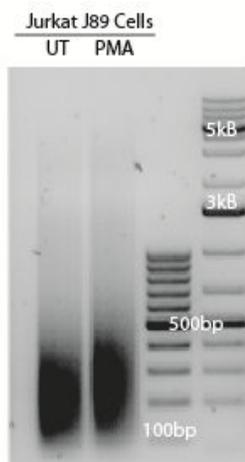


Chromatin Shearing – Jurkats, HEK293

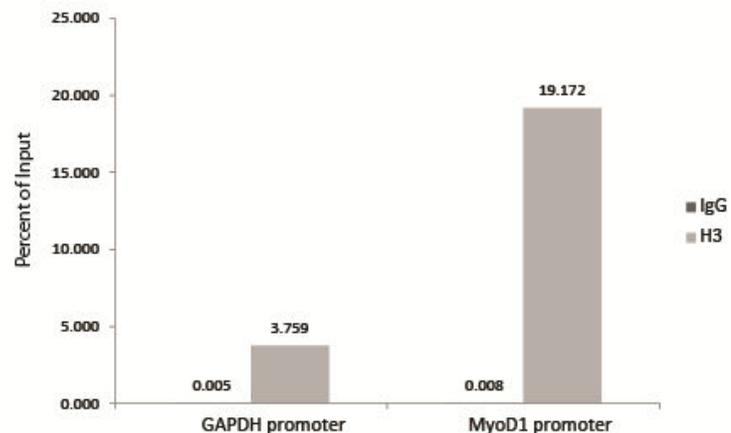
Protocol

Cell Type:

Lysis Buffer and Concentration:	0.5% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1, protease/phosphatase inhibitors
Sample volume:	300uL
Sample Concentration Details:	10 ⁶ cells/tube
Formaldehyde Concentration:	0.75%
Fixation Time:	10 min
Sonicator Amplitude Setting:	70%
Total Sonication ON Time:	22 minutes
Pulse Mode:	15 seconds On/ 30 seconds Off



PMA treated and untreated Jurkat J89 cells crosslinked with 0.75% formaldehyde for 10min followed by glycine quench at 0.25M for 5min. 10E6 cells in 300uL 0.5% SDS lysis buffer were sonicated in thin wall 0.5mL PCR tubes at 70% amplitude for 22 minutes of total ON time, 15sec on/30sec off in Q800R. Samples were reverse crosslinked, DNA was recovered via phenol chloroform extraction and run on a 1.5% agarose gel.



ChIP of total H3 (Abcam ab1791) at active GAPDH promoter versus inactive MyoD1 promoter in J89 cells.

Customer Notes

Start with a fixed number of cells and run various total sonication 'on' times to find the ideal sonication pattern. For ChIPs, make sure to properly process samples including reverse crosslinking and proteinase K treatment prior to a phenol/chloroform extraction to recover sonicated DNA. The starting points provided by other owners of the Q800R worked well as initial starting points for these cell lines.

The Q800R has provided consistent sonication and is quite easy to use. ChIP data shows differential enrichment of total H3 at genomic loci with differing transcriptional activity and gel analysis of DNA shows good sonication with the predominant products between 100-500bp.

Having used probe and water-bath sonicators, the Q800R is by far the easiest and most consistent. The amount of cells that can be sonicated at once beat any water-bath type sonicator, you can run a good amount of samples (12 for the 0.5mL thin wall), and you don't have to sonicate samples one-by-one. It does take a while to run as the ON time is longer than other types of sonicators, but it's worth it to be able to leave your samples and multi task. Another big selling point was the ability to replace just the cup horn as it is expected to lose power over time. This is a much better alternative to having to replace the entire machine.