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

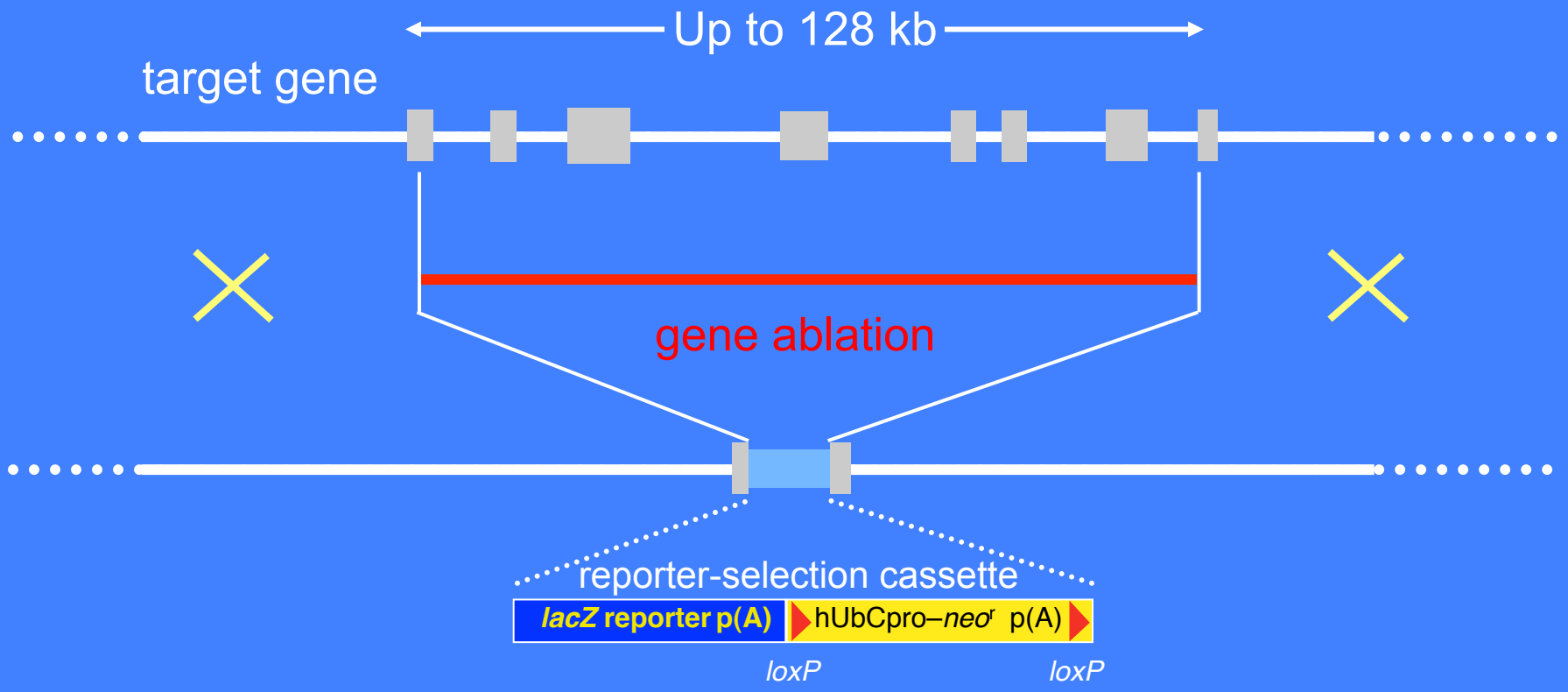
   We developed new robotic automation methods for nearly every step in BacVec construction.

   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.

