Module 2: Expression Engineering

20.109
Lecture 5
October 25th, 2007
Expression Engineering Experiment

Day 1

Day 2

Day 3

Image of glowing luciferase in a microcentrifuge tube, removed due to copyright restrictions.

Day 4

Day 5

Day 6

Figure by MIT OpenCourseWare.


Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
<table>
<thead>
<tr>
<th>Lecture 1</th>
<th>Lecture 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>• intro to cell culture</td>
<td></td>
</tr>
<tr>
<td>• intro to gene exp’n/RNAi</td>
<td></td>
</tr>
<tr>
<td>Lecture 3</td>
<td>Lecture 4</td>
</tr>
<tr>
<td>• off-target/nonspecific RNAi</td>
<td></td>
</tr>
<tr>
<td>Lecture 5</td>
<td>Lecture 6</td>
</tr>
<tr>
<td>• measuring gene express’n</td>
<td></td>
</tr>
<tr>
<td>Lecture 7</td>
<td>Lecture 8</td>
</tr>
<tr>
<td>• high throughput technologies or RNAi applications (no lab)</td>
<td></td>
</tr>
</tbody>
</table>

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
DNA/RNA/Protein Compass
### Figure 2 Western Blot

<table>
<thead>
<tr>
<th>Sample</th>
<th>Marker</th>
<th>M13k07 (positive control)</th>
<th>M13 Candidate 1 (colony #3)</th>
<th>M13 Candidate 2 (colony #4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume loaded (µl)</td>
<td>5</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

Figure 2 Protein samples from bacteria infected with modified and unmodified M13 virus ran in a polyacrylamide gel. Antibodies with alkaline phosphatase were used to identify virally encoded protein p3. We see strong bands at the expected length of p3 for our control and experimental samples which suggests our modified viruses successfully induced p3 production in their hosts. AB α used: Primary: mouse anti-P3, Secondary: Goat anti-mouse with alkaline phosphatase.

Courtesy of Augusto Tentori. Used with permission.

from Augusto Tentori

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
Figure 2 Protein samples from bacteria infected with modified and unmodified M13 virus ran in a polyacrylamide gel. Antibodies with alkaline phosphatase were used to identify virally encoded protein p3. We see strong bands at the expected length of p3 for our control and experimental samples which suggests our modified viruses successfully induced p3 production in their hosts. AB Ó used: Primary: mouse anti-P3, Secondary: Goat anti-mouse with alkaline phosphatase.

Figure 2 Western Blot

<table>
<thead>
<tr>
<th>Sample</th>
<th>Marker</th>
<th>M13k07 (positive control)</th>
<th>M13 Candidate 1 (colony #3)</th>
<th>M13 Candidate 2 (colony #4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume loaded (ul)</td>
<td>5</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

50 KD

37 KD

P3 (~48KD)

Blot: DNA
Probe: DNA (P³²)
Measures: ______

Courtesy of Augusto Tentori. Used with permission.

from Augusto Tentori

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
Figure 2 Protein samples from bacteria infected with modified and unmodified M13 virus ran in a polyacrylamide gel. Antibodies with alkaline phosphatase were used to identify virally encoded protein p3. We see strong bands at the expected length of p3 for our control and experimental samples which suggests our modified viruses successfully induced p3 production in their hosts. ABs used: Primary: mouse anti-P3, Secondary: Goat anti-mouse with alkaline phosphatase.

Blot: RNA
Probe: DNA (P^{32})
Measures: _____

Blot: DNA
Probe: DNA (P^{32})
Measures: _____

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray

Mark Schena,* Dari Shalon,† Ronald W. Davis,
Patrick O. Brown‡

A high-capacity system was developed to monitor the expression of many genes in parallel. Microarrays prepared by high-speed robotic printing of complementary DNAs on glass were used for quantitative expression measurements of the corresponding genes. Because of the small format and high density of the arrays, hybridization volumes of 2 microliters could be used that enabled detection of rare transcripts in probe mixtures derived from 2 micrograms of total cellular messenger RNA. Differential expression measurements of 40 Arabidopsis genes were made by means of simultaneous, two-color fluorescence hybridization.

fluorescein (root)
lissamine (leaf)
2 scans
+pseudocolor-->
Microarray
the array

Spot diameter: 10-150 um

Content: $\sim 10^9$ molecules/um$^2$

http://www.youtube.com/watch?v=S8Cwy71nMNU
Courtesy of André Silva. Used with permission.

http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html
Courtesy of A. Malcolm Campbell. Used with permission. © Copyright 2003 Department of Biology, Davidson College.

