Protocol: Making Libraries for ChIP-Seq

Protocol from Seth Frietze and the Farnham lab ChIP-Seq protocols. This protocol is written for use with the Illumina Sequencing platform. For adaptation to other sequencing platforms, consult the specific information for preparation of libraries for your sequencing platform.

General Considerations before you start:

Good ChIP-Seq results depend on good ChIP elutes: Enrichment over background! Test all ChIP elutes w/ Pos and Neg primers in qPCR before making (& sequencing) ChIP-Seq Libraries.

Negative control seq sample (input): You need to prepare Libraries also for input gDNA (same samples, after sonication), in order to set the background signal for your cell type. This input sample can be used for later Sequencing experiments from same cell type.

DNA Purification: The protocol requires purification of the DNA at several steps. This can be done with Qiagen PCR purification kits, OR with Ampure Magnetic Beads. For magnetic beads, it is important to read instructions and use correct ratio of beads to sample, as the size of DNA that is purified depends on the concentration of the buffer solution of the beads.

Size selection: After Adapter ligation (and after PCR), it is critical to do a size-selection step in order to remove excess adapter-dimers (if present, these will compete out your real samples in the sequencing). This can be done by blind-cutting (too little DNA to see with normal gels) from a gel and subsequent gel-purification (Qiagen kit), OR by using a 1:1 ratio (1x) of the Ampure magnetic beads (read reagent instructions for further information and protocol).

General Outline of Steps:
1. End-repair
2. A-tailing
3. Adapter Ligation + Size selection
4. PCR (+ Size selection and quality control)

Assumes 30 µl ChIP elute that is tested and found to have significant enrichment at positive control regions. For input: Use approx. 200 ng sonicated input genomic DNA.

1. End repair: Master Mix:	1x ____	___ x
ChIP Elute (or diluted input gDNA):	30 µl	na
10 x End-repair buffer (from kit)	5 µl	___ µl
2.5 mM dNTP Mix	5 µl	___ µl
10 mM ATP	5 µl	___ µl
ddH2O (adjust to total 50 µl reaction)	4 µl	___ µl
END-Repair Enzyme Mix (from kit)	1 µl	___ µl
Total rxn volume	50 µl	20 µl to add/rxn

→ RT for 45 min: @ _____ am/pm → _____ am/pm
Purify DNA over Qiagen PCR purification column OR using 1.8x Ampure Beads. Elute in 34 µl.

2. A-tailing:	Master Mix:	1x ____	___ x
	DNA (from above)	34 µl	na
	10 x NEB buffer 2	5 µl	___ µl
	1 mM dATP	10 µl	___ µl
	Klenow Fragment (exo-) Enzyme	1 µl	___ µl
	Total rxn volume	50 µl	16 µl to add/rxn

→ 37 °C for 30 min: @ _____ am/pm → _____ am/pm
Purify DNA over Qiagen PCR purification MinElute column OR using 1.8x Ampure Beads. Elute in 10 µl.

3. Adapter Ligation: Master Mix:		1x ____	___ x
	DNA (from above)	10 µl	na
	LigaFast 2x buffer	14 µl	___ µl
	Adapter (note Barcode, if multiplexing, MP)	1 µl	___ µl (not if MP)
	LigaFast Ligase Enzyme	3 µl	___ µl
	Total rxn volume	28 µl	18 µl to add/rxn

→ RT for 15 min: @ _____ am/pm → _____ am/pm
+ add 22 µl water = 50 µl ==> Purify DNA w/ Qiagen PCR purification column PLUS gel-purify (cut blind 200-500 bp range and subsequent gel-purification with Qiagen kit) OR **purify and size-select** is same step using 1x (!) Ampure Beads (50 µl). Elute in 30 µl.

4. PCR Amplification: Master Mix:		1x ____	___ x
	DNA (from above)	30 µl	na
	5x Phusion HF buffer	10 µl	___ µl
	Primer-mix	1 µl	___ µl
	10 mM dNTP	0.5 µl	___ µl
	ddH2O	8 µl	___ µl
	Phusion Pfu Enzyme	0.5 µl	___ µl
	Total rxn volume	50 µl	20 µl to add/rxn

PCR-Program:
98 °C for 30 sec
Cycle: 98 °C for 10 sec
65 °C for 30 sec
72 °C for 30 sec
Repeat 14 times for total 15 cycles.
72 °C for 5 min
4 °C for ever

Save 1 µl of PCR reaction (back-up: Can amplify further if required)
Purify and size select DNA after PCR reaction: Qiagen Gel-purification (200-500 bp) OR with 1x Ampure magnetic beads: Elute in 30 µl.
Measure DNA concentration (Nanodrop ok for initial measurement, but need better before sequencing: Qubit or other quantification). Run out about 100 ng (if you have enough, 50-70 ng is sufficient) on agarose gel in narrow wells to check for library size and quality (no "ladder").
Test libraries (1:20 dilutions) with qPCR for enrichment over background.
If Ok ==> Dilute (mix if multiplexing) and submit for sequencing.

Reagents:	**Vendor**		**Cat#**
End-It DNA End Repair Kit	Epicentre		ER0720
Klenow (3'->5' exo-)	NEB		M0212s
LigaFast	Promega		M8221
Phusion DNA polymerase	NEB		F-531
Agencourt AMPure XP (Magnetic beads)	Beckman Coulter	5 ml	A63880
NEXTflex™ DNA Barcodes - 6	Bioo Scientific	48 rxns	514101
Or: Illumina Adapters and primers	Illumina		??
Qiagen PCR purification columns	Qiagen		