Fermentation Workflow

**DAY 1**
- *Target Selection: Mol Bio Lists & Specials*
- *Set up for week’s work*
- *Transformations (P.M.)*

**DAY 2**
- *Innoculation (A.M.)*
- *Media Preparation*
- *1st Dilution (P.M.)*

**DAY 3**
- *2nd Dilution*
- *Induction*

**DAY 4**
- *Harvest*
- *Run Gels*

**DAY 5**
- *Gel Analysis*
- *SPiNE Upload*
Information Transfer: Mol Bio → Fermentation

- Molecular Biology analyzes and scores small scale expression SDS-PAGE gels
- Promising targets (ES ≥ 8) compiled on a list
- We go over list together; I view gels
  - Eliminate oversights and mistakes
  - Double check scoring
  - Discard targets that are extremely poor
    - Band of interest is superimposed on background → this makes band seem more intense
  - Any questionable targets?? Seek Li’s opinion
  - I rate the targets that will continue on in the pipeline (K-Score) (+, -)
    - “+” → above average solubility
    - “-” → below average solubility
    - Bookkeeping mechanism to track discrepancies between small scale and large scale solubility
    - Also, targets with a “-” rating will start with 2L of fermentation to increase yield

- Mol Bio compiles a FINAL list with the approved targets
  - Stored in Spins Webserver

- Each week, I select targets from the FINAL list to Ferment
Day 1: Target Prioritization

– Collect e-mails from Li, Tom, & Rong for *SPECIAL* fermentation requests

– Select targets from Molecular Biology Team
  • Targets >25 kD require one fermentation (SeMet labeled)
  • Targets <25 kD require two fermentations (SeMet and N/NC5 labeled)

*Total capacity: ~23 Fermentations per 5 day cycle
*Simultaneous cycles are possible when spaced 24 hours apart
Day 2: Inoculations (A.M)

- Inoculate the target into LB Media
  - Transformation
  - Glycerol Stock

*Use Archive Plate Master to determine location of expression glycerol stock and/or Target DNA for transformation
Day 2 (cont.): Media Prep

- NC (100%N15, 100%C13)
- NC5 (100%N15, 5%C13, 95% C12)
- N (100%N15, 100%C12)
- SeMet (100%N14, 100%C12; Seleno-Methionine)
- 14N (100%N14, 100%C12)
- LB

*SPECIAL* requests will specify media type
Mol Bio Targets >25 kD are SeMet labeled
Mol Bio Targets >15 kD and <25 kD are SeMet labeled and N labeled
Mol Bio Targets <15 kD are SeMet labeled and NC5 labeled

*All cultures require 1L of media and are grown in 2L baffled flasks*
Day 2 (cont.): 1st Dilution (P.M.)

- Overnight starter culture for each fermentation is prepared using the LB inoculation from A.M. and an aliquot of the target’s appropriate MJ9 media
- 1:1000 fold dilution
- Grown in 250mL flasks; shaking at 37°C o/n
Day 3: 2\textsuperscript{nd} Dilution

- Each overnight starter culture is added to its corresponding 2L flask
- 1:25 fold dilution
- Shake at 37°C until OD reaches A600 0.6-0.8 (approximately 2 hours)
- Record OD (Initial OD)
Day 3 (cont.): Induction

- At OD A600 0.6-0.8, 2L flasks are moved to the 17°C Induction Room
- Seleno-Methionine and Amino Acid Solution (K, F, T, L, I, V) are added to SeMet fermentations
- Record a second OD (Induction OD)
- Each fermentation is induced with 1M IPTG stock (final concentration of IPTG = 1mM)
- Shake at 17°C o/n
Day 4: Harvest & Gels

- Take an aliquot of induced culture before Spin Down
  - For SeMet and NC: 540uL
    - 140uL → Glycerol Stock
    - 70uL → DNA Sequencing Plate
    - 70uL → Fermentation Archive
  - 100uL → Final OD
  - 300uL → Mini-Harvest (Gel Analysis)

- For N, NC5, 14N, LB, etc.: 400uL
  - 100uL → Final OD
  - 300uL → Mini-Harvest (Gel Analysis)
Day 4 (cont.): Harvest & Gels

