Regeneron’s KOMP Production

Year 5: Final Annual Review

28 September 2011
Regeneron’s KOMP Challenges

Creating gene-ablating definitive null alleles for 3,500 genes

- **Target gene and allele design**
  - Regeneron was assigned the difficult genes that were not targetable by the knockout first conditional allele design used by the CSD and EUCOMM.
  - Deleting the entire coding sequence from start to stop codons
  - Insertion of a robust and effective lacZ reporter gene precisely at the start codon whose expression avoids trap-like artificial splicing and the use of viral translational signals such as IRES and T2A.

- **C57BL/6 ES cell line development**
  - Derive and develop a robust, genomically stable B6 line that targeted at a reasonable rate and retained high germline transmission (GLT) potential after manipulation in the setting of a high throughput production pipeline.
  - Develop stringent quality assurance methods for all materials prior to shipment to the KOMP Repository

- **Production**
  - Build high quality BAC-based targeting vectors at a rate of 40–50 per week.
  - Develop high throughput ES cell electroporation methods.
  - Increase the speed, throughput, and accuracy of ES cell screening.
  - Enhance database functions for data acquisition, archiving, analysis, and communication.
Meeting the Challenges

1. First Priority: B6 ES Cell Line Development
   We devoted considerable effort in our first two years of production to develop, test, and validate our VGB6 C57BL/6NTac ES cell line.
   We established that the line was genomically stable even after multiple passages. We developed a growth medium that maintains VGB6 cells in an undifferentiated state and promoted excellent GLT efficiency.
   We had to develop all new production methods specifically adapted to the VGB6 line.

2. Robotics
   We developed new robotic automation methods for nearly every step in BacVec construction.
   These methods increased speed and throughput while improving BacVec quality and reproducibility that led to higher gene targeting efficiencies.
   We applied novel robotic solutions to the automation of ES cell growth and manipulation.
   These methods improved throughput and productivity while maintaining high GLT efficiency.
Regeneron’s KOMP KO Allele Design
Gene-ablating Definitive Null

- LacZ fused at the start codon in nearly all alleles.
- All clones are in one ES cell line: VGB6.
93% of the alleles are complete coding sequence deletions.
Regeneron’s KOMP Goals and Results

### Promised Yearly Production Milestones:

<table>
<thead>
<tr>
<th>Year</th>
<th>Correctly Targeted Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>175</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
</tr>
<tr>
<td>3</td>
<td>940</td>
</tr>
<tr>
<td>4</td>
<td>942</td>
</tr>
<tr>
<td>5</td>
<td>943</td>
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<tr>
<td>Total</td>
<td>3,500</td>
</tr>
</tbody>
</table>

### Production Goal:
A rate of 1,000 correctly targeted genes per year by Year 3

### Actual Reported Production Results:

<table>
<thead>
<tr>
<th>Period</th>
<th>Alleles Designed</th>
<th>Genes &quot;Designed for&quot;</th>
<th>Vectors Constructed</th>
<th>ES Cell Electroporations</th>
<th>Genes Electroporated</th>
<th>Colonies Screened</th>
<th>Correctly Targeted Clones</th>
<th>Number of Genes Screened</th>
<th>Correctly Targeted Genes: at least 2 clones</th>
<th>Success Rate (%) (&gt;1 clones)</th>
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<tbody>
<tr>
<td>Summary 2006-2007</td>
<td>1030</td>
<td>1023</td>
<td>418</td>
<td>514</td>
<td>366</td>
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<td>1226</td>
<td>141726</td>
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<td>1964</td>
<td>1840</td>
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<td>Feb-11</td>
<td>158</td>
<td>158</td>
<td>158</td>
<td>149</td>
<td>148</td>
<td>13216</td>
<td>747</td>
<td>127</td>
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<td>Mar-11</td>
<td>187</td>
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<td>134</td>
<td>147</td>
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<td>14480</td>
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<td>Apr-11</td>
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<td>190</td>
<td>170</td>
<td>147</td>
<td>147</td>
<td>16800</td>
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<td>May-11</td>
<td>194</td>
<td>194</td>
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<td>17056</td>
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<td>Jun-11</td>
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<td>142</td>
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<td>171</td>
<td>16992</td>
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<td>Jul-11</td>
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<td>Sep-11</td>
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<td>119</td>
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<td>123</td>
<td>12960</td>
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<td>136</td>
<td>110</td>
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<td>81</td>
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<td>6947</td>
<td>6345</td>
<td>692468</td>
<td>28617</td>
<td>5001</td>
<td>3869</td>
<td>77</td>
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Summary of Regeneron’s Five Years of KOMP Production

