MEDICOVER
GENETICS

TarCET Kit

Instructions For Use

CE IVD

For Diagnostic Procedure Only
Proprietary Document: WI-03-100-TT-ENG
Version 6.0

Medicover Genetics Ltd.
31 Neas Engomis Str, 2409 Engomi,
Nicosia - Cyprus

www.medicover-genetics.com



Revision History

Date (YYYY-MM-DD)	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 Medicover
2023-04-07	4.0	Clarifications on Workflow Steps and Rebranding
2023-12-18	4.1	Edits on the User-Supplied Equipment List
2024-02-15	5.0	Modifications to Simplify the Targeted Capture Enrichment Procedure and Restructuring for Compliance with IVDR
2024-09-18	5.1	Edits on the User-Supplied Consumables List to Add a Reagent
2024-11-29	5.2	Edits to introduce sequencing on Element Biosciences platforms. Changes in Page 8 for clarification purposes and removal of Summary of Safety and Performance (SSP) in Page 36
2025-04-04	6.0	Refinement for clarification purposes

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Symbols


Keep dry


Consult instructions for use (IFU)


Temperature


Contains sufficient for N tests


Expiration date


GHS07 health hazard


1.5mL tube


20mL tube



Item number


Keep away from sunlight



In-Vitro diagnostic medical device


Manufacturer


Telephone number


Email address



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


Box number


10mL tube


30mL tube



Lot number

Trademarks and Disclaimers

Use of the TarCET 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 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
PH	Pulmonary Hypertension
Pk	Package
P/N	Product Number
Qty	Quantity
RT	Room Temperature
Rxn	Reaction
SNVs	Single Nucleotide Variants
SSP	Summary of Safety and Performance

Intended Use

TarCET Kit is a reagent kit including library preparation and targeted capture enrichment. The kit is intended for use with genomic DNA from human cells for library preparation and capture enrichment for Next-Generation Sequencing (NGS) applications. The kit can be used to target specific regions of the genome with validated DNA hybridization probe panels supplied in the kit. All further downstream analyses can be performed in conjunction with the IVD VEGA analysis software, not provided in this kit, or using an independent IVD analysis software.

TarCET Portfolio

TarCET Kits are reagent kits designed for DNA preparation for downstream analysis via NGS sequencing. However, TarCET Kits cannot be used directly to identify a genetic condition, but only in combination with an IVD analysis software.

The following validated DNA hybridization probe panels can be supplied in the kit:

- TarCET Metabolic Panel (Cat # ET201-00-2016)
- TarCET Aortopathy Panel (Cat # ET203-00-2016)
- TarCET Arrhythmia Panel (Cat # ET204-00-2016)
- TarCET Cardiomyopathy Panel (Cat # ET205-00-2016)
- TarCET Congenital Heart Defects Panel (Cat # ET206-00-2016)
- TarCET Familial Hypercholesterolemia (FH), Pulmonary Hypertension (PH) and RASopathy (RAS) Panel (Cat # ET207-00-2016)
- TarCET Cardiac Comprehensive Panel (Cat # ET210-00-2016)
- TarCET Hereditary Cancer Panel (Cat # ET211-00-2016)
- TarCET Infertility Panel (Cat # ET212-00-2016)
- TarCET Neonatal Panel (Cat # ET213-00-2016)
- TarCET Carrier Screening Core Panel (Cat # ET214-00-2016) and TarCET Carrier Screening Comprehensive Panel (Cat # ET215-00-2016)

Principles of Procedure

Medicover Genetics TarCET Kit can be used in conjunction with the SIRIUS sample information and visualization software. The kit includes library preparation reagents and in solution hybridization reagents for DNA sequencing of multigene panels. It is used with unique indices allowing combination of multiple libraries onto a single sequencing run. The kit includes enough reagents for 16 library preparation and in-solution hybridization reactions.

