

# Comparing DNA libraries prepared with the Qsonica and Branson Instruments

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# Experimental Outline

- We are using formaldehyde fixed K562 cells
- We will shear cells using the Branson Probe Sonifier or the Qsonica Q800 System and compare results
- We will perform Chromatin Immunoprecipitation using antibodies to H3K4me3 and H3K27me3
- We prepare and sequence libraries to assess how shearing instruments and parameters may affect outcome overall
- ChIPs for each condition were prepared in parallel with the same batch of cross linked cells, same antibody concentration, and the same number of cells per antibody

# Protocol Outline

Cell Lysis 20mM Tris pH 8.0, 85mM KCl, 0.5% NP40

Nuclear Lysis by two methods

Qsonica Q800 System

1% (SDS) Buffer

50mM Tris pH 8.0, 1% SDS, 10mM EDTA

Closed 500ul thin walled tubes  
4°C maintained  
0.3ml, 3e6 cells/tube

Amplitude 70  
15s on, 45s off  
3.6e7 cells  
"On" time ~ 30'

Qsonica 1

Amplitude 50  
30s on, 30s off  
3.6e7 cells  
"On" time ~7.5'

Qsonica 2

Branson Probe sonicator

0.1% (SDS) Buffer

10mM Tris pH 8.0, 0.5% NaDOC, 1% NP40, 0.1% SDS

Open 1.5ml tubes  
4°C (inconsistent)  
1ml, 1e7 cells/tube

Amplitude ~ 36-40  
0.7s on, 1.3s off  
4e7 cells  
"On" time = 6'

Branson

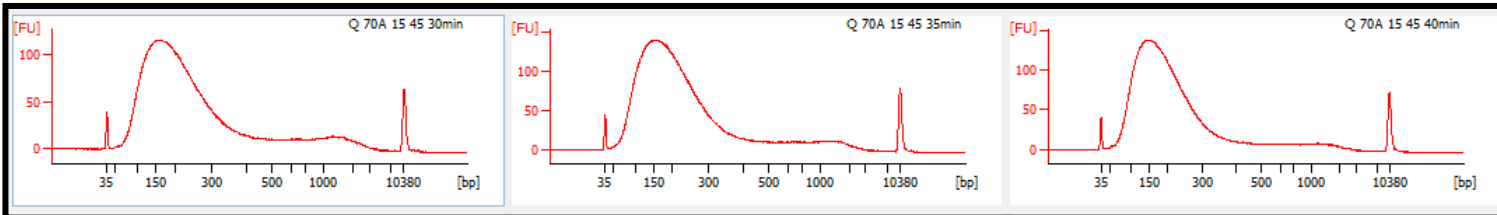
# BioAnalyzer Traces of Sheared Chromatin

**Qsonica 1: 70A, 15s on, 45s off**

**“On” time = 30’**

**“On” time = 35’**

**“On” time = 40’**

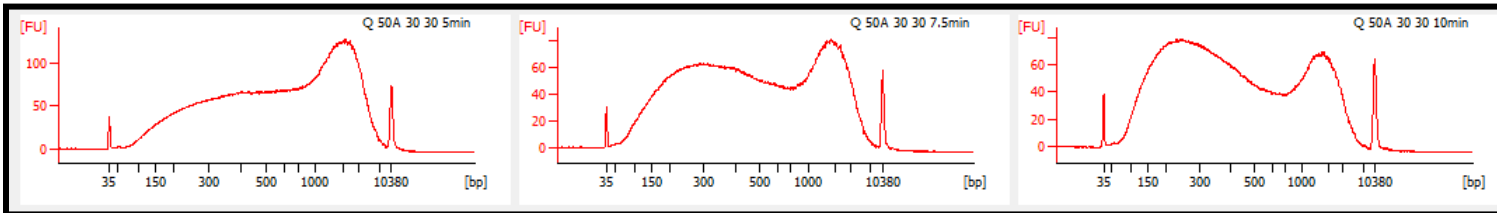


**Qsonica 2: 50A, 30s on, 30s off**

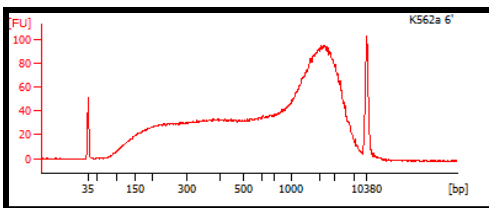
**“On” time = 5’**

**“On” time = 7.5’**

**“On” time = 10’**



**Branson: 40A, 0.7s on, 1.3s off, “On” time = 6’**



fragment size  
Small  $\longleftrightarrow$  Large

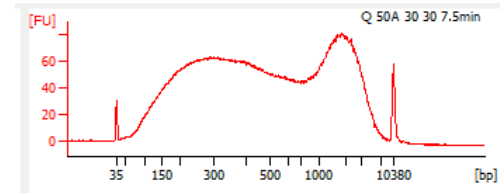
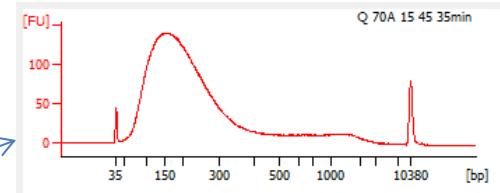
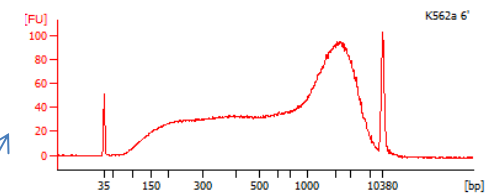
# Chromatin sheared with the Qsonica 1 (35'), Qsonica 2 (7.5') and the Branson Sonifier (6') was used in ChIP

