Qsonica

Q800R3 超声波破碎仪

Protocols

版本号：V1.3

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目　录

Comparing DNA libraries prepared with the Qsonica and Branson Instruments

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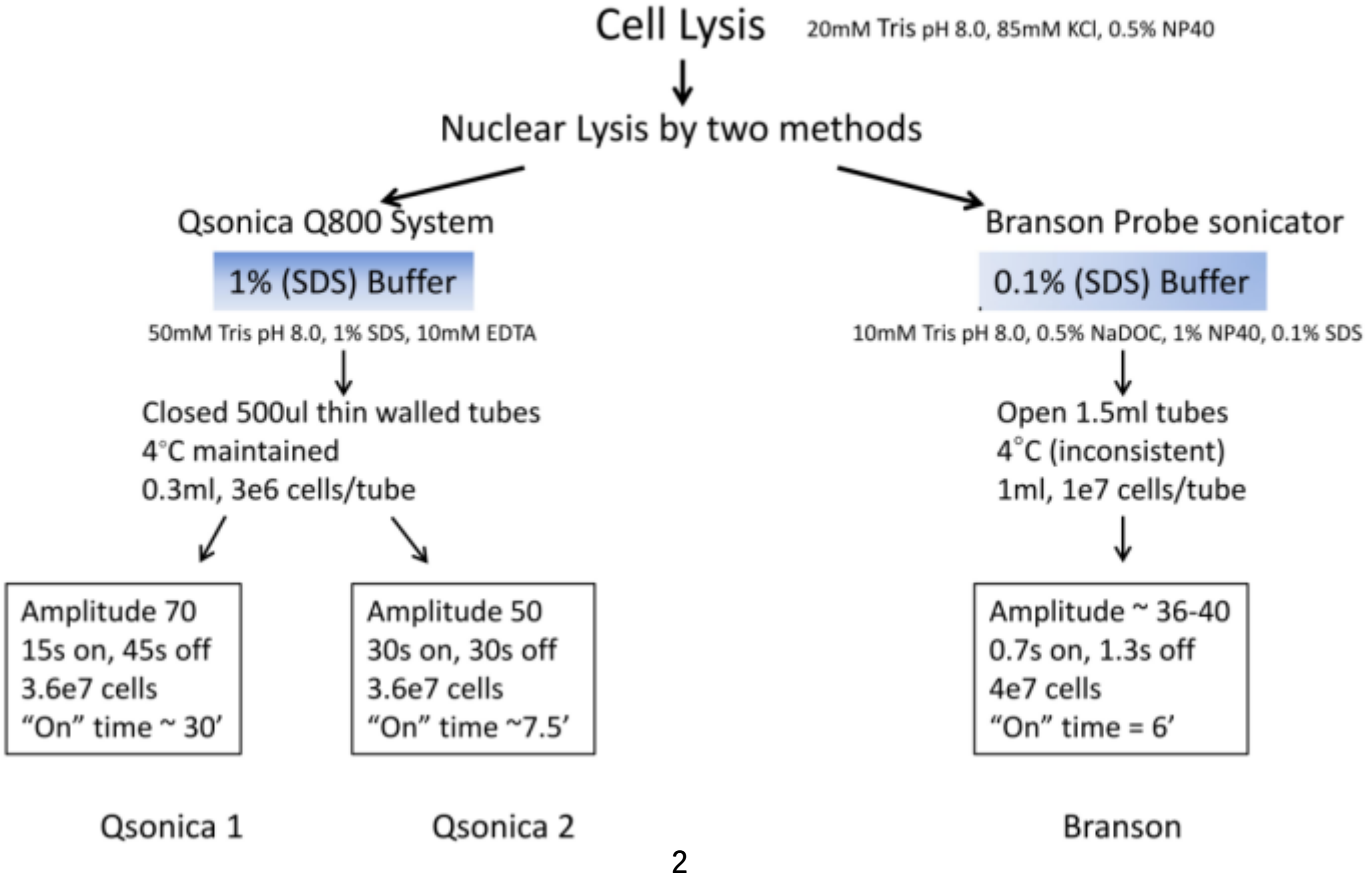
April, 2016

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

QSonica Evaluation R.Issner Broad Institute

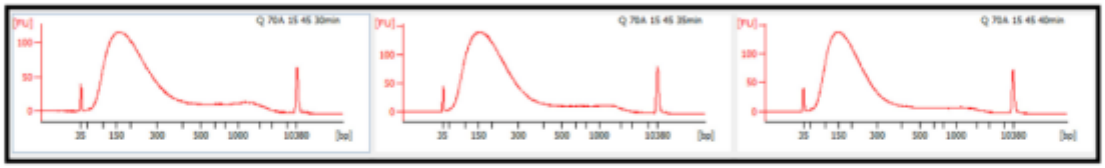
Protocol Outline



BioAnalyzer Traces of Sheared Chromatin

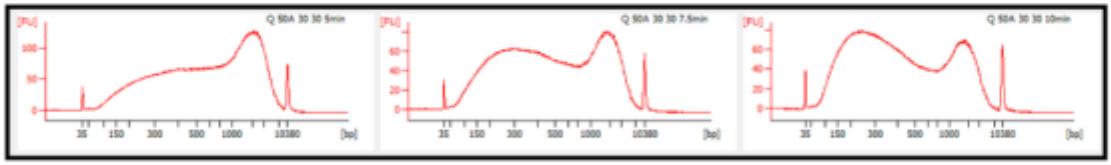
Qsonica 1: 70A, 15s on, 45s off

"On" time = 30' **"On" time = 35'** **"On" time = 40'**

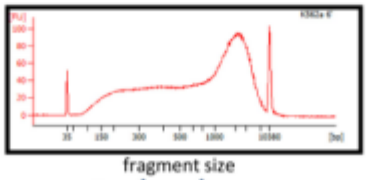


Qsonica 2: 50A, 30s on, 30s off

"On" time = 5' **"On" time = 7.5'** **"On" time = 10'**



Branson: 40A, 0.7s on, 1.3s off, "On" time = 6'



fragment size

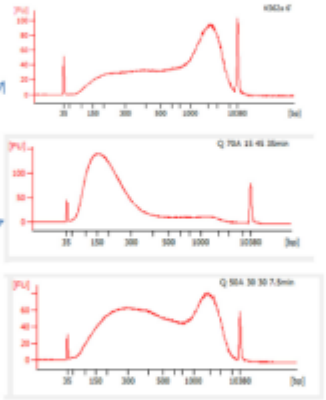
Small ⟷ Large

QSonica Evaluation R.Issner Broad Institute

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

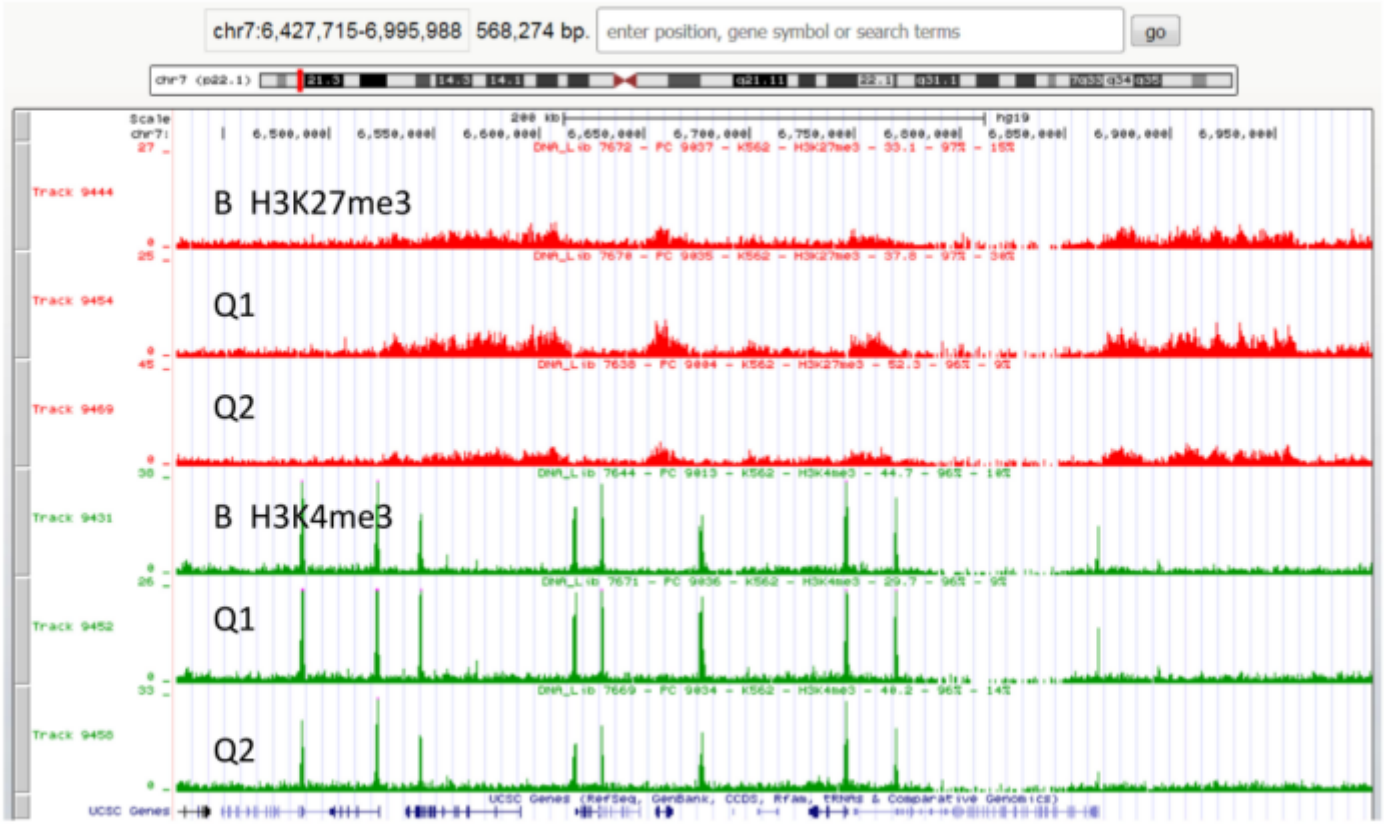


% material in size range

	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

QSonica Evaluation R.Issner Broad Institute

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.

ChIP (Whetstine Lab)

Chromatin

- Crosslink cells by adding 1% formaldehyde to the media, 13 min 37 degrees. Stop by adding Glycine to 0.125M.
- Wash cold PBS. Add PBS on dish (2.5 ml for 15 cm dish). Scrape, collect in 15 ml tube, spin 2 min 800 rpm 4 degrees. Resuspend the pellet in cellular lysis buffer-proteases inhibitors (typically 2.5 ml for 1 15 cm dish 293T, 1 ml for 1 10 cm dishes 293T-2 10 cm RPE-1 15 cm RPE), incubate 5 min on ice and spin 2 min 800 rpm 4 degrees. Discard the supernatant and resuspend the pellet in nuclear lysis buffer-proteases inhibitors (typically 300 µl for 1 10 cm dishes 293T-2 10 cm RPE- 1 15 cm RPE). It corresponds to 1-1.2x107 cells per 300 µl.
- **Sonication:** Q800R. 300 µl of chromatin in 0.5 ml thin wall PCR tubes (Brandtech #781312). To get the chromatin <300bp: sonicator at 70% amplitude 15 sec on 45 sec off (RPE in 0.2% SDS 40min. time on; in 1% SDS, 30min. time on). Sonication test. Spin 10 min 14000 rpm 4 degrees for clear the chromatin.