The workflow consists of the following procedures:

- Sample Batching: Prior to starting the procedure, samples are grouped into batches created via the SIRIUS sample information management tool, where samples are assigned indices and the sequencing output is estimated for the run.
- DNA Fragmentation: Genomic DNA is fragmented to an average length of 200-250 bp either mechanically via sonication or enzymatically.
- Library Preparation: The DNA fragments undergo an end repair process to fill in 5' and 3' overhangs and convert them to blunt ends. Adapters are then ligated onto the DNA fragments. Adapters contain sequences that enable the capture of the samples on the surface of a sequencing flow cell for cluster generation and subsequent sequencing. Upon adapter ligation 5' overhangs are filled-in using an adapter fill-in enzyme. Next the DNA fragments are amplified using indexing primers, which assign a unique barcode to each sample and thus allow sample identification in subsequent sequencing. The indexed DNA fragments are purified using solid phase reverse immobilization beads.
- Targeted Capture Enrichment: Blocking agents are mixed with the purified library fragments to prevent adapter-to-adapter hybridization. Blocked library fragments are then hybridized to the predesigned TarCET Capture Mix, which is specific to each TarCET Kit, to enrich the target regions. The resulting complexes are coupled to magnetic Capture Beads, any unbound library fragments are washed out and the captured DNA fragments are eluted by heating. The post-capture DNA fragments are amplified using outer-bound adapter primers and subsequently the Post-Capture PCR products are purified using solid phase reverse immobilization beads.
- Sequencing: The enriched post-captured samples are pooled as determined by the WI-30 SIRIUS user guide instructions. Recommendations for DNA denaturation and sequencing conditions are provided in documents WI-46A DNA Denaturation Guidelines for Illumina Platforms and WI-46C DNA Denaturation and Sequencing Conditions for Element Biosciences Platforms. Sequencing results can be communicated through the IVD VEGA analysis software, not provided in the kit, to SIRIUS in qualitative or quantitative formats. Otherwise, an independent IVD analysis software device can be used for result interpretation.

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, and for the results to be considered valid. Additionally, control genetic material with known variants can be included to evaluate the laboratory process.

Storage and Handling

- Room temperature is defined as 15°C to 30°C.
- 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.
- 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 handling beads:
    1. The appropriate magnetic stand for the plate/tube should be used.
    2. Do not remove the plate/tube from the magnetic stand before the specified time.
    3. Do not disturb the plate/tube while on the magnetic stand, and do not agitate the bead pellet.
    4. 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.
    5. If there is liquid on the side of the plate/tube, briefly centrifuge.
    6. Ensure that all ethanol is removed from the bottom of the wells during wash steps. Residual ethanol may impact results.
    7. 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 (1L8), 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, except of the Hybridization Buffer (1L8), which must thaw on bench.

Warnings and Precautions

- The procedures 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.
- 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).
- 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.
- Any serious incident that has occurred in relation to the device must be reported to Medicover Genetics Ltd. via ivdsupport.genetics@medicover.com, providing all the necessary information to properly identify the medical device and the incident that has occurred.

Kit Contents

TarCET Kit Panels

Panels	Number of Tests	P/N
Metabolic	16	ET201-00-2016
Aortopathy	16	ET203-00-2016
Arrhythmia	16	ET204-00-2016
Cardiomyopathy	16	ET205-00-2016
Congenital Heart Defects	16	ET206-00-2016
FH, PH and RAS	16	ET207-00-2016
Cardiac Comprehensive	16	ET210-00-2016
Hereditary Cancer	16	ET211-00-2016
Infertility	16	ET212-00-2016
Neonatal	16	ET213-00-2016
Carrier Screening Core	16	ET214-00-2016
Carrier Screening Comprehensive	16	ET215-00-2016

TarCET Kit Reagents (Box 1)

Name	ID in Box	Container	Volume (µL)	Qty	Storage	P/N
ATP	1R1	1.5 mL	128	1	-20°C	ET100-01-01R1
dNTP	1R2	1.5 mL	14	1	-20°C	ET100-01-01R2
End-repair Buffer	1R3	1.5 mL	68	1	-20°C	ET100-01-01R3
End-repair Enzyme 1	1R4	1.5 mL	8	1	-20°C	ET100-01-01R4
End-repair Enzyme 2	1R5	1.5 mL	8	1	-20°C	ET100-01-01R5
Ligation Enzyme	1G1	1.5 mL	16	1	-20°C	ET100-01-01G1
Adapters	1G2	1.5 mL	28	1	-20°C	ET100-01-01G2
Adapter Fill-in Buffer	1B1	1.5 mL	4	1	-20°C	ET100-01-01B1
Adapter Fill-in Enzyme	1B2	1.5 mL	32	1	-20°C	ET100-01-01B2
Indexing Mix	1W5	1.5 mL	308	1	-20°C	ET200-01-01W5
Blocking Oligos 1	1L1	1.5 mL	20	1	-20°C	ET200-01-01L1
Blocking Agent 1	1L4	1.5 mL	80	1	-20°C	ET200-01-01L4
Blocking Buffer	1L7	1.5 mL	32	1	-20°C	ET200-01-01L7
Hybridization Buffer	1L8	1.5 mL	204	1	-20°C	ET200-01-01L8
Post-capture Primer 1	1Y1	1.5 mL	32	1	-20°C	ET200-01-01Y1
Post-capture Primer 2	1Y5	1.5 mL	32	1	-20°C	ET200-01-01Y5
Post-capture Mix	1Y6	1.5 mL	384	1	-20°C	ET200-01-01Y6

