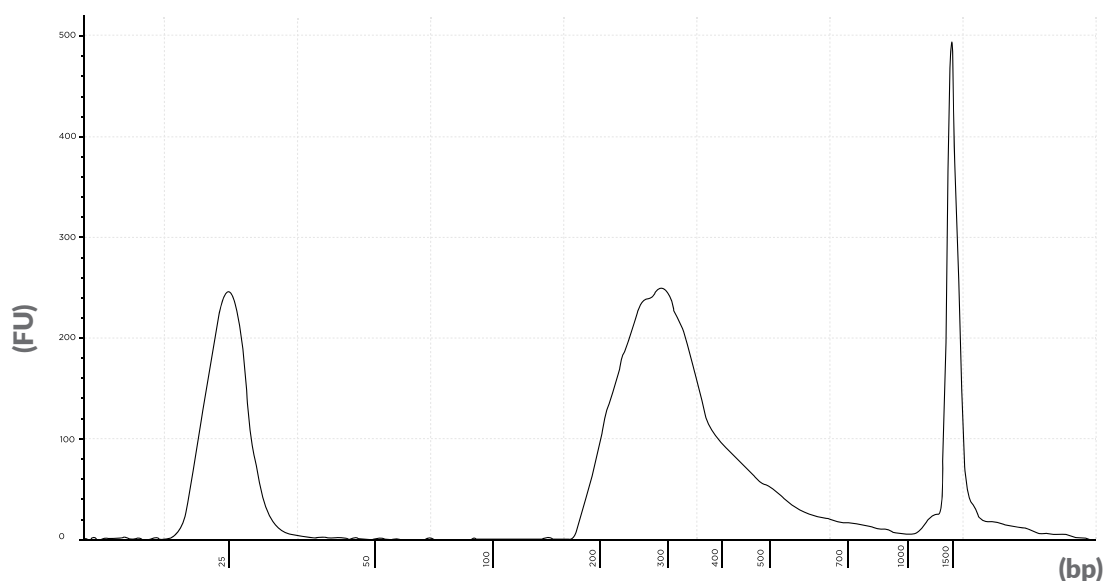


Figure 2: Representative electropherogram of Post-captured DNA sample.  
Accepted fragment size peak > 250 bp.

## Sample Preparation for Sequencing

The assay has been validated on Illumina sequencing platforms.

### Sample Pooling Guidelines:

- In preparation for loading the samples for sequencing, please refer to the SIRIUS user guide for instructions on loading quantities per assay and on how to proceed with importing data information necessary for the analysis.
- Pool all samples in a single 1.5 mL tube to sequence together according to the SIRIUS user guide instructions and loading quantities in ng mentioned in the Sirius application.
- Samples are given a unique identification run number in this format: ABC\_1234. The first letters refer to the laboratory's ID number that is generated during Sign Up on the SIRIUS web application. The four digits at the end are given by the lab for each sample.

### DNA Denaturation Guidelines:

#### For MiSeq System sequencing runs

1. Concentrate the pooled samples using a vacuum concentrator set at 30°C. Follow the manufacturer's instructions to reduce the volume of the pooled samples to  $\leq 10$   $\mu$ L. Adjust volume to 10  $\mu$ L with HPLC water if necessary. Avoid total desiccation.
2. Thaw a tube of 10 nM PhiX Control stock (Illumina, FC-110-3001) and dilute the PhiX Control stock to 2 nM by combining the following volumes in an empty 1.5 mL tube and mix by pipetting up and down:
  - 2  $\mu$ L of 10 nM PhiX
  - 8  $\mu$ L of HPLC water
3. Denature the 10  $\mu$ L of the concentrated pooled samples by adding 10  $\mu$ L of freshly diluted 0.1 N NaOH and mix by pipetting up and down. Incubate at room temperature for 5 minutes.

