# PREPARING SAMPLES FOR THE SOLEXA PIPELINE*:
## GENOMIC DNA & Chromatin IP DNA

### PROLEGOMENON

You have the choice between 2 options: purchase the Illumina kit for preparing genomic DNA samples or follow these guidelines using considerably cheaper components from Epicentre and Stratagene. In any case it is a good idea to read the corresponding Illumina protocol before starting. It is available on Nemo's web - thanks to the diligence of the crew. Please go to http://www.umassmed.edu/nemo and choose the Services option in the left nav.

The present protocol begins after you have some kind of cleaned genomic (or otherwise) DNA fraction that you obtained by any procedure of your choice including ChIP, The DNA will be dissolved in a small volume of water and it is a good idea to start with at least 5-10 ng DNA. Our experience is that a precise quantification of the starting material is not absolutely required but starting with less DNA may lead to disproportionate losses on the multiple Qiagen column runs needed between each step. Qiagen column runs should be performed carefully, taking care to avoid carry over of washing solutions in the elution step. We found that switching column type between steps as in the Illumina protocol is not necessary and the Qiaquick gel extraction components can be used at every step.

The gel purifications steps fulfill two equally important roles:

1. Ensure that all fragments you will give for a Solexa* run are distributed narrowly around a modal size ( less than 1Kb and ideally less than 500bp, with only a 50bp range of variance within the population, e.g. 450bp $\pm$ 50bp). This is important for the downstream processing of your samples.
2. Ensure the complete removal of adapters and adapter-dimers after the ligation of the adapters which are in large excess. The bulk of them are washed through the Qiagen column used after the ligation step but a significant portion of dimers ( which are minimized by design but are nevertheless produced ) remain and must be eliminated by careful agarose gel purification.

* Illumina's Solexa 1G Genome Analyzer ~ Deep Sequencer

## JUST DO IT

### BLUNTING THE FRAGMENTS

[ Obtain an END-IT DNA REPAIR KIT # ER0720 $75 Epicentre ]

1-34 µl DNA ( less than 5µg )
5µl 10x buffer
5µl dNTP mix
5µl ATP solution
*x* µl $H_2O$ (to volume)
1µl enzyme mix
Incubate 45-60 minutes at room temperature

Add 50µl water + 400µl Qiagen bufferQG
Transfer to Qiaquick Column
Centrifuge 2min 2000 RPM
Give a pulse full speed
Wash 2 times with PE full speed
Elute with 15µl EB
Elute a second time with 15µl EB (reversing the orientation of the column in the centrifuge)

### "A TAILING" OF THE FRAGMENTS

[ Obtain Klenow Exo-minus 50u/µl #KL06041K $55 Epicentre ]

30 µl of DNA solution( check volume )
5 µl Klenow buffer
1 µl 10mM dATP
*x* µl $H_2O$ (to volume)
1µl Klenow
Incubate 45-60min room temperature

Option1

Add 50µl water+ 400µl Qiagen buffer QG
Transfer to Qiaquick Column
Centrifuge 2min 2000 RPM
Give a pulse full speed
Wash 2 times with PE full speed
Elute with 15µl EB
Elute a second time with 15µl EB (reversing the orientation of the column in the centrifuge)
Speed vac to reduce the volume to 10µl

Option2

You may elect to use the Min elute PCR purification kit (Qiagen) at this point and elute with 10µl of EB

**LIGATION OF ADAPTERS TO THE FRAGMENTS**

[ Obtain a FAST LINK KIT #LK11025 $75 Epicentre ]

10 µl of DNA solution
1.5 µl 10X buffer
0.75 µl of 10mM ATP
1 µl of adapters ( for any quantity <100ng; 2 µl for 100ng-1µg; 2 µl for every additional µg )
*x* µl of $H_2O$ (to volume)
1 µl Ligase

1-2 hr room temperature

Then add another 10µl of the mix:
7.5 µl $H_2O$
1 µl 10X buffer
0.5 µl 10mM ATP
1 µl Ligase

3 hr-16hr 16°C
add 75ul of water+ 400ul Qiagen bufferQG
Transfer to Qiaquick Column
Centrifuge 2min 2000 RPM
Give a pulse full speed
Wash 2 times with PE full speed
Elute with 15 µl EB
Elute a second time with 15 µl EB (reversing the orientation of the column in the centrifuge)

Quantity > 50ng proceed to gel purification then to PCR

Quantity < 50ng proceed directly to 15 cycles PCR as described below then proceed to Qiagen purification and gel purification.

**GEL PURIFICATION**

Load your sample on a purification 1.5-2.5% agarose minigel poured in TAE+ Ethidium Bromide depending on the size of your starting fragments - the smaller the size, the higher the % agarose needed).

Run 2-4 V/centimeter 1-2 hrs.

Minimize exposure to UV ( shield with bottom tray if possible)
(Nobody from Nemo's crew will blame you for taking a picture of your gel only after you removed the bands of interest.)

Cut the band of interest with clean razor blade - keep the volume as low as possible ( around 100 µl ).

Add 400 µl Qiagen buffer QG
Transfer to Qiaquick Column
Centrifuge 3 min 2000 RPM
Give a pulse full speed
Wash one time with buffer QG full speed
Wash 2 times with PE full speed
Elute with 15 µl EB
Elute a second time with 15 µl EB (reversing the orientation of the column in the centrifuge)

**PCR Amplification**

[ Obtain Stratagene PfuUltra™ II Fusion HS DNA Polymerase # 600670 40 rxn $99 ]

*X* µl of DNA solution (as determined)
2 µl of Solexa PCR primers
10 µl of 10X buffer
10 µl of dNTP 2.5mM
1 µl of enzyme
*Y* µl of $H_2O$ (to volume)
100µl total

Cycling
95°C 1min
( 95°C 50sec 65°C 1min 72°C 30sec ) repeat 15 – 18 times
72°C 5 min
8°C as needed

Add 400 µl Qiagen buffer QG
Transfer to Qiaquick Column
Centrifuge 2min 2000 RPM
Give a pulse full speed
Wash 2 times with PE full speed
Elute with 15 µl EB
Elute a second time with 15 µl EB (reversing the orientation of the column in the centrifuge)
Take 10% to run on an analytical agarose gel

**Provide one picture to Nemo** (submit with your analysis ticket for these samples)

Take 10% for quantification.

Take 2-5% for "A tailing" with Taq in a 20ul reaction with 0.25mM dATP and 0.2 µl Taq 72°C for 45 min. Ligate into the TA cloning easy vector transform (or TopoTA Cloning System, or TA Cloning system of your choice). Sequence 20 individual clones to estimate your % of dimers and productively adaptered fragments. (Add this information to the analysis ticket for Nemo)

Dilute 20-50% of your sample as needed in water or Qiagen EB buffer to a final concentration of 10nM. THIS IS YOUR “Library” or “Sample” for analysis. Store at -20 °C

**Provide at least 25ul of the 10nM dilution to Nemo.**

For each sample submitted for analysis, you will need 25μl of 10nM library and a completed analysis ticket with supporting information. The genomic DNA and Chromatin IP DNA protocols use the Illumina DNA Primer set, please be sure to indicate this on the analysis ticket, as this determines which sequencing primer is used during cluster generation.

Thank you,

NEMO and Crew

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Protocol courtesy of Ned Land, Harpooner
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