Name	ID in Box	Container	Volume (µL)	Qty	Storage	P/N
Target Capture Mix Metabolic	1L6	1.5 mL	64	1	-20°C	ET201-01-01L6
Target Capture Mix Aortopathy	1L6	1.5 mL	64	1	-20°C	ET203-01-01L6
Target Capture Mix Arrhythmia	1L6	1.5 mL	64	1	-20°C	ET204-01-01L6
Target Capture Mix Cardiomyopathy	1L6	1.5 mL	64	1	-20°C	ET205-01-01L6
Target Capture Mix Congenital Heart Defects	1L6	1.5 mL	64	1	-20°C	ET206-01-01L6
Target Capture Mix FH, PH and RAS	1L6	1.5 mL	64	1	-20°C	ET207-01-01L6
Target Capture Mix Cardiac Comprehensive	1L6	1.5 mL	64	1	-20°C	ET210-01-01L6
Target Capture Mix Hereditary Cancer	1L6	1.5 mL	64	1	-20°C	ET211-01-01L6
Target Capture Mix Infertility	1L6	1.5 mL	64	1	-20°C	ET212-01-01L6
Target Capture Mix Neonatal	1L6	1.5 mL	64	1	-20°C	ET213-01-01L6
Target Capture Mix Carrier Screening Core	1L6	1.5 mL	64	1	-20°C	ET214-01-01L6
Target Capture Mix Carrier Screening Comprehensive	1L6	1.5 mL	64	1	-20°C	ET215-01-01L6

TarCET Kit Reagents (Box 2)

Name	ID in Box	Container	Volume (µL)	Qty	Storage	P/N
Purification Beads	2K1	10 mL	2760	1	+4°C	ET200-02-02K1
Capture Beads	2L1	1.5 mL	640	1	+4°C	ET200-02-02L1

TarCET Kit Reagents (Box 3)

Name	ID in Box	Container	Volume (µL)	Qty	Storage	P/N
Wash Buffer A (2X)	3C3	30 mL	6400	1	RT	ET200-03-03C3
Wash Buffer B	3L1	20 mL	1760	1	RT	ET200-03-03L1
Stringent Wash Buffer	3Y2	20 mL	6400	1	RT	ET200-03-03Y2
Elution Buffer	3Y3	1.5 mL	320	1	RT	ET200-03-03Y3
Enhancer	3Y7	1.5 mL	32	1	RT	ET200-03-03Y7

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

TarCET 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 Kit should be stored at the temperatures indicated on the box labels.

User-Supplied Consumables and Equipment

Consumables

Item	Supplier	Catalogue Number
UltraVerse Index Oligos (Type A-D)	Medicover Genetics	EU100-00-100 (A-D)
dsDNA Shearase™ Plus	Zymo Research	E2018-200
Microseal® 'B' PCR Plate Sealing Film, adhesive, optical*	Bio-Rad	MSB 1001
PCR Plate Heat Seal, foil, pierceable*	Bio-Rad	1814040
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, 1 Pk TapeStation*	Agilent	5067-5598
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 Scientific	Q33226
Bioruptor® Pico*†	Diagenode	B01060010
NanoDrop™ 8000 Spectrophotometer* ‡	Thermo Fisher Scientific	ND8000LAPTOP
PX1™ PCR Plate Sealer*	Bio-Rad	1814000
2 µL Pipettes	General Laboratory Supplier	Varies

Item	Supplier	Catalogue Number
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
12-Tube Magnetic Separation Rack*	NEB	S1509S
Magnum FLX Magnet 96 Well plate* or DynaMag™-96 Side Skirted Magnet	Alpaqua or Thermo Fisher Scientific	A000400 or 12027
4150 TapeStation system*	Agilent	G2992AA

