Using the TapeStation 4200 to Assess Nucleic Acids and NGS Libraries

Adapted from Agilent's ScreenTape Quick Guides and TapeStation User Guide.
Updated 29-Feb-2024 by Katie Murphy

BACKGROUND:

Our primary TapeStation instruments are TapeStation 4200s located at both NMNH and MSC (the latter is in the WRBU space). There is also a TapeStation 2200 at NMNH. This protocol is for the TapeStation 4200; there is a separate protocol published for the TapeStation 2200.
We regularly stock four types of ScreenTapes at the LAB, each of which is good for different applications. Each tape contains 16 lanes. Depending on the type of tape, you may or may not need to run a ladder in one of the lanes every time you use the tape.

- ***High Sensitivity (HS) D1000 DNA ScreenTapes***
  - Used for samples between 35 – 1000 bp.
  - If samples are > 10 nM, you may need to dilute prior to running.
  - Often used to check the fragment size of NGS libraries or sheared DNA, especially when expected to be < 700 bp.
  - Running a full tape will take approximately 20 minutes.
  - Running a ladder is optional with this tape.
  - Maximum number of samples per tape is 16.

- ***High Sensitivity (HS) D5000 DNA ScreenTapes***
  - Used for samples between 100 – 5000 bp.
  - If samples are > 10 nM, you may need to dilute prior to running.
  - Ideal if you expect your NGS libraries to possibly be ~700 bp or larger, to avoid your library peak overlapping the upper marker in the D1000 assay.
  - Must always run a ladder in one lane.
  - Maximum number of samples per tape is 15.

- ***Genomic DNA ScreenTapes***
  - Often used to assess HMW gDNA quality.
  - Requires higher concentrations than the HS tapes. Sample concentration should ideally be 10 – 100 ng/µL.
  - Must always run a ladder in one lane.
  - Maximum number of samples per tape is 15.

- **High Sensitivity (HS) RNA ScreenTapes**
  - Outputs a RIN that can be used to assess integrity of eukaryotic or prokaryotic RNA.
  - Running a ladder is optional with this tape.
  - Maximum number of samples per tape is 16.
  - Run time for a full tape is approximately 30 minutes.

There are additional types of ScreenTapes, but they will need to be purchased directly from Agilent if needed for your projects.

HELPFUL HINTS:

- *MAKE SURE TO REMOVE CAPS FROM YOUR TUBES PRIOR TO RUNNING ON THE TAPESTATION!*
- ***Make sure your reagents match your tape type!***
- Allow reagents and tape to come to room temperature for 30 minutes prior to use.
- Some reagents are light sensitive – make sure to keep in a dark location.
- Store partially used tapes upright (the same orientation it's run in) in its original packaging at 4°C.
    - We suggest taping it upright inside your fridge.
- Tapes expire two weeks after opening, but they can still be run after this point. We have successfully run tapes that were months old (but proceed at your own risk).
- Agilent tubes, plates, and tips are kept in the drawers below the TapeStation 2200 and the 4200.
- While the TapeStation 4200 can run individual samples, the TapeStation 2200 only runs samples in pairs. This means that even if you run an odd number of samples, you will need to use up one extra lane (e.g. If you want to run 7 samples, you'll need to use 8 lanes on your tape).
- *Partially used tapes must be run on the same instrument on which they were initially run.*
    - E.g. If the tape is first run on the TapeStation 4200, remaining lanes on the tape must also be run on the TapeStation 4200. The software keeps track of each tape and which lanes have been used, and this information is not shared between the TapeStation 2200 and 4200.

MATERIALS & REAGENTS:

- Tape(s) - make sure to use the appropriate type
- Reagents – *make sure to use the ones that match your tape type!!*
    - Sample buffer
    - Ladder (if needed)
- Agilent PCR plate and foil seal, and/or strip tubes and caps
- TapeStation tips (for loading the instrument)

PROTOCOL:

Step 1 – Sample Preparation
Follow the protocol that is relevant to your tape type.

1A. Preparing to run a HS D1000 DNA tape

1A.1 Retrieve your tape and associated reagents from 4°C and equilibrate at room temperature for 30 minutes. Keep the buffer in a dark location (box, drawer, etc.).