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
Microarray
the arrays we’ll be using

Catalog Oligo Microarrays

Agilent’s non-contact industrial inkjet printing process uniformly deposits oligo monomers onto specially-prepared glass slides. Both the catalog and custom microarrays are manufactured using Agilent’s non-contact in situ synthesis process of printing 60-mer length oligonucleotide probes, base-by-base, from digital sequence files. This is achieved with an inkjet process which delivers extremely small, accurate volumes (picoliters) of the chemicals to be spotted. Standard phosphoramidite chemistry used in the reactions allows for very high coupling efficiencies to be maintained at each step in the synthesis of the full-length oligonucleotide. Precise quantities are reproducibly deposited “on the fly.” This engineering feat is achieved without stopping to make contact with the slide surface and without introducing surface-contact feature anomalies, resulting in consistent spot uniformity and traceability.

Agilent’s in situ
Oligonucleotide Microarray

Traditional "In-lab"
Pin Spotted Microarray

Courtesy of Agilent Technologies, Inc.
Used with permission.
Microarray
the arrays we’ll be using

Catalog Oligo Microarrays

Agilent’s non-contact industrial inkjet printing process uniformly deposits oligo monomers onto specially-prepared glass slides. Both the catalog and custom microarrays are manufactured using Agilent’s non-contact in situ synthesis process of printing 60-mer length oligonucleotide probes, base-by-base, from digital sequence files. This is achieved with an inkjet process which delivers extremely small, accurate volumes (picoliters) of the chemicals to be spotted. Standard phosphoramidite chemistry used in the reactions allows for very high coupling efficiencies to be maintained at each step in the synthesis of the full-length oligonucleotide. Precise quantities are reproducibly deposited “on the fly.” This engineering feat is achieved without stopping to make contact with the slide surface and without introducing surface-contact feature anomalies, resulting in consistent spot uniformity and traceability.

4x44K spots “features”

41,000+ mouse genes and transcripts represented
Each 60-mer in length

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
Microarray
sample preparation

two samples to compare

isolate RNA

reverse transcribe and label

http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html

 Courtesy of A. Malcolm Campbell. Used with permission.
© Copyright 2003 Department of Biology, Davidson College.

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
Microarray fluorescent tags

Cy3 ex em
Cy5 ex em

Courtesy of Qubit Systems. Used with permission.

www.qubitsystems.com

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
Microarray wash and scan

http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html

Courtesy of A. Malcolm Campbell. Used with permission.
© Copyright 2003 Department of Biology, Davidson College.

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
Microarray

wash and scan

http://www.bio.davidson.edu/people/macampbell стратегий/чипсintro.html

Courtesy of A. Malcolm Campbell. Used with permission.
© Copyright 2003 Department of Biology, Davidson College.

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
Microarray controls

Self-Self Microarrays

S. cerevisiae

Courtesy of A. Malcolm Campbell. Used with permission. © Copyright 2003 Department of Biology, Davidson College.
Microarray controls

Self-Self Microarrays

4 Dye Swap Microarrays

S. cerevisiae

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
Microarray controls

Self-Self Microarrays

Dye Swap Microarrays

Courtesy of Agilent Technologies, Inc. Used with permission.
Microarray

indirect labeling method

http://www.genisphere.com/about_3dna.html
MIAME

Minimal Information About a Microarray Exp’t

Provide:
1. Raw data for each hybridization
2. Final processed data for the set of hybridizations
3. Experimental factors and their values (e.g., compound and dose in a dose response experiment)
4. Experimental sample relationships (e.g., which raw data file relates to which sample, which hybridisations are technical, which are biological replicates)
5. Array annotation (e.g., commercial array catalog number)
6. Data processing protocols (e.g., normalization method used)
Diagnostic Tool: is it cancer?

1 in 3 women will develop a cancer in their lifetime, 1 in 8 breast cancer

Of all breast cancers diagnosed in the U.S., only 5 to 10% are related to genetics and family history of breast cancer.

Available treatments surgery, chemo, radiation, hormone

Science 2007 315:924

Cancergeek.com

Figure removed due to copyright restrictions.

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
Treatment Evaluation Tool: will tumor be hormone-responsive?

~75% of all breast cancers are ER+ (estrogen receptor positive), with remaining 25% negative or an unknown status.

~ About 65% of all ER+ are also PR+ (progesterone receptor positive).

~ 10% of breast cancers are ER+ and PR-.

~ 5% of breast cancers are ER- and PR+.
Treatment Evaluation Tool: how likely is it to spread?

Most commonly spreads to lymph tissue

“Chemotherapy and/or hormonal therapy reduce the risk of distant metastases by approximately one-third; however, 70-80% of patients receiving this treatment would have survived without it.”

Agenda

Courtesy of National Cancer Institutes.
Illustration by Jane Hurd.

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (http://ocw.mit.edu), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].
FDA approved “MammaPrint” assay
Summary

1. Mechanics of microarrays

2. Microarrays for molecular medicine
the end