Biomarkers

Fragmentation

Protocol:
Beginning notes: cRNA must be at a minimum concentration of 0.6 □g/□l.

1. Combine reagents in RNase-free, thin-walled PCR tube.

Component	**Volume**
20 □g cRNA (0.6 □g/□l to 2.0 □g/□l.)	1-32 □l
5X Fragmentation Buffer	8□l RNA
DEPC water	Bring volume to 40 □l

2. Incubate at 94°C for *exactly* 35 minutes. *If possible, use heated lid to prevent liquid evaporation. In addition, foil can be placed around the tube to prevent possible evaporation.* Put on ice following the incubation.

3. Store sample at –20°C until ready for shipping to NSUH.

Sample ID	Amount of cRNA to be fragmented	CRNA volume	5x Fragmentation Buffer	DEPC Water	Total Volume