

Standard Operating Procedure (SOP)	
SOP Title	Agilent TapeStation
SOP number	GbS04
SOP Version	2

1. SCOPE

The Agilent 2200 TapeStation system is an automated platform for simpler, faster and more reliable electrophoresis. The Genomic DNA High Sensitivity ScreenTape system is designed for analysing genomic DNA samples in the size range from 35 bp to 1000 bp.

It is made up of three elements:

- 2200 TapeStation system.
- Genomic DNA High Sensitivity D1000 ScreenTape with Genomic DNA High Sensitivity Reagents (Ladder and Sample Buffer).
- Agilent Software packages (2200 TapeStation Controller Software, and TapeStation Analysis Software).

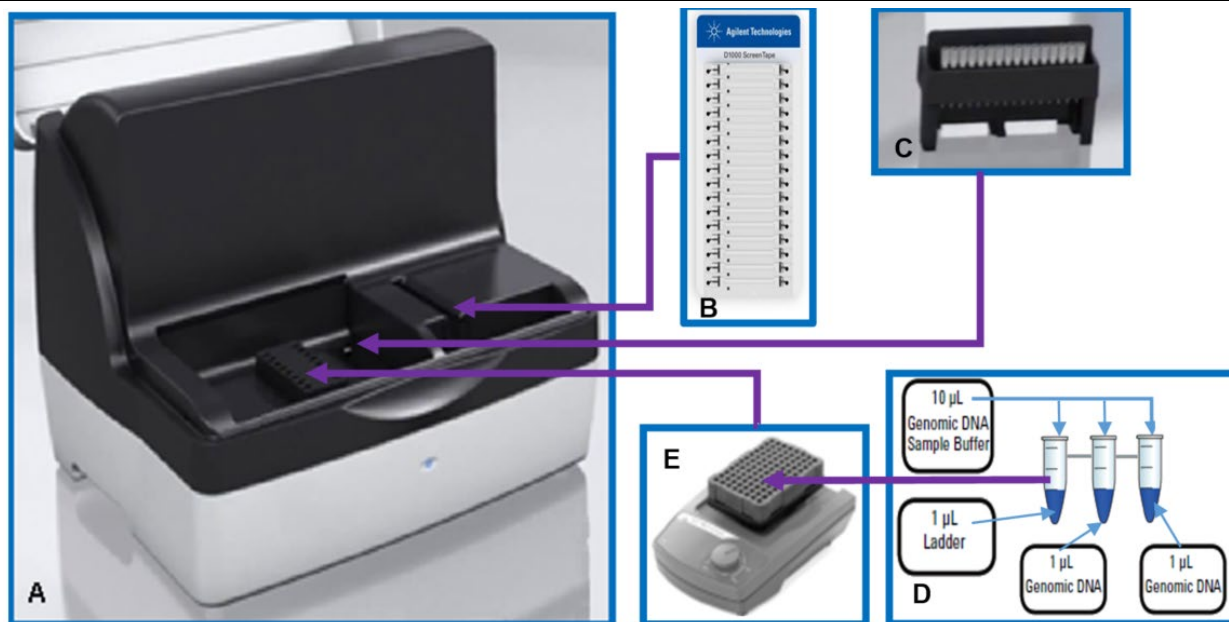
2. MATERIALS REQUIRED:

2.1 REAGENTS, LIBRARIES AND MATERIALS		
<i>Reagents</i>	<i>Supplier</i>	<i>Cat. No.</i>
Genomic DNA High Sensitivity D1000 ScreenTape	Agilent	5067-5584
Genomic DNA High-sensitivity reagents	Agilent	5067-5585
<i>Prepared Libraries (Generated from Library preparation)</i>		
Pooled amplified GbS samples GRC1.		
Pooled amplified GbS samples GRC2.		
Pooled amplified GbS samples Speciation.		
<i>Materials</i>	<i>Supplier</i>	<i>Cat. No.</i>
Agilent TapeStation System	Agilent	
Agilent – tube vortexer (IKA MS3)	included with TapeStation system	
Agilent consumables (loading tips, optical tube, optics cap, optical 96 well plate and foil seals)	Specific to TapeStation system	
Tube Vortexer		
20 µL pipette		
1 µL pipette		