IPs

- Prebind the beads with antibody. In a 1.5 ml tube: 900 µl dilution IP buffer-proteases inhibitors + 25 µl protein A or G magnetic beads (A for rabbit polyclonal Ab, G for mouse monoclonal) + 2 µg Ab. Incubate at least 6h rotator 4 degrees.
- Add 100 µl of wanted amount of chromatin (in nuclear lysis buffer) in the tube, usually 10-30
- µg. Incubate o/n rotator 4 degrees. A preclear step can be added before the IP.
- Washes: two times 1 ml dilution IP buffer, one time TSE buffer, one time LiCl buffer, two times TE. Vortex + incubate couple min each time.
- Elution: add 150 µl elution buffer + 1 µl RNase A 200 mg/ml, incubate 30 min 37 degrees 1000 rpm. Add 1 µl proteinase K 10 mg/ml, 1h 55 degrees 1000 rpm (or vortex sometimes). Treat inputs the same way from this step. The RNase treatment is optional.
- Decrosslinking: remove the samples from the beads and incubate 4h 65 degrees.
- Purification DNA with a PCR clean up system kit.

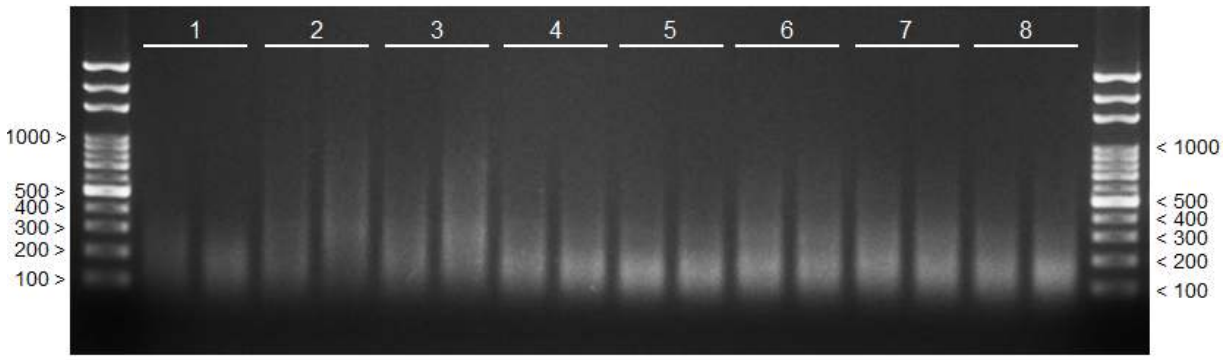
Buffers

- Cellular lysis: 5 mM PIPES pH8, 85 mM KCl, 0.5% NP40.
- Nuclear lysis: 50 mM Tris pH8, 10 mM EDTA pH8, 0.2% or 1% SDS.
- Dilution IP: 16.7 mM Tris pH 8, 1.2 mM EDTA pH8, 167 mM NaCl, 0.01% SDS, 1.1% Triton X100.
- TSE: 20 mM Tris pH8, 2 mM EDTA pH8, 500 mM NaCl, 1% Triton X100, 0.1% SDS.
- LiCl: 100 mM Tris pH8, 500 mM LiCl, 1% deoxycholic acid, 1% NP40.
- TE: 10 mM Tris pH8, 1 mM EDTA pH8.
- Elution: 50 mM NaHCO3, 140 mM NaCl, 1% SDS.

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

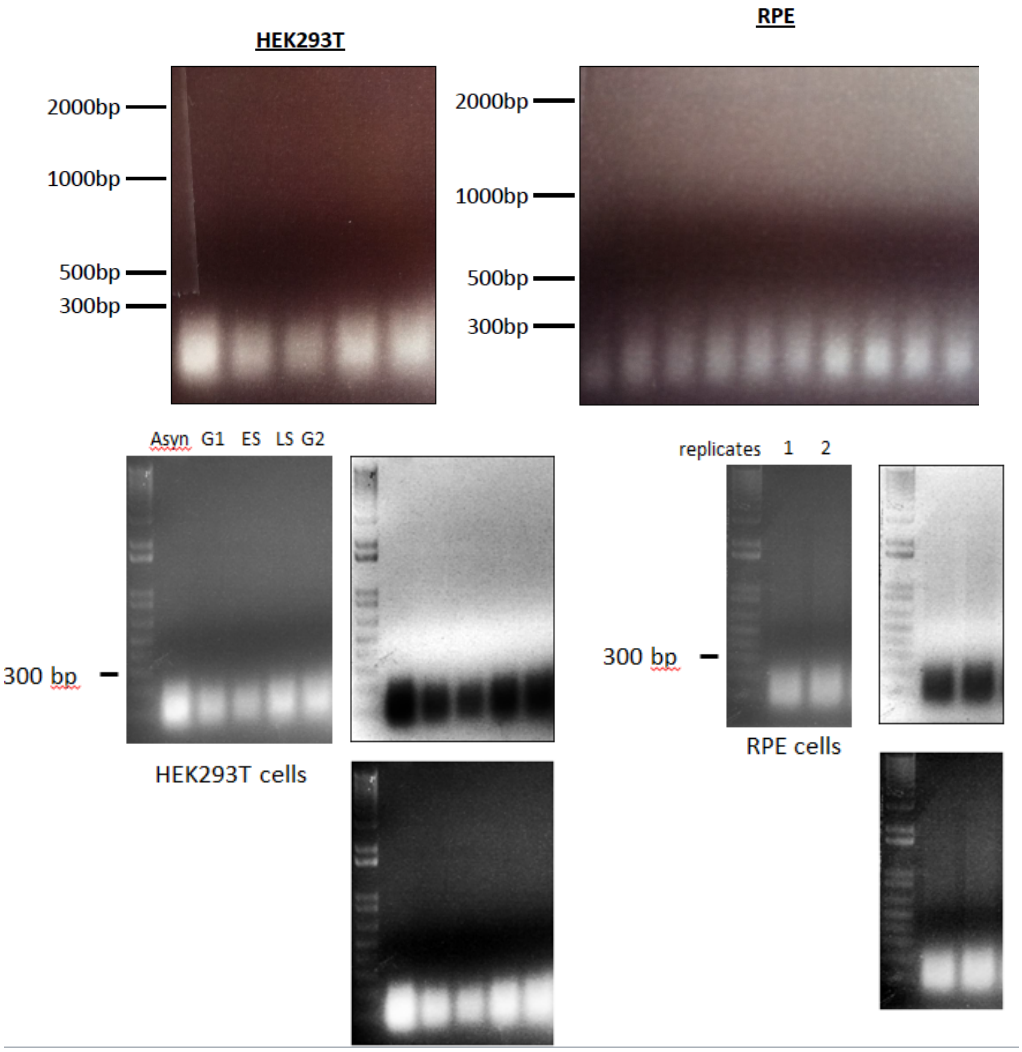
- Total time of sonication seems to be most important, as the tdifference between 20 and 30 min. is most noticeable, regardless of amplitude or pulse cycle.
- The sonication seems to plateau at 30% amplitude with no extra benefit of soncating at 40%.

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.

Human Cell Line Chromatin Prep

Example protocols and results are based on customer feedback.



Ladder : 1kb plus Invitrogen

Protocol:

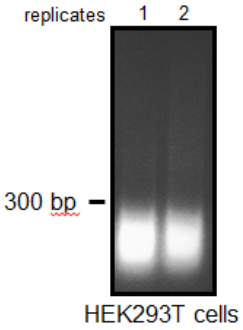
Cell Type: HEK 293T (2) 10cm dishes 70-80% confluent ; RPE (1) 15cm dish 70-80% confluent
Total Sample volume: 300ul
Fixation Time: 1% Formaldehyde, 13 min
Sonicator Amplitude Setting: 70%
Sonication Pulse Rate: 15 seconds On, 45 seconds Off
Total Sonication On Time: 30 min.
Sample Process Temperature: 4°C

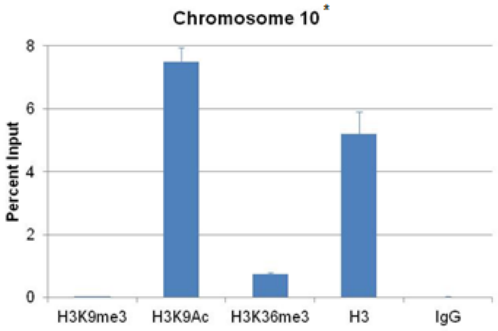
Customer Notes :

- Samples were processed in Brand Tech 0.5ml thin walled PCR tubes (Ref. #781312)
- The sonication is highly reproducible from time to time.
- This is a really good sonicator which allows one to sonicate a lot of samples at the same time.
- A cup horn is better than a probe because nothing is in contact with the sample, and it allows to sonicate much smaller volumes.
- Sonication time is needed to get all DNA under 300bp (one nucleosome)
- If more cells are used it will need a longer sonication.
- Tested as low as 50ul sample volumes successfully, > 300ul produces inefficient results for these samples

Mammalian Chromatin Prep & ChIP

Example protocols and results are based on customer feedback.





*Average of two independent replicate chromatins in panel A.