- Harvest induced cultures and store pellets at -20°C
When Mol Bio has a Totals gel that is ambiguous due to a smear effect brought on by a high cell density, the solubility rating then becomes less subjective because you cannot see the band for expression. They then rate the soluble portion on a 0-5 scale.
Day 4 (cont.): Harvest & Gels

- **Glycerol Stock**
  - DNA Sequencing
  - Fermentation Archive

- **Final OD (Record)**
  - 1:10 fold dilution A600

- **Mini-Harvest**
  - Spin down
  - Re-suspend pellet with Lysis Buffer
  - Sonicate
  - Run Harvest Total and Harvest Soluble on SDS-PAGE Gel

- Glycerol Stock (SeMet and NC only)
- Mini-Harvest
- Final OD
Day 5: Gel Analysis & SPiNE Upload

• Photograph gel and label well lanes using Adobe
Day 5 (cont.): Gel Analysis & SPiNE Upload

- Prepare Fermentation Mass Upload Sheet

<table>
<thead>
<tr>
<th>Expression ID</th>
<th>Label</th>
<th>Researchers</th>
<th>Exp Date</th>
<th>Host Strain</th>
<th>Grow</th>
<th>Ind</th>
<th>Media</th>
<th>Exp Scale</th>
<th>Exp</th>
<th>Ind O.D.</th>
<th>Fin O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR525-21.1</td>
<td>SeMa</td>
<td>KC, LM</td>
<td>2/15/2006</td>
<td>BL21(DE3)</td>
<td>37</td>
<td>17</td>
<td>MJ9 SeMet</td>
<td>Preparative</td>
<td>5</td>
<td>0.6985</td>
<td>3.255</td>
</tr>
<tr>
<td>VR68-21.2</td>
<td>SeMa</td>
<td>KC, LM</td>
<td>2/15/2006</td>
<td>BL21(DE3)</td>
<td>37</td>
<td>17</td>
<td>MJ9 SeMet</td>
<td>Preparative</td>
<td>5</td>
<td>0.6991</td>
<td>3.464</td>
</tr>
<tr>
<td>ZR29-21.2</td>
<td>SeMa</td>
<td>KC, LM</td>
<td>2/15/2006</td>
<td>BL21(DE3)</td>
<td>37</td>
<td>17</td>
<td>MJ9 SeMet</td>
<td>Preparative</td>
<td>5</td>
<td>0.8117</td>
<td>3.476</td>
</tr>
<tr>
<td>ZR32-21.1</td>
<td>SeMa</td>
<td>KC, LM</td>
<td>2/15/2006</td>
<td>BL21(DE3)</td>
<td>37</td>
<td>17</td>
<td>MJ9 SeMet</td>
<td>Preparative</td>
<td>5</td>
<td>0.7822</td>
<td>3.228</td>
</tr>
<tr>
<td>ER29-21</td>
<td>LBb</td>
<td>KC, LM</td>
<td>2/15/2006</td>
<td>BL21(DE3)</td>
<td>37</td>
<td>17</td>
<td>LB</td>
<td>Preparative</td>
<td>4</td>
<td>0.7173</td>
<td>2.68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vol</th>
<th>Flask</th>
<th>SDS Gel ID</th>
<th>Comments</th>
<th>Phase</th>
<th>Sol Gel #</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>[link](<a href="http://spine.nesg.org/private/upload/files/GEL_FERMENTATION/Gel">http://spine.nesg.org/private/upload/files/GEL_FERMENTATION/Gel</a> 895.jpg)</td>
<td>Soluble+Inclusion</td>
<td>4  895</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>[link](<a href="http://spine.nesg.org/private/upload/files/GEL_FERMENTATION/Gel">http://spine.nesg.org/private/upload/files/GEL_FERMENTATION/Gel</a> 895.jpg)</td>
<td>Soluble</td>
<td>5  895</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>[link](<a href="http://spine.nesg.org/private/upload/files/GEL_FERMENTATION/Gel">http://spine.nesg.org/private/upload/files/GEL_FERMENTATION/Gel</a> 895.jpg)</td>
<td>Soluble</td>
<td>5  895</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>[link](<a href="http://spine.nesg.org/private/upload/files/GEL_FERMENTATION/Gel">http://spine.nesg.org/private/upload/files/GEL_FERMENTATION/Gel</a> 895.jpg)</td>
<td>Soluble</td>
<td>5  895</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>[link](<a href="http://spine.nesg.org/private/upload/files/GEL_FERMENTATION/Gel">http://spine.nesg.org/private/upload/files/GEL_FERMENTATION/Gel</a> 895.jpg)</td>
<td>Soluble</td>
<td>5  895</td>
<td></td>
</tr>
</tbody>
</table>
Day 5 (cont.): Gel Analysis & SPiNE Upload