Kit Components			
Part Number	Name	Colour	Amount
5067-5584	Genomic DNA ScreenTape		7 ScreenTape devices
5067-5585	Genomic DNA Reagents		2 vials
	• Genomic DNA Ladder	YELLOW	25 µL
	• Genomic DNA Sample Buffer	GREEN	1350 µL

3. METHODOLOGY

3.1 METHODOLOGY



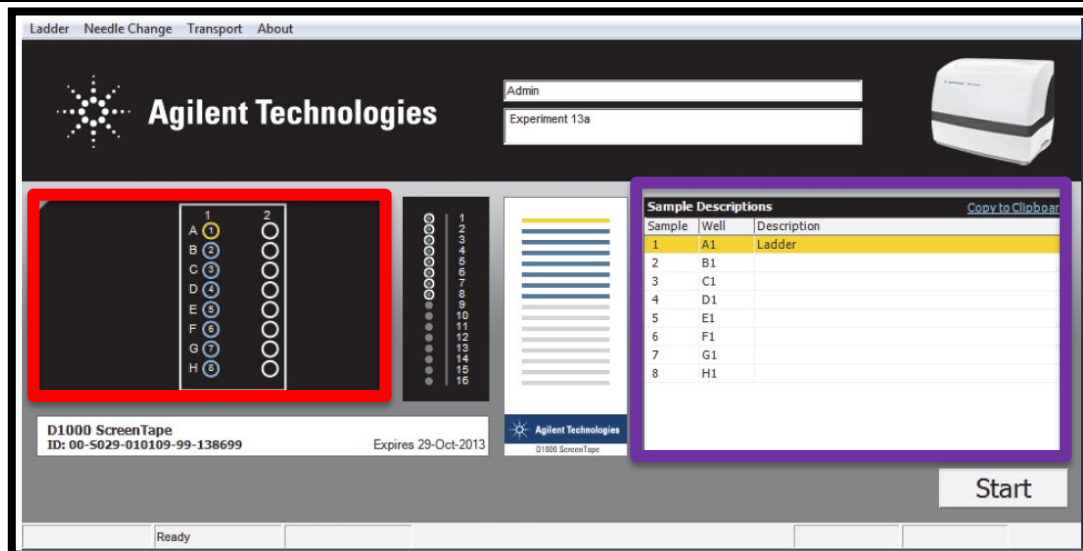
Methodology PCR_1 – Pre-PCR room

3.1.01	Remove reagents from fridge and allow to equilibrate at room temperature for 30 min.
3.1.02	Turn on the Agilent TapeStation (Power button on rear).
3.1.03	Launch the 2200 TapeStation Controller Software.
3.1.04	Open the lid on the 2200 TapeStation instrument (A).
3.1.05	Load Genomic DNA ScreenTape device (B) into the TapeStation.
3.1.06	Load the “loading tips” into the tip rack (C).
3.1.07	Load the tips rack (C) into the 2200 TapeStation instrument.
3.1.08	Vortex to mix reagents briefly before use.
3.1.09	Place the required number of “optical tube strips” (8x Strip) onto the Agilent – tube shaker (D). 1 Strips = 1 ladder and 7 samples 2 Strips = 1 ladder and 15 samples
3.1.10	LADDER - In the first tube add 10µL Genomic DNA Sample Buffer (●) and add 1µL Genomic DNA Ladder (●).

NOTE: Use a fresh ladder for each run. No electronic ladder is available for the Genomic DNA assay.

3.1.11	Add 10µL of Genomic DNA Sample Buffer (●) into the number of tubes required.
3.1.12	Add 1µL of each individual library sample to the corresponding tube.
3.1.13	Cap all the tubes using optical cap strip once all the dilutions are completed.
3.1.14	Vortex using the IKA vortexer and adaptor at 2000 rpm for 1 min (E).
3.1.15	Spin down to position the sample at the bottom of the tube.
3.1.16	Place the 8 strip tubes into the TapeStation

3.2 SAMPLE ANALYSIS



3.2.01 Select the required samples on the 2200 TapeStation Controller Software.

N.B. sample descriptions can be manually entered into the software before the instrument is started and whilst the instrument is operating.

