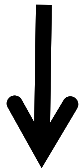
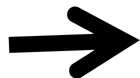
# Post-Gene-Trap Era at TIGM: Genome Editing

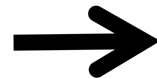
**Dr. Ben Morpurgo, Executive Director**

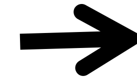
**Dr. Andrei Golovko, Senior Scientist**

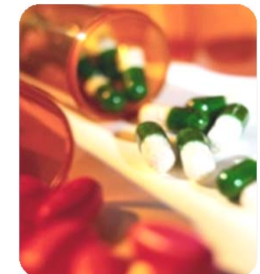
TIGM provides access to powerful research technologies and creates an opportunity to evaluate and validate new concepts in genetically relevant model systems

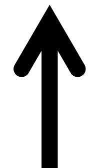












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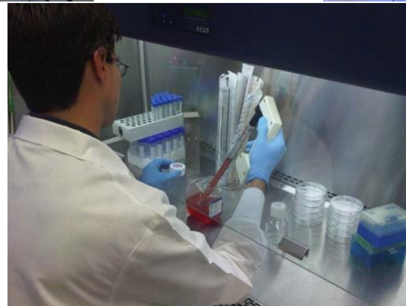
www.TIGM.org

# Headquarters and Research Facility

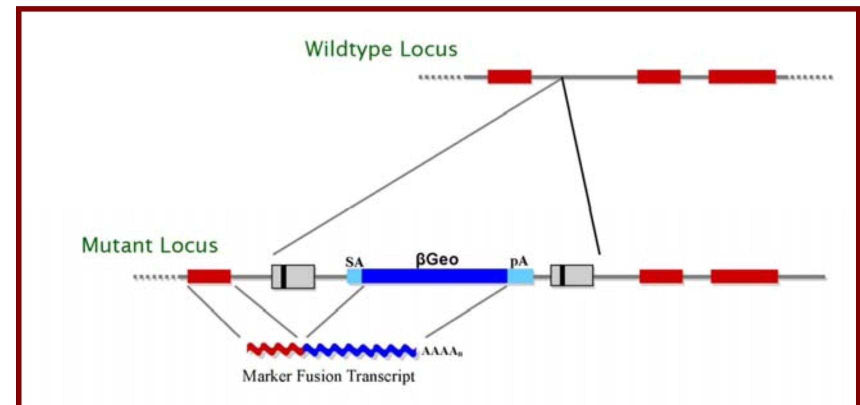
## College Station, Texas

- Up to 80,000 mice
- Full shower-in barrier and an open-access research core, including animal surgery, necropsy, and procedure rooms.



# Mutant ES Cell Resources

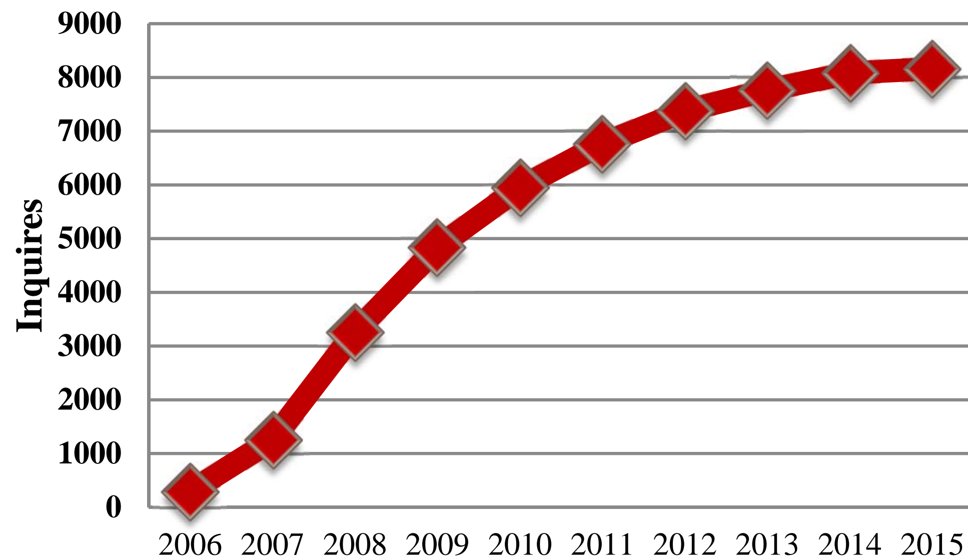
- Retroviral insertion of gene trapping vector containing a promoter-less marker/reporter gene (Neo, or β-Geo)
- World's largest library of mouse Gene Trapped ES cell clones in C57BL/6 background
  - >350,000 ES cell clones
  - >10,000 unique genes