4. Denature the 10  $\mu\text{L}$  of the diluted PhiX Control by adding 10  $\mu\text{L}$  of freshly diluted 0.1 N NaOH in the tube and mix by pipetting up and down. Incubate at room temperature for 5 minutes.
5. Add 980  $\mu\text{L}$  of HT1 Buffer to each tube. Mix by inverting the tubes several times. The total volume is now 1 ml in each tube.
6. Perform a dilution of the denatured sample by combining 100  $\mu\text{L}$  HT1 buffer and 900  $\mu\text{L}$  of the denatured sample in a new 1.5 mL tube. Mix by inverting the tubes several times.
7. Perform a dilution of the denatured PhiX Control by combining 100  $\mu\text{L}$  HT1 buffer and 900  $\mu\text{L}$  of the denatured PhiX in a new 1.5 mL tube. Mix by inverting the tubes several times.
8. In a new 1.5 mL tube, combine 800  $\mu\text{L}$  of the diluted sample and 200  $\mu\text{L}$  of the diluted PhiX Control.
9. Refer to the Illumina Miseq User Guide for instructions on how to perform the preparation of the Miseq sequencing reagents, sample loading and how to operate the MiSeq System.
10. Refer to the Sequencer Setup chapter in Sirius User Guide on how to setup the sequencing run, using the Manual Option Setup.

#### For NextSeq System sequencing runs

1. Concentrate the pooled samples using a vacuum concentrator set at 30°C. Follow the manufacturer's instructions to reduce the volume of the pooled samples to  $\leq 10$   $\mu\text{L}$ . Adjust volume to 10  $\mu\text{L}$  with HPLC water if necessary. Avoid total desiccation.
2. Thaw a tube of 10 nM PhiX Control stock (Illumina, FC-110-3001) and dilute the PhiX Control to 2 nM by combining the following volumes in an empty 1.5 mL tube and mix by pipetting up and down:
  - 2  $\mu\text{L}$  of 10 nM PhiX
  - 8  $\mu\text{L}$  of HPLC water
3. Denature the 10  $\mu\text{L}$  of the concentrated pooled samples by adding 10  $\mu\text{L}$  of freshly diluted 0.2 N NaOH and mix by pipetting up and down. Incubate at room temperature for 5 minutes.
4. Denature the 10  $\mu\text{L}$  of the diluted PhiX Control by adding 10  $\mu\text{L}$  of freshly diluted 0.2 N NaOH and mix by pipetting up and down. Incubate at room temperature for 5 minutes.
5. Once the incubation period is over, add 10  $\mu\text{L}$  of 200mM Tris-HCl, pH 7, to each tube.
6. Add 970  $\mu\text{L}$  of HT1 Buffer to each tube. Mix by inverting the tubes several times. The total volume is now 1 ml in each tube.
7. Perform a dilution of the denatured sample by combining 1287  $\mu\text{L}$  HT1 buffer and 13  $\mu\text{L}$  of the denatured sample in a new 1.5 mL tube. Mix by inverting the tubes several times.
8. Perform a dilution of the denatured PhiX Control by combining 1287  $\mu\text{L}$  HT1 buffer and 13  $\mu\text{L}$  of the denatured PhiX in a new 1.5 mL tube. Mix by inverting the tubes several times.
9. In a new 1.5 mL tube, combine 1040  $\mu\text{L}$  of the diluted sample and 260  $\mu\text{L}$  of the diluted PhiX Control.

10. Refer to the Illumina Nextseq User Guide for instructions on how to perform the preparation of the Nextseq sequencing reagents, sample loading and how to operate the NextSeq Sequencer.
11. Refer to the Sequencer Setup chapter in Sirius User Guide on how to setup the sequencing run, using the Manual Option Setup.

#### For NovaSeq 6000 sequencing runs

1. Add the appropriate volume of 10mM Tris-HCl, pH 8.5, in the pooled samples to result in 100  $\mu$ L final volume.
2. Spike-in 1% nondenatured PhiX as follows:
  - a) Dilute the 10 nM PhiX Control stock, (Illumina, FC-110-3001), to 2.5 nM using 10 mM Tris-HCl, pH 8.5, by adding 3  $\mu$ L of 10 mM Tris-HCl, pH 8.5, to 1  $\mu$ L of 10 nM PhiX.
  - b) Add 0.6  $\mu$ L of nondenatured 2.5 nM PhiX to the tube of diluted nondenatured pooled samples. You may store the remaining diluted PhiX at -20°C for up to 3 months.
3. Denature the pooled samples including PhiX by adding 25  $\mu$ L of freshly diluted 0.2 N NaOH.
4. Vortex briefly and spin.
5. Incubate at room temperature for 8 minutes to denature.
6. Once the incubation period is over add 25  $\mu$ L of 400 mM Tris-HCl, pH 8, to the tube of the denatured pooled samples and PhiX.
7. Vortex briefly and spin.
8. Transfer the full volume of the denatured pooled samples and PhiX to the library tube provided with the NovaSeq 6000 Reagent Kit.
9. Immediately proceed to loading the library tube into the cluster cartridge and setting up the run. The reagent cartridges, including the library tube, must be loaded onto the instrument within 30 minutes.
10. Refer to the Illumina Novaseq 6000 User Guide for instructions on how to perform the preparation of the Novaseq 6000 sequencing reagents, sample loading and how to operate the Novaseq 6000 Sequencer.
11. Refer to the Sequencer Setup chapter in Sirius User Guide on how to setup the sequencing run, using the Manual Option Setup.

