Fragment Analyzer HS Genomic DNA 50 Kb Analysis Kit

INSTRUMENT

Manufacturer: Advanced Analytical Technologies
Distributor: Agilent Technologies (customercare_finland@agilent.com, +358 10 855 2465)
Technical support: genomics_tech_europe@agilent.com

Owner: Tampere University / LAS
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RESERVATIONS (billing according to reservations) in AGENDO: https://tuni.agendo.science/

ANALYSIS KIT: HS Genomic DNA 50 Kb Analysis Kit, #DNF-468-0500

- Sizing range: 75 – 60 000 bp
- Input concentration range: 0.3 – 12 ng/µl

REAGENTS:

Stored at +4 °C E218 cold room	Stored at -20°C E217 freezer room	Stored at RT E215
Genomic DNA Separation Gel (#DNF-270-0240)	Intercalating Dye (#DNF-600-U030, same for all FA Analysis Kits)	5x Capillary Conditioning Solution (#DNF-475-0050, same for all FA Analysis Kits)
1x & 5x 930 dsDNA Inlet Buffer (#DNF-355-0125, same for all FA Analysis Kits)		
BF-25 Blank Solution (#DNF-300-0008)	HS Genomic DNA Diluent Marker (DM) (#DNF-375-0003)	Gel/Dye Mixture (at FA)
0.25X TE Rinse Buffer (#DNF-497-0125)		Capillary Storage Solution (#GP-440-0100-100/Kem-En-Tech)
1x Capillary Conditioning Solution		
HS Extended Genomic DNA Ladder (#DNF-364-U125)		

- Reagents are stored in room E215 (RT), E217 freezer room or E218 cold room. Opened reagents and dilutions (1x Capillary Cond. & 1x Inlet buffer) are marked with opening date and/or ”X”.
- Please open a new reagent tube or bottle in order of the expiration dates and mark the opening date and/or “X” to the tubes
- Take all the other reagents to warm up to room temperature (RT) at least 30 minutes prior to use
- All other reagents than Inlet Buffer are harmless and can be discarded by pouring down the sink

OTHER SUPPLIES: (stored at the shelf or drawer under the instrument table)

- Capillary Storage Solution (AATI #GP-440-0100-100)
- Specified 96-well semi-skirted PCR plates for samples (drawers 1-3) and Rinse buffer (drawer M)
- 96-well deepwell plate (Fisherbrand #12-566-120) (drawer W)
- BD Falcon 50 ml centrifuge tubes (BD #352070, #734-0448/VWR)
- Pipettes and pipette tips
- Electronic dispenser (Biohit) and dispenser tips (0.5/1.0/2.5/5 ml)
- Serum pipettes (for discarding 1x Inlet Buffer from the 96 DeepWell 1 mL Plate in row A) and serological 5 & 10 ml pipettes
- Microplate seals (#4ti-0510/Biotop)

QUICK CHECK LIST (for more instructions, see the specific sections)

1. Take the reagents to RT at least 30 minutes prior to performing the run

   From +4 C° cold room:
   - 1x (or 5x) Inlet Buffer
   - 0.25x TE Rinse Buffer
   - Genomic DNA Separation Gel (in case of new gel/dye mixture is required)
   - BF-25 Blank solution if required
   - HS Genomic DNA Ladder
   - 1x Capillary Conditioning Solution

   From –20 °C freezer room:
   - Intercalating dye (in case of new gel/dye mixture is required)
   - HS Genomic DNA Diluent Marker Solution (DM)

2. Turn on the computer and the instrument
3. Turn on the Fragment Analyzer Software (User ID: "Administrator", no password).
4. If necessary, insert (or add) the Gel/Dye mix solution to the desired Gel fluid line (1 or 2) and update the solution level to the software (Utilities – Solution levels).
5. Check the level of Capillary Conditioning Solution and add or replace if necessary and update the solution level to the software (Utilities – Solution levels).
6. Replace the 1x Inlet Buffer in the 96-well Midi plate at row A in the Buffer drawer (B) (once a day). Press "Park" – replace the buffer – place the plate back – press "Buffer"
7. Place the 0.25x Rinse buffer into drawer M.
8. Prepare the sample plate and load into one of the three sample trays. If you place a plastic seal on the plate in order to spin the plate, remember to remove the seal before running the plate to avoid capillary damage!

NOTE! When using the instrument for the first time, contact the person(s) responsible for introduction!