**With over 10,000 unique genes, it represents over 40% of known mouse genome.**

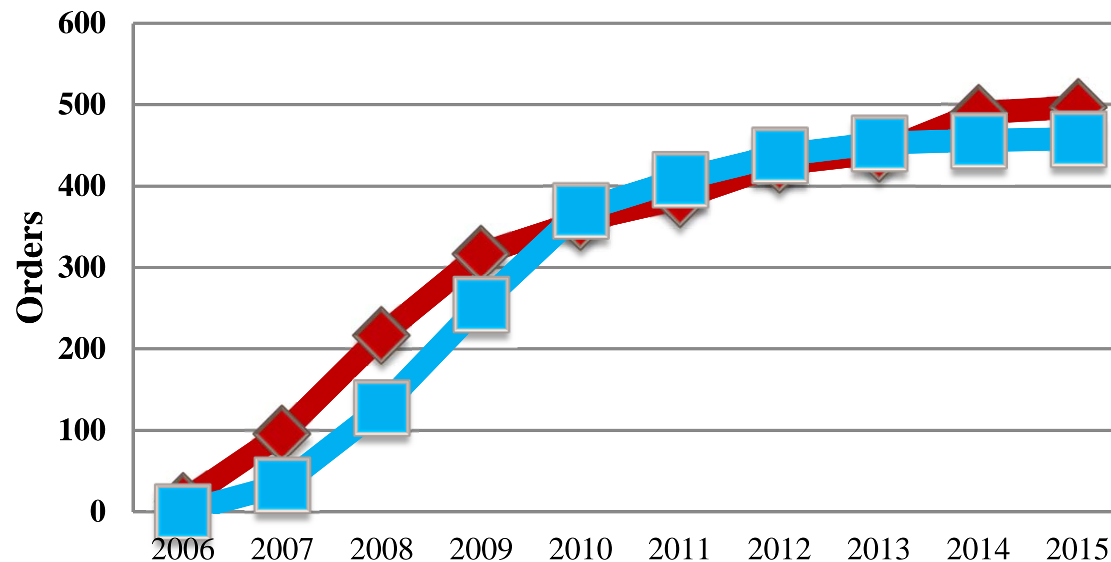
*Large-scale gene trapping in C57BL/6N mouse embryonic stem cells.* Hansen GM, et al. **Genome Res**. 2008 Oct;18(10):1670-9.

# Cumulative Inquires




More than 700 different academic, research and commercial institutions from 40 countries

# Cumulative Orders



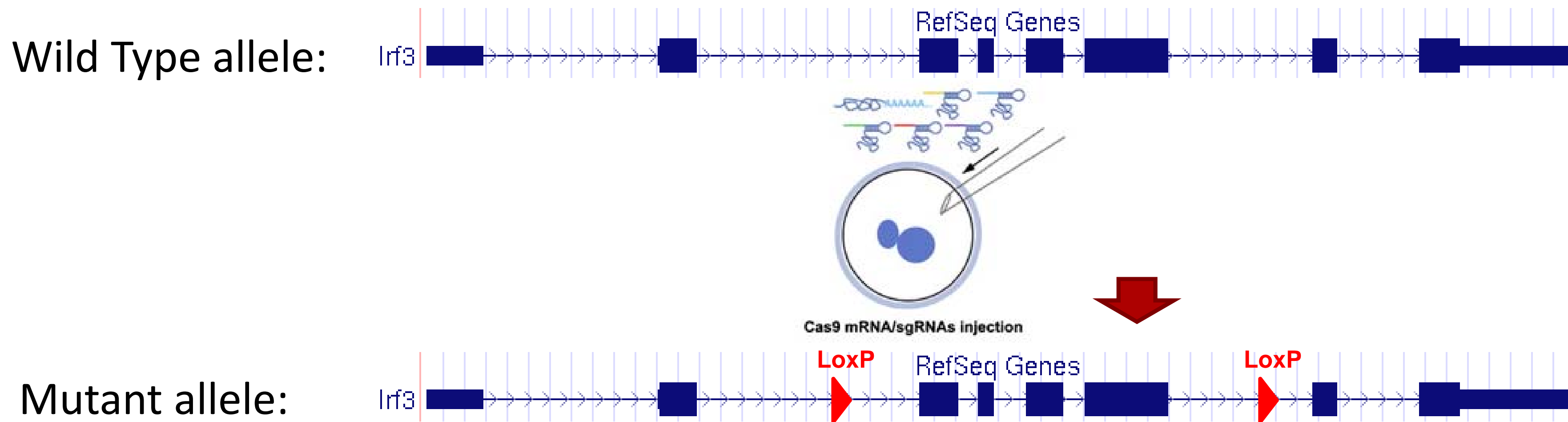

- Mouse orders were halted Q2 of 2010 in preparation for transition to CS
- Red = Mice; Blue = ES cells