Year 1
- Year 1 Goal: 175 (21-Nov-07)

Year 2
- Year 2 Goal: 675 (17-Nov-08)

Year 3
- Year 3 Goal: 1675 (19-Oct-09)

Year 4
- Year 4 Goal: 2675 (27-Sep-10)
- Project Goal: 3500 (03-Jun-11)

Year 5
- Projection of production growth

Production Week
- Correctly Targeted Genes
- Production Goal Milestones
- Week range: 0 to 240
## Efficiency Improvements from Years 1 to 5

<table>
<thead>
<tr>
<th></th>
<th>Y1</th>
<th>Y2</th>
<th>Y3</th>
<th>Y4</th>
<th>Y5</th>
</tr>
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<tbody>
<tr>
<td><strong>Targ. Freq. (%)</strong></td>
<td>2.6</td>
<td>2.8</td>
<td>4.8</td>
<td>4.7</td>
<td>4.9</td>
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<tr>
<td><strong>Weekly Averages</strong></td>
<td></td>
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<tr>
<td><strong>Targ. Genes</strong></td>
<td>4.1</td>
<td>11.6</td>
<td>22.0</td>
<td>21.8</td>
<td>25.7</td>
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<tr>
<td><strong>Targ. Gene Success Rate (%)</strong></td>
<td>61.1</td>
<td>66.6</td>
<td>76.5</td>
<td>81.7</td>
<td>82.9</td>
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</tbody>
</table>
Shipments to the KOMP Repository

- Targeting vectors for 4,730 genes
- >15,000 Targeted ES cell clones for 3,752 genes
  - (≥2 clones for 3,696 genes)
  - average of 4 clones/gene; mode = 6 clones
- 214 mouse lines for 169 genes
# Performance of Regeneron’s Targeted KOMP ES Cells

## Data Set

<table>
<thead>
<tr>
<th>Data Set</th>
<th>Method</th>
<th>Injected Clones with Complete Data</th>
<th>Strain of Injected Host Embryo</th>
<th>Clonal GLT Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regeneron’s KOMP Production QC</td>
<td>VelociMouse®</td>
<td>300</td>
<td>Swiss Webster</td>
<td>74.7%</td>
</tr>
<tr>
<td>KOMP Repository Results</td>
<td>Blastocyst Injection</td>
<td>233</td>
<td>BALB/c</td>
<td>77.0%</td>
</tr>
</tbody>
</table>

*P = 0.95 that at least one clone will achieve GLT from two injected*  
*P = 0.99 for three clones injected*
Conclusions

Regeneron met its quantitative promised goals for the KOMP

- 3,500 correctly targeted genes
- All ES cells shipped to the Repository before 31-Aug-11
- Finished ahead of time and under budget

We will continue to target genes for about another month and then prepare them for shipment to the Repository by the end of the year — we expect our total to be near 4,000.

Beyond the numbers, Regeneron has been committed to providing the highest quality product.

- Our targeted VGB6 ES cells have the highest GLT rate of any in KOMP or EUCOMM.
- Our cells have the highest QC success rate at the KOMP Repository: karyotypic stability, allele structure, copy number.

We have created a resource that will prove its value for years to come.
Summary of Regeneron’s Five Years of KOMP Production

- **Year 1 Goal**: 175 genes, 21-Nov-07
- **Year 2 Goal**: 675 genes, 17-Nov-08
- **Year 3 Goal**: 1675 genes, 19-Oct-09
- **Year 3 Goal**: 2675 genes, 27-Sep-10
- **Project Goal**: 3500 genes, 03-Jun-11

The graph shows the production weeks and the number of correctly targeted genes for each year from Year 1 to Year 5.