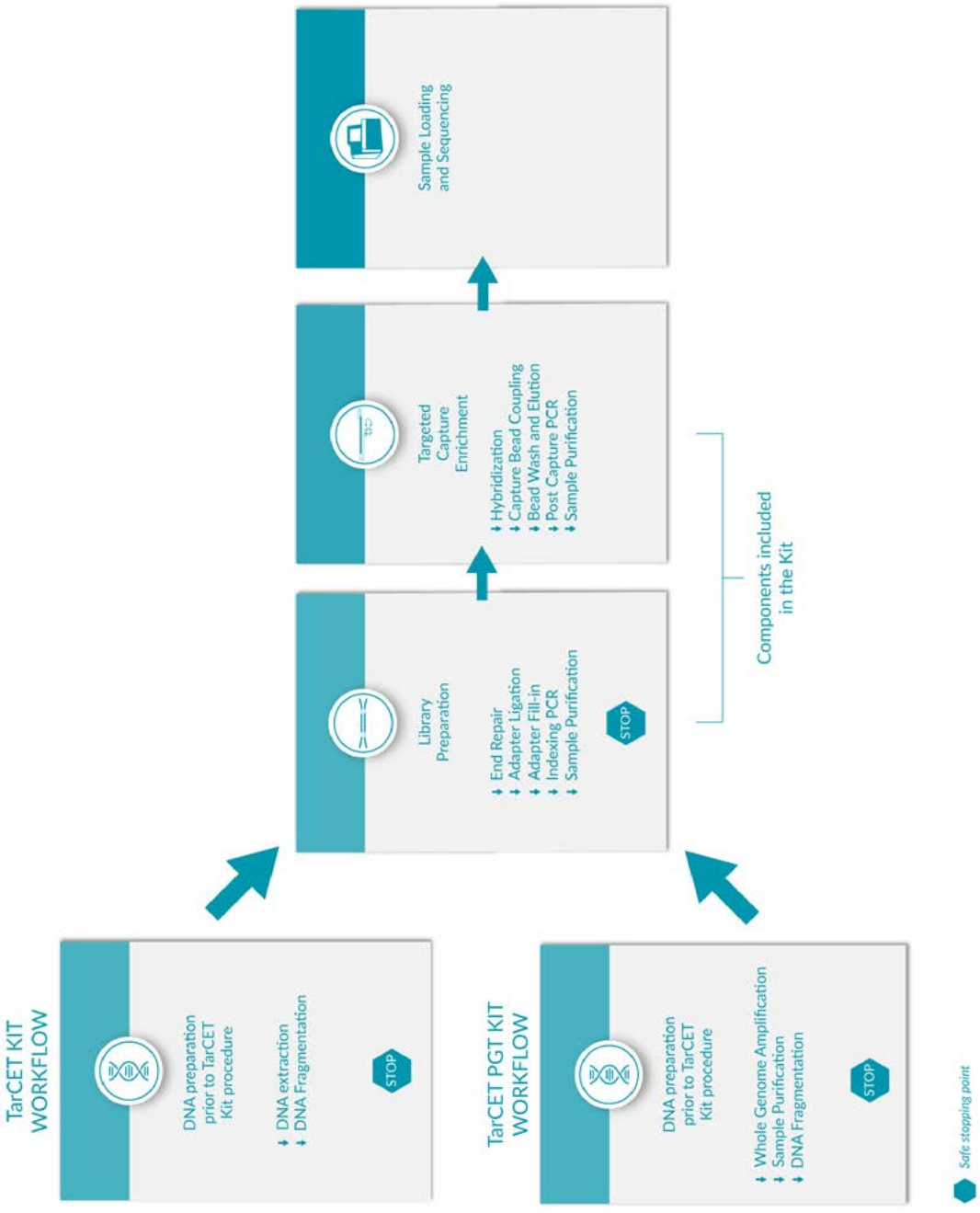
(*or equivalent) († Bioruptor® Pico, Cat # B01060010, has been discontinued. The new equivalent device is Bioruptor® Pico sonication device, Cat # B01080010) (‡ NanoDrop™ 8000 Spectrophotometer, has been discontinued. The new equivalent device is NanoDrop™ Eight Spectrophotometer, Cat # 13-400-527)

Sample Specification and Storage

- Genomic DNA of >250ng must be collected.
- To assess the purity of DNA, for an absorbance ratio of 260nm and 280nm, the acceptable ratio is ~1.8. Additionally, as a secondary measure, the 260/300 values are usually in the range of 2.0-2.2.
- Samples should be stored at -20°C.

Test Procedure

Workflow



Sample Batching

Prior to starting the test procedure, please refer to the WI-30 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. In case TarCET assays are sequenced in combination with the VERACITY/VERACITY HC assays please refer to WI-30 SIRIUS user guide for instructions.