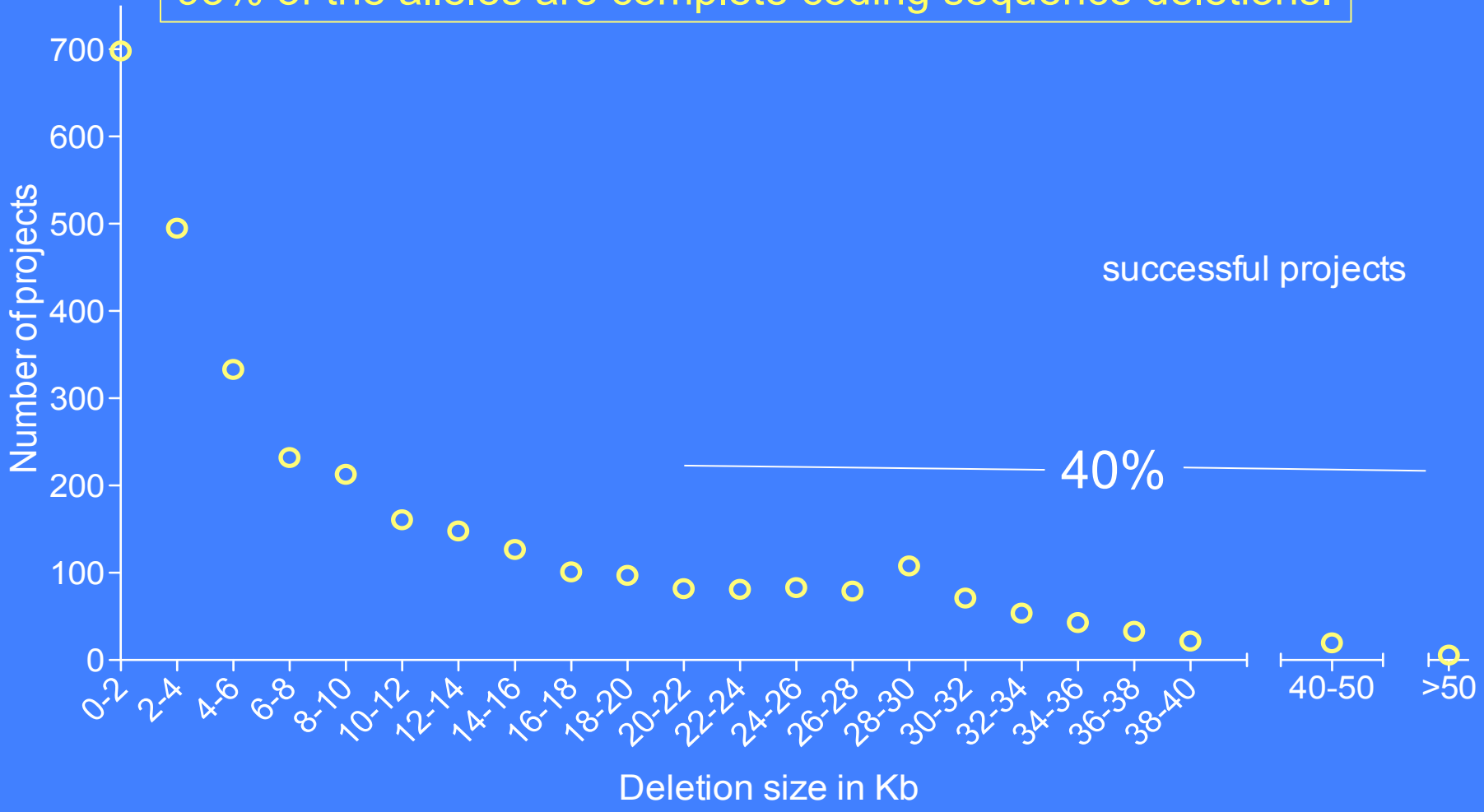
Regeneron's KOMP KO Allele Design
Gene-ablating Definitive Null



- *LacZ* fused at the start codon in nearly all alleles.
- All clones are in one ES cell line: VGB6.

Deletion Sizes for Correctly Targeted Genes

93% of the alleles are complete coding sequence deletions.



Regeneron's KOMP Goals and Results

Promised Yearly Production Milestones:

Year	Correctly Targeted Genes
1	175
2	500
3	940
4	942
5	943
Total	3,500

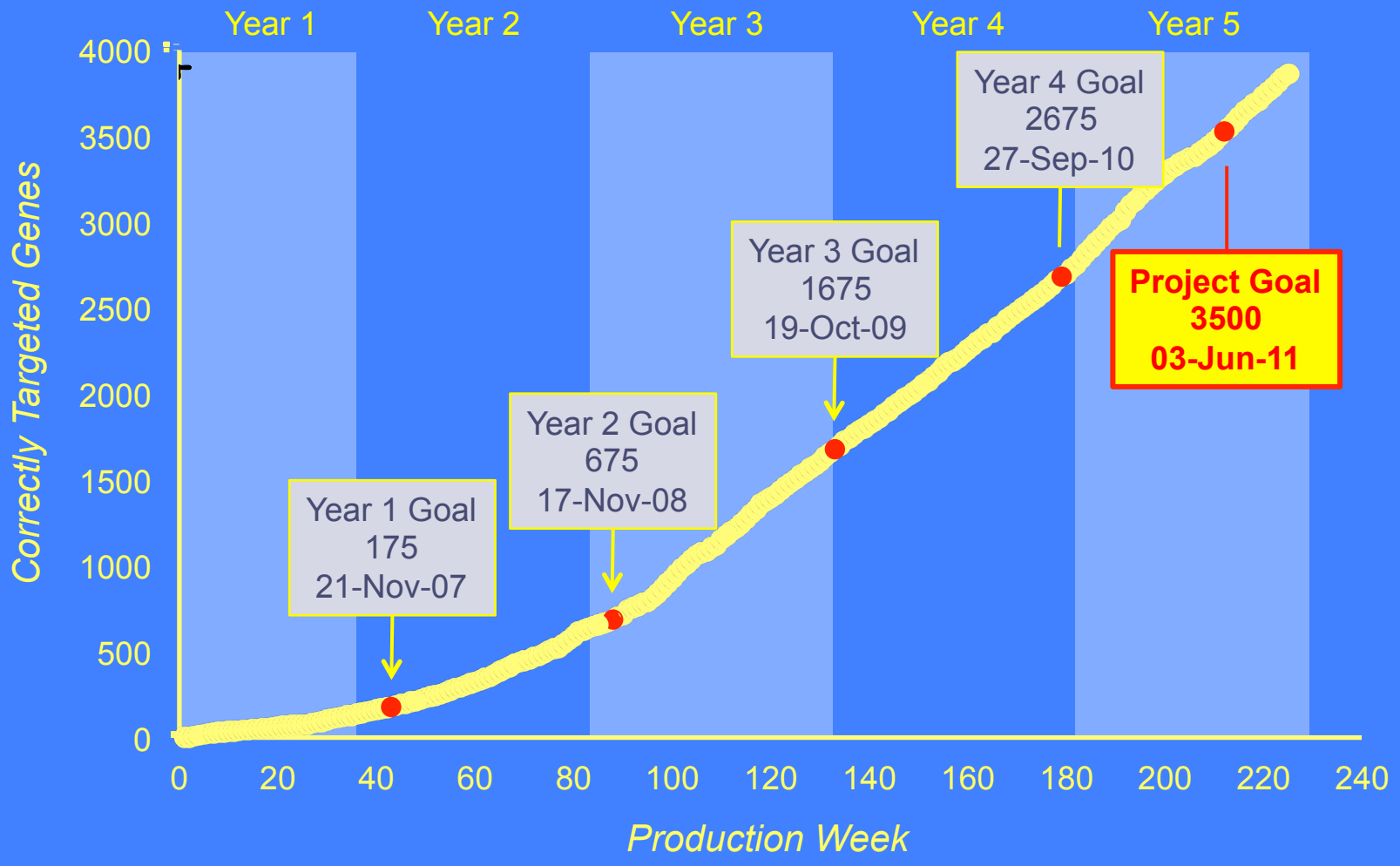
Production Goal:

A rate of 1,000 correctly targeted genes per year by Year 3

Actual Reported Production Results:

Period	Alleles Designed	Genes "Designed for"	Vectors Constructed	ES Cell Electroporations	Genes Electroporated	Colonies Screened	Correctly Targeted Clones	Number of Genes Screened	Correctly Targeted Genes: at least 2 clones	Success Rate (%) (>1 clones)
Summary 2006-2007	1030	1023	418	514	366	82475	2654	334	212	63
Summary 2008	1652	1652	970	1526	1226	141726	3995	863	579	67
Summary 2009	1875	1875	1356	1964	1840	181735	8990	1475	1104	75
Summary 2010	1868	1868	1459	1712	1687	168100	7983	1286	1131	88
Feb-11	158	158	158	149	148	13216	747	127	111	87
Mar-11	187	187	134	147	147	14480	764	146	111	76
Apr-11	190	190	170	147	147	16800	527	108	92	85
May-11	194	194	128	206	206	17056	533	168	92	55
Jun-11	133	133	142	172	171	16992	504	106	97	92
Jul-11	62	62	158	116	116	14256	733	136	124	91
Aug-11	20	20	123	168	168	12672	668	116	106	91
Sep-11	0	0	119	126	123	12960	519	136	110	81
Current Summary 2011	944	944	1132	1231	1226	118432	4995	1043	843	81
Grand Total	7369	7362	5335	6947	6345	692468	28617	5001	**3869**	77