- Upload labeled Gel pictures into SPiNE using SPiNE Database File Upload tool
  [http://spine.nesg.org/private/upload/](http://spine.nesg.org/private/upload/)

- **File:**
  - Browse for appropriate file:
    Gel ### copy.jpg
- **Record ID:** GEL_FERMENTATION
- **Name of file:** Gel ###

- Click Submit
Day 5 (cont.): Gel Analysis & SPiNE Upload

File Upload Successful

The submitted file is at http://spine.nesg.org/private/upload/files/GEL_FERMENTATION/Gel_###.jpg

Enter this link into SPINE database as: none

Primary ID: GEL_FERMENTATION  Submit

Click here to access all uploaded files for this target

• Click Submit

** Jessica/Mike Wilson** → Batch Upload for Gel pictures using spins webserver
Day 5 (cont.): Gel Analysis & SPiNE Upload

- Upload Fermentation Mass Upload Sheet into SPiNE using SPiNE Bulk Data Upload
  http://spine.nesg.org/private/bulk_ferm.pl
- Copy and Paste Fermentation Mass Upload Sheet from Excel into the area provided
  - Include only columns ‘Construct ID’ through ‘Solubility’
  - Do not include row with the column headers

Paste Fermentation Excel spreadsheet here:

Expected column format:

<table>
<thead>
<tr>
<th>Construct ID</th>
<th>Label</th>
<th>Researcher</th>
<th>Expression Date</th>
<th>Host Strain</th>
<th>Growth Temp</th>
<th>Induct. Temp</th>
<th>Media</th>
<th>Expr. Scale</th>
<th>Expr. Level</th>
<th>Induction OD</th>
<th>Final OD</th>
<th>Ferm. Volume</th>
<th>Flask Volume</th>
<th>SDS-Gel ID</th>
<th>Comments</th>
<th>Phase</th>
<th>Solubility</th>
</tr>
</thead>
</table>

• Click Submit
Day 5 (cont.): Gel Analysis & SPiNE Upload

If the columns line up properly, create records by clicking "SUBMIT". "OK" entries will be inserted into the database. Entries with "Errors" will not be inserted into the database until the errors are corrected.