## Results Interpretation

SIRIUS can be used to visualize the results after sequencing in a graphical representation or tabular form. Please use the SIRIUS user guide to perform the post-analytical data management.

## Limitations of the Procedure

All TarCET NGS assays aim to detect all variants relevant to the genes listed in [www.medicover-genetics.com](http://www.medicover-genetics.com). Variants that fall outside of the targeted regions are not intended to be detected by this assay. Unless otherwise noted, sequence changes (single nucleotide variants, SNVs, and small insertions and deletions, INDELs) in the promoter and other non-coding regions are not covered by these tests. In cases where two variants are identified in a gene, the test does not distinguish whether these are on one chromosome (in cis) or on different chromosomes (in trans). Certain types of genetic abnormalities such as inversions, rearrangements, polyploidy and epigenetic modifications are not covered by these tests. Certain sequence changes (SNVs and INDELs) in targeted regions adjacent to or within repeats, sequences of high homology such as segmental duplications and pseudogenes, as well as regions of high/low GC-content may not be detected. The test can detect copy number variants (CNVs) down to a few exons level resolution. All positive CNVs should be confirmed using an orthogonal method. The test cannot detect CNVs at genomic regions with either low mappability or containing repeats, pseudogenes and high/low GC-content. Detection of CNVs using NGS has lower sensitivity/specificity than orthogonal quantification methods, therefore the absence of reported CNVs does not guarantee the absence of CNVs. The lack of disease-causing variants in the targeted genes diminishes but does not exclude the possibility of a disease associated syndrome. Although the tests are highly accurate there is still a possibility for false positive and false negative results.

## Clinical Validation

The clinical validation of the TarCET Metabolic, TarCET Cardiac, TarCET Hereditary Cancer, TarCET Infertility, TarCET Neonatal and TarCET Carrier screening assays was performed on genomic DNA isolated from buccal swabs from consented individuals. The following two groups were included in the validation studies: a) Individuals of unknown variant status and b) Individuals who carry a genetic disorder previously identified by an independent laboratory. SNVs, INDELs and CNVs detected in individuals of unknown variant status (group a) were confirmed by Sanger sequencing (for SNVs/INDELs) and qPCR or MLPA (for CNVs). Variants were classified based on the American College of Medical Genetics and Genomics 2015 guidelines.

# Appendix

The tables with all the genes that are included in each Kit can be found online at [www.medicover-genetics.com](http://www.medicover-genetics.com).

## TarCET Metabolic Clinical Validation

Analytical concordance*	Estimate (95% CI)
Single Nucleotide Variants (SNVs) + Insertions and Deletions (INDELs)	
Sensitivity (SNVs + INDELs)	100% (88-100%)
Specificity (SNVs + INDELs)	100% (99.9-100%)
Copy Number Variations (CNVs)	
Sensitivity (CNVs)	100% (81.5 – 100%)
Specificity CNV calling	>99.9%
Simulated sensitivity (1 exon)	70.4%
Simulated sensitivity (2 exons)	85.2%
Simulated sensitivity (3 exons)	94.7%

Table 14 Validation table for SNVs, INDELs and CNVs of TarCET Metabolic panel

\* Analytic concordance table between the Metabolic assay and other orthogonal methods (i.e. Sanger sequencing, qPCR, or other NGS-based assays).