Recommendation for DNA Extraction

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

- QIAamp Blood Mini Kit (Qiagen, Cat # 51104).
- Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (Omega, Cat # M6399).

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. Fragmentation can be achieved mechanically using a sonicator or enzymatically.

3a. Sonication

i. For the development and validation of TarCET Kit, mechanical fragmentation was performed using the Bioruptor® Pico (Diagenode, Cat # B01060010), which has been discontinued, according to the manufacturer's instructions. The new equivalent device for sonication is Bioruptor® Pico, Cat #B01080010.

ii. Following sonication, quantify the sonicated 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.

OR

3b. Enzymatic Digestion

i. For the development and validation of TarCET Kit, fragmentation was performed using dsDNA Shearase™ Plus, according to the manufacturer's instructions.

ii. Prior to library preparation add 10 µL of HPLC water in order to have a total of 20 µL.



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

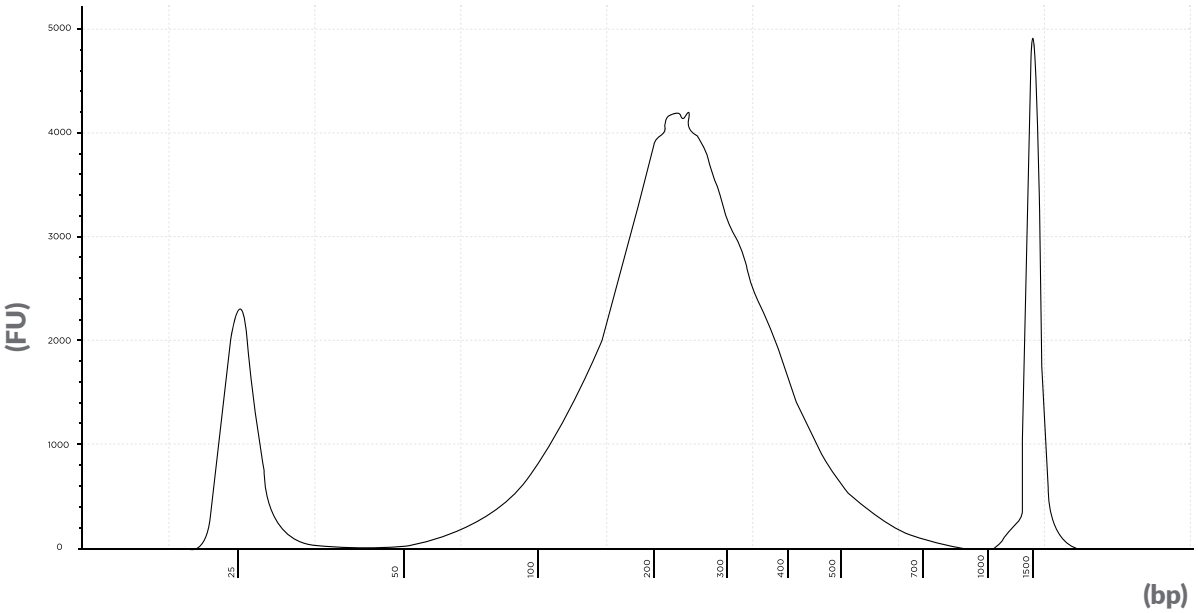


Figure 1: Representative electropherogram of post-sonicated DNA sample.
Accepted fragment size peak ~ 200 bp - 250 bp.

Library Preparation

1. Thaw all Library Preparation reagents on ice and program a thermal cycler as described in Table 2 below.
2. Transfer 20 µL 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 (µL)	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 µL of the End Repair reaction mix to each well/tube containing DNA samples to a final volume of 35.3 µL and seal the plate/tubes. When using a plate, seal with adhesive film.
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 85°C

Temperature (°C)	Time
20	30 min
75	20 min
16	∞

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 and seal the plate/tubes. When using a plate, seal with adhesive film.
9. Thoroughly vortex to mix well and briefly centrifuge the samples.
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 Adapter Fill-in reaction Master Mix

12. Add 4 µL of the Adapter Fill-in reaction master mix to each well/tube containing the samples to a final volume of 42 µL and seal the plate/tubes. When using a plate, seal with adhesive film.
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	∞

Table 6 Adapter Fill-in reaction conditions

Indexing PCR

15. Add 19.2 µL of the Indexing Mix (●1W5, TarCET Kit Box 1) to each well/tube containing the samples to a final volume of 61.2 µL.
16. Pierce the foil of the index plate (UltraVerse Index Oligos), add to each well/tube containing the samples 3.8 µL of pre-mixed unique index primer set and seal the plate/tubes. When using a plate, heat-seal with foil.