PROTOCOL

1. GEL/DYE MIX PREPARATION

- A gel/dye mix is valid for up to 2 weeks after preparation when stored in the instrument.
- For both Genomic DNA kits the Separation Gel is the same.
- If necessary, prepare a fresh mixture:
  1. Bring the Genomic DNA Separation Gel and Intercalating Dye (same for all kits) to RT prior to mixing.
  2. Add an appropriate volume of Intercalating Dye to Separation Gel in a 50 mL tube according to the table below.
     - NOTE! When switching applications between different analysis kits at the certain Gel fluid line 1 or 2 you need to do 5 mL more of the mix due to priming – see the example for 12 samples at the table and the step 7.

# of samples	Volume of Intercalating Dye	Volume of Separation Gel
12	1,0 µl (1,5 µl for priming)	10 mL (15 mL for priming)
24	1,5 µl	15 mL
36	2,0 µl	20 mL
48	2,5 µl	25 mL
60	3,0 µl	30 mL
72	3,5 µl	35 mL
84	4,0 µl	40 mL
96	4,5 µl	45 ml

  3. Mix by gently inverting the tube – do not vortex!
  4. Mark the date on the tube.
     - NOTE! If there is still less than 2 weeks old mixture left at the instrument, a fresh gel/dye mixture can be mixed with the old one to achieve the required total amount of the mixture (e.g. if there's still 5 mL of the mixture left, add 0,5 µl of Intercalating Dye and 5 mL Separation Gel to obtain required 10 mL for 12 samples). Do not change the date!
  5. Place the gel/dye mixture onto the instrument and insert into the desired Gel fluid line (1 or 2). Ensure that the fluid line is positioned at the bottom of the conical tube.
  6. Update the solution levels in the Fragment Analyzer instrument control software. From the Main Menu, select Utilities – Solution Levels. A menu will be displayed to enter in the updated fluid levels.
  7. PRIMING: When switching applications between different analysis kits on the certain Gel fluid line 1 or 2, prime the fluid line after loading fresh gel/dye mix. From the Main Menu select Utilities – Prime – select the desired fluid line 1 or 2.
  8. Leave the gel/dye mixture in the instrument after the run.

2. CAPILLARY CONDITIONING (CC) SOLUTION PREPARATION

- Capillary Conditioning Solution is the same for all kits.
- Place the 1x CC Solution onto the instrument in the Conditioning Solution location in a 50 mL Falcon tube (a typical 12-capillary experiment cycle consumes less than 4 mL but the instrument requires at least 10 mL in the tube).
- Update the solution levels in the Fragment Analyzer instrument control software. From the Main Menu, select Utilities – Solution Levels. A menu will be displayed to enter in the updated fluid levels.
- 1x CC Solution should be added to the system as use demands or if it hasn't been changed within 2 weeks.
  - NOTE! If there is less than 2 weeks old 1x CC Solution left at the instrument, new 1x CC Solution can be mixed into the same tube to achieve the required total amount (e.g. if there's still 5 mL of the 1x CC solution left, add 5 mL of new 1x CC solution to obtain required 10 mL for 12 samples). Do not change the date on the tube!
- If necessary, prepare the 1x Capillary Conditioning Solution to a 250 mL glass bottle by adding 50 mL of the 5x Capillary Conditioning Solution to 200 mL of Milli-Q water. Mix by gently inverting the bottle. Mark the date and your initials. 1x CC Solution can be used up to 2 weeks after preparation when stored in the instrument or 3 months when stored at +4 °C. Store most of the 1x buffer at +4 °C and leave an appropriate aliquot in the instrument.

3. INLET BUFFER PREPARATION

- Inlet Buffer is the same for all kits. Bring the 1x or 5x Inlet Buffer to RT prior mixing and use.
- 1x Inlet Buffer in the 96-well Deep Well Plate in the Buffer drawer (B) has to be replaced daily (the first user of the day):
  - From the Main Menu press "Park"-icon to bring the plate to the tray B.
  - Remove the old buffer from the row A by pipetting it to a waste bottle with a serum pipette. Add 1 ml of 1x Inlet Buffer to every well at row A in the 96 DeepWell Plate. Do not overfill!
    - Note! The Storage Solution is at the same plate at row H and is replaced only once in a month, so empty only the row A!
  - Place the plate back to the tray B and press "Buffer".
- If necessary, prepare the 1x Inlet Buffer. Bring the 5x Inlet Buffer to RT prior diluting. Add 50 mL of 5x Inlet Buffer to 200 mL of Milli-Q water in a 250 mL glass bottle. Mix by gently inverting the bottle. Aliquot the 1x Inlet Buffer into 50 ml conical tubes. Mark the date and your initials on the tube. 1x buffer can be used up to 3 months after preparation when stored at +4 °C.
- Replace the 96-well Deep Well Plate and Capillary Storage solution monthly. When taking a new Deep Well Plate, add 1.1 ml of Capillary Storage Solution to the wells at row H. Mark the date to the plate. Row H is used for the store location and the array moves to this position at the end of the experimental sequence.