Chr10 Primer sequences:
Forward- TCCTTCTCCCAACAATCAGC
Reverse- GATGTCGCTCCGAATCTTG

Antibodies Used: H3K9me3 (abcam ab8898), H3K9Ac (upstate 07-352), H3K36me3 (abcam ab9050), H3 (abcam ab1791)

Protocol Information

Cell Type: HEK 293T (2) 10cm dishes 70-80% confluent
Total Sample Volume: 300ul
Fixation Time: 1% Formaldehyde, 13 min
Sonicator Amplitude Setting: 70%
Sonication Pulse Rate: 15 seconds On, 45 seconds Off
Total Sonication On Time: 30 min.
Sample Process Temperature: 4°C

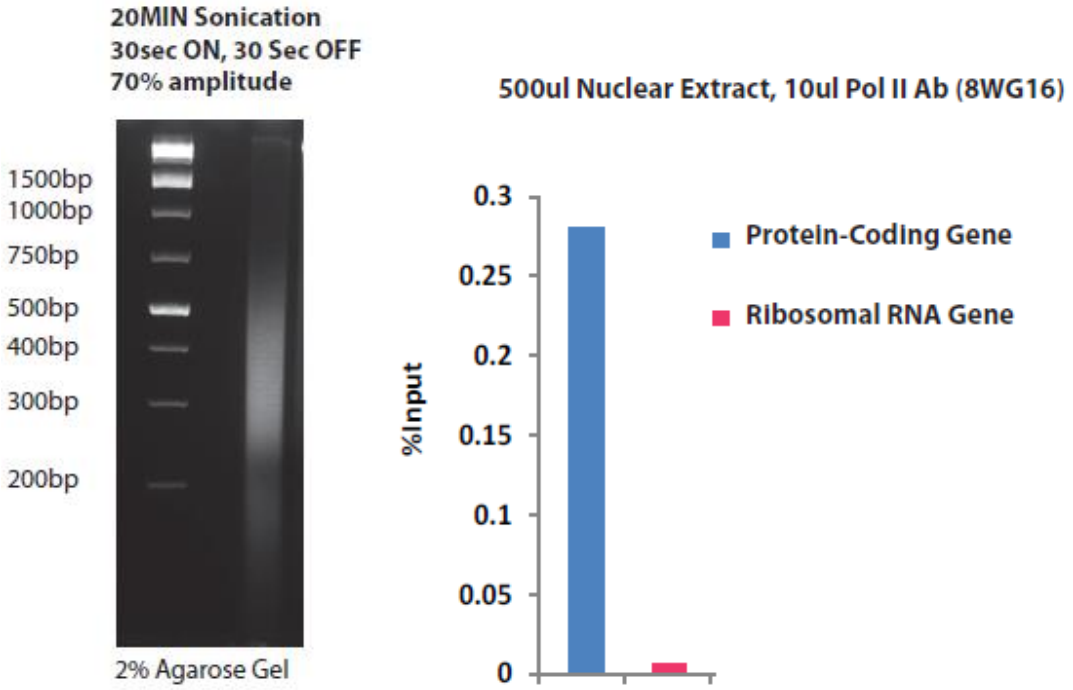
1. Crosslink cells by adding 1% Formaldehyde to the media, 13 min 37°C.
2. Stop by adding pHed Glycine to 0.125M.
3. Wash cold PBS.
4. On ice, add PBS on dish (2.5 ml).
5. Scrape, collect in 15 ml tube, spin for 2 min at 800 rpm, 4°C.
6. Resuspend pellet in cellular lysis buffer-protease inhibitors (Example: 2.5 ml for (1) 15cm dish, 1 ml for (2) 10cm dishes)
7. Incubate 5 min on ice and then spin for 2 min at 800 rpm 4°C.
8. Discard the supernatant and resuspend the pellet in nuclear lysis buffer-protease inhibitors (2 ml for four 15 cm dishes, 300 ml for two 10 cm dishes 293T-3 10 cm RPE).
   - Cellular lysis: 5 mM PIPES, 85 mM KCl, 0.5% NP40.
   - Nuclear lysis: 50 mM Tris pH8, 10 mM EDTA pH8, 0.2 or 1% SDS (depends on application).
9. Sonication : For two 10 cm dishes (293T), using Q800R System Sonicate for 30 min at 70%. Sonication test. Spin for 10 min at 14000 rpm, 4 °C to clear chromatin.

Customer Notes:
- To obtain chromatin less than 300bp. Aliquot 300ul of chromatin in thin walled PCR tubes from Brandtech #781312.
RPE cells :
- In 1% SDS : 35 min of sonication time (total on).
- In 0.2% SDS (or other weaker detergent): 45 min of sonication time (total on).

*Total time is longer than a probe alone but 12 samples can be processed at one time and results are very consistent.

C. elegans Chromatin Prep

Example protocols and results are based on customer feedback.

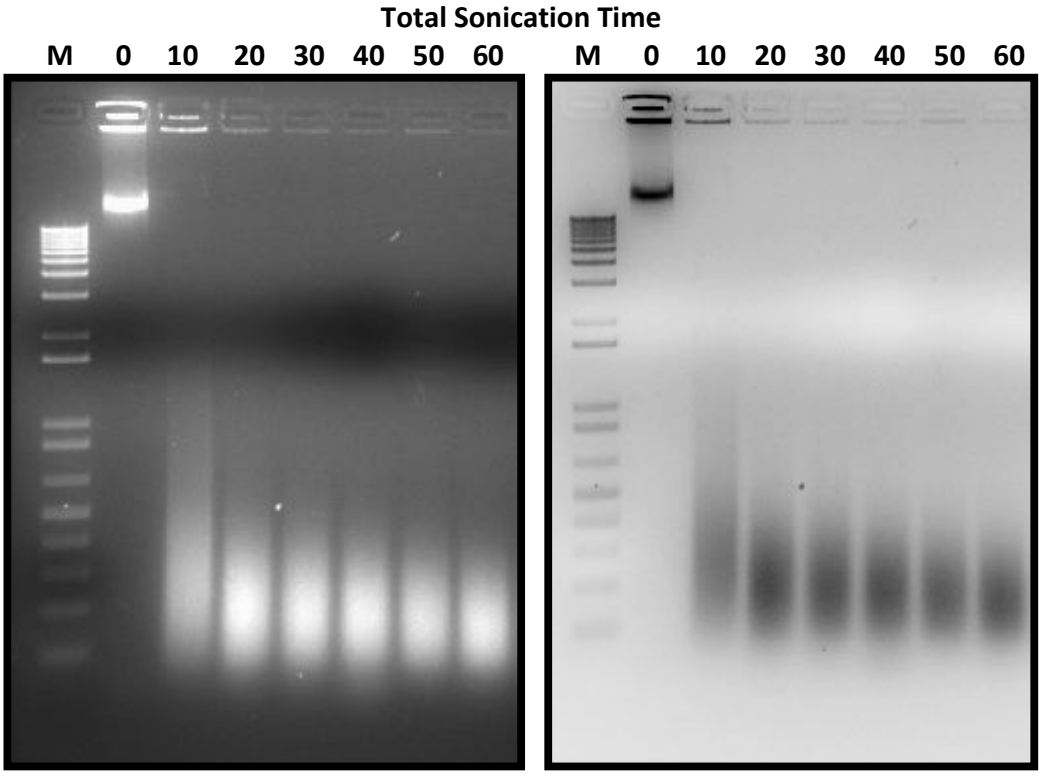


Protocol:

Cell Type: *C. elegans* culture; Nuclear extract 1mg/ml
Total Sample Volume: 500ul
Fixation Time: 2% Formaldehyde, 20 min
Sonicator Amplitude Setting: 70%
Sonication Pulse Rate: 30 seconds On, 30 seconds Off
Total Sonication On Time: 20 min
Sample Process Temperature: 4°C; Sonicator is set up in a 4°C Cold Room.
Customer Notes: ChIP using anti-Pol II antibody

Yeast Chromatin Prep

Example protocols and results are based on customer feedback.



No clarification of sample *Zymolyase treated cells*

Protocol

Cell Type: Wild type *S. pombe* cells grown to an OD of 1.3-1.5 in YEA
Total Sample Volume: 250-300ul
Fixation Time: 1% Formaldehyde, 15 min
Sonicator Amplitude Setting: 70%
Sonication Pulse Rate: 20 seconds On, 40 seconds Off
Total Sonication On Time: 12 minutes
Sample Process Temperature: 4°C

Customer Notes

Use Zymolase for lysis

- Cross-link 50 - 200ml of S. pombe cells grown to a density of OD600 @ 1.3-1.5 with 1% formaldehyde for 15 minutes
- Quench cross-linking, wash and spin cells down to 0.16-0.18g per pellet
- Resuspend cells in 1.0ml of room temperature PEMS buffer and add 20-40ul of Zymolase per pellet
- Incubate at 32^0 for one hour in an end-over-end nutator
- Wash cells 2X in PEMS buffer
- Resuspend cells in 250ul of lysis buffer and transfer to the 0.5ml PCR tube (Brand Tech #781312)
- Sonication (settings listed above)

Zymolase concentration may require standardization for each new batch. Manufacturers report batch to batch variability for enzyme activity.

- DNA samples shown are prior to centrifugation and clearing of the lysate, the entire genome is represented.
- We use 10X less SDS in our lysis buffer, so 0.1% SDS.

Lower SDS concentration is hugely beneficial to us for 3 reasons:

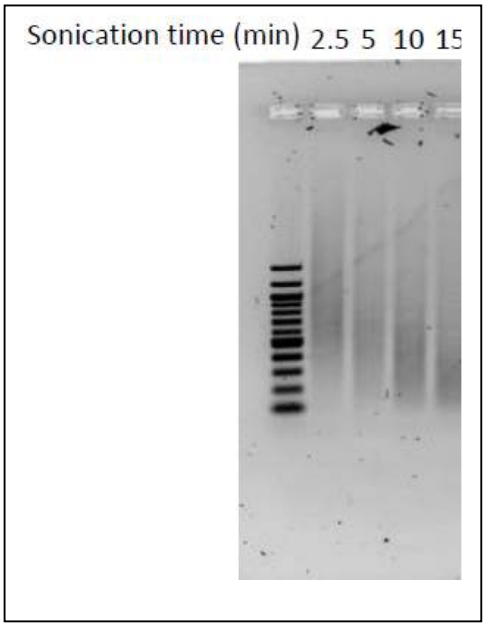
1. We don't have to decrease SDS concentration afterwards, so our protein concentration remains high for the IP part of ChIP (especially relevant for rare proteins)
2. Some proteins' structures do not tolerate such high SDS levels, unfold and don't refold properly. This could affect their interactions with other proteins or DNA, thus affecting their localization. Also note that for the proteins that miss-fold and don't refold following lowering the SDS concentration, the antibody no longer recognizes the protein, even once the SDS level is reduced to the normal 0.1%.
3. High SDS concentration (1%) interferes with antibody antigen interaction, and thus must be lowered for the IP part of ChIP experiments.

Yeast and Mammalian Chromatin Prep

Example protocols and results are based on customer feedback.

Yeast Protocol:

Cell Type: Budding yeast; 5 -20 ml worth of culture at OD around 1.0 per sample
Sample Lysis Solution: 50 mM Tris, pH 7.5, 300 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 10% Glycerol
Total Sample volume: 500ul
Fixation Time: 1% Formaldehyde, 5-15 min
Sonicator Amplitude Setting: 50%
Sonication Pulse Rate: 30 seconds On, 30 seconds Off
Total Sonication On Time: 5-30 min.
Sample Process Temperature: 4°C



Mammalian Cell Protocol:

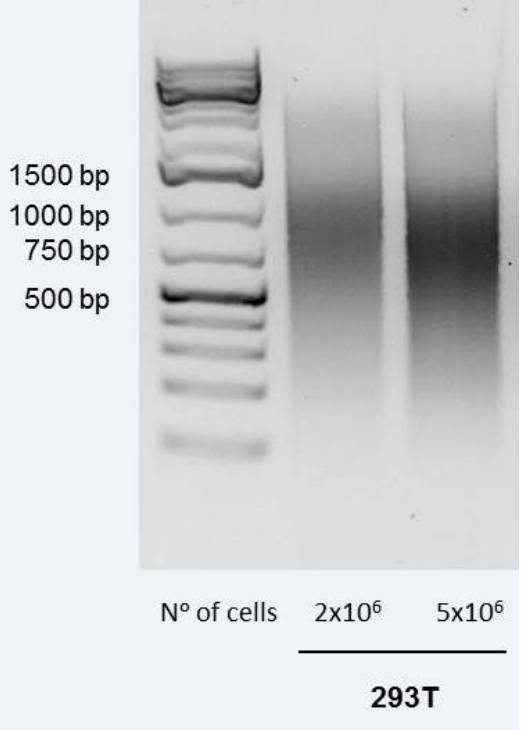
Cell Type: Mammalian cells (mouse and human); 1-5 million cells per sample
Sample Lysis Solution: RIPA buffer
Total Sample volume: 500ul
Fixation Time: 1% Formaldehyde, 5-15 min
Sonicator Amplitude Setting: 50%
Sonication Pulse Rate: 30 seconds On, 30 seconds Off
Total Sonication On Time: 5-30 min.
Sample Process Temperature: 4°C

Customer Notes:

- In general, we use 5 min for ChIPping histones and histone modifications.
- For cultured human fibroblasts and MSCs, 15-20 min sonication is enough.
- You will need longer time for mouse or human tissues, and MEFs.
- Crosslinking time should also be empirically determined.
- The shortest effective time is the best because crosslinking can cause aggregation of soluble proteins and make chromatin harder to solubilize.
- It is not necessary to keep it in the cold room. We keep it in room temp on a bench and use the chiller.
- To reduce the time needed to chiller the water at the start of a cycle, we keep several bottles of water chilled in a cold room and pour into the water bath prior to use.
- We wrap the tubing to and from chiller in absorbent pads for insulation. It helps the chiller to cool the water more efficiently and prevents condensation from dripping all over the bench.
- Samples processed using 1.5ml polystyrene tubes (Evergreen).
- The Q800R has a more consistent and efficient sonication performance when compared to Bioruptor.

