NeuroLINCS Total RNAseq protocol

Overview

The neuroLINCS group utilizes total RNA-seq to quantify the global transcriptional profile, coding and non-coding RNAs, of a cell. These data can then be used to generate transcriptional signatures and assess differential RNA expression and alternative splicing between multiple sample groups. This protocol has been generated based current (2016) RNA-Seq accepted standards and best practices in the bioinformatics community and modified to fit our specific usage from our previous successful RNA-seq studies that show high rigor and reproducibility. The step outlined here will briefly summarize the necessary information for any end user to reproduce our results and analyze other datasets using similar methodology. Figure 1 shows the outline of our RNA-seq procedure.

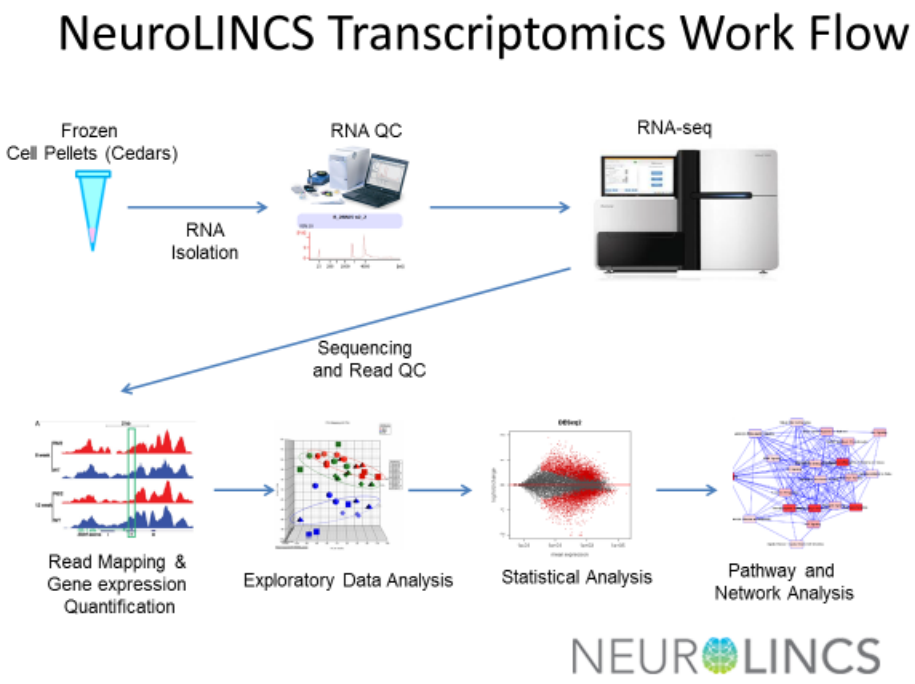

Figure 1. Outline of RNA-Seq steps described in this protocol.

Materials and equipment

1. RNeasy mini kit, Qiagen, Cat #: 74106
2. QIAshredder, Qiagen, Cat #: 79656
3. Agilent 2100 Bioanalyzer, Agilent Technologies, Cat #: G2939AA

4. ERCC ExFold RNA Spike-in Mixes, Thermo Fisher Scientific, Cat #: 4456739
5. Ribo-Zero Gold rRNA Removal Kit, Illumina, Cat #: MRZG12324
6. TruSeq Stranded Total RNA Library Prep Kit, Illumina, Cat #: RS-122-2203

Procedure

1. RNA isolation

- **a.** RNeasy Mini Kit RNA Extraction Protocol
  - **i.** RNA extraction using QIAGEN RNeasy Mini Kit (cat# 74104) and QIAshredders (cat# 79654). Protocol according to QIAGEN RNeasy Mini Handbook.
  - **ii.** Harvest up to 1x10^7 cells. ****5ug** is needed from extraction to give a 2.5ug prep after digestion for RNAseq. **1x10^6** cells should be sufficient, **2.5x10^6** would be ideal because ½ could be prepped for RNAseq and ½ could be kept for later analysis, we also like to freeze back an extra pellet at the same time for possible later analysis.***
  - **iii.** Collect growing cells as needed (trypsin, accutase, etc.) and spin out culture medium. Aspirate of culture medium and re suspend in DPBS (-Ca, -MG), spin out PBS and aspirate of as much as possible. Use Immediately or flash freeze and store for later use.
  - **iv.** **Analyze samples by Agilent and only use those with a RIN greater than 7.5. **

2. Library Prep

- **a.** TruSeq Stranded Total RNA Library Prep Kit Protocol
  - **i.** Protocol according to Illumina Truseq total RNA library Prep Kit Handbook.
  - **ii.** Total RNA is monitored for quality control using the Agilent Bioanalyzer Nano RNA chip and Nanodrop absorbance ratios for 260/280nm and 260/230nm.
  - **iii.** The input quantity for total RNA is 1ug. ERCC spike-ins are added.
  - **iv.** rRNA depletion following Ribo-zero Gold protocol.
  - **v.** cDNA is cleaned after second strand synthesis using AMPure XP beads
  - **vi.** Adapter ligated fragments are enriched by nine cycles of PCR.
  - **vii.** Lib are sized by Agilent Bioanalyzer DNA high sensitivity chip @ ~200-300bp.