## TarCET Cardiac Clinical Validation

Analytical concordance*	Estimate (95% CI)
Single Nucleotide Variants (SNVs) + Insertions and Deletions (INDELs)	
Sensitivity (SNVs + INDELs)	100% (79.4-100%)
Specificity (SNVs + INDELs)	100% (99.8-100%)
Copy Number Variations (CNVs)	
Sensitivity (CNVs)	100% (66.4–100%)
Specificity CNV calling	>99.9%
Simulated sensitivity (1 exon)	76.5%
Simulated sensitivity (2 exons)	87.1%
Simulated sensitivity (3 exons)	91.6%

Table 15 Validation table for SNVs, INDELs and CNVs of TarCET Cardiac panels

\* Analytic concordance table between the Cardio-panels assay and other orthogonal methods (i.e. Sanger sequencing, qPCR or other NGS-based assays).

TarCET Hereditary Cancer Clinical Validation

Analytical concordance*	Estimate (95% CI)
Single Nucleotide Variants (SNVs) + Insertions and Deletions (INDELs)	
Sensitivity (SNVs + INDELs)	100% (94-100%)
Specificity (SNVs)	100% (99.999-100%)
Copy Number Variations (CNVs)	
Sensitivity (CNV 1 exon)	100% (69-100%)
Sensitivity (CNV 2 exons)	100% (54-100%)
Sensitivity (CNV 3-7 exons)	100% (78-100%)
Specificity CNV calling	100%
Simulated sensitivity (2 exons)	90.4%
Simulated sensitivity (4 exons)	98%
Simulated sensitivity (10 exons)	99.9%

Table 16 Validation table for SNVs, INDELs and CNVs of TarCET Hereditary Cancer panel

\* Analytic concordance method between the Hereditary assay and other orthogonal methods (i.e. Sanger sequencing, qPCR).



## TarCET Infertility Clinical Validation

Analytical concordance*	Estimate (95% CI)
Single Nucleotide Variants (SNVs) + Insertions and Deletions (INDELs)	
Sensitivity (SNVs + INDELs)	100% (87-100%)
Specificity (SNVs + INDELs)	100% (99.9-100%)
Copy Number Variations (CNVs)	
Sensitivity (CNVs)	100% (40–100%)
Specificity CNV calling	>99,99%
Simulated sensitivity (1 exon)	80.1%
Simulated sensitivity (2 exons)	92.5%
Simulated sensitivity (3 exons)	98.8%

Table 17 Validation table for SNVs, INDELs and CNVs of TarCET Infertility panel

\* Analytical concordance table between this assay and other orthogonal methods (i.e. Sanger sequencing, qPCR, MLPA etc).

TarCET Neonatal Clinical Validation

Analytical concordance*	Estimate (95% CI)
Single Nucleotide Variants (SNVs) + Insertions and Deletions (INDELs)	
Sensitivity (SNVs + INDELs)	100% (87-100%)
Specificity (SNVs + INDELs)	100% (99.8-100%)
Copy Number Variations (CNVs)	
Sensitivity (CNV 1 exon)	100% (74-100%)
Sensitivity (CNV 2 exons)	100% (72-100%)
Sensitivity (CNV > 2 exons)	100% (82-100%)
Specificity CNV calling	>99.99%
Simulated sensitivity (1 exon)	88.6%
Simulated sensitivity (2 exons)	90.1%
Simulated sensitivity (3 exons)	96.4%

Table 18 Validation table for SNVs, INDELs and CNVs of TarCET Neonatal panel

\* Analytical concordance table between this assay and other orthogonal methods (i.e. Sanger sequencing, qPCR, MLPA etc).

## TarCET Carrier Clinical Validation

Analytical concordance*	Estimate (95% CI)
Single Nucleotide Variants (SNVs) + Insertions and Deletions (INDELs)	
Sensitivity (SNVs + INDELs)	100% (87-100%)
Specificity (SNVs + INDELs)	100% (99.8-100%)
Copy Number Variations (CNVs)	
Sensitivity (CNV 1 exon)	100% (74-100%)
Sensitivity (CNV 2 exons)	100% (72-100%)
Sensitivity (CNV > 2 exons)	100% (82-100%)
Specificity CNV calling	>99.99%
Simulated sensitivity (1 exon)	88.6%
Simulated sensitivity (2 exons)	90.1%
Simulated sensitivity (3 exons)	96.4%

Table 19 Validation table for SNVs, INDELs and CNVs of TarCET Carrier panel

\* Analytical concordance table between this assay and other orthogonal methods (i.e. Sanger sequencing, qPCR, MLPA etc).

## Support Contact Info

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