> 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.

17. Gently vortex and briefly centrifuge the samples.
18. Place the samples in a thermal cycler and perform the PCR reaction using the following conditions as described in Table 7.

Heated Lid at 105°C

Temperature (°C)	Time	Cycles
98	3 min	x1
98	30 sec	x12
60	30 sec	
72	30 sec	
72	3 min	x1
4	∞	

Table 7 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. If the PCR-amplified libraries are in 0.2 mL tubes, transfer them to 1.5 mL LoBind tubes. If they are in a PL2 plate, you may proceed without transferring.
21. Add 97.5 µL of well resuspended Purification Beads to each PCR amplified library and mix well by pipetting 20 times.
22. Incubate for 5 minutes at RT.
23. Place the plate/tubes on a magnetic stand for 5 minutes or until the solution is clear.
24. Without disturbing the beads, remove and discard the supernatant.
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. With the plate still on the magnetic stand, remove any residual ethanol using a 10 µL pipette.
29. 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.
30. 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.
31. Quantify the libraries using a nanodrop spectrophotometer.

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

Targeted Capture Enrichment

Hybridization

1. Add the appropriate amount of library (Table 8) 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 3.

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 8 Amount of Library required for Target Capture Reaction

2. Prepare the Hybridization reaction master mix according to Table 9. Mix carefully to avoid bubbles and briefly centrifuge the tube.

Reagent Code	Reagent	1 rxn (µL)	Storage
1L1	Blocking Oligos 1	1.25	TarCET Kit Box 1 (-20°C)
1L4	Blocking Agent 1	5	
1L7	Blocking Buffer	2	
1L8	Hybridization Buffer*	12.75	
1L6	Target Capture Mix	4	
Total Master Mix Volume		**25**	

Table 9 Hybridization reaction Master Mix
*Thaw the Hybridization Buffer on the bench, do not place on ice. Make sure it appears homogenous before use by slowly pipetting up and down, to minimize foaming

3. Add 25 µL of the Hybridization Master mix per library sample in the PL3 plate containing the libraries from step 1.
4. Heat-seal the plate with foil, thoroughly vortex to mix well and briefly centrifuge the plate.
5. Place the plate in a thermal cycler with the Hybridization reaction program as described in Table 10.

Heated Lid at 105°C

Temperature (°C)	Time
98	4 min
65	16 hr
65	∞

Table 10 Hybridization reaction conditions

Preparation of Buffers

6. Dilute 400 µL of Wash Buffer A (3C3, TarCET Kit Box 3) (2X) with 400 µL of HPLC water, for a total volume of diluted Wash Buffer A (3C3) of 800 µL (1X) per sample.
7. Set a thermal cycler at 60°C with a heated lid set at 70°C.
8. Pre-heat 400 µL per sample of Stringent Wash Buffer (3Y2, TarCET Kit Box 3) in the pre-heated thermal cycler set at 60°C.

Capture Bead Coupling

9. Add 40 µL of Capture Beads (● 2L1, TarCET Kit Box 2) to a new LoBind PCR plate (PL3), filling a well for every sample being captured.
10. Add 200 µL of diluted Wash Buffer A (3C3) (1X) to the beads and pipette up and down 10 times.
11. Place the plate on a magnetic stand for 1 minute, or until the solution is clear, and carefully remove the supernatant.
12. Remove the plate from the magnetic stand and resuspend the beads with 100 µL of Wash Buffer B (● 3L1, TarCET Kit Box 3).
13. Place the plate on the magnetic stand for 1 minute, or until the solution is clear and carefully remove the supernatant.
14. Remove the plate from the magnetic stand and add to the beads 10 µL of Wash Buffer B (3L1). Do not attempt to resuspend the beads by pipetting up and down.
15. After the 16 hour incubation is complete, remove the sample plate from the thermal cycler, spin down and unseal.
16. Transfer the samples to the wells of the PL3 plate containing the beads.
17. Heat-seal and gently vortex the plate until the mixture is homogenous.
18. Place the plate in a thermal cycler with the Coupling reaction program as described in Table 11.

Heated Lid at 75°C

Temperature (°C)	Time
65	35 min
65	∞

Table 11 Capture Bead Coupling conditions

19. During the incubation, remove the plate every 12 minutes to **gently vortex** until the mixture appears fully homogenous.