293T Chromatin Prep

Example protocols and results are based on customer feedback.



Protocol:

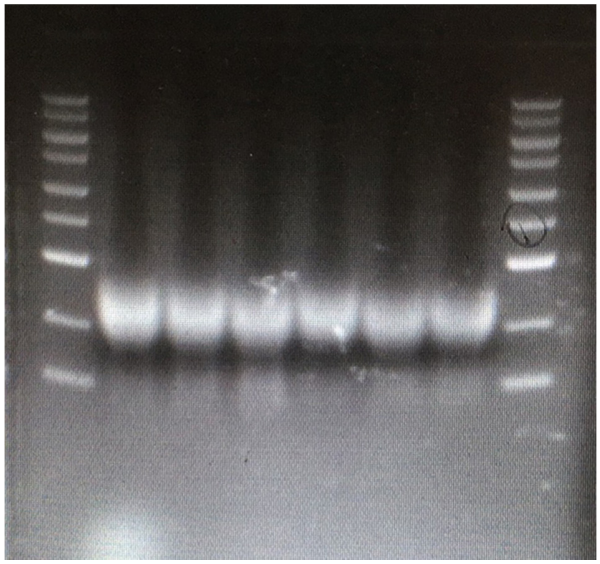
Cell Type: 293T
Sample Lysis Solution: 50 mM Tris-HCl pH 8, 10 mM EDTA, 1% SDS, protease inhibitor cocktail Set I (Calbiochem)
Sample Details: Normally we use 20 million cells per ChIP. During the sonication we divide the pellet in 6 tubes with a final volume of 200 or 300µL of Lysis buffer
Total Sample volume: 200-300ul
Fixation Time: 1% Formaldehyde, 10 min
Sonicator Amplitude Setting: 30%
Sonication Pulse Rate: 30 seconds On, 30 seconds Off
Total Sonication On Time: 15 min.
Sample Process Temperature: 4°C; Sonicator is set up in a 4°C Cold Room.

Customer Notes:

- Once you set up your protocol follow it step by step.
- Use same number of cells, lysis buffer and final volume.
- Samples processed using 1.5ml polystyrene tubes (Evergreen).
- System works better than Sonicator tips and chiller is better than competitor's unit.
- Also uses Qsonica system for Neonatal rat ventricular myocytes (NRVMs), HeLa, C2C12 and 3T3 cell lines

Chromatin Shearing

Example protocols and results are based on customer feedback.



Marker: 100bp, 250bp, 500bp, 750bp starting from the bottom.

Protocol:

Sample Type: *293T cell, 10e6*
Sample Volume: *300µl*
Sonicator Amplitude Setting: *80%*
Sonication Pulse Rate: *20 seconds ON, 40 seconds OFF*
Total Sonication ON Time: *8 minutes*
Sample Process Temperature: *8C - controlled by Chiller*

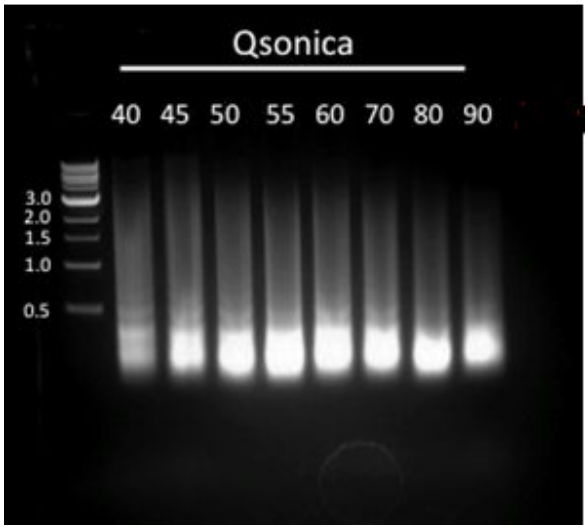
Customer Notes:

Target fragment: 200-500bp
Using 500ul pcr tubes
Image from Dr. Shao

Chromatin Shearing – T Cells

Protocol

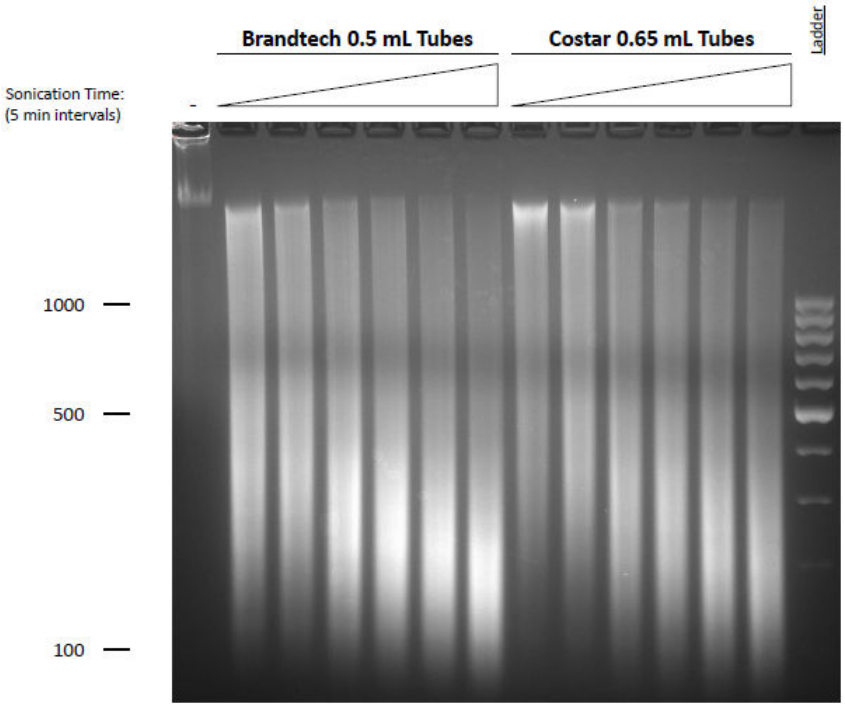
Cell Type:	Mouse DC4 T Cells
Lysis Buffer and Concentration:	Nuclear lysis buffer (1%SDS, 10mMEDTA, 50mM Tris, pH 8.1)
Sample volume:	200ul
Sample Concentration Details:	5 X10^6
Formaldehyde Concentration:	1%
Fixation Time:	10 min at 37^{0}C
Sonicator Amplitude Setting:	85%
Total Sonication ON Time:	60 minutes
Pulse Mode:	30 seconds On/Off



Customer Notes

- Processed up to 12 samples at a time.
- Used 500ul Qubit assay tubes.
- Chiller held temperature well.

Chromatin Shearing
HCT116



Customer Notes

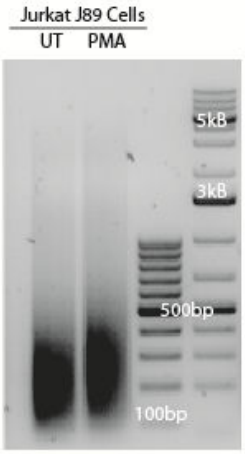
- Chromatin from **HCT116** cells was prepared according to ChIP-seq protocol Schmidt et al. 2009.
- Sonication was performed in 5 minute intervals at 50% amplitude setting.
- DNA was purified according to the protocol and run on a 2% agarose gel containing 0.01% ethidium bromide.
- Tubes tested were Brandtech (Cat. #781310) and Corning (Cat. #3208)

References:
Schmidt, Dominic et al. "ChIP-Seq: Using High-Throughput Sequencing to Discover Protein-DNA Interactions." *Methods (San Diego, Calif.)* 48.3 (2009): 240–248. *PMC*. Web. 3 May 2016.

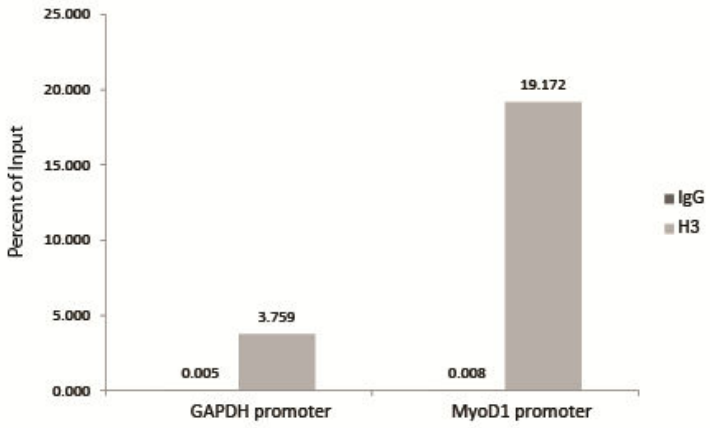
Chromatin Shearing – Jurkats, HEK293

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% amplitute 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

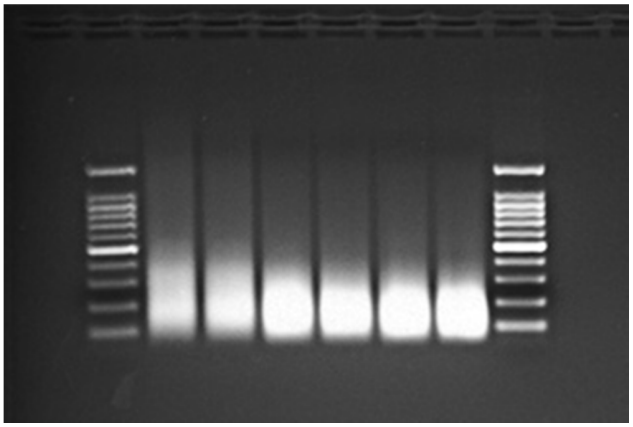
Start with a fixed number of cells and run various total sonication 'on' times to find the ideal sonication pattern. For ChIPs, make sure to properly process samples including reverse crosslinking and proteinase K treatment prior to a phenol/chloroform extraction to recover sonicated DNA. The starting 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 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.

