Determining quality of *Chlamydomonas reinhardtii* RNA

1. The simplest way to have a rough check of RNA quality is to run a gel. A typical picture of intact RNA is shown below (Figure 1).

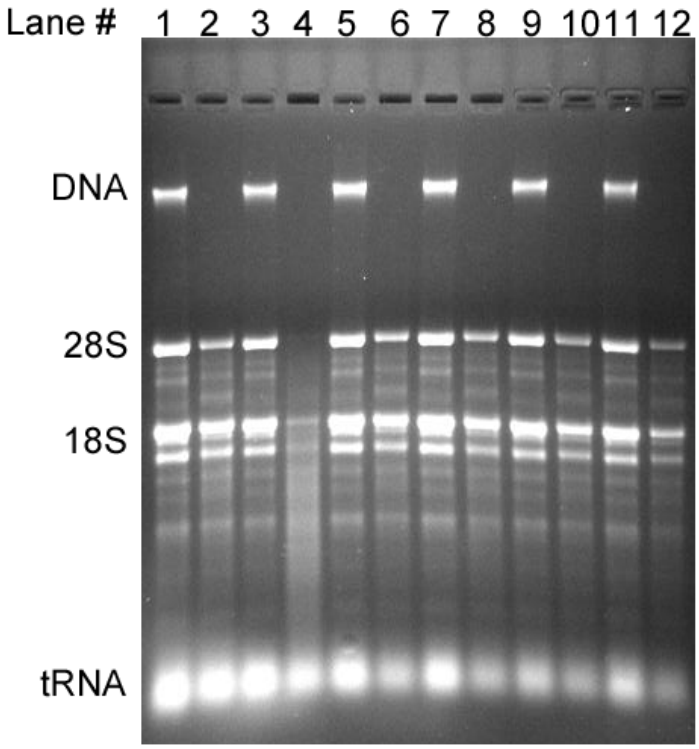

Figure 1. Typical RNA gel of intact RNA. Lanes 1, 3, 5, 7, 9, and 11 are total RNA samples; lanes 2, 4, 6, 8, 10, and 12 are DNase treated RNA samples. **Lane 4** is an example of **degraded RNA**. The sample in lane 4 was DNase treated and EDTA was *NOT* added prior to inactivation of DNase at 75ºC. The DNA, 28S and 18S ribosomal bands, as well as the tRNA band are labeled. Note that the 18S band is actually a triplet due to the prokaryotic nature of plastid and mitochondrial ribosomes which are slightly smaller. The smear in the background is the mRNA. If the RNA is degraded, the smear will not be there and there will be a large/bright band on the bottom of the gel (smaller pieces of RNA move faster). Gel image courtesy of Anja Hermschemeier.

2. Next, to ensure that the RNA you have is from the conditions you wanted it to be from, you need to check RNA abundance of sentinel genes. This requires some *a priori* knowledge of sentinel gene expression in the treatment so you can make sure that the genes you expect to be induced or down regulated actually are. (Refer to the qPCR protocol on the Merchant Lab website.

3. The third step is to hybridize the RNA with *CβLP*, a housekeeping gene. For this, we are looking for nice sharp bands for each sample. If the bands appear smeared or tear-drop shaped then the RNA is degraded.