## Conditions of each Chromatin Prep

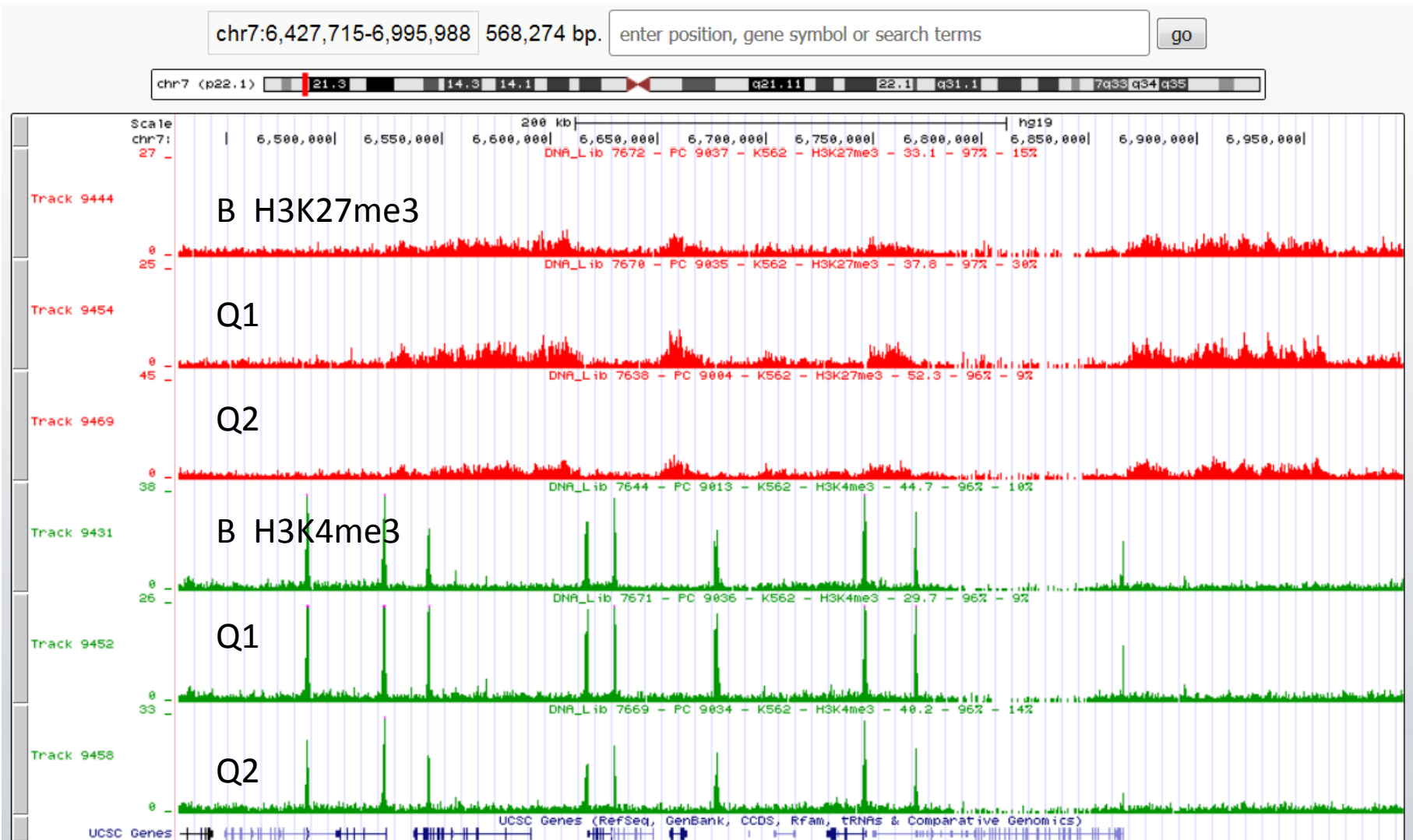
	Sonication min	amplitude	time on	time off
Branson	6	40	0.7s	1.3s
Qsonica 1	35	70	15s	45s
Qsonica 2	7.5	50	30s	30s

## % material in size range

	Sonication min	80-150bp	151-700bp	701-8500bp
Branson	6	6	59	26
Qsonica 1	35	30	63	4
Qsonica 2	7.5	12	44	43



# Comparison of Tracks obtained



# Summary and Conclusion

- The Qsonica instrument is capable of producing a range of fragment size distributions, depending on the operating parameters.
- Excellent ChIP-seq results can be obtained using the Qsonica instrument, for both “active” and “repressive” histone modifications. These are similar to the results obtained using Branson probe sonification.
- Mononucleosome enriched chromatin obtained using the Qsonica (Q1) may represent the ideal parameter set for repressive histone modifications.