MEDICOVER
GENETICS

TarCET Kit

DNA Denaturation Guidelines & Sequencing Conditions for Element Biosciences Platforms

- Metabolic Panel
- Aortopathy Panel
- Arrhythmia Panel
- Cardiomyopathy Panel
- Congenital Heart Defects Panel
- FH, PH and RAS Panel
- Cardiac Comprehensive Panel
- Hereditary Cancer Panel
- Infertility Panel
- Neonatal Panel
- Carrier Screening Core Panel
- Carrier Screening Comprehensive Panel

CE IVD

For Diagnostic Procedure Only
Proprietary Document: WI-46C
Version 1.1

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Revision History

Date (YYYY-MM-DD)	Version	Description of Change
2024-11-20	1.0	Initial Document Issue
2025-03-12	1.1	Cover Modifications

Planned Run Creation:

1. Create a planned run on ElemBio Cloud by selecting the “Plan A Run” button on the top right corner of the platform
2. Select “Sequencing with Cloudbreak”
3. Save the Planned Run as AVITIRunID (i.e. AVITI001)
4. Enter the run’s information as shown in the table below:

Run Configuration

Library type	Third Party	
Library structure	Linear	
Sequencing kit	e.g. 2x75 CB Freestyle High	
Low-Diversity High-Multiplex option	No	
Library Pools	1	
Cycles		
AVITI 2x75 Sequencing Kit Cloudbreak FS	**Med**	**High**
Index 1	10	10
Index 2	10	10
Reads 1	76	63
Reads 2	76	63

5. Select “Advanced Run Settings” and for Polony Density select “High Density”
6. Save the Run

DNA Denaturation Guidelines:

1. Follow loading instructions for pooling and dilution of the samples in a DNA LoBind 2.0 ml tube
2. Dilute the PhiX Control stock by combining the following in an empty DNA LoBind 2.0 ml tube:
   - 2ul of 1nM PhiX
   - 18ul of HPLC H_2O
3. In a new Safe lock 1.5ml tube, perform a 1N dilution of NaOH by transferring 2ul of 10N NaOH and 18ul HPLC H_2O. Vortex the tube to mix
4. In a new Safe lock 1.5ml tube, perform a 0.2N dilution of NaOH by transferring 10ul of 1N NaOH and 40ul HPLC H_2O. Vortex the tube to mix
5. Add 15ul HPLC H_2O and 2.8ul of diluted PhiX to the final pooled samples from Step 1
6. Denature the pooled samples and PhiX from step 5 by:
   a. Adding 27.8ul of 0.2N NaOH and
   b. Briefly vortex and spin down
7. Incubate for 5 minutes at RT
8. Add 27.8ul of 200mM Tris-HCl buffer pH 7 and gently flick by hand to mix
9. Briefly spin down the sample
10. Add 1317ul of Library loading buffer to reach the final volume of 1400ul
11. Transfer the full volume of denatured library and PhiX to the library well of the sequencing cartridge

> ! Avoid introducing air bubbles when dispensing the library to the sequencing cartridge.

12. Discard all other tubes
13. Proceed immediately to the next step

Sequencing Run:

1. On the Home Page of the Sequencer select New Run -> Side to run (A or B) -> Sequence -> Planned Run and select the run form the form the list of planned runs (i.e. AVITI001)

2. Review the run configuration based on the Table below:

Run Configuration

Library type	Third Party	
Library structure	Linear	
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Library Pools	1	
Cycles		
AVITI 2x75 Sequencing Kit Cloudbreak FS	**Med**	**High**
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Advanced Run Settings		
Polony Density	High Density	

Make sure that the Polony density option under Advanced Run Settings is set to High.

3. Select storage space

4. Please refer to the Element AVITI System User Guide for instructions on how to load the required reagents to the sequencer

5. Initiate the sequencing run

Support Contact Info

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

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

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