Bead Wash and Elution

20. After the 35 minute incubation is complete, remove the plate from the thermal cycler, spin down, and unseal.
21. Place the plate on a magnetic stand for 1 minute and carefully remove the supernatant.
22. Remove the plate from the magnetic stand.
23. Perform the following wash with Wash Buffer A (3C3) (1X):
    a. Add 200 µL of diluted Wash Buffer A (3C3) (1X) to each well containing the beads and pipette up and down 10 times.
    b. Place the plate on the magnetic stand for 1 minute, or until the solution is clear, and remove the supernatant.
24. While the plate is on the magnetic stand repeat step 23, without pipetting up and down 10 times.
25. Remove the plate from the magnetic stand.
26. Perform the following wash with Stringent Wash Buffer (3Y2):
    a. Add 200 µL of preheated Stringent Wash Buffer (3Y2) to each well containing the beads and pipette up and down 10 times.
    b. Seal the plate with adhesive film and incubate at 60°C for 2 minutes in the thermal cycler.
    c. Remove the plate from the thermal cycler and unseal.
    d. Place the plate on the magnetic stand for 1 minute, or until the solution is clear, and carefully remove the supernatant.
27. While the plate is on the magnetic stand repeat step 26, without pipetting up and down 10 times.
28. While the plate is on the magnetic stand perform the following wash with Wash Buffer A (3C3) (1X):
    a. Add 200 µL of diluted Wash Buffer A (3C3) (1X) to each well containing the beads.
    b. Incubate on the magnetic stand for 1 minute, or until the solution is clear, and remove the supernatant.
29. Remove the plate from the magnetic stand.
30. Resuspend the beads in 20 µL of Elution Buffer (3Y3, TarCET Kit Box 3) and transfer the resuspended beads in a clean PCR column or a new LoBind PCR plate (PL3).
31. Heat-seal and incubate at 98°C for 3 minutes in a thermal cycler with a heated lid set at 105°C.
32. Vortex the plate thoroughly for 10 seconds and briefly centrifuge the plate.
33. Place the plate on the magnetic stand for 1 minute, or until the solution becomes clear. Then, transfer the supernatant to a new PCR plate (PL2) or into 0.2 mL PCR tubes.

Post-Capture PCR

34. Prepare the Post-capture PCR reaction master mix according to Table 12.

Reagent Code	Reagent	1 rxn (µL)	Storage
1Y1	Post-capture Primer 1	2	TarCET Kit Box 1 (-20°C)
1Y5	Post-capture Primer 2	2	
1Y6	Post-capture Mix	24	
3Y7	Enhancer*	2	TarCET Kit Box 3 (RT)
Total Master Mix Volume		**30**	
Template DNA		**20**	

Table 12 Post-capture PCR reaction Master Mix
* Do not place on ice.

35. Add 30 µL of the Post-capture PCR reaction master mix to each sample to a final volume of 50 µL and heat-seal the plate with foil.
36. Gently vortex and briefly centrifuge the samples.
37. Place the plate/tubes in a thermal cycler with the Post-capture PCR reaction program as described in Table 13.

Heated Lid at 105°C

Temperature (°C)	Time	Cycles
98	3 min	x1
98	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

38. 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.
39. If the Post-capture PCR samples are in 0.2mL tubes, transfer them to 1.5mL Lobind tubes. If they are in a PL2 plate, you may proceed without transferring.
40. Add 75 µL of well resuspended Purification beads to each Post-capture PCR sample and mix well by pipetting 20 times.
41. Incubate for 5 minutes at RT.

42. Place the plate/tubes on a magnetic stand for 5 minutes or until the solution is clear.
43. Without disturbing the beads, remove and discard the supernatant.
44. 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.
45. Repeat step 44 for a total of 2 washes. Remove as much excess ethanol as possible.
46. Incubate the beads on the magnetic stand for 2 minutes to dry.
47. With the plate still on the magnetic stand, remove any residual ethanol using a 10 µL pipette.
48. 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.
49. 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

50. Quantify the amplified Post-captured sample using a High-Sensitivity fluorometric method (Qubit Fluorometer).
51. Assess the size distribution of the Post-captured sample using Agilent Tapestation.