Sample information must be added before analysis software is launched.

3.2.02 Select the tubes in the red box and add the description to the purple box

3.3 SAVING FILES

3.3.01	<p>Click Start and specify a filename with which to save the results. This will produce a Save As window.</p> <p>As a default the file name starts with the date, in reverse order, and a run counter. When run continuously, the save function auto increments the counter part of the file name.</p>
3.3.02	<p>Type in the name that you wish the analysis to be saved as. NOTE: Do not include a full stop in file names.</p>

3.4 FINAL CHECK

3.4.01	Lift the lid of the 2200 TapeStation instrument.
3.4.02	Ensure that there are fresh tips in the tip holder and that all the samples have been correctly loaded with lids removed and correspond to the sample selection on the screen.
3.4.03	Close the lid.

3.5 COMPLETE THE RUN

<p><u>When finished, a pop up will ask for removal of the tip cartridge and ScreenTape device.</u></p>	
3.5.01	Remove tip cartridge and ScreenTape device.
3.5.02	Empty tip buckets.
3.5.03	Click OK .

4. TAPESTATION ANALYSIS

4.1 AGILENT TAPESTATION

The figure consists of three numbered screenshots of the Agilent TapeStation Analysis Software interface:

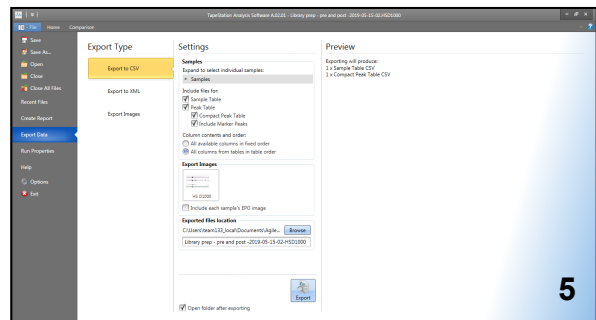
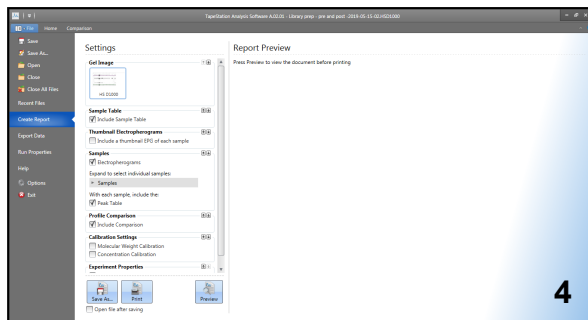
- 1:** Shows the 'Data Analyze' window with a chromatogram and a 'Peak Table' containing the following data:

Sample	Area	Height	Peak	Retention Time (min)	Peak Width (min)	Peak Intensity (cps)	% Integrated Area	Peak Comment	Observation
38	460	2700							Lower Marker
50	56.3	2700							
100	47.3	707							
200	46.2	254							
300	47.4	243							
400	47.8	246							
500	48.4	152							

- 2:** Shows the 'Comparison' window with a chromatogram comparing multiple samples.
- 3:** Shows the 'Comparison' window with a 'Save Comparison File' button highlighted.

4.1.01	The comparison software will open once the run is complete.
4.1.02	The analysis software requires the Ladder to have worked (see 1).
4.1.03	For comparison select the comparison tab (see 2).
4.1.04	Specific samples can be compared by selecting and de-selecting sample within the “comparison” window (see 2 and 3).
4.1.05	The comparison can be saved by selecting “Save Comparison File” and following the instructions.

4.2 REPORTING AND SAVING FILES



4.2.01	A report can be completed by selecting the “File” and then “Create Report”.
4.2.02	The Agilent “Report” can be previewed and saved using the icons at the bottom of the setting segment (see 4)
4.2.03	The data can be exported as either a peak information (CSV) and/or images of the individual samples (Export Images).
4.2.04	The file can be saved by selecting “File” then “Save As”. Choose a file name and folder, then save the file (see 5).