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TarCET Kit

DNA Denaturation Guidelines for Illumina 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
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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)
2024-02-02	1.0	Initial Document Issue
2024-11-29	1.1	Updated Title to Clarify the NGS Platform Described

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 ≤10 µL. Adjust volume to 10 µL 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 µL of 10 nM PhiX
   - 8 µL of HPLC water

3. Denature the 10 µL of the concentrated pooled samples by adding 10 µL 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 µL of the diluted PhiX Control by adding 10 µL 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 µL 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 µL HT1 buffer and 900 µL 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 µL HT1 buffer and 900 µL 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 µL of the diluted sample and 200 µL 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 500/550 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 ≤10 µL. Adjust volume to 10 µL 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 µL of 10 nM PhiX
   - 8 µL of HPLC water

3. Denature the 10 µL of the concentrated pooled samples by adding 10 µL 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 µL of the diluted PhiX Control by adding 10 µL 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 µL of 200mM Tris-HCl, pH 7, to each tube.

6. Add 970 µL 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 µL HT1 buffer and 13 µL 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 µL HT1 buffer and 13 µL 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 µL of the diluted sample and 260 µL 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, in the pooled samples to result in 100 µL 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, by adding 3 µL of 10 mM Tris-HCl, pH 8, to 1 µL of 10 nM PhiX.

   b) Add 0.6 µL 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 µL 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 µL 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.

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 Engomi,
Nicosia - Cyprus