TracSeq Manifest Help Guide

The manifest that you can download is generated based on the responses to steps 1- steps 6. It will be based on the material you submit, if there will be a pool created, loading extra PhiX and if you made libraries… the barcode type you used. The manifest is downloaded in Step #7 on TracSeq via the *"Download manifest template based on these selections"* blue link.

Tips on filling out a Manifest:

KEY POINTS for filling out manifest

Never modify the manifest you download from TracSeq. This manifest will interact with a number of databases when you upload it to TracSeq and when processing your samples. The manifest will not upload and/or function as intended if you modify header, move columns, add or delete columns and add/or delete rows

Never allow your spell check to "check" your manifest
* disable your spell check
* spell checked manifest will have significant issues when you try to submit it

Each line on the manifest will be a new sample and should have all fields filled out

Not all field discussed below may be on your manifest

1- Creating a Sample ID

- Sample Names must be between 5-16 characters long
- ID cannot start with a Zero
- Use both alpha + numeric
- ONLY use hyphens or underscore
  o Do not use period, brackets, and other special characters
- Do not use generic names: i.e.- "sample1"
- Watch out for spaces within a name or at the end

2- External Code Field

- This is not a required field
- Use this field to track other necessary info for your sample.
- The Sample ID and the external code are linked and visible at all times for the lab and on the name of your final data
- A mix of 20 alpha or numeric, no special characters are allowed in this field

3- Naming a pool

- Start pool names with PL_abbreviation of account name_"
- Follow with a unique number or name from all other pools submitted on your account
- A mix of 16 alpha or numeric, Hyphen or underscore allowed, no special characters are allowed in this field
- Do not use generic names: i.e.- "PoolA"
- List the same name after all libraries that either are or will be a member of the same pool
- Watch out for spaces within a name or at the end

4- Sample Data fields (Conc., Vol., A260/280, A260/230, RIN, Ave fragment size)

- ALWAYS enter conc. and volume for a sample
- A260/280, A260/230 – please enter these fields
- RIN – only for RNA samples
- DV200 – please enter value if known
- Ave Fragment Size – this is required for all libraries and pools that are submitted to the HTSF
- For unknown fields, enter 9999 – this will indicate to us you do not know the values

5- Concentration Procedure

- List the procedure or equipment used to determine the concentration of the submitted material
- Do not list how you may have concentrated it

6- Index Number

- SINGLE barcodes
  - List kit index #
- DUAL BARCODES
  - list a dual index separated with a hyphen
  - Index Number field = i7 index#-i5index#

7- Barcodes

- Single barcodes
  - List full nt seq for barcode (ie 8mer of nt)
  - DO not list the adapters
- DUAL BARCODES
  - list a dual barcodes separated with a hyphen (ie 8mer of nt)

o   NT Sequence field = 5' NNNNNNNN- 3' NNNNNNNN

8- Number of Lane
- List the number of lanes each pool will be run on
- Do not list the overall number of lane being requested to be sequenced

9- Species
- not required, but HTSF prefers if you indicate this in case we need to troubleshoot a seq run

10- Tissue
- Not required, but if you are submitting RNA from tissue, please note if it is FFPE or fresh Frozen

11- Special needs
- These are notes on a SMAPLE basis. If you need to place a note that pertains to the entire batch, please list in batch special needs on the submission Step #7

12- Percent PhiX
- If more than the standard 1% PhiX is required, list it here

13- Customer Guid and Customer Cohort
- Only required for MAND or LCCC samples submissions where the data is necessary to link back to patient information.

SUBMITTING FOR PLATES: if you are submitting a plate. Please ensure:

1- Plate loading order is in columns, <u>NOT ROWS</u>
    i. First sample is in A1
    ii. Second sample in B1 ,
    iii. Third is in C1 , 4th = D1 and so
    iv. Last sample in a plate is H12

2- The manifest will be prelabeled with Row and positions,
    - please do not modify the order of the rows on the manifest
    - 1 plate can be submitter per manifest

3- Fill in the rows on the manifest in the order on the plate
    i. First sample in A1 = row 2 on manifest
    ii. Second sample in B1 , = row 3
    iii. Third is in C1 = row 4
    iv. Last sample in a plate is H12 = row 97 on the manifest