Before your run:

- **Purchase tape(s)** [through minilims at least 24-48 hours before](#) your reservation time
    - Reference tape specs on the front of the instruments or [see here for an online version.](#)
- **Reserve instrument** in [SPINAL](#)
- **Warm tape(s) and reagents to room temperature** before starting instrument set-up (~30 mins)

Instrument Set-Up:

- Instrument, Reagents, Consumables:
    - Available on bench for users to use: pipettes, cycler, microfuges, other items in the surrounding area
    - Plates & foil seals, strip tubes & caps, tips in drawer
        - **Always use** Agilent designated consumables, especially foil seal.
        - **Always remove caps from strip tubes prior to starting instrument**
        - **Do Not cut strip tubes**
    - Quick Guides are in the drawer -always use for reference
        - Volume of buffer & ladder/sample differs depending on tape used
        - See last pages for images of each set-up
    - Using the instrument
        - Power button & power cycling
            - Turn instrument on & then software, do reverse to turn off
        - Instrument deck set-up
            - Important to make sure tape waste & tip waste are always cleaned prior to starting run
    - Reagents located in mini-fridge below the middle bench
        - DNA has a ladder provided by Core
        - RNA does not
            - **Ladder will always be in position A1 of strip tube**
            - Ladder is run on every new tape for DNA - 15 samples per tape
            - RNA will need to use electronic ladder - 16 samples per tape
        - Order tapes through minilims & pick up in drop off fridge
        - Orient tape in instrument with the barcode in the bottom right corner, facing the back so that you see the blue or green label
            - Ensure no bubbles in lanes before use
                - Lightly tap to dislodge any bubbles in the lane
        - Partial tapes can't be used on a different instrument
            - Use color-coding stickers and labeling to ensure you do not mix your tapes
            - Orange #1
            - Red #2
        - When samples are set up, vortex **using designated vortexer set at 2000 rpm** & spin down

Laptop log-in:

- Log in with RC account
- Open controller software
    - First time use will need to change settings to **allow expired tapes**
    - Can select wells for both strip tubes or plate format
        - Recall: DNA ladder is always in A1 location of the strip tube
        - For RNA, right click and select "Electronic Ladder"
    - Import .txt file for descriptions
        - Create an excel .txt file with the "sample description" in second column
            - No need to add in any well locations, it will automatically be set by file
        - **Save as .txt** to RC account to import at instrument – see details below for how to access the location
    - Prefix is the file name – unique to your labeling and organizing process
        - Don't need to include date if it is part of output under settings
    - Required box will show how many tips, tapes, & ladder/buffer need for run
        - Important to doublecheck this prior to starting run
    - When ready select start, then proceed, & **wait for run to start**
        - If you walk away too soon, it could error out, so wait for the status bar

Analysis software

- First under File, Save As to RC folder
    - This will save the original data file
- De-select align to check alignment
    - If there are issues see troubleshooting guide
- For DNA, view the samples under the Region tab
    - Also select scale to sample
    - Add region(s)
        - Can either right click over electropherogram and select "add region"
          OR
        - Can go to region settings and set a standard region across the entire file
- For RNA, stay under Electropherogram tab
    - May need to right click and select the sub unit peaks if not appropriately called
- Scroll through each profile to see if generally everything looks good
    - No major tape or sample related issues
- Can re-assign, add, remove peaks while under Electropherogram tab
- Export file
    - RNA: File → Export → for RNA select sample table only
    - DNA: File → Export → for DNA select compact region table only
- Create report
    - Select/deselect any samples in the "samples" location
    - Deselect Peak table for DNA – region table will have the necessary information

When the run is complete:

- o Save partial tape
  - ▪ Wrap up with original foil seal and tape
  - ▪ Write the latest date on the foil so the core does not toss it out during a tape clean out – note: anything 4 weeks or older will be discarded as well as anything not labeled
  - ▪ Unused, old tapes will be recovered if older than 4 weeks
- o Clean up bench area
  - ▪ Put things away and wipe down with 70% EtOH
  - ▪ Log out of your windows account, DO NOT lock the profile

Accessing and Saving to Your RC Account: Data Storage and Transfer Policy

Note: Data storage on local hard drives is prohibited, and any files found on the hard drives may be deleted without warning.

- • **Users with lab folders on the Research Computing Network Storage Space:**
  - o Can connect directly to their folders on RC file systems (\\rcfs1, \\rcfs2, \\rcfs3, etc...) and should use these servers to store their data.
  - o There are no time limits for these servers as they belong to the individual labs.

- • **Users without Lab Folders:**
  - o Can temporarily store data generated on the center's instruments in a folder on [\\rcstore02.rc.fas.harvard.edu\data](\\rcstore02.rc.fas.harvard.edu\data) you can access this through the file explorer
  - o Within this area, users can create folders and store up to 2.5 GB of data.
  - o If you don't have a lab folder, this will be mapped as your Z: drive in Windows Explorer.
    - ▪ These data will be accessible to download remotely for 90 days by either HTTPS or SFTP. See instructions below.
    - ▪ You will need to authenticate with your Active Directory (RC) username and password.
- • **HTTPS:**
  - o Point your web browser to [https://data .rc.fas.harvard.edu](https://data.rc.fas.harvard.edu)
  - o Log in using your Research Computing username and your password.
    Open your subfolder and click on files to open or save them.
    This will only allow you to open/save a single file at a time.
- • **SFTP:**
  - o Follow the instructions at https://rc.fas.harvard.edu/resources/access-and-login/#Odyssey_access_requires_the_OpenAuth_tool_for_two_factor_authentication to establish your OpenAuth two-factor authentication tool.
  - o Use an FTP client such as Filezilla (freeware).
    Under Site Manager, create a new site (Host: data.rc.fas.harvard.edu)
    - ▪ Protocol: SFTP   Logon Type: Interactive
  - o Under Advanced, Default remote directory: /data
  - o Under Transfer Settings, check the box for "Limit number of simultaneous connections" and set the maximum number of connections to 1.
    - ▪ See the following page for more details: https://rc.fas.harvard.edu/resources/documentation/transferring-data/sftp-file-transfer/
  - o Browse for your data in the folder in the lower right field.
  - o Browse for the desired destination in the lower left field.
  - o Drag files from the right hand field to the left hand field to copy them from the data folder to your computer.