These are examples of the bioanalyzer traces shown as a gel files. The total RNA from the top gel image is of excellent quality. The lower gel image is of poorer quality. The green band covers a marker which is put into each well with your sample. The concentration and sizing is determined from the standard ladder loaded in lane one.
28S/18S Ratio

• The 28S/18S ratio may be indicative of problems with the RNA, but not always. Even though the ideal ratio is 2.0, the bioanalyzer rarely reflects this ratio. If the ratio is greater than 2.0 it may indicate the presence of sheared single stranded genomic DNA which can run around the 28S band. Usually if the ratio is less than 1.0, there are definite degradation problems with the RNA. More important than an absolute number is the visual sign of degradation.
RIN number

• The Agilent Expert software assigns a RIN number to each trace. It assigns a number according to how much signal is found between the 5S and 18S band, between the 18S and 28S bands, and after the 28S band. A RIN number of 10 is perfect score. The software does not always call RIN numbers for prokaryotic RNA and the RIN can be misleading for samples containing additional RNA bands such as those from chloroplasts or a symbiotic RNA. The following slides show some examples of total RNA run on the bioanalyzer.
RIN 10.0

Marker RNA

5S RNA

18S RNA

28S RNA

Total RNA Examples p. 4-14
Other RNA Bands p. 15-22
Spikes p 23-24
Contaminants p. 25-31
Baseline Problems p 32-34
Sample Shift p 35-36
cRNA and Fragmented p 37-39
RIN 9.2

Still excellent quality RNA but the small RNA peaks before 18S cause the RIN number to be less than 10.
RIN 9.4

Still very high quality RNA
RIN 7.9

Marker RNA  5S RNA

18S  28S
Total RNA below a RIN of 7.0 is not recommended for microarrays.
RIN 5.3

Marker RNA

Quite a bit of degradation

18S
28S

Total RNA Examples p. 4-14
Other RNA Bands p. 15-22
Spikes p 23-24
Contaminants p. 25-31
Baseline Problems p 32-34
Sample Shift p 35-36
cRNA and Fragmented p 37-39
RIN 2.8

Marker RNA

18S  28S

Total RNA Examples p. 4-14
Other RNA Bands p. 15-22
Spikes p 23-24
Contaminants p. 25-31
Baseline Problems p 32-34
Sample Shift p 35-36
cRNA and Fragmented p 37-39
RIN 2.2
RIN 2.0

Fully degraded RNA

Marker RNA
Two very good total RNAs

Different organisms may have 18S and 28S bands that run at different sizes.
A peak past the 28S peak may be due to undenatured RNA or possibly genomic DNA.
Douglas Fir total RNA

The bands before the 18S RNA are from chloroplast RNA bands – the RNA is still very good quality but the RIN number, will be low or not called.
Brassica Leaf Total RNA

RNA from chloroplasts

Marker RNA

RNA 5S

18S

28S

Total RNA Examples p. 4-14
Other RNA Bands p. 15-22
Spikes p 23-24
Contaminants p. 25-31
Baseline Problems p 32-34
Sample Shift p 35-36
cRNA and Fragmented p 37-39
Other RNA bands
Symbiotic RNA

Marker RNA

Extra RNA Bands from a symbiotic organism
Very rarely the 28S peak is seen as a double peak. This may be real or possibly due to the extraction method, or as a non-denatured peak. We have seen this in snail and zebrafish.
5S RNA

[Graph showing 5S RNA, 18S, 28S, and Marker RNA levels on the x-axis (nt) and y-axis (FU)].

Total RNA Examples p. 4-14
Other RNA Bands p. 15-22
Spikes p 23-24
Contaminants p. 25-31
Baseline Problems p 32-34
Sample Shift p 35-36
cRNA and Fragmented p 37-39
Primarily 5S RNA

Marker RNA
5S RNA
18S 28S
Spikes

Total RNA Examples p. 4-14
Other RNA Bands p. 15-22
Spikes p 23-24
Contaminants p. 25-31
Baseline Problems p 32-34
Sample Shift p 35-36
cRNA and Fragmented p 37-39
A spike could be due to bumping the instrument during the run or interference in the electrical current while the run is going. A contaminant such as glove dust, or other particle in the tube may also cause the spike.
A contaminant such as salt can interfere with the run and cause peaks to disappear. The missing 28S peak may be caused by salt in the prep.
Contaminant caused by metal shard, obscured presence of RNA that was actually there
Metal contaminant

Hump caused by contaminating metal shard from forceps

5S RNA

18S RNA

28S RNA

Marker RNA
Contaminant + split 28S peak
Degraded RNA + Genomic DNA
Contaminant - Genomic DNA

Smaller sheared single stranded genomic DNA can show up around the 28S peak. In this instance the 28S/18S ratio was well above 2.0, close to 3.0.
Contaminant - Genomic DNA

Marker RNA

5S RNA

18S RNA

28S RNA

Genomic DNA can cause rise and fall in baselines, especially in lanes 7-11

Contaminant - Genomic DNA

Total RNA Examples p. 4-14
Other RNA Bands p. 15-22
Spikes p 23-24
Contaminants p. 25-31
Baseline Problems p 32-34
Sample Shift p 35-36
cRNA and Fragmented p 37-39
This baseline irregularity may be caused by the presence of longer single stranded genomic DNA fragments. Irregular baselines are most often seen in lanes 7-11, because contaminating fragments continue to run and interfere with later lanes.
Wavy Baseline

Also probably cause by genomic DNA

Also probably cause by genomic DNA
Wavy Baseline

Probably caused by a contaminant
Shifted peaks

In this particular instance, the shift in peaks was due to a contaminant in a previous lane.
A shift and wider peaks may also be due to old or poorly mixed reagents. An unanalyzed gel file would show progressively shifted lanes.
Good quality cRNA

Marker RNA

Affymetrix one-cycle cRNA synthesis
2-cycle cRNA

Shorter products from 2 cycle cRNA synthesis
Fragmented cRNA

[Graph showing Fragmented cRNA and Marker RNA]

Total RNA Examples p. 4-14
Other RNA Bands p. 15-22
Spikes p 23-24
Contaminants p. 25-31
Baseline Problems p 32-34
Sample Shift p 35-36
cRNA and Fragmented p 37-39