Why RNA-Seq and ChIP-Seq are better than microarrays

Presenters

Vincent Funari, PhD - Director of Genomics Core at Cedars-Sinai
*Overview of nextgen sequencing services at Cedars-Sinai*

Joe Aman – Illumina Field Application Scientist
*Get better data from sequencing than microarrays without paying a lot more.*

Questions; snack break

Quoclinh Nguyen, PhD – Research Scientist at Genomics Core
*How to request service; how raw data is filtered and accessed*

Cecile Boysen – CLC Bio Field Application Scientist
*How to do your analysis and extract biological meaningful data.*

Friday, October 7th, 2011
1 pm to 3:30 pm
Davis Room 1004

*FREE RNA-SEQ or ChIP-SEQ preparations and bioinformatic analysis will be given away to 10 applicants that are present.*

PLEASE RSVP if you are interested in organizing a second hands-on library making and analysis workshop with your own samples.
Human genome at ten: The sequence explosion

“Data first” – Todd Golub

• “Large, unbiased genomic surveys are taking cancer therapeutics in directions that could never have been predicted by traditional molecular biology.”

*Nature* (1 April 2010)
TEAM

GENOMICS.CSMC.EDU
genomics@csmc.edu
Why switch to RNA-Seq and ChIP-Seq?
Why do we always have to validate with qPCR?
• Dynamic Range here at Cedars and Abroad.
Dynamic range can be a real problem in Science too

- **Dynamic Range**
- Arrays 4 orders of magnitude
- qPCR has 9 orders of magnitude

![Bar chart showing expression levels of different genes across different conditions.](chart.png)
Microarray vs total RNA Seq

- Ribosomal removal*
How do we identify binding sites for human transcription factors on a genome-wide scale?

**ChIP-chip and ChIP-seq**

1. Formaldehyde treat cells
2. Isolate and sonicate nuclei
3. Immunoprecipitate
4. Reverse crosslinks/purify DNA

ChIP-chip

ChIP-seq

```
actcatgcatgaaacctgacgcagg
ccgatatgatgaggaqctcicagga
gctagtcgatgaccaagtgcagtcag
......
```
Overview of CHIP library construction.
Flow Cell hybridization and Amplification
## To switch or not to switch?

<table>
<thead>
<tr>
<th>Feature</th>
<th>Arrays</th>
<th>RNAseq/Chipseq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute &amp; Relative Expression</td>
<td>NA</td>
<td>X</td>
</tr>
<tr>
<td>Dynamic Range</td>
<td>4-5</td>
<td>Unlimited</td>
</tr>
<tr>
<td>False Positives/Insensitive</td>
<td>+++</td>
<td>NA</td>
</tr>
<tr>
<td>Future for FFPE/Single cell</td>
<td>NA</td>
<td>X</td>
</tr>
<tr>
<td>Novel Transcripts/Splicing</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Transcripts from +/- strand</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Mutations</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>RNA-RNaseq</td>
<td>100-1ug</td>
<td>1ug-5ug</td>
</tr>
<tr>
<td>Array compatible</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Time</td>
<td>4-5 days</td>
<td>2-3 weeks</td>
</tr>
</tbody>
</table>
ChIP-Chip vs ChIP-Seq

**ChIP-chip vs ChIP-seq**

- Cost more for an entire genome
- Can focus only on promoters, specific genes, or certain chromosomal regions
- Can omit repeats from array
- Can use a lot fewer cells
- Easy bioinformatics

- Cost less for an entire genome
- Can’t focus only on specific regions, entire genome must be analyzed each time
- Repeats are sequenced
- Requires more cells
- Harder bioinformatics
Human genome at ten: The sequence explosion
Current Technologies

• Microarrays
  – mRNA and miRNA
  – Methylation(2012)*

• Next Generation Sequencing
  – Targeted Resequencing
  – Expression profiling (Total RNAseq)
  – Epigenomics (ChIP-Seq)
  – Methylation(2012)
  – Genome Sequencing (3-5 years away)

• qPCR(Nov 2011)*
  – Low (96 – 384 well qPCR)
  – Mid (2400 qPCR)
  – High (9600 qPCR)
  – Single Cell qPCR
Announcements

**Sequencing Laragen**

Cost: $3.75/sample (premixed) if less than 48 samples
$3.50/sample if more than 48 samples

**Sample Submission:**
1. Register samples on line at Laragen.com
2. Use code **CSGC2011** (Cedars Sinai Genomics Core) for discount
3. Drop off in **Genomics Core 142A** / 5th floor of Davis Rm 5022.

**Just Do it! FREE** Next Gen Sequencing sponsored by Illumina and Genomics Core

5 Pilot RNAseq and 5 ChIp-Seq projects

Hypothesis to test?
- Scientist name:
- PI lab:

What is the question you are asking?

Is this a pilot project for a currently funded project?

What do you intend to do with the data?

**IDT Oligos**

100 dollars of free oligos

How many oligos do you use in 6 months

Name