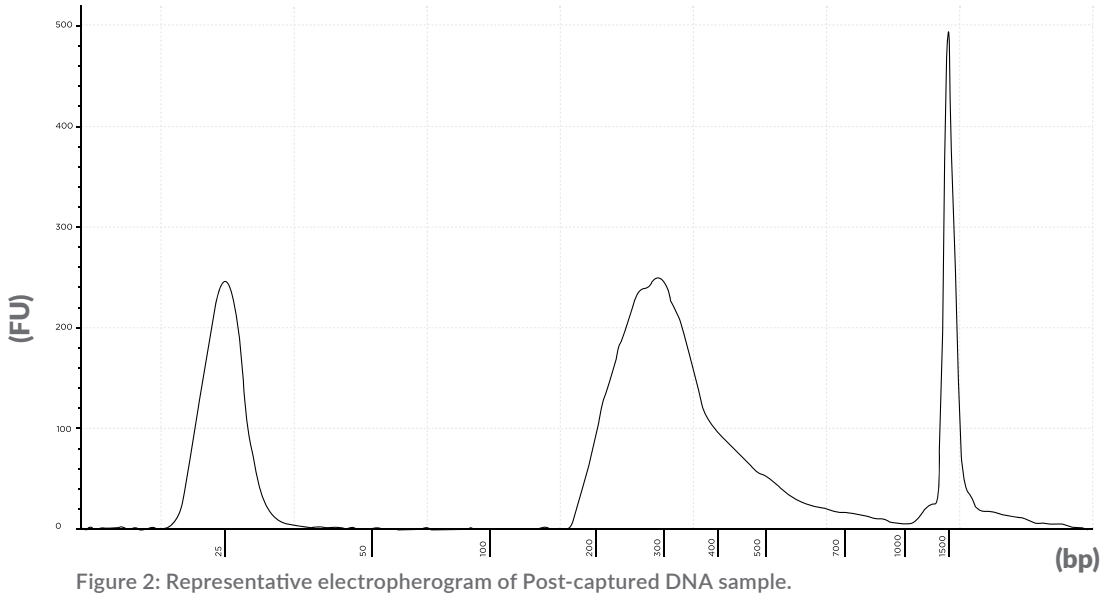


Figure 2: Representative electropherogram of Post-captured DNA sample. Accepted fragment size peak > 250 bp.

Sample Preparation for Sequencing

The assay has been validated on NGS platforms.

Sample Pooling Guidelines:

- In preparation for loading the samples for sequencing, please refer to the WI-30 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 WI-30 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.

To proceed with NGS the appropriate DNA Denaturation Guidelines protocol must be followed (document #WI-46A or #WI-46C).

Results Visualization

SIRIUS can be used to visualize the results after sequencing in a graphical representation or tabular form. Please use the WI-30 SIRIUS user guide to perform the post-analytical data management.

Limitations of the Procedure

- For In-Vitro Diagnostic (IVD) use.
- This kit is not intended as a standalone tool for disease diagnosis, prevention, or treatment.
- TarCET Products are compatible with genomic DNA (gDNA) extracted from human cells, including whole blood and buccal swab.
- TarCET Products support high-quality, double-stranded gDNA inputs ranging from 180 to 250 ng. The use of inputs outside this range may not guarantee reliable results.
- TarCET Products do not include reagents for DNA extraction and DNA fragmentation. Their performance has been evaluated using DNA from whole blood and buccal swab samples, using the specified DNA extraction kit and fragmentation methods.
- Device performance may be influenced by factors such as sample collection, storage and transportation.
- For diagnostic applications, end users must conduct additional validation before integrating TarCET Products into their workflows.

Performance Characteristics

The following data were generated from genomic DNA collected from buccal swabs and blood, as outlined in Clinical Validation section from multiple batches, performed by multiple users to ensure reproducibility.

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 and/or blood, 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.

TarCET Kit Clinical Validation

Analytical concordance*	Estimate (95% CI)
Single Nucleotide Variants (SNVs) + Insertions and Deletions (INDELs)	
Sensitivity (SNVs + INDELs)	100% (96.5-100%)
Specificity (SNVs + INDELs)	100% (99.98-100%)
Copy Number Variations (CNVs)	
Sensitivity (CNVs)	100% (93.6-100%)
Specificity (CNVs)	96.3% (94.3-97.8%)

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

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

Support Contact Info

For Technical Support inquiries: ivdsupport.genetics@medicover.com

For Customer Support inquiries: customersupport.genetics@medicover.com

For more information visit our website: www.medicover-genetics.com

Medicover Genetics Ltd.
31 Neas Engomis Str, 2409, Nicosia, Cyprus
Phone number: + 357-22266888