<table>
<thead>
<tr>
<th>Errors</th>
<th>Construct ID</th>
<th>Label</th>
<th>Researcher</th>
<th>Expression Date</th>
<th>Host Strain</th>
<th>Growth Temp</th>
<th>Induct. Temp</th>
<th>Media</th>
<th>Expr. Scale</th>
<th>Expr. Level</th>
<th>Induction OD</th>
<th>Final OD</th>
<th>Ferm. Volume</th>
<th>Flask Volume</th>
<th>SDS-Gel ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Already in SPiNE</td>
<td>SRS25-21:1</td>
<td>SeMa</td>
<td>Kelcie Cunningham, Li-Chung Ma</td>
<td>2/15/2006</td>
<td>BL21 (DE3)  + Magic</td>
<td>37</td>
<td>17</td>
<td>M9 SeMet</td>
<td>Preparative</td>
<td>5</td>
<td>0.6985</td>
<td>3.255</td>
<td>1</td>
<td>2</td>
<td><a href="http://spine.nesg.org/private/upload/file185.jpg">http://spine.nesg.org/private/upload/file185.jpg</a></td>
</tr>
<tr>
<td>Already in SPiNE</td>
<td>VR68-21:2</td>
<td>SeMa</td>
<td>Kelcie Cunningham, Li-Chung Ma</td>
<td>2/15/2006</td>
<td>BL21 (DE3)  + Magic</td>
<td>37</td>
<td>17</td>
<td>M9 SeMet</td>
<td>Preparative</td>
<td>5</td>
<td>0.6991</td>
<td>3.464</td>
<td>1</td>
<td>2</td>
<td><a href="http://spine.nesg.org/private/upload/file185.jpg">http://spine.nesg.org/private/upload/file185.jpg</a></td>
</tr>
<tr>
<td>Already in SPiNE</td>
<td>ZK29-21:2</td>
<td>SeMa</td>
<td>Kelcie Cunningham, Li-Chung Ma</td>
<td>2/15/2006</td>
<td>BL21 (DE3)  + Magic</td>
<td>37</td>
<td>17</td>
<td>M9 SeMet</td>
<td>Preparative</td>
<td>5</td>
<td>0.6117</td>
<td>3.476</td>
<td>1</td>
<td>2</td>
<td><a href="http://spine.nesg.org/private/upload/file185.jpg">http://spine.nesg.org/private/upload/file185.jpg</a></td>
</tr>
<tr>
<td>Already in SPiNE</td>
<td>ZK32-21:1</td>
<td>SeMa</td>
<td>Kelcie Cunningham, Li-Chung Ma</td>
<td>2/15/2006</td>
<td>BL21 (DE3)  + Magic</td>
<td>37</td>
<td>17</td>
<td>M9 SeMet</td>
<td>Preparative</td>
<td>5</td>
<td>0.7822</td>
<td>3.220</td>
<td>1</td>
<td>2</td>
<td><a href="http://spine.nesg.org/private/upload/file185.jpg">http://spine.nesg.org/private/upload/file185.jpg</a></td>
</tr>
<tr>
<td>Already in SPiNE</td>
<td>ZR41-21:2</td>
<td>SeMa</td>
<td>Kelcie Cunningham, Li-Chung Ma</td>
<td>2/15/2006</td>
<td>BL21 (DE3)  + Magic</td>
<td>37</td>
<td>17</td>
<td>M9 SeMet</td>
<td>Preparative</td>
<td>4</td>
<td>0.6383</td>
<td>1.774</td>
<td>1</td>
<td>2</td>
<td><a href="http://spine.nesg.org/private/upload/file185.jpg">http://spine.nesg.org/private/upload/file185.jpg</a></td>
</tr>
</tbody>
</table>

- If the left column reads ‘OK’, then you can click submit at the bottom of the page; if errors are present, re-visit the Fermentation Mass Upload sheet in Excel to fix entries.
- Error checking mechanism
  - ‘Already in SPiNE’?
  - ‘Error’ → Unrecognizable value?
- Standardization of Fermentation Mass Upload fields
  - Allows me to prevent data entry mistakes from being uploaded into the database
Information Transfer: Fermentation → Purification

- Purification performs expression search in SPiNE

http://spine.nesg.org/private/view.html#expression

- E-mail Purification Team comments on specific proteins
  - i.e. if multiple fermentations for the same construct were done (i.e. due to low solubility), then a comment to co-purify these fermentations is sent
Sample Search

Expression search

Quick Link: View records from the past one month or three months.

Select search criteria and display parameters:

- **Target Organism:** Any
- **Expression Scale:** Preparative
- **Host Strain:** Any
- **Growth Medium:** Any
- **Expression level at least:** Any
- **Solubility at least:** Any
- **Purified:** N
- **Expression ID:**
- **Construct ID:**
- **Target ID:**
- **pI**
- **Molecular Weight**
- **Extinction Coefficient**
- **Ext Coef. / MW Ratio**
- **Date range:** Jan 17, 2006 to Mar 17, 2006

Sort by:

- Date expressed
- Order: Descending

[Create Table] [Reset] [Form Excel Spreadsheet]
### Expression Viewer

Click on a column header to sort downwards by this value.