4. INSTRUMENT PREPARATION
   - Prepare a new 96-well plate filled with 200 µl/well of 0.25x TE Rinse Buffer at row A daily. If necessary, spin the plate shortly to reduce air bubbles.
   - Use only specified semi-skirted 96-well PCR plates (Appendix A) in the instrument. Using wrong kind of plates may lead to capillary damage!
   - Place the prepared Rinse Buffer plate into drawer "M". Ensure the plate is loaded with well A1 towards the back left on the tray.

5. SAMPLE PLATE PREPARATION
   - The total input genomic DNA sample concentration should be within a range of 0.3 ng/µl – 12 ng/µl for optimal sizing and quantification.
   - High Sensitive Extended Genomic DNA Ladder is aliquoted in 10 µl aliquots (for 4-times use) in Eppendorf LoBind 0.5 ml tubes.
   - Allow the Genomic DNA Diluent Marker (DM) solution, HS Genomic DNA Ladder and the genomic DNA samples to warm up to RT prior to use.
   - Use only specified semi-skirted 96-well PCR plates (Appendix A) in the instrument. Using wrong kind of plates may lead to capillary damage!
   - LADDER PREPARATION:
     1. Add 22 µl of DM solution into a new 0.5 mL Eppendorf DNA LoBind tube (included in the kit, stored in the drawer under the instrument table)
     2. Gently vortex and spin the Ladder aliquot, then pipette 2 µl of the HS gDNA Ladder to the 22 µl of the DM solution. If you are going to run several rows at the same plate, prepare as many working gDNA Ladder Solution tubes as you will have rows to run.
     3. Mix the working gDNA Ladder solution only by gently vortexing in the vortex mixer!
     4. Pipette the entire 24 µl of the working gDNA Ladder solution into Well 12 of the Sample plate in each row to be analyzed.
   - SAMPLE PREPARATION:
     5. Before sampling, bring the DNA samples to RT and mix them by vortexing.
     6. Pipette 22 µl of DM solution to each well in a row that is to contain sample. Do not add any DM solution to the well reserved for the Genomic DNA Ladder (well 12).
     7. Fill any unused wells within the row with 24 µl/well of BF-25 Blank Solution.
     8. Pipette 2 µl of each sample into the wells 1-11 containing 22 µl DM solution and mix by pipetting up/down 3-4 times.
     9. Mix well using a separate pipette tip with the pipettor set to ~20 µl volume and pipette each well up/down about 10 times.
     10. Check that there are no air bubbles trapped in the bottom of the wells. If necessary, remove the bubbles by spinning the plate shortly in a mini plate spinner (seal the plate with a microplate seal prior centrifuging, remember to remove the seal before loading the plate into the instrument) or using a clean pipette tip.
     11. Run the samples immediately once prepared or cover the plate with a cover film, store the plate at +4 °C and run ASAP. Alternatively, to prevent evaporation, place a mineral oil overlay on each sample (50 µl/well).
     12. Place the Sample plate in one of the three sample trays (drawers 1, 2 and 3). Load the experimental method.