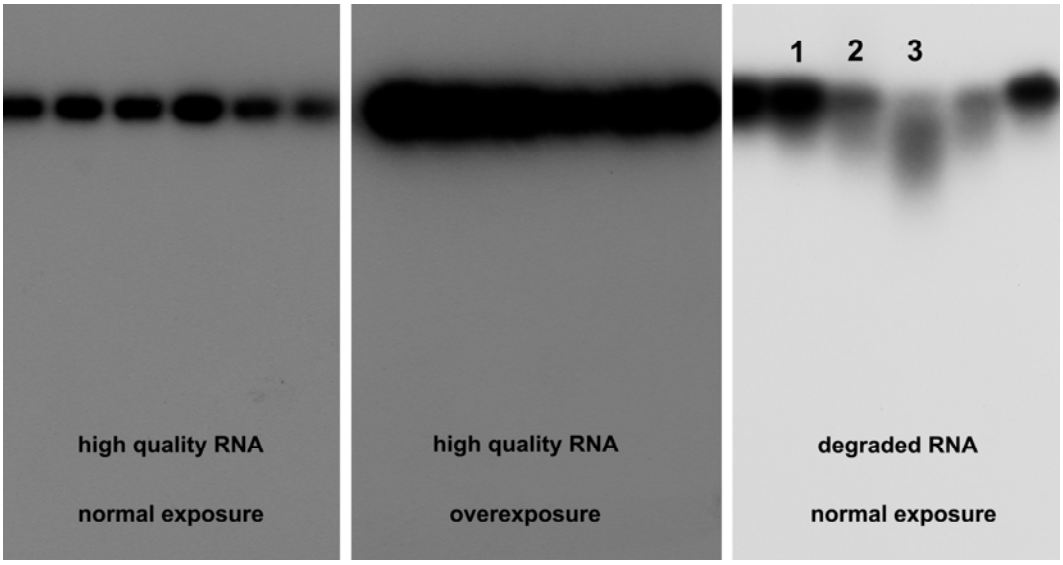

Figure 2. RNA hybridized with *CϑLP* probe, the pictures on the left are high quality RNA and the one on the right is degraded RNA. The bands on the left film are from a typical RNA blot and are sharp and well defined. When the same film is overexposed, smears from degradation are not seen. In the blot of degraded RNA, lane 1 depicts a small amount of degradation, lane 2 shows significant degradation and lane 3 has almost no intact RNA. If there is no signal, that indicates total degradation of the sample.

4. The next step is to analyze your total RNA samples on the Agilent 2100 bioanalyzer. Below are figures showing good quality RNA and degraded RNA for *C. reinhardtii* (this is an important distinction because each organism varies slightly in the shape of the electropherogram).

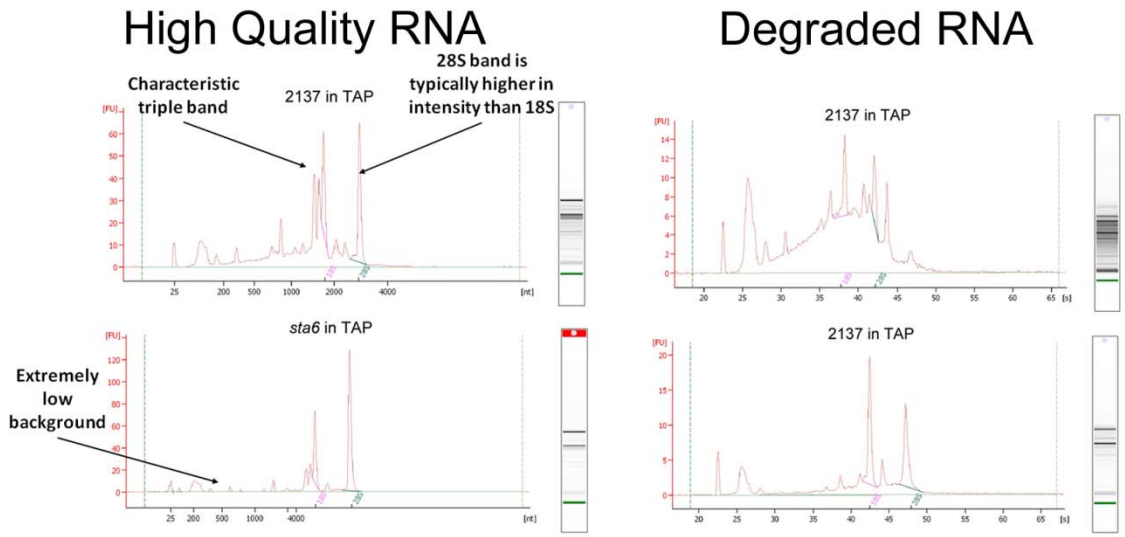

Figure 3. Results of RNA analysis for *C. reinhardtii* on the Agilent Bioanalyzer 2100. The left panel shows what high quality RNA electropherograms and gel pictures look like for both 2137 and *sta6* strains. Note that instead of just two ribosomal bands (18S and 28S), *C. reinhardtii* has a triple band around 18S due to the prokaryotic nature of the plastidic and mitochondria ribosomes. The baseline of high quality RNA tends to be quite straight unless the RNA concentration exceeds that recommended by Agilent (500 ng/µl). Also, the peak at 28S is usually higher in intensity that the peak for the 18S ribosomes. Degraded RNA (shown in the right hand panel) is missing the triple peak at 18S, doesn't have a straight baseline (also can be caused by high DNA content in the sample) or the 28S peak is significantly lower than 18S. Also note the gel pictures to the right of the electropherogram show significantly higher background than that for high quality RNA.