Chromatin Shearing

Example protocols and results are based on customer feedback.



Marker: 100bp, 200bp, 300bp, 400bp, 500bp starting from the bottom.

Protocol:

Sample Type: CH12 cell, 1.2×10e7
Sample Volume: *300µl*
Sonicator Amplitude Setting: *80%*
Sonication Pulse Rate: *20 seconds ON, 40 seconds OFF*
Total Sonication ON Time: *10min, 14min, 18min, 22min, 26min, 30min from left*
Sample Process Temperature: *8C - controlled by Chiller*

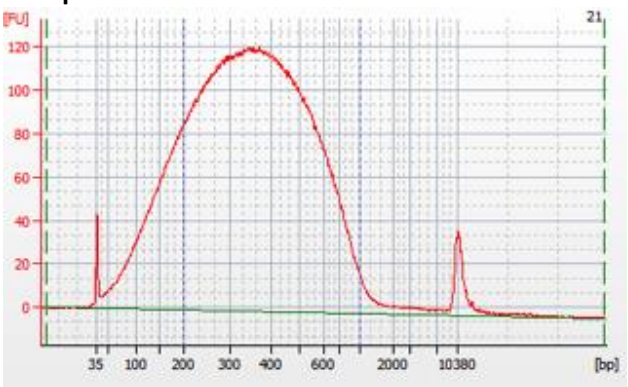
Customer Notes:

Target fragment: 100-300bp
Using 500ul pcr tubes
Image from Dr. Luo

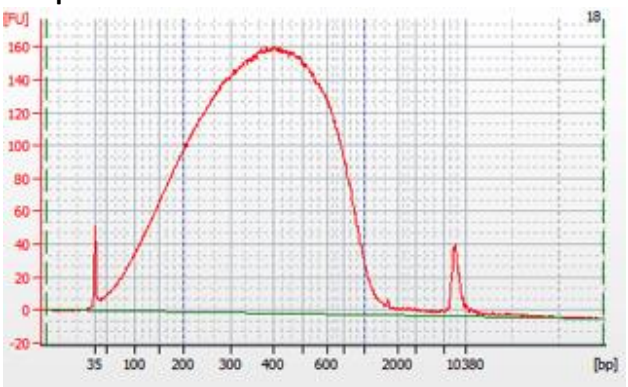
Avian Blood and Tissue Genomic DNA Shearing

Example protocols and results are based on customer feedback.

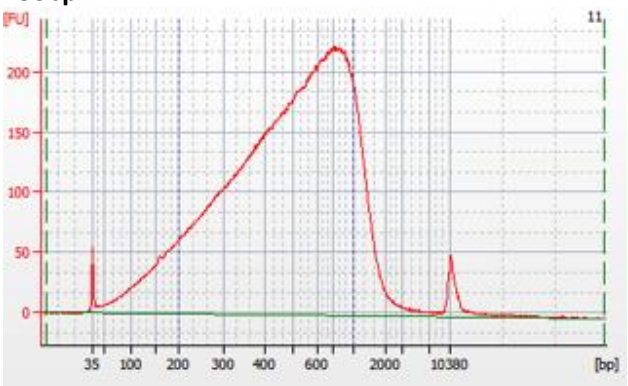
350bp



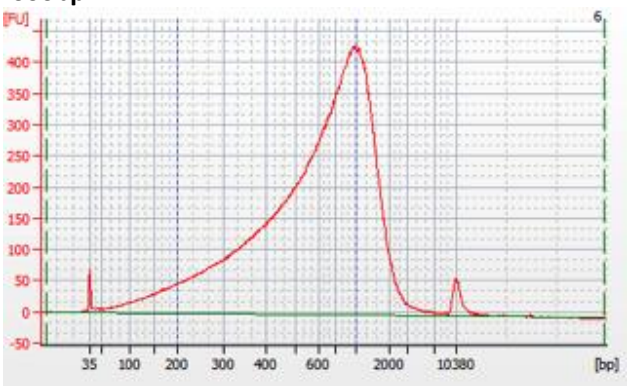
450bp



750bp



1000bp



Protocol Information

Cell Type: Avian Blood and Tissue samples
Total Sample Volume: 100ul
Sonicator Amplitude Setting: 25%
Sonication Pulse Rate: 15 seconds On, 15 seconds Off
Total Sonication On Time: Desired fragment size dependent. See Reference guide below
Sample Process Temperature: 3°C

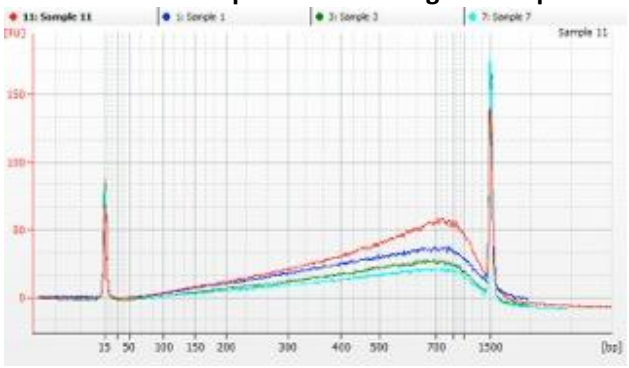
Fragment Size Reference Guide:

Fragment size (bp)	1000	750	450	350
Amplitude (25)	25	25	25	25
Pulse (on:off)	(15:15)	(15:15)	(15:15)	(15:15)
Duration (Seconds)	60	105	150	180

Customer Notes:

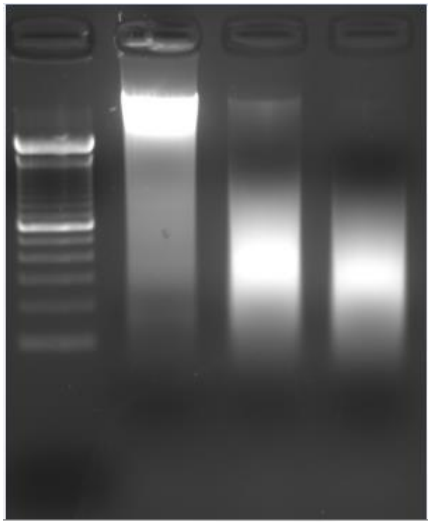
1) Turn on chiller and set to 3 °C
2) Adjust water level in cup horn to match the sample level in the tube.
3) Aliquot 100 µl high molecular weight DNA (TE buffer or sterile water) into individual 0.2 ml PCR tubes
4) Chill the DNA aliquots to 4 °C
5) When chilled, spin down PCR tubes for 30 seconds at 1000 RPM
6) Carefully place tubes into sample cradle, making sure not to splash sample onto the tube walls
7) Carefully attach loaded sample cradle to the rotator, place into instrument and close the lid
8) Sonicate for desired duration, amplitude and pulse
9) Sheared DNA may now be used as starting material for library preparation

Concentration Independent shearing to 750bp



Bacterial Genomic DNA Shearing

Example protocols and results are based on customer feedback.



Sonication Conditions:
Lane 1 – unsheared
Lane 2 – 5min Total Sonication
Lane 3 – 10min Total Sonication
**Samples run on 1% agarose gel, 100bp ladder.*

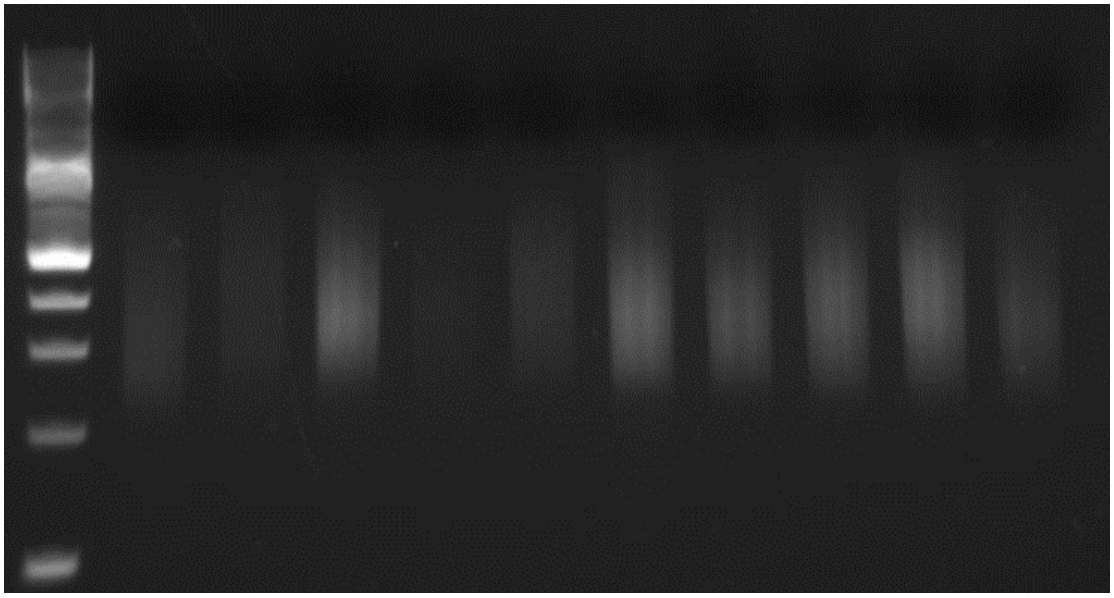
Protocol Information

Cell Type: Bacterial Genomic DNA, 1ug/ml
Total Sample Volume: 500ul
Sonicator Amplitude Setting: 60%
Sonication Pulse Rate: 10 seconds On, 10 seconds Off
Total Sonication On Time: 5min or 10min. Desired fragment size dependent. See resulting gel image above
Sample Process Temperature: 3°C

Customer notes:
-Sonicated in 1.5ml mirocentrifuge tube – polypropylene (commercially available).
-Sample was in TE buffer
-We use Illumina for all sequencing. NEB next gen kits work well with sonicated DNA using the Q800R.
-System worked very well.

Bacterial Genomic DNA Shearing 2

Example protocols and results are based on customer feedback.