1A.2 Retrieve an Agilent PCR plate and foil seal from the drawers under the instrument. If you are running < 16 samples, you could alternatively use Agilent strip tubes and caps. Label the strips with a sharpie so you know which sample is where.
1A.3 Briefly vortex the buffer and ladder (if using) to mix, then spin down.
1A.4 If running a ladder, mix 2 µL buffer with 2 µL of ladder for each tape you plan to run. We generally recommend mixing this in the first tube of a strip or in an empty plate well.
1A.5 Prepare samples in your Agilent PCR plate or strip by mixing 2 µL buffer with 2 µL of sample.
1A.6 Seal the plate with foil, and/or cap your strip(s). Briefly spin down.
1A.7 Mix by vortexing at approximately 2000 rpm for 1 minute.
    a. The vortex next to the TapeStation 4200 will vortex your plate at exactly 2000 rpm and stop automatically after 1 minute. Ensure your plate is firmly secured to the vortex adapter.
1A.8 After vortexing, briefly spin down the plate and/or tubes. If using strips, carefully remove the platic caps. Foil seals should remain in place.
1A.9 Inspect each tube. If there are any bubbles or liquid splattered on the sides, repeat the spin. If there are no bubbles and all liquid is collected at the bottom of the tubes, you're ready to run.

1B. Preparing to run a HS D5000 DNA tape

1B.1 Retrieve your tape and associated reagents from 4°C and equilibrate at room temperature for 30 minutes. Keep the buffer in a dark location (box, drawer, etc.).
1B.2 Retrieve an Agilent PCR plate and foil seal from the drawers under the instrument. If you are running < 16 samples, you could alternatively use Agilent strip tubes and caps. Label the strips with a sharpie so you know which sample is where.
1B.3 Briefly vortex the buffer and ladder (if using) to mix, then spin down.
1B.4 To prepare the ladder, mix 2 µL buffer with 2 µL of ladder for each tape you plan to run. Generally this should be done in the first tube of your strip, or in a new PCR strip if your samples will be run in a plate.
1B.5 Prepare samples in your Agilent PCR plate or strip by mixing 2 µL buffer with 2 µL of sample.
1B.6 Seal the plate with foil, and/or cap your strip(s). Briefly spin down.
1B.7 Mix by vortexing at approximately 2000 rpm for 1 minute.
    a. The vortex next to the TapeStation 4200 will vortex your plate at exactly 2000 rpm and stop automatically after 1 minute. Ensure your plate is firmly secured to the vortex adapter.
1B.8 After vortexing, briefly spin down the plate and/or tubes. If using strips, carefully remove the plastic caps. Foil seals should remain in place.
1B.9 Inspect each tube. If there are any bubbles or liquid splattered on the sides, repeat the spin. If there are no bubbles and all liquid is collected at the bottom of the tubes, you're ready to run.

1C. Preparing to run a Genomic DNA tape

1C.1 Retrieve your tape and associated reagents from 4°C and equilibrate at room temperature for 30 minutes. Keep the buffer in a dark location (box, drawer, etc).
1C.2 Retrieve an Agilent PCR plate and foil seal from the drawers under the instrument. If you are running < 16 samples, you could alternatively use Agilent strip tubes and caps. Label the strips with a sharpie so you know which sample is where.
1C.3 Briefly vortex the buffer and ladder to mix, then spin down.
1C.4 To prepare the ladder, mix 10 µL buffer with 1 µL of ladder for each tape you will be running. We recommend mixing this in the first tube of your Agilent strip or in an empty plate well.
1C.5 Prepare samples in your Agilent plate or strip(s) by mixing 10 µL buffer with 1 µL of sample.

1C.6 Seal your plate with foil and/or cap your strips and briefly spin down.
1C.7 Mix by vortexing at approximately 2000 rpm for 1 minute.
   a. The vortex next to the TapeStation 4200 will vortex your plate at exactly 2000 rpm and stop automatically after 1 minute. Ensure your plate is firmly secured to the vortex adapter.
1C.8 After vortexing, briefly spin down the plate and/or tubes. If using strips, carefully remove the plastic caps. Foil seals should remain in place.
1C.9 Inspect each tube. If there are any bubbles or liquid splattered on the sides, repeat the spin. If there are no bubbles and all liquid is collected at the bottom of the tubes, you're ready to run.

1D. Preparing to run a HS RNA tape

1D.1 Allow the high sensitivity RNA sample buffer and your ScreenTape to equilibrate at room temperature for 30 minutes.
1D.2 Retrieve an Agilent PCR plate and foil seal from the drawers under the instrument. If you are running < 16 samples, you could alternatively use Agilent strip tubes and caps. Label the strips with a sharpie so you know which sample is where.
1D.3 Thaw the High Sensitivity RNA Ladder and any RNA samples on ice.
1D.4 Briefly vortex the buffer to mix, then spin down.
1D.5 If this is the first time the ladder is being used, it must be diluted. Add 10 µL RNase-free water to the ladder vial. Mix thoroughly and spin briefly.
1D.6 Mix 2 µL of the diluted ladder and 1 µL of sample buffer for each tape you will be running; use your first Agilent tube (tube A1), or an empty plate well.
1D.7 Prepare your samples by pipetting 1 µL of sample buffer and 2 µL RNA sample into the designated well(s).
1D.8 Seal your plate with foil and/or cap your strips and briefly spin down.
1D.9 Mix by vortexing at approximately 2000 rpm for 1 minute.
   a. The vortex next to the TapeStation 4200 will vortex your plate at exactly 2000 rpm and stop automatically after 1 minute. Ensure your plate is firmly secured to the vortex adapter.
1D.10 After vortexing, briefly spin down the plate and/or tubes.
1D.11 Denature the ladder and samples as follows:
   a. Heat the ladder and samples at 72°C for 3 min.
   b. Place ladder and samples onto ice for 2 min.
1C.10 Briefly spin down the plate and/or strips.
1C.11 If using strips, carefully remove the plastic caps. Foil seals should remain in place.
1D.12 Inspect each tube. If there are any bubbles or liquid splattered on the sides, repeat the spin. If there are no bubbles and all liquid is collected at the bottom of the wells, you're ready to run.

