

Fragmenting the cRNA for Target Preparation

5X Fragmentation Buffer is supplied with the GeneChip Sample Cleanup Module.

Fragmentation of cRNA target before hybridization onto GeneChip probe arrays has been shown to be critical in obtaining optimal assay sensitivity.

Affymetrix recommends that the cRNA used in the fragmentation procedure be sufficiently concentrated to maintain a small volume during the procedure. This will minimize the amount of magnesium in the final hybridization cocktail. The cRNA must be at a minimum concentration of 0.6 $\mu\text{g}/\mu\text{L}$. Fragment an appropriate amount of cRNA for hybridization cocktail and gel analysis (see Section 2, Chapter 3, Table 2.3.1).

1. Add 2 μL of 5X Fragmentation Buffer for every 8 μL of RNA plus H_2O . The fragmentation buffer has been optimized to break down full-length cRNA to 35 to 200 base fragments by metal-induced hydrolysis.

The final concentration of RNA in the fragmentation mix can range from 0.5 $\mu\text{g}/\mu\text{L}$ to 2 $\mu\text{g}/\mu\text{L}$. The following table shows an example of a fragmentation mix for a 20 μg cRNA sample at a final concentration of 0.5 $\mu\text{g}/\mu\text{L}$.

For fragmentation, use **adjusted** cRNA concentration, as described in *Step 2: Quantification of the cRNA* on page 2.1.20.

Example for 0.5 $\mu\text{g}/\mu\text{L}$ final concentration:

Table 2.1.9
Example of Fragmentation Reaction

Component	Volume
20 μg cRNA	1 to 21 μL
5X Fragmentation Buffer	8 μL
RNase-free water	to 40 μL

2. Incubate at 94°C for 35 minutes. Put on ice following the incubation.

3. Save an aliquot for gel analysis.

At least 1 μg fragmented cRNA is needed if using ethidium bromide for staining the gel. Less RNA can be used with SYBR Green II staining. See *Step 3: Checking Unfragmented Samples by Gel Electrophoresis* on page 2.1.21, for information regarding gel electrophoresis. The standard fragmentation procedure should produce a distribution of RNA fragment sizes from approximately 35 to 200 bases. An example of a gel with cRNA samples before and after fragmentation is shown below.

4. Store undiluted, fragmented sample RNA at -20°C until ready to perform the hybridization, as described in Section 2, Chapter 3.

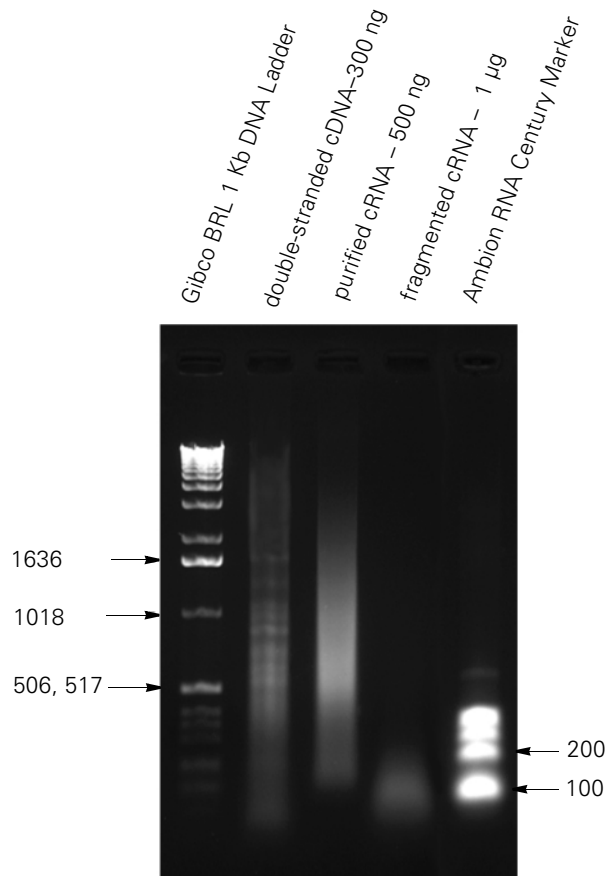


Figure 2.1.1
Monitoring of target preparation by agarose gel electrophoresis