Summary of Regeneron's Five Years of KOMP Production

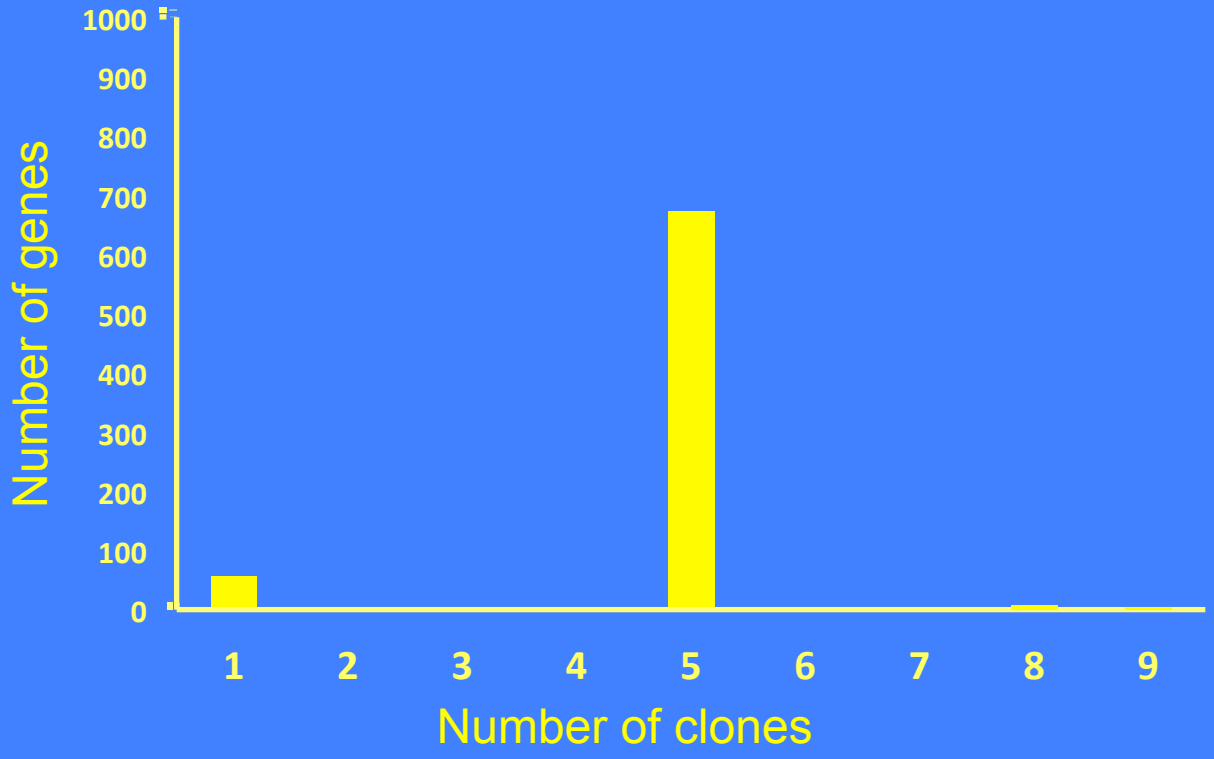


Efficiency Improvements from Years 1 to 5

		Y1	Y2	Y3	Y4	Y5
Weekly Averages	Targ. Freq. (%)	2.6	2.8	4.8	4.7	4.9
	Targ. Genes	4.1	11.6	22.0	21.8	25.7
Targ. Gene Success Rate (%)		61.1	66.6	76.5	81.7	82.9

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	Method	Injected Clones with Complete Data	Strain of Injected Host Embryo	Clonal GLT Frequency
Regeneron's KOMP Production QC	VelociMouse®	300	Swiss Webster	74.7%
KOMP Repository Results	Blastocyst Injection	233	BALB/c	77.0%

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