3. Sequencing and Data Analysis

- **a.** Sequencing
  - **i.** Libraries are sequenced on the HiSeq 2500 or 4000 multiplexing and using enough lanes to obtain >50M reads/sample. Paired end 100.
- **b.** Data analysis
  - **i.** See Figure 2.
  - **ii.** FASTQC(v. 0.11.2),
  - **iii.** Trimmomatic(v.0.32)

iv. Alignment to Human hg19. Tophat2(v.2.0.12), Bowtie2(v.2.2.3), Samtools(v.0.1.19).
v. Cufflinks(v. 2.1.1)
vi. HTSeq(v.0.6.1p1.)
vii. DESeq2

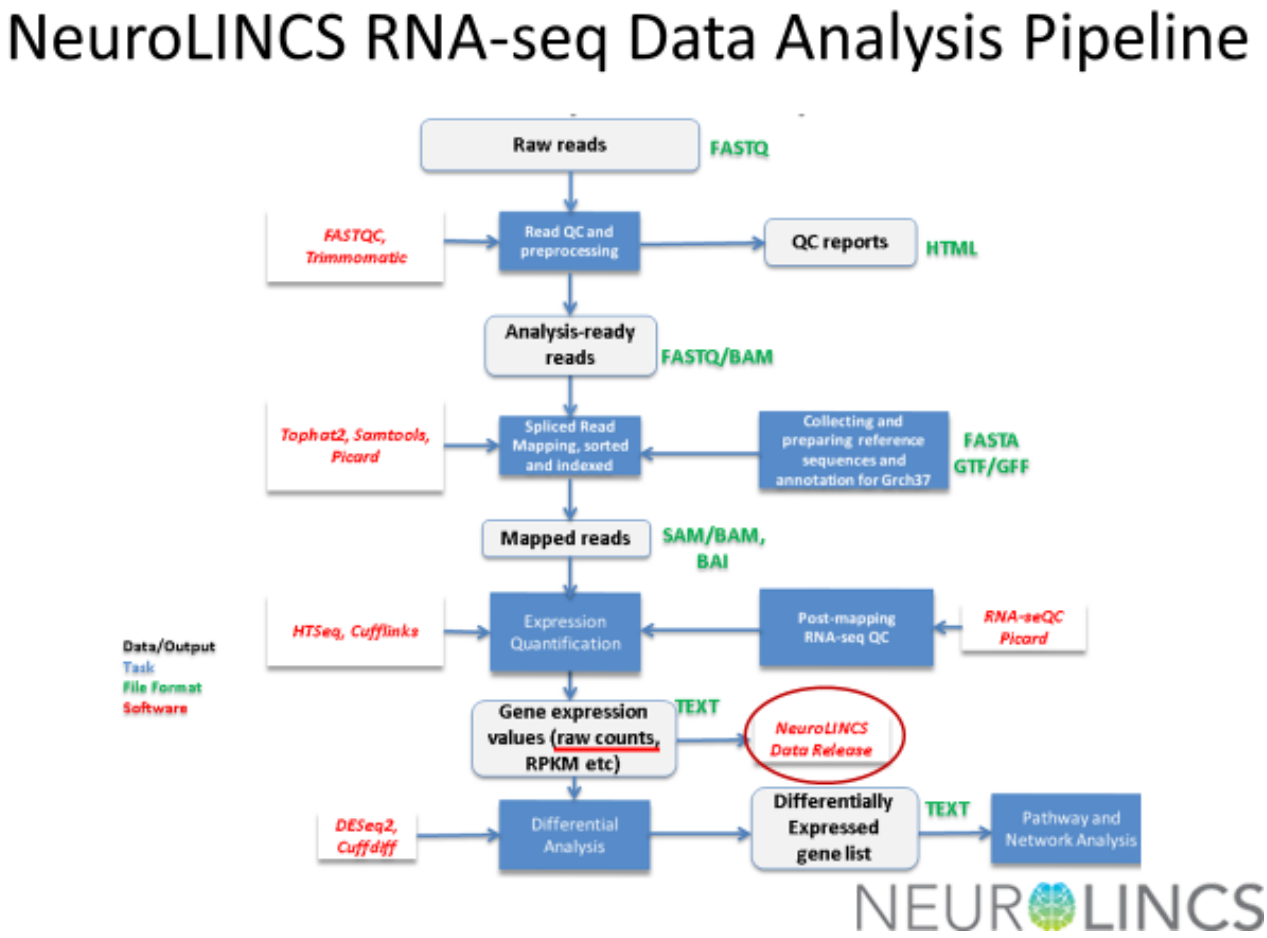

Figure 2. Flowchart of neuroLINCS RNA-Seq data analysis pipeline.