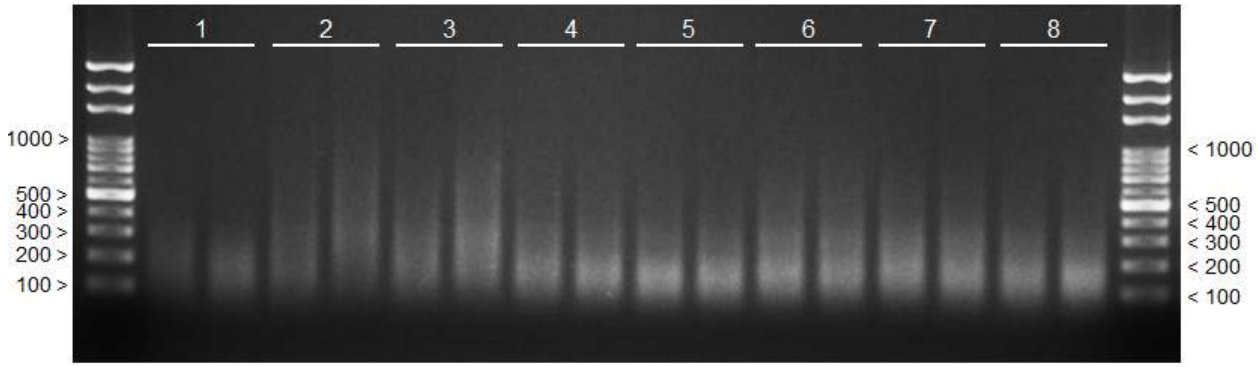
Chromatin Shearing - Tissue

Protocol

Cell Type: Mouse Whole Hippocampi
Lysis Buffer and Concentration: SDS, 1% (Millipore)
Sample volume: 100ul
Sample Concentration Details: ~25mg per hippocampus
Formaldehyde Concentration: 1%
Fixation Time: 15 minutes
Sonicator Amplitude Setting: 30%
Total Sonication ON Time: 30 minutes
Pulse Mode: 10 seconds ON, 10 seconds OFF



Code	Sonicator	Amplitude	On/Off	Total Sonication Time	Quality Notes
1	SLPe probe	50	20s/60s	1 min 20 sec	Probe created a lot more bubbles in 1st replicate vs. 2nd
2	Qsonica	20	10s/10/s	20 min	65-70 Watts (~8 Watts per tube)
3	Qsonica	20	15s/15s	20 min	65-70 Watts (~8 Watts per tube)
4	Qsonica	20	10s/10/s	30 min	65-70 Watts (~8 Watts per tube)
5	Qsonica	30	10s/10/s	30 min	120-126 Watts (~15 Watts per tube)
6	Qsonica	30	15s/15s	30 min	120-126 Watts (~15 Watts per tube)
7	Qsonica	40	10s/10s	20 min	178-190 Watts (~22 Watts per tube)
8	Qsonica	40	10s/10s	30 min	178-190 Watts (~22 Watts per tube)

Comparison of Probe and Q800R

- Both probe and Q800R can create fragment peaks at 150-200bp.
- Probe is more powerful than Q800R but the probe creates smears with less overall brightness.
- Inter-sample variability is an issue with probe.

For Q800R

- Total time of sonication seems to be most important, as the tdifference between 20 and 30 min. is most noticeable, regardless of amplitude or pulse cycle.
- The sonication seems to plateau at 30% amplitude with no extra benefit of soncating at 40%.

Customer Notes

- Used 12 Tube rack with GeneMate 0.65mL tubes (C-3259-1)
- Sonicator provided consistent results over a variety of settings.
- Q800R processes 12 samples at a time vs. probe with 1 single sample.
- Generated fragments of 150-200bp.