<table>
<thead>
<tr>
<th>Expression ID</th>
<th>Scale</th>
<th>Date expressed</th>
<th>Strain</th>
<th>Media</th>
<th>Expr. Level</th>
<th>Solubility</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER397-21.1-NcA</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 100%N15</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>ER476-21.6-SeMa</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ER476-21.3-SeMa</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>ER474-21.2-SeMa</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>ER469-21.4-SeMb</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>ER469-21.4-SeMa</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>ER461-21.3-SeMa</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>ER104-21.11-SeMb</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ER184-21.11-SeMa</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>VR78-21.1-SeMa</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>VR94-21.5-SeMa</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>MaR30-21.1-NcB</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 100%N15 100%C13</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>SrR39-21.1-Nc5a</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 100%N15 5%C13</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>HdrR14-21.2-Nc5a</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 100%N15 5%C13</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>ZR55-21.2-SeMc</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ZR55-21.2-SeMb</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>VR95-21.2-SeMb</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>VR95-21.2-SeMa</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>VR94-21.5-SeMb</td>
<td>Preparative</td>
<td>2006-03-08</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 SeMet</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>HR2879A-28.1-Nc5b</td>
<td>Preparative</td>
<td>2006-03-02</td>
<td>BL21(DE3)+ CodonplusRIL</td>
<td>M9 100%N15 5%C13</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HR2879A-28.1-Nc5c</td>
<td>Preparative</td>
<td>2006-03-02</td>
<td>BL21(DE3)+ CodonplusRIL</td>
<td>M9 100%N15 5%C13</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HR2879A-28.1-Nc5d</td>
<td>Preparative</td>
<td>2006-03-02</td>
<td>BL21(DE3)+ CodonplusRIL</td>
<td>M9 100%N15 5%C13</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HR2879A-28.1-Nc5e</td>
<td>Preparative</td>
<td>2006-03-02</td>
<td>BL21(DE3)+ CodonplusRIL</td>
<td>M9 100%N15 5%C13</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HR2873A-14.3-Ncg</td>
<td>Preparative</td>
<td>2006-03-01</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 100%N15 100%C13</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HR2873A-14.3-Ncf</td>
<td>Preparative</td>
<td>2006-03-01</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 100%N15 100%C13</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>HR2873A-14.3-Nce</td>
<td>Preparative</td>
<td>2006-03-01</td>
<td>BL21(DE3)+ Magic</td>
<td>M9 100%N15 100%C13</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Help from PLIMS?

• LIMS archival for glycerol stocks
  – DNA Sequencing
  – Fermentation Archive

• Batch Upload for Gel Pictures
  – (in the works)

• Label Production
  – Spreadsheet of targets → print labels for flasks, bottles, tubes, etc. with target names
Improvements Needed For Fermentation Efficiency

• Plate Reader
  – Time saver
  – Configure with Excel to export readings to Fermentation Mass Upload Sheet

• Expression/ Solubility Ratings
  – Automated?

• Bench Tablets
Freezer Organization

• SeMet/14N/LB Freezer

• NC/NC5 Freezer

- Three shelves in each freezer will be dedicated to pellets, each corresponding to a specific week of Fermentation.

- There will be lists on the outside of the freezer for each shelf that have exactly those proteins that correspond to that shelf’s week.

- When a pellet is removed from a shelf, Purification should cross out that protein on the list, so everyone knows it has been removed.

- When a new week is done, the pellets located on the shelf with the oldest fermentation date will be re-suspended into 50mL tubes and archived, thus making room for the new bottles. A new list for that week will be provided on the freezer door, and the old list will be removed. When re-suspending, pellets can be cross referenced with the corresponding list to make sure everything matches up. Discrepancies can be discussed.
Freezer Organization

SHELF 1: 1/1/2006
SHELF 2: 1/8/2006
SHELF 3: 1/15/2006

SeMet Freezer

SHELF 1: 1/1/2006
ER1-21.1-SeMa
FR1-21.1-SeMa
SR23-21.1-SeMa
GR12-21.2-SeMa
HR2018-14.1-SeMb
PfR16-21.3-SeM-R1

N Freezer

SHELF 1: 1/1/2006
ER1-21.1-NC5a
FR1-21.1-NCa
SR23-21.1-NC5a
GR12-21.2-NC5a
ER411-21.1-NCa
SeMet Freezer

SHELF 1: 1/1/2006
- ER1-21.1-SeMa
- FR1-21.1-SeMa
- SR23-21.1-SeMa
- GR12-21.2-SeMa
- HR2018-14.1-SeMb
- PIR16-21.3-SeM-R1

Pellets that have not been purified will be re-suspended, put into 50mL tubes, and archived. SHELF 1 will now be empty and ready for the next batch: 1/22/2006. The list on the freezer door will be replaced with one that corresponds to 1/22/2006.