6. PERFORMING AN EXPERIMENT

1. To set up an experiment, from the Main Menu of the Fragment Analyzer instrument control software, select the Operation tab.
2. Select the sample tray location (1, 2 or 3) by left-clicking the Sample Tray # dropdown or by clicking the appropriate sample plate tab and choosing the appropriate location.
3. Left click a well of the desired sample plate row. Enter the sample name if desired into the respective Sample ID cell by left clicking the cell and typing the name or import the sample information from .txt or .csv file by selecting the Load from File... -option.
4. Under the Run Selected Group -field press Add to queue. The Separation Setup pop-up form will appear to the screen.
5. In the Separation Setup pop-up form:
   - From the Method dropdown menu select the right method:
     DNF-468-33 – HS Genomic DNA 50Kb.mthds
   - Select the Gel line (1 or 2)
   - The Tray Name can be entered if it's necessary to identify the sample plate
   - The Folder Prefix can be entered to amend the folder name for the results
   - The results can be copied to another directory location by checking the Copy Results box and selecting the desired Copy Path
   - Any Notes can be entered regarding the experiment
6. Once all information has been entered, press OK to add the method to queue. After a row (or tray) has been added to the queue, the method(s) will be listed on the main screen under the Method Queue. Repeat the steps 4-5 for any remaining sample rows. Additional experiments can also be programmed and added to the Method Queue at any time while the instrument is running.
7. Once the experiment has been loaded onto the queue, the method can be viewed or edited by pressing the Method Summary field.
8. <u>Prior to starting the experiment, verify all trays (buffer/storage, rinse, waste, sample, etc.) have been loaded into their respective drawer locations!</u>
9. Press the Play icon to start. To clear the run queue of all loaded runs, press the Clear icon.

7. AFTER COMPLETION OF THE EXPERIMENT

- After completion of the last queued experiment, the instrument stage will automatically move to the Store location.
- Throw away both the Sample plate and the Rinse Buffer plate (from drawer M) and empty and rinse the waste tray (from drawer W). Update the waste solution level in the Fragment Analyzer instrument control software. From the Main Menu, select Utilities – Solution Levels. A menu will be displayed to enter in the updated fluid levels.
- Turn off the instrument and the computer.

8. PROCESSING EXPERIMENTAL DATA

The data is viewed and processed using PROSize software.

1. Open your run by clicking File – Open File. Select the correct file and click 'Open'.
2. Look at the ladder first. In principle, as long as the ladder looks good, the results are reliable (refer to the user guides to see the representative ladder results). If the ladder is not ok, you can import ladder from a previous successful run. Select Analysis – Show size calibration. Choose 'Export' and save the ladder to your folder. Then open the run where the ladder was not optimal. Select Analysis – Show size calibration. Select 'Use imported ladder profile' and choose the saved ladder from your folder.
3. Remember to change the dilution factor if your dilution is not 200 (1 µl sample + 199 µl Diluent Marker Solution). It can be changed by clicking a tool icon on the upper right corner of a peak table that says 'Set individual parameters'. Select 'Quantification', change the dilution factor and press 'Apply to selected' or 'Apply to all'.
4. If the software hasn't placed the lower and upper marker correctly for a certain sample, you can manually correct this (for example, some small contaminants may cause the software to interpret the contaminant as a lower marker). Select the individual sample that has the incorrect peak(s) called as markers and right-click on top of the correct peak(s). Choose 'Set as lower marker' or 'Set as upper marker'.
5. The data is given in three different concentrations: TIC = total integrated concentration in ng/µL, TIM = total integrated molarity in nmole/L and total concentration in ng/µL. Be aware that based on your application, you may want to use for example TIC rather than the total concentration.
6. If you want to analyze peaks with certain sizes, go to 'Set individual parameters' and select 'Smear analysis'. Determine the start and end size and press 'Apply to selected' or 'Apply to all'.
7. Exit the program by selecting File – Exit. There is no need to save your changes. The changes are automatically saved when you exit the program. In case you want to return to the original raw data without any changes made by you, go to drive C – AATI – Data and delete .ANAI and .PKS files from your run's folder. This will delete your changes and you can start analyzing the raw data from the beginning.

Appendix A

List of approved Sample/Marker PCR Plates (semi-skirted):
Approved Vendor/Part Number; Description

- VWR # 83007-374; VWR® 96-Well PCR Plates, Half-Skirted Plates, Natural
- VWR # 89049-178; VWR® 96-Well Thermal Cycling Plate
- Eppendorf # 951020303 (various colors); Eppendorf* 96-Well twin.tec* PCR Plates, Semiskirted
- MidSci Pryme # AVRT1; Pryme PCR Ergonomic Plates, 96x0.2ml, Semi-Skirted, Natural
- BioRad Hard-Shell # HSS-9601; Hard-Shell® Full-Height 96-Well Semi-Skirted PCR Plates
- Greiner Bio-One # 652280; 96W PCR Microplate, Polypropylene, Half-Skirt, Natural, No Lid
- 4titude Framestar # 4ti-0900, -0950, -0770/C; FrameStar® 96 semi-skirted
- Scientific Specialty # 3450-00; 96-Well "Semi Skirt" UltraFlux® PCR Plate
- Neptune # 3742.X; Semi-Skirted 96-Well PCR Plates