NARG 2014-2015 Study/Survey

The influence of DNA fragment size on ChIP-Seq results from low amounts of DNA

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ChIP-Seq

Chromatin Immuno Precipitation & Sequencing

1. Cell Nucleus
2. Crosslink Protein and Shear DNA
3. Add Protein-Specific Antibody
4. Immunoprecipitate and purify complexes
5. Reverse Crosslinks, Purify DNA and prepare for sequencing
6. Sequence DNA fragment and map to genome

10 ng
>10^6 cells
1 ng DNA worked fine

All chemistries tell a similar story
100 pg: Plenty of False Positives

<table>
<thead>
<tr>
<th>10 ng</th>
<th>Diagenode</th>
<th>Illumina</th>
<th>NEB</th>
<th>NuGEN</th>
</tr>
</thead>
</table>

In the hands of the NARG members
120 pg: “Perfect”

Differences: NARG vs. None-NARG? Chemistry? DNA?
NARG DNA

“Real world” samples

1 “Qubit” ng input

1 “Qubit” ng ChIP

DNA diluted from large scale ChIP

Lets blame the DNA – Not us!
How to Blame DNA for Bad Results?

1) IP
Worked well from 10 ng and 1 ng

2) Purity
Worked well from 10 ng and 1 ng

3) Size
Worked well from 10 ng and 1 ng

100 pg = $10^8$ molecules at 1,000 nucleotides

$10^4$ promoters $\rightarrow 10^4$ molecules/promoter

2% of ChIP DNA comes from peaks

$\rightarrow$ 200 molecules/promoter

? Efficiency of de-crosslinking, repair, ligation...

$\rightarrow$ 3 actively participating molecules/promoter?
Experimental Design

1 chromatin prep

3 fragmentations: +/-200, +/-500, +/-1,000 nts

3 IPs with the same antibody

3 library preps from 100pg

3 x sequencing

Problem solved if 200 nts work well and 1,000 not
Let’s Bring in the Pros for Fragmentation

Academia could not do it

A “fragmentation company” said: we do it

No time
Did not work
Did not work well
Not enough
Forgotten
Product launch
Tech had car accident

ChIP fragmentation is tough – What do you think?
Experience with Larger Fragments

Q3 Did you try 500-1,000 nts?
Answered: 20  Skipped: 13

Q5 Did you try >1,000 nts?
Answered: 20  Skipped: 13

Q4 Did you like the 500-1000 results?
Answered: 20  Skipped: 13

Q6 Did you like the >1,000 results?
Answered: 19  Skipped: 14
Experience with Low Input

Like NARG

Size matters at low input
Do the Ultra Low Answers have Something in Common?

+/-200, +/-500 nucleotides

Chemistries
SPRIworks
NEB Ultra
TruSeq
BioO
Kapa Hyper
Summary

DNA Size for Low Input ChIP-Seq

1 ng, 200 – 500 nucleotides seems save
Little is know about influence of size
People feel that size is more important at low input
Difficult to make a well controlled experiment
No agreement if one chemistry works better than others
ENCODE guidelines do not mention fragmentation
Acknowledgement

NARG members

Charlie Nicolet

Survey participants
Gold Standard Results

H3K4me3 at active promoters