The next recent fermentation batch will assume position on SHELF 2. Any left over pellets on SHELF 2 from week 1/8/2006 not purified by the completion of the that week’s batch will be re-suspended so room can be made to accommodate the new bottles. Etc, etc, etc.
## Sequencing results

<table>
<thead>
<tr>
<th>ID</th>
<th>WELL</th>
<th>LENGTH</th>
<th>EXPECTED_SEQ</th>
<th>ACTUAL_SEQ</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZR142-21.1</td>
<td>A01</td>
<td>306</td>
<td>atgtgacagttaccaaattagacc</td>
<td>ATGTGGACAGTACAAAT</td>
<td>T159C Correct</td>
</tr>
<tr>
<td>ZR149-21.1</td>
<td>B01</td>
<td>360</td>
<td>atgtcagtaatttctatttttatttt</td>
<td>ATGTCGAAAAGAAATT</td>
<td>37 mismatches Correct</td>
</tr>
<tr>
<td>ZR199-21.1</td>
<td>C01</td>
<td>406</td>
<td>atgtcagaaaaaagttaattttactaa</td>
<td>ATGTGACAGTACAAAT</td>
<td>C83T Correct</td>
</tr>
<tr>
<td>SaR13-21.1</td>
<td>H01</td>
<td>253</td>
<td>atgagagtttaacattttccatgaac</td>
<td>ATGGAGATTATAGCAATT</td>
<td>Correct</td>
</tr>
<tr>
<td>OR5D-14.1</td>
<td>B02</td>
<td>387</td>
<td>atgcctgtgccttcgcatcaacgta</td>
<td>ATGCGTGGTTCAGTACAT</td>
<td>Correct</td>
</tr>
<tr>
<td>OR6D-14.2</td>
<td>C02</td>
<td>387</td>
<td>atgcctgtgccttcgcatcaacgta</td>
<td>ATGCGTGGTTCAGTACAT</td>
<td>G39T Correct</td>
</tr>
<tr>
<td>OR7D-14.1</td>
<td>D02</td>
<td>387</td>
<td>atgcctgtgccttcgcatcaacgta</td>
<td>ATGCGTGGTTCAGTACAT</td>
<td>Correct</td>
</tr>
<tr>
<td>OR8D-14.1</td>
<td>E02</td>
<td>387</td>
<td>atgcctgtgccttcgcatcaacgta</td>
<td>ATGCGTGGTTCAGTACAT</td>
<td>Correct</td>
</tr>
<tr>
<td>ER336-21.1</td>
<td>A04</td>
<td>381</td>
<td>atgagaggctttgcttgattttataag</td>
<td>ATGGAGAGTGCTAAGAATG</td>
<td>Correct</td>
</tr>
<tr>
<td>ER353-21.2</td>
<td>B04</td>
<td>267</td>
<td>atgaaatgtgtaacctgcttgatt</td>
<td>ATGAAATGTGTAAGCTG</td>
<td>Correct</td>
</tr>
<tr>
<td>ER356-21.3</td>
<td>C04</td>
<td>297</td>
<td>atgagaggctttgcttgattttataag</td>
<td>ATGGAGAGTGCTAAGAATG</td>
<td>Correct</td>
</tr>
<tr>
<td>ER364-21.4</td>
<td>D04</td>
<td>300</td>
<td>atgagaggctttgcttgattttataag</td>
<td>ATGGAGAGTGCTAAGAATG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR424-21.1</td>
<td>A06</td>
<td>249</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>A145G Not match</td>
</tr>
<tr>
<td>SR426-21.1</td>
<td>B06</td>
<td>255</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR437-21.1</td>
<td>C06</td>
<td>435</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR438-21.2</td>
<td>D06</td>
<td>291</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR439-21.2</td>
<td>E06</td>
<td>324</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR445-21.1</td>
<td>F06</td>
<td>447</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR446-21.2</td>
<td>G06</td>
<td>441</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR489-21.1</td>
<td>H06</td>
<td>246</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR499-21.1</td>
<td>A07</td>
<td>363</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR506-21.1</td>
<td>B07</td>
<td>387</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR607-21.2</td>
<td>C07</td>
<td>361</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR481-21.2</td>
<td>A09</td>
<td>339</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>SR482-21.1</td>
<td>B09</td>
<td>327</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>Correct</td>
</tr>
<tr>
<td>BcR29-21.1</td>
<td>C09</td>
<td>646</td>
<td>tgtgtacctaaacagcttgccat</td>
<td>TGGTTACTAAAGACGCTAG</td>
<td>T224C, pending the other strand Correct</td>
</tr>
</tbody>
</table>