Functional Genomics Research Stream

Research Meeting: April 20, 2010
PCR Saturation, Cell Synchrony & RT-PCR

One on One Meetings

• Continue to Find Me ....
• Talk Grades
• Talk Fall

Business Issues

Research Progress Report V

• Final Report
• PDF Posted to Course Website
• Online Submission
• Due: Friday, May 7 @ 10:00 PM
  the last day of classes, no final exam
Fall Courses

- All Research, All The Time
- Progress Reports Similar to Now
- See Me if Questions, Concerns

Conceptual Issues

PCR Saturation
PCR Evaluation of ChIP

<table>
<thead>
<tr>
<th>PCR</th>
<th>Targets</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non Targets</td>
<td>Non Targets</td>
</tr>
</tbody>
</table>

A Saturation Issue

Double the volume of water in these tubs 10 times.

How does this relate to PCR?

tub a

tub b

Moryan, K 2009

<table>
<thead>
<tr>
<th>LANE 1</th>
<th>LANE 2</th>
<th>LANE 3</th>
<th>LANE 4</th>
<th>LANE 5</th>
<th>LANE 6</th>
<th>LANE 7</th>
<th>LANE 8</th>
<th>LANE 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMPLE</td>
<td>Ladder</td>
<td>ChIP Sample</td>
<td>ChIP Sample</td>
<td>empty</td>
<td>Input DNA</td>
<td>Input DNA</td>
<td>empty</td>
<td>Genomic DNA</td>
</tr>
<tr>
<td>PRIMERS</td>
<td>target primers for SWI5-1</td>
<td>non-target primers ACT1-S</td>
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<td>non-target primers ACT1-S</td>
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Double the volume of water in these tubs 10 times.

How does this relate to PCR?

cycle course, increasing cycle count
comparing 1 to 1/10 mass DNA amplification

Moryan, K 2009
Cell Synchrony

Synchrony Experiments

- How do these experiments work?
- What is the generalized process?
- What are we measuring and when? (think morphology and percentages)
**Synchrony: Hydroxyurea**

- What process does hydroxyurea inhibit?
- Where do hydroxyurea treated cells arrest?
- Is there a morphological marker for cell-cycle arrest at this position? What is its name and what does it look like?

**Synchrony: Nocodazole**

- What process does nocodazole inhibit?
- Where do nocodazole treated cells arrest?
- Is there a morphological marker for cell-cycle arrest at this position? What is its name and what does it look like?

**Synchrony: α Factor**

- Do all yeast cells arrest with α factor treatment?
- Where do α factor treated cells arrest?
- What is the term for this position in the cell cycle?
- Is there a morphological marker for cell-cycle arrest at this position? What is its name and what does it look like?

**Cell Morphology**

- the schmoo
A Killer Issue: BAR1

- What is BAR1?
- How might it affect our ability to effectively synchronize cell cultures?
- What can we do about it?
- How would we do it?
Studying & Documenting Cell Synchrony

RT-PCR

• What is RT-PCR?
• Is it one reaction or two?
• What primers are used for each?
• What might we use RT-PCR for with respect to judging cell synchrony and synchronized cultures?

Gene Markers

• **CLN2** - G1/S Boundary
  - G1 cyclin involved in regulation of the cell cycle; activates Cdc28p kinase to promote the G1 to S phase transition; late G1 specific expression depends on transcription factor complexes, MBF (Swi6p-Mbp1p) and SBF (Swi6p-Swi4p).

• **H2A** - S Phase
  - Histone H2A, core histone protein required for chromatin assembly and chromosome function; one of two nearly identical subtypes (see also HTA2); DNA damage-dependent phosphorylation by Mec1p facilitates DNA repair; acetylated by Nat4p.

• **CLB4** - G2
  - B-type cyclin involved in cell cycle progression; activates Cdc28p to promote the G2/M transition; may be involved in DNA replication and spindle assembly; accumulates during S phase and G2, then targeted for ubiquitin-mediated degradation.

• **CLB2** - M Phase
  - B-type cyclin involved in cell cycle progression; activates Cdc28p to promote the transition from G2 to M phase; accumulates during G2 and M, then targeted via a destruction box motif for ubiquitin-mediated degradation by the proteasome.

• **EGT2** - M/G1 Boundary
  - Glycosylphosphatidylinositol (GPI)-anchored cell wall endoglucanase required for proper cell separation after cytokinesis, expression is activated by Swi5p and tightly regulated in a cell cycle-dependent manner.
Step 1: Experiment

- BAR1 knockout strain (BAR1Δ) grown.
- Synchronized.
- Synchronization visualized.
- Synchrony released.
- Time-points: 0, 20, 40, 60, 80, 100 minutes.

Step 2: RNA Preps

- Time-points: 0, 20, 40, 60, 80, 100 minutes.

Step 3a: RT-PCR

CLN2

How do I know if this is the correct result?
Step 3a: RT-PCR

CLN2 BASIC INFORMATION

- **Standard Name**: CLN2
- **Systematic Name**: YPL265C
- **Feature Type**: ORF, Verified
- **Description**: G3 cyclin involved in regulation of the cell cycle; activates GSK3b kinase to promote the G1 to S phase transition; late (S) specific expression depends on transcription factor complexes, MBP (Mbp1-Mbp5) and SBF (Swe1p-Sbe1p) (1, 2, 3, 4 and see Summary Paragraph)
- **GO Annotations**
  - All CLN2 GO evidence and references
  - View Computational GO annotations for CLN2
- **Molecular Function**
  - cyclin-dependent protein kinase regulator activity (TAS)
- **Biological Process**
  - negative regulation of transcription, RNA-mediated (IMP)
  - entry into mitotic cell cycle after pheromone arrest (G1)
  - regulation of cyclin-dependent protein kinase activity (TAS)
- **Cellular Component**
  - cyclin-dependent protein kinase holoenzyme complex (GOA)
  - cytoplasm (GOA)
  - nucleus (GOA)
- **Mutant Phenotype**
  - All CLN2 Phenotype details and references
  - cell cycle passage through START: increased rate
  - cell cycle progression: abnormal
  - cell cycle progression in G1 phase: increased duration
  - mitotic cell size at START: increased cell size (checkpoints); decreased
  - phenotype in cell cycle arrest: decreased
  - invasive growth: decreased
  - invasive

CLN2 RESOURCES

- **Literature**
  - Literature Code
- **Retrieve Sequences**
  - Genomic DNA
- **Sequence Analysis Tools**
  - BLAST
- **Protein Info & Structure**
  - Protein Info
- **Localization Resources**
  - Yeast localization database
- **Interactions**
  - BioGRID (Torres)
- **Phenotype Resources**
  - REGSITE
- **Maps & Displays**
  - Chromosomal Proteins Map
- **Comparison Resources**
  - Cellular phenotypes
- **Functional Analysis**
  - Cell cycle transcript profile

Step 3b: RT-PCR

CLB2

Fairchild, 2010

Step 3c: RT-PCR

ACE2

Fairchild, 2010
Step 3d: RT-PCR

What is the point of these experiments?

ChIP Results & Analysis
Lane 1 – Ladder
Lane 2 – ChIP and FKH2 – 1 primers
Lane 3 – ChIP and ACT1 short primers
Lane 4 – Skipped
Lane 5 – Positive control with genomic DNA and ACT1 short primers
Lane 6 – Negative control
Lane 7 – Skipped
Lane 8 – Input and FKH2 – 1 primers
Lane 9 – Input and ACT1 short primers

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Pre-Clearing

Luo, 2010