Lonza 1.2% Gel, NEB 2-log ladder, sheared samples

Protocol Information

Cell Type: Bacteria and purified DNA (*Vibrio cholera* and *E. coli)*
Total Sample Volume: 100-500ul
Sample Concentration: 50-100ng/ul
Sample Lysis Solution: PBS + 0.1% Tween
Sonicator Amplitude Setting: 60-80%
Sonication Pulse Rate: 15 seconds On, 15 seconds Off
Total Sonication On Time: 20-30 min.
Sample Process Temperature: 4°C *(Chiller Temperature setting; System placed in cold room)*

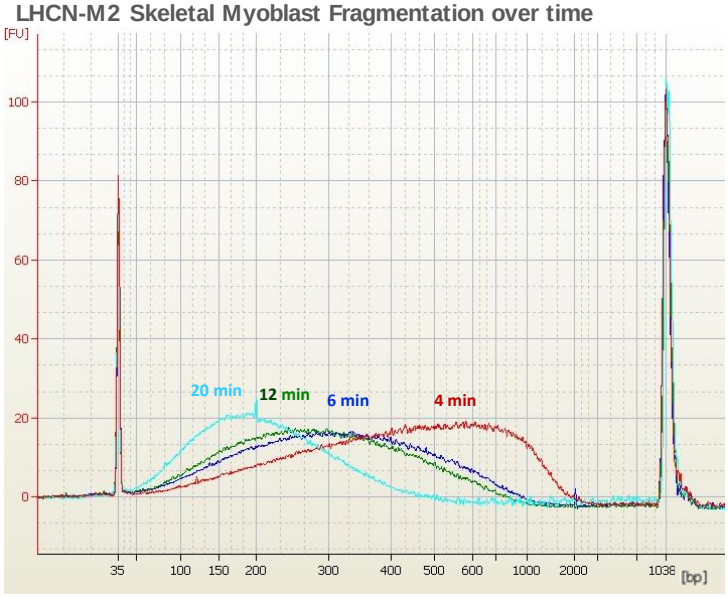
Customer Notes: We use the Q800R for cellular lysis and DNA shearing (*Vibrio cholera* and *E. coli)* for Illumina next generation sequencing library preparation. Using my protocol I always get uniform shearing to ~250-400bp. This system is better than probe sonicators for lysing small aliquots as the sample stays cooled and the process is automated. We use Denville 1.7ml microcentrifuge tube (cat# C2170).

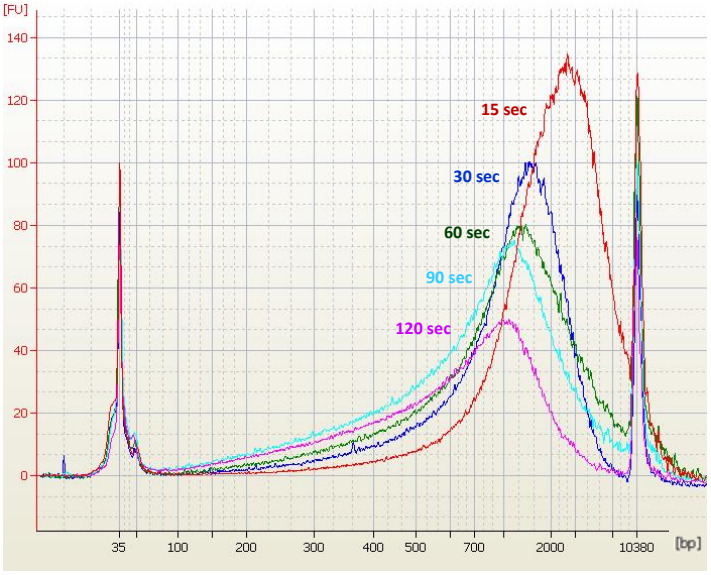
Sequencing platform: Illumina

Human Skeletal Myoblast Genomic DNA Shearing

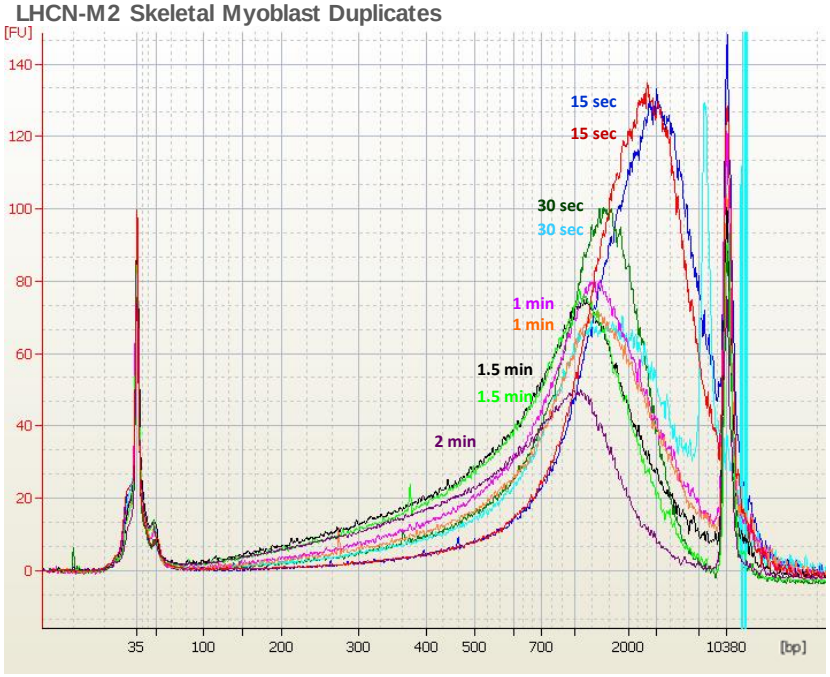
Example protocols and results are based on customer feedback.

LHCN-M2 Skeletal Myoblast Fragmentation over time





LHCN-M2 Skeletal Myoblast Duplicates



Protocol Information

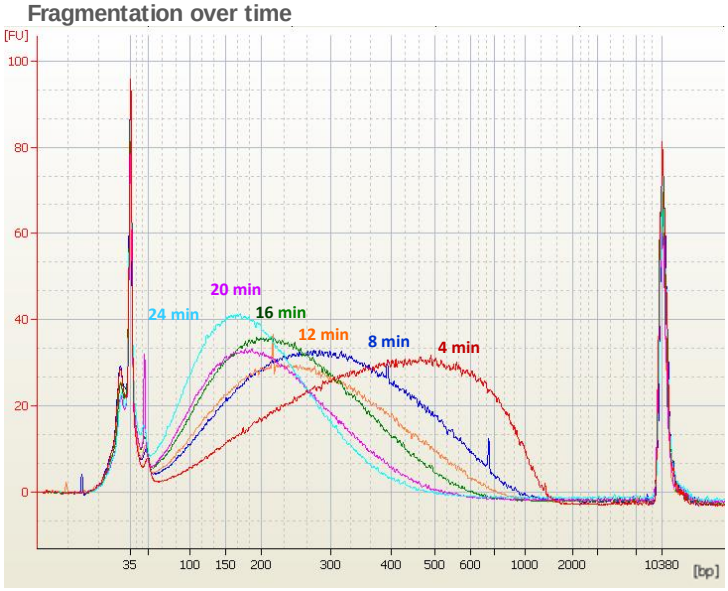
Cell Type: LHCN-M2 Skeletal Myoblast / 200ng
Total Sample Volume: 200ul (200ng of DNA) in Brand Tech 0.5 ml tubes
Sonicator Amplitude Setting: 20%
Sonication Pulse Rate: 15 seconds On, 15 seconds Off
Total Sonication On Time: As indicated on each bio-analyzer image
Sample Process Temperature: 3°C

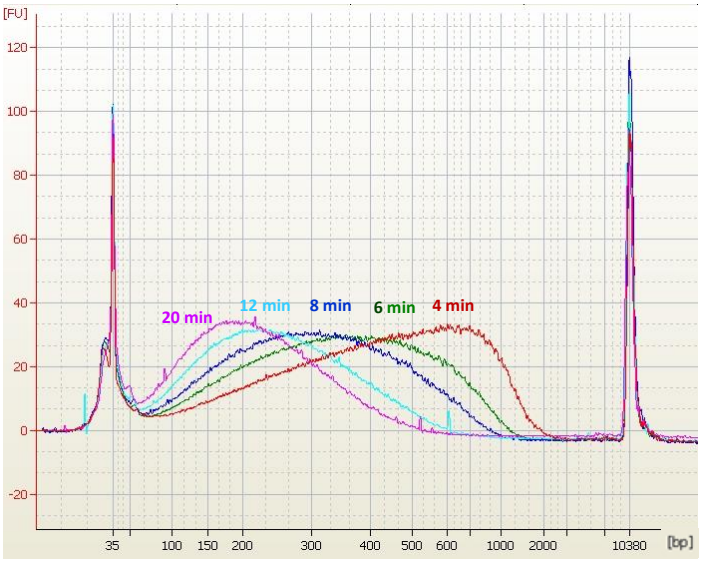
Customer Notes: The size distributions were consistently moving towards smaller sizes and the replicate-to-replicate reproducibility was very good. All samples were done as duplicates. 15 sec produces a reasonably compact peak of about 3 kb. At 30 sec, the peak size is about 1.5 kb, and it drops to ~1 kb at 1 and 1.5 min. There is relatively little change in peak sizes from 1.5 to 2.

Bacterial Genomic DNA Shearing

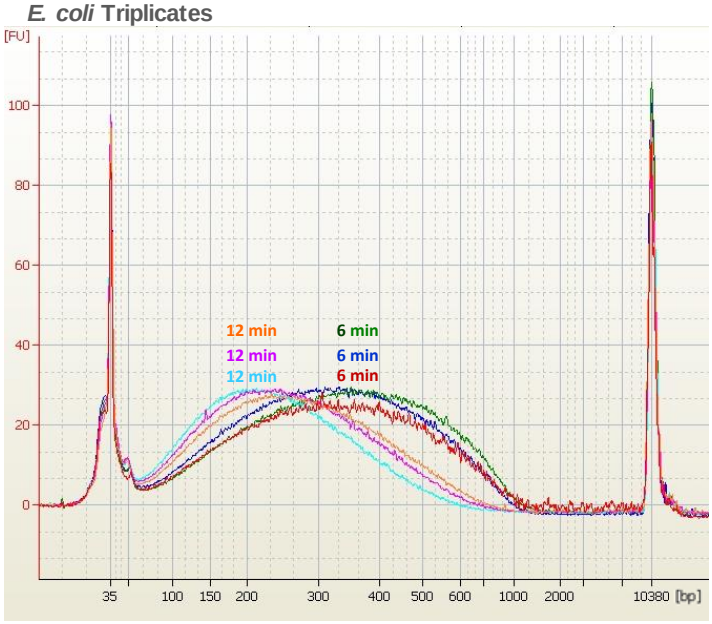
Example protocols and results are based on customer feedback.

