Module 2: Expression Engineering

20.109
Lecture 5
October 25th, 2007

Expression Engineering Experiment

<table>
<thead>
<tr>
<th>Lecture 1</th>
<th>Lecture 2</th>
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<tbody>
<tr>
<td>• intro to cell culture</td>
<td>• transfection</td>
</tr>
<tr>
<td>• intro to gene exprn/RNAi</td>
<td>• luciferase</td>
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<table>
<thead>
<tr>
<th>Lecture 3</th>
<th>Lecture 4</th>
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</thead>
<tbody>
<tr>
<td>• off-target/nonspecific RNAi</td>
<td>• Writing lecture</td>
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<td></td>
<td>(Neal Lerner)</td>
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<table>
<thead>
<tr>
<th>Lecture 5</th>
<th>Lecture 6</th>
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<tr>
<td>• measuring gene exprn</td>
<td>• microarray analysis</td>
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<td></td>
<td>(Rebecca Fry)</td>
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<thead>
<tr>
<th>Lecture 7</th>
<th>Lecture 8</th>
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<tr>
<td>• high throughput technologies or RNAi applications (no lab)</td>
<td>• review of your data</td>
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DNA/RNA/Protein Compass

Microarray

Spot diameter: 10-150 um
Content: ~10^9 molecules/um^2

http://www.youtube.com/watch?v=S8Cwy71nMNU
http://www.bio.davidson.edu/people/macambell/strategies/chipsintro.html

Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray

Mark Schena, Dart Shalon, Ronald W. Davis, Patrick O. Brown

Science 1995 270:467
Microarray sample preparation
- two samples to compare
- isolate RNA
- reverse transcribe and label

Microarray fluorescent tags
- Cy3
- Cy5

Microarray wash and scan

Microarray controls
- Self-Self Microarrays
- Dye Swap Microarrays

Microarray indirect labeling method

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

http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html
http://www.qubitsystems.com
http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html
http://www.genisphere.com/about_3dna.html
Science 2007 315:924
Cancergeek.com
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+

Cancergeek.com

Treatment Evaluation Tool: how likely is it to spread?

“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.”

Agendia

Summary

1. Mechanics of microarrays

2. Microarrays for molecular medicine