

Modify RNA Fragmentation Time for Intact RNA

To modify the fragmentation of the RNA to allow for longer RNA fragments, the time of fragmentation can be shortened. This is accomplished during the *Ribo-Zero Deplete and Fragment RNA* procedures by modifying the thermal cycler **Elution 2 - Frag - Prime** program: 94°C for X minutes followed by a 4°C hold for the thermal cycler where X is determined by the length of RNA desired. A range of suggested times and sizes is described in Table 25.

Table 25 Library Insert Fragmentation Time

Time at 94 °C (minutes)	Range of Insert Length ^a (bp)	Median Insert Length ^a (bp)	Average Final Library Size (Bioanalyzer bp)
0 ^b	130–350	200	467
1	130–310	190	439
2	130–290	185	410
3	125–250	165	366
4	120–225	160	326
8	120–210	155	309
12	115–180	140	272

a. Insert length determined after clustering and sequencing with a paired-end sequencing run.
b. Start without bringing up to temperature.