Step 2 – Operating the TapeStation 4200 Instrument at NHB

2.1 Power on the laptop and the TapeStation 4200. The power switch for the TapeStation is on the front left. Log on to the computer using our LAB login.
   a. There's a sticker on the laptop with the password and username if you forget.
2.2 Open the TapeStation Controller software using the desktop icon and wait for the system to initialize.
2.3 Hold your ScreenTape with the label facing you and gently flick the top of the ScreenTape. If there are any small bubbles present, this will move them to the top of the chamber.
2.4 Lift the lid on the TapeStation instrument.

2.5 Insert your first tape into the slot on the back right of the deck, with the 2D barcode down and facing.
   a. If the tape is not inserted correctly, the software will pop up a warning saying that it did not recognize the tape.
   b. If the tape has been open for more than 2 weeks, there will be a popup saying it's expired. You can still run the tape; just close the popup.

2.6 In the software, select the well(s) you wish to run by clicking and dragging. The wells you select directly in the software correspond to their physical placement in the TapeStation.

2.7 The software will automatically calculate how many tapes you need for your sample number, how many tips are needed, and how much ladder to prep (if using). These numbers will be in the top middle panel of the software.

2.8 Load any additional required tapes in the rack on the back left of the deck. The rack has labeled slots (1-6); start with slot 1. Tapes should be placed in the same orientation as the initial tape (see **Step 5**).

2.9 The software automatically assigns the first strip tube well as ladder. If your ladder is in a different well, select the correct well. If you are running a HS D1000 tape with no ladder, select "use electronic ladder" instead.

2.10 If using a PCR plate, place the foil-sealed plate onto the sample block in the lower left of the TapeStation deck. Push the plate all the way down – it should fit snugly. A1 should be on the upper left.

2.11 **Remove caps from any strip tubes**, then place onto the tube block in the middle of the TapeStation deck.

2.12 Remove the lid from the on-deck tip box (if present), and make sure sufficient tips are loaded. Partial tip columns are acceptable, but please don't leave gaps.

2.13 Close the lid of the TapeStation.

2.14 Optional: Add sample names for each in the "Description" column.
   a. You can add sample names in the Analysis software later, after the TapeStation runs your samples.

2.15 Click "Start".

2.16 Final Check: A pop-up will appear asking you to verify everything is loaded correctly and caps are removed. Click okay.

2.17 Do NOT try to open the TapeStation instrument while the run is going!

2.18 When the run finishes, open the instrument, discard the plate and tubes, empty the tip bucket, and store or discard your used tape(s).

2.19 If you have no further tapes to run, close the Controller Software and power off the TapeStation.

Step 3 – Assessing your data in the TapeStation Analysis Software

3.1 After the run finished, the TapeStation Analysis software should have launched automatically. If not, double-click the desktop icon to launch the software and navigate to your file.

3.2 Verify that the ladder has been assigned to the correct lane. To assign or unassign a ladder lane, highlight the lane and select "Assign" or "Unassign" from the menu bar along the top. If you did not run a ladder, click "Insert" to add an electronic ladder.

3.3 Verify that all marker bands look correct on the electropherogram. You can add or remove peaks by right-clicking on them in the electropherogram.

3.4 For each sample, verify that each peak has been correctly identified. Add or remove peaks as needed.

3.5 For amplicon libraries or other samples where you expect to have a single peak, make note of the peak size.

3.6 For many sample types (such as sheared DNA and genomic libraries), it's important to know the **average** fragment size. To calculate this, do the following:

a. Click on "Region" in the menu bar.
b. Select the region of interest. Drag the left and right vertical bars until the region encompasses the entire peak or region of interest.
c. In the region table below the figure, the average BP will be calculated.

3.7 To save your data in a PDF format, click "Create Report" from the file menu.

a. If you've added Region data, this will automatically be included in the report.

3.8 Alternatively, you can also export your data to excel. I also recommend saving your edited file in case you want to re-open it later in the Analysis Software.