Fragmentation over time

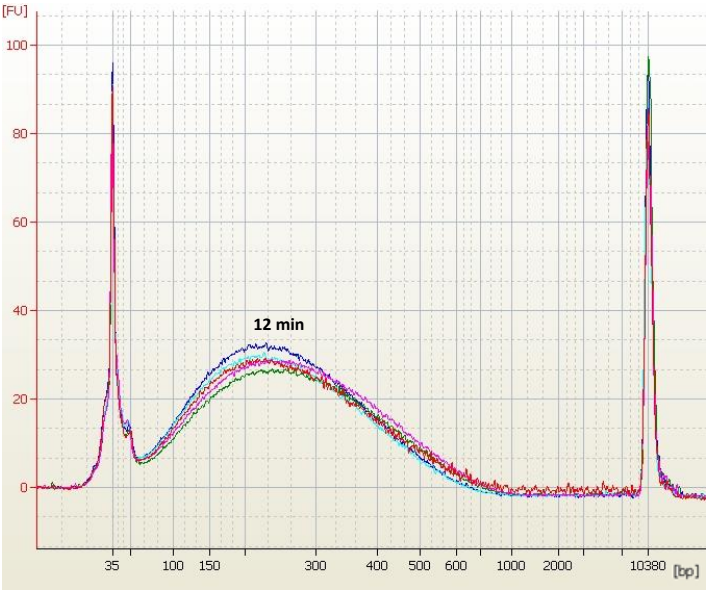




***E. coli* Triplicates**



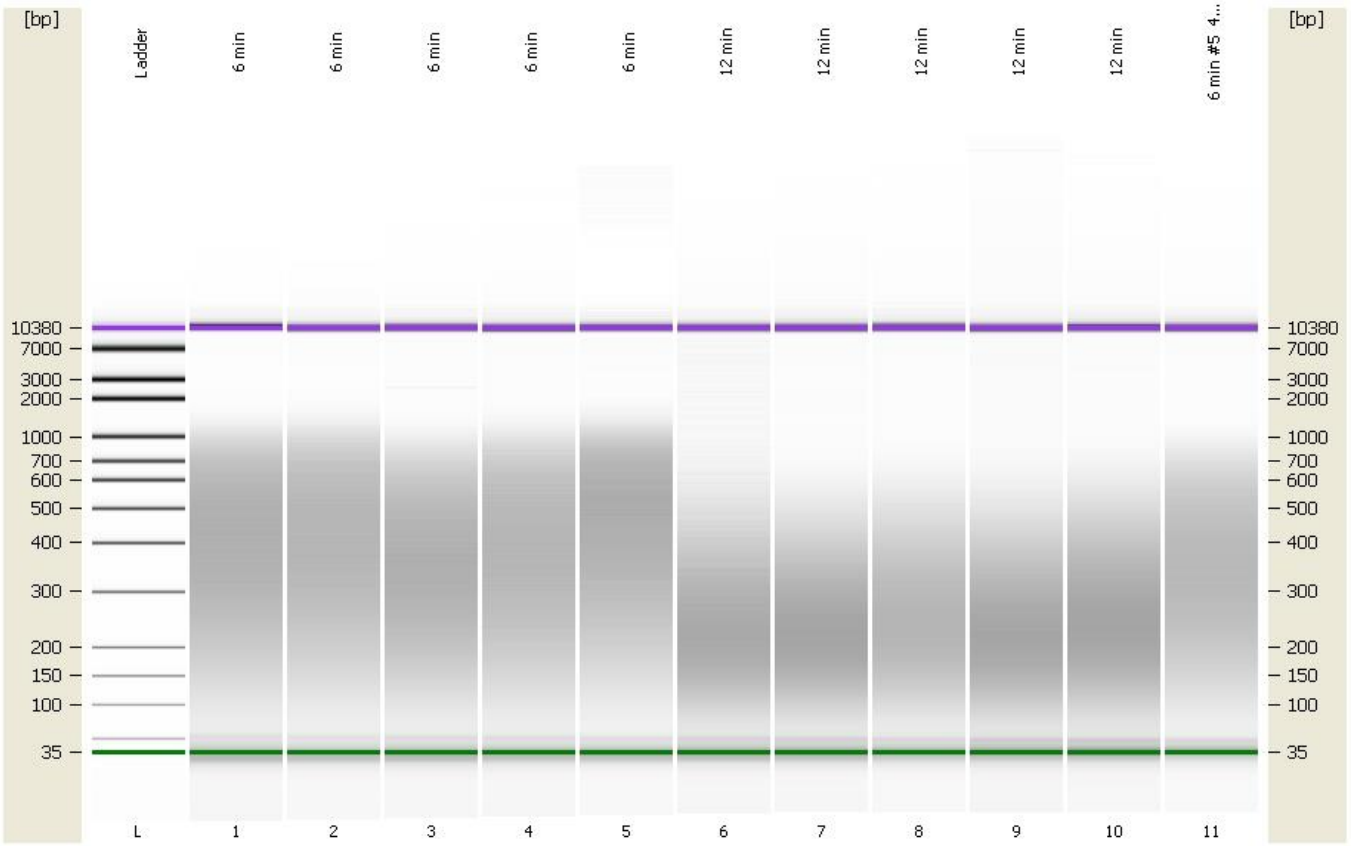
***E. coli* 5 Replicates**



Protocol Information

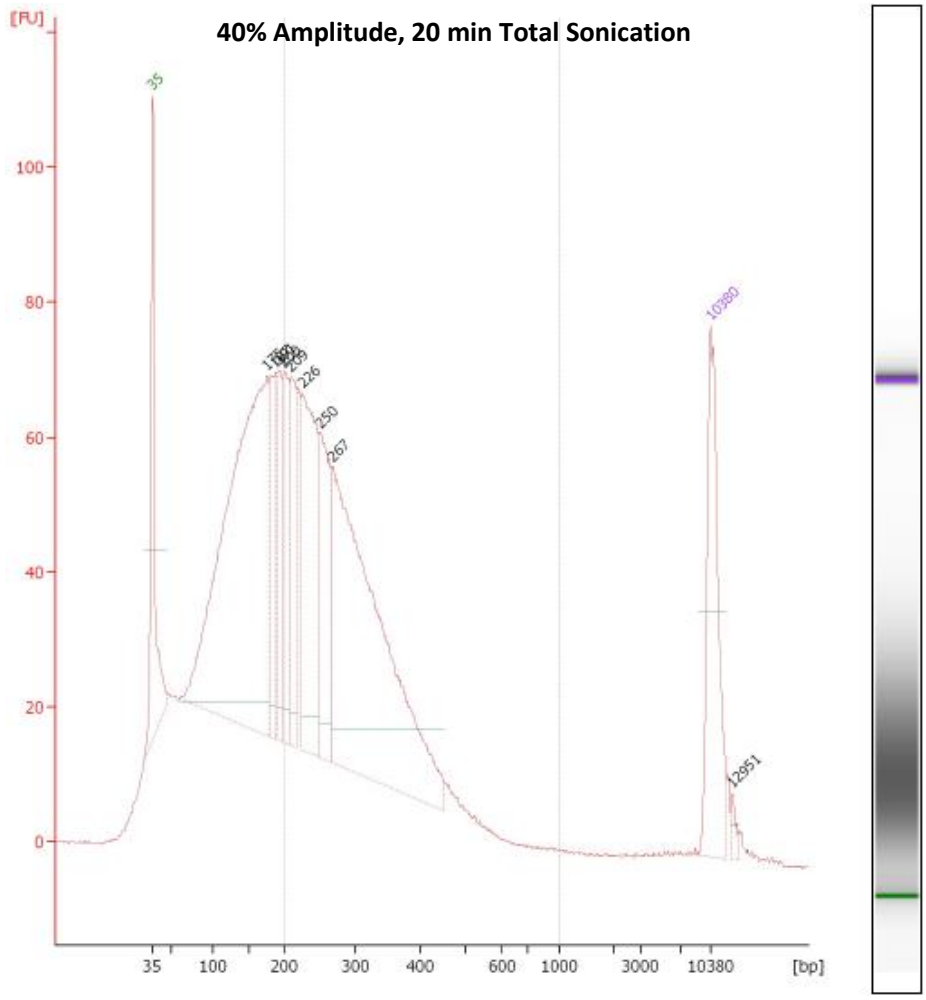
Cell Type: *E. coli* genomic DNA
Total Sample Volume: 200ul (250ng of DNA) in Brand Tech 0.5 ml tubes
Sonicator Amplitude Setting: 20%
Sonication Pulse Rate: 15 seconds On, 15 seconds Off
Total Sonication On Time: As indicated on each bio-analyzer image
Sample Process Temperature: 3°C
Customer Notes: The size distributions were consistently moving towards smaller sizes and the replicate-to-replicate reproducibility was very good

E. coli 6 Minute and 12 Minute 5 Replicates



Human Genomic DNA Shearing

Example protocols and results are based on customer feedback.



Protocol Information

Cell Type: Human gDNA *(Male Genomic DNA from Zyagen (#GH-180M))*
Total Sample Volume/Concentration: 100ul / (4ug of gDNA)
Number of Samples Sonicated per run: 18
Sonicator Amplitude Setting: 40%
Sonication Pulse Rate: 15 seconds On, 15 seconds Off
Total Sonication On Time: 20 min.
Sample Process Temperature: 3°C

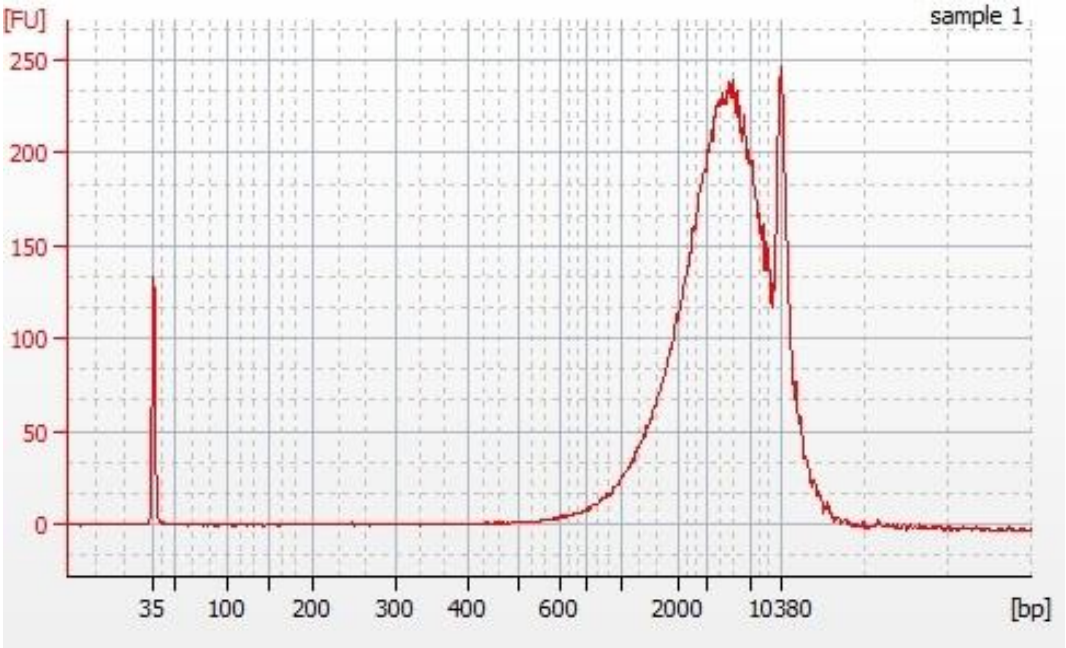
Customer Notes: We sonicated purified genomic DNA for the purpose of preparing sequencing libraries for different NGS sequencing platforms that require different target lengths. We use up to 4 micrograms gDNA in 100 ul water. I found the higher the amplitude, the tighter the distribution and I was able to achieve a fragment size of 150 bp to 200 bp.
This sonicator is very easy to use and versatile. We are able to put more DNA per tube which greatly reduces time of sample processing pre and post sonication (i.e. no vacuum centrifugation to concentrate sample). We can use standard 0.2 ml tubes (BrandTech #781305) which reduces cost and the temperature is regulated to decrease the risk of degradation.

