RNA QC

Total RNA QC (Please refer to “Sample Prep Services”)

1. Quantitation and estimation of RNA purity using NanoDrop spectrophotometer readings of A_{260}, A_{260}:A_{280} and A_{260}:A_{230} ratios.
2. Assessment of RNA integrity using Denaturing Formaldehyde Gel Electrophoresis and/or Agilent 2100 Bioanalyzer (measure RNA integrity number, RIN).

- **Quality Control of RNA sample**

  Accurate measurement of RNA quality is essential for further analysis, especially for good data from high throughput sequencing. All samples prepared by Arraystar Services are routinely qualified with the NanoDrop ND-1000 spectrophotometer, Gel Electrophoresis and/or the Agilent 2100 Bioanalyzer. Regardless of downstream application, detailed QC reports including nucleic acid purity, concentration and yield, RNA integrity (RIN) electropherograms (where applicable) are provided on each sample. If you have chosen to use Arraystar Services, a sample does not pass the recommended QC metrics for analysis, you will be contacted promptly to discuss possible solutions. These may include replacing the sample or re-isolating the RNA, excluding the sample from further analysis, or proceeding at-risk.

- **RNA Purity:**

  RNA purity can be determined by NanoDrop ND-1000 Spectrophotometer readings of A_{260}:A_{280} and A_{260}:A_{230} ratios.

- **RNA Concentration and Yield:**

  a. RNA concentration can be determined by NanoDrop Spectrophotometer reading of A_{260}. An A_{260} of 1 is equivalent to 40μg/ml.

  A_{260} X dilution factor X 40 = μg RNA / ml

  b. RNA yield widely varies according to the type and amount of samples.

  Warning---Any DNA contamination in the RNA sample will lead to an overestimation of yield, since all nucleic acids absorb at 260 nm.

- **RNA Integrity:**

  RNA integrity can be determined by Gel Electrophoresis and/or Agilent 2100 Bioanalyzer.

  a. **Gel Electrophoresis:** Total RNA integrity can be assessed by Denaturing Formaldehyde Gel Electrophoresis. High quality total RNA will have fairly compact rRNA bands, and the 28S rRNA band will be about twice as intense as the 18S rRNA band.

  Warning---RNA degradation will cause smearing of the rRNA bands.

  DNA, if present, will be evident as a high molecular weight smear or band migrating more slowly than the 28S rRNA band.

  b. **Bioanalyzer:** The integrity of total RNA can also be evaluated by Agilent 2100 Bioanalyzer. RNA Integrity Number (RIN) algorithm assigns a 1 to 10 RIN score, where level 10 RNA is completely intact. For best results from high throughput sequencing, RIN score should be greater than 7, and a 28S:18S rRNA peak ratio should be at or near 2:1.