Sequencing platform: LifeTechnologies - Ion Proton Torrent

Human Genomic DNA Shearing

Example protocols and results are based on customer feedback.

100% Amplitude Setting, 10 seconds total Sonication time



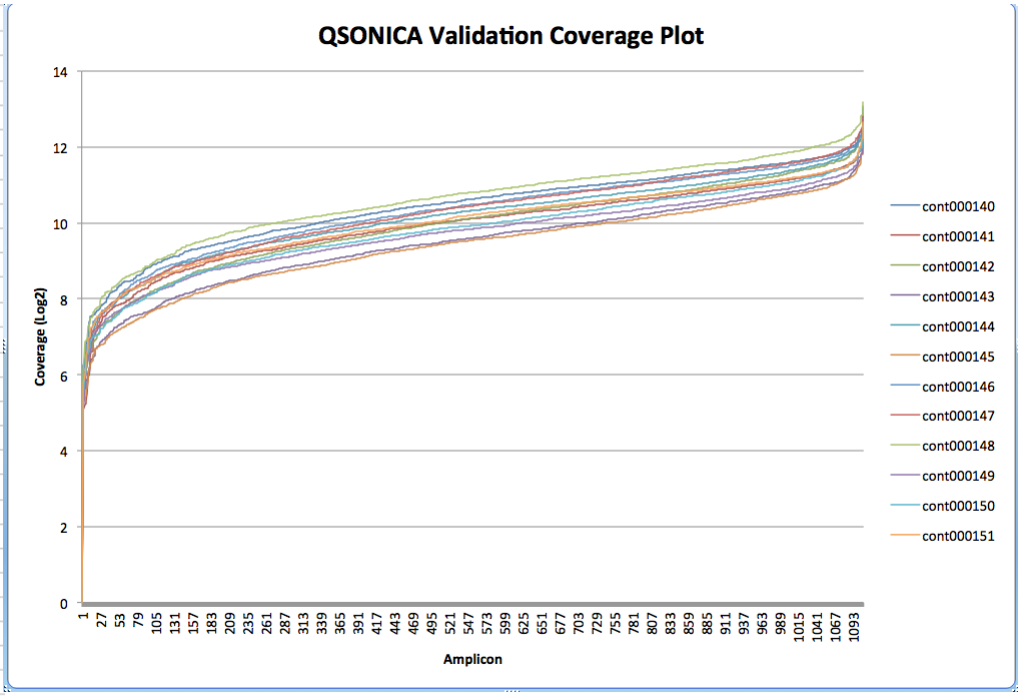
Protocol Information

Cell Type: Human genomic DNA
Total Sample Volume: 4.5ug of gDNA in 150ul TE
Sample Tubes used: 1.5 ml polystyrene tubes from Evergreen Scientific
Sonicator Amplitude Setting: 100%
Sonication Pulse Rate: 5 seconds. Stop sonication, quickly spin down samples and then sonicate for another 5 seconds
Total Sonication On Time: 10 seconds
Sample Process Temperature: 4°C
Sequencing Platform: Raindance - Thunderstorm

Customer Notes: The QSONICA instrument can be used instead of COVARIS to shear gDNA prior to loading Thunderstorm.
Advantages of the Q800R:
-Compatible with commercially available sample tubes as opposed to Covaris (requires custom tubes made exclusively by Covaris). Tubes: $0.06 per tube for QSONCA vs $7.20 per tube for COVARIS!
-Capable of processing up to 24 samples at once
-Shearing time: Seconds vs hours for Covaris (4hours per 24 samples)

Bioanalyzer Fragment Size Results

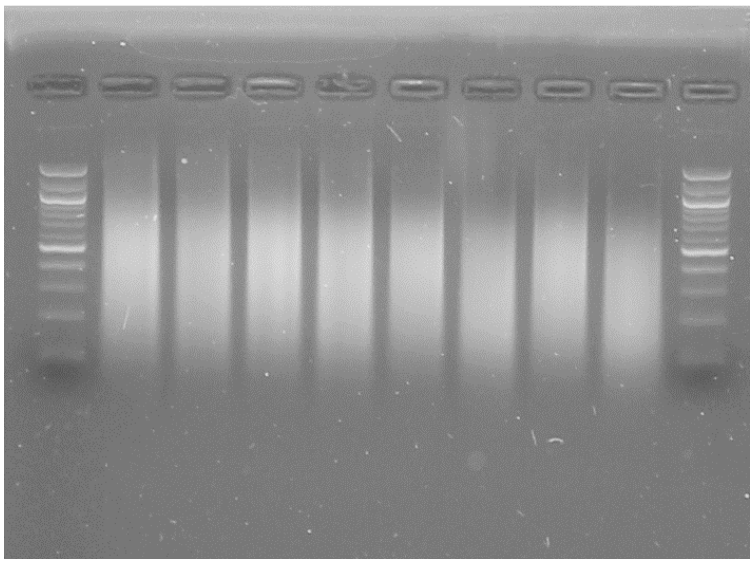
Sample ID	Detected fragments (bp)	% Total
cont000140	1,993	39.8
	2,363	12.2
	3,266	45.8
	11,347	2.2
cont000141	1,924	100
cont000142	1,822	55.2
	2,766	44.8
cont000143	582	4.2
	1,591	56.4
	3,193	39.4
cont000144	184	2.2
	380	3.4
	572	5.4
	785	6.3
	1,549	45.4
	2,959	37.3
cont000145	1,718	44.5
	2,720	55.5
cont000146	374	10.8
	492	9
	532	2.6
	586	3.5
	1,471	57.3
	6,072	16.8
cont000147	447	1.3
	475	0.8
	536	3
	589	1.8
	638	2
	741	4.2
	1,620	39.9
	2,688	7
	3,546	40
cont000148	1,667	51.1
	2,802	48.9
cont000149	1,644	75.6
	2,877	24.4
cont000150	769	5
	1,606	49.8
	2,963	45.2
cont000151	1,670	67.8
	6,278	10.6
	7,351	21.6



Sample ID	# LC amplicons
cont000140	1
cont000141	2
cont000142	1
cont000143	2
cont000144	2
cont000145	5
cont000146	3
cont000147	2
cont000148	1
cont000149	2
cont000150	3
cont000151	2

Plant Tissue Genomic DNA Shearing for SNP Enrichments

Example protocols and results are based on customer feedback.



Protocol:

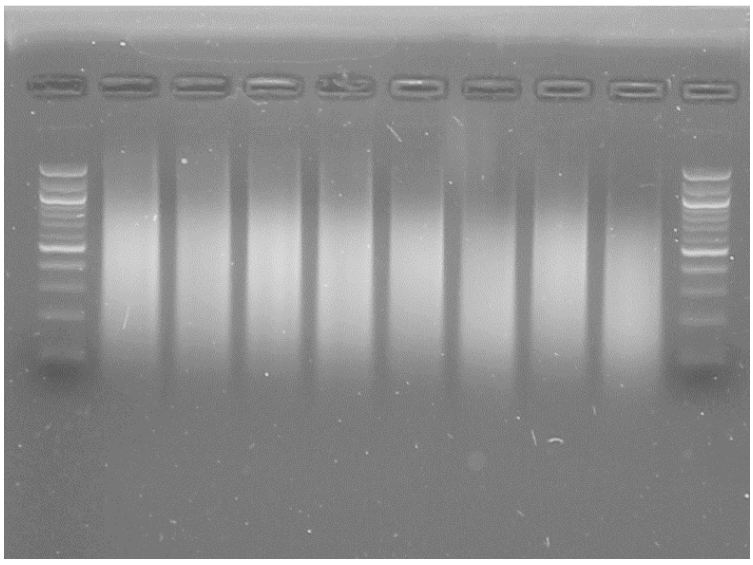
Cell Type: Plant Tissue Genomic DNA (Leaves)
Sample Lysis Solution: ddH_20
Total Sample Volume: 1ug of DNA in 100ul
Sonicator Amplitude Setting: 25%
Sonication Pulse Rate: 10 seconds On, 10 seconds Off
Total Sonication On Time: 3 minutes, 30 seconds
Sample Process Temperature: 3°C

Customer Notes:

- The Sonication is easily done and repeatable. Once in a while I would get a sample that was highly degraded which could not be sonicated, but that was due to the initial sample quality.
- A total volume of 1ug was placed in the tubes and standardized to 100ul total volume.
- Used USA Scientific, 0.2ml Tubes, Ref# 1402-3900

Plant Tissue Genomic DNA Shearing for SNP Enrichments

Example protocols and results are based on customer feedback.



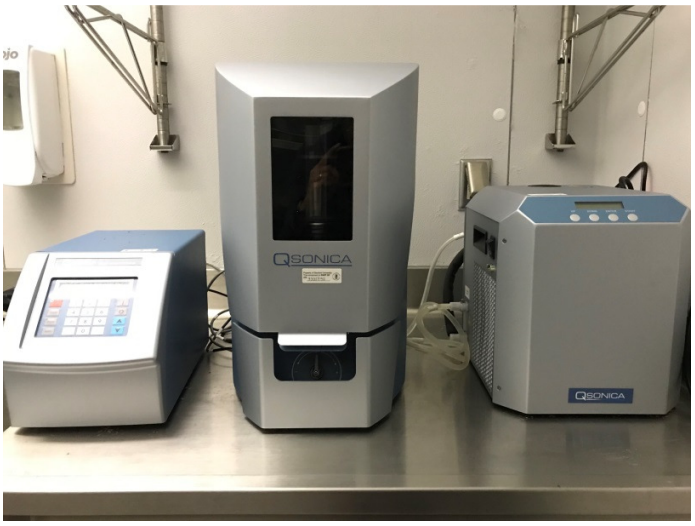
Protocol:

Cell Type: Plant Tissue Genomic DNA (Leaves)
Sample Lysis Solution: ddH_20
Total Sample Volume: 1ug of DNA in 100ul
Sonicator Amplitude Setting: 25%
Sonication Pulse Rate: 10 seconds On, 10 seconds Off
Total Sonication On Time: 3 minutes, 30 seconds
Sample Process Temperature: 3°C

Customer Notes:

- The Sonication is easily done and repeatable. Once in a while I would get a sample that was highly degraded which could not be sonicated, but that was due to the initial sample quality.
- A total volume of 1ug was placed in the tubes and standardized to 100ul total volume.
- Used USA Scientific, 0.2ml Tubes, Ref# 1402-3900

Cell Fractionation & Chromatin Shearing for Solubilization of Proteins in Chromatin Fraction



Protocol

Sample Type: *Human RPE cells*
Sample Details: *30 million cells pelleted and lysated with 2 different buffers to separate cells into 3 fractions: Cytoplasm, Nucleus and Chromatin*
Lysis Buffer: *9mM EDTA pH8, 0.2mM EGTA pH8, 0.1% Triton, 1mM DTT, Protease Inhibitors*
Sample Volume: *200ul*
Amplitude Setting: *70%*
Sonication Pulse Rate: *30 seconds ON, 30 seconds OFF*
Sonication ON Time: *5 minutes*
Sample Tubes: *0.5ml Eppendorf thin wall*

Customer Notes

I appreciate the light inside the sonicator for this experiment, since you can see the insoluble chromatin "disappear" after sonication.