# TIGM Services

- Mouse production from TIGM or external ES cells
- ES cell manipulation
- Animal production, breeding and maintenance
- Blastocyst injections
- Pronuclear injection
- IVF and embryo transfer rederivation
- Sperm and embryo cryopreservation
- Molecular biology services
- Animal studies
- CRISPR/CAS9-based genome editing

# CRISPR/CAS9-Based Genome Editing

Test run project: Production of a floxed mutation




- All work was done according to protocols described by Rudolf Jaenisch's group (Yang, Cell, 2014; Yang, Nature Protocols, 2014)
- Donor oligos: 60 nt each arm, 180 nt total
- Embryo donors: C57BL/6 N (CRL), both ordered and internal colonies
- Cas9: started injecting as RNA, switched to protein
- Both pronuclear and cytoplasmic

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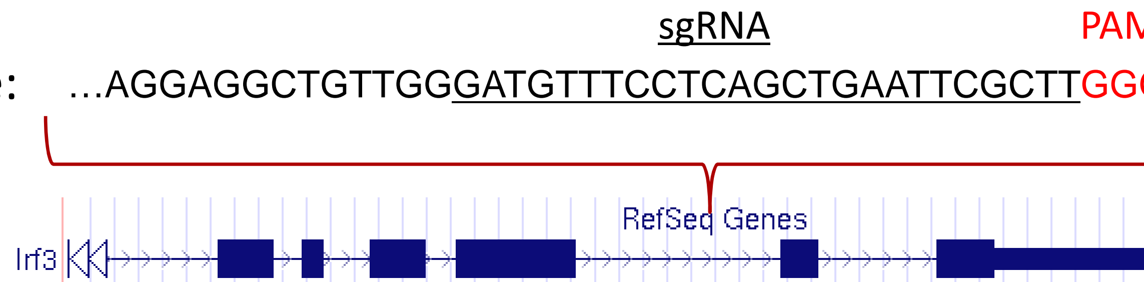
# CRISPR/CAS9-Based Genome Editing

Test run project: Production of a small insertion mutation

sgRNA PAM

Wild Type allele: ...AGGAGGCTGTTGGGATGTTTCCTCAGCTGAATTCGCTTGGGGCCCAA...




Mutant allele:

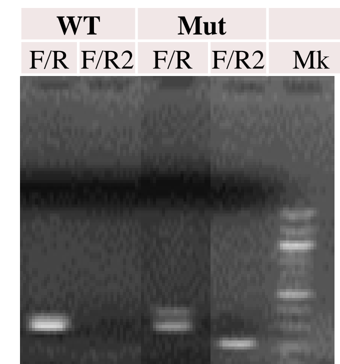
...AGGAGGCTGTTGGGATGTTTCCTCAGCT*ataacttcgtatagcatacattatacgaagttatgaattc*GCTTGGGGCCCAA...

PCR Confirmation:

Irf3 F/R, bands: 287bp (wt) + 328bp (mut)
Irf3 F/R2, bands: 219bp (mut)

The mutation was also sequence-confirmed

Targeting efficiency: 5-7% (confirmed)





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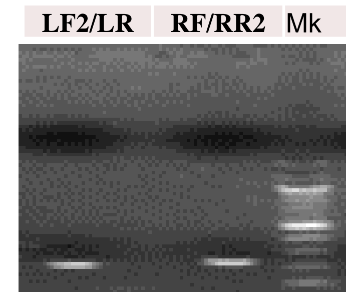
# CRISPR/CAS9-Based Genome Editing

## Test run project: Production of a floxed mutation

PCR Confirmation:

Irf3 LF2/LR mutant band (upstream LoxP): 189 bp
Irf3 RF/RR2 mutant band (downstream LoxP): 219bp

The mutations have been sequence-confirmed




- Timeline: 12 months (injections only)

| embryos injected | # survived injection | transferred as 2-cell | transferred as 1-cell | # Pups | # Survived |
|---|---|---|---|---|---|
| 1294 | 854 | 462 | 189 | 78 | 75 |

- Targeting efficiency: ~2%
- Most single LoxP insertions were produced from Cas9 RNA injections
- The only double LoxP insertions was produced from the Cas9 protein
- Mutations are confirmed germline

# CRISPR/CAS9-Based Genome Editing

## Current efforts

NHEJ-mediated deletions in Ube3a, both mice and gene trapped ES cell clone

- **Embryo injections: 11 pups screened, optimizing genotyping conditions**
- **ES cell transfections: screening colonies**

Several point mutations in Ube3a, ES cells

- **ES cell transfections in progress**

Simultaneous knock-in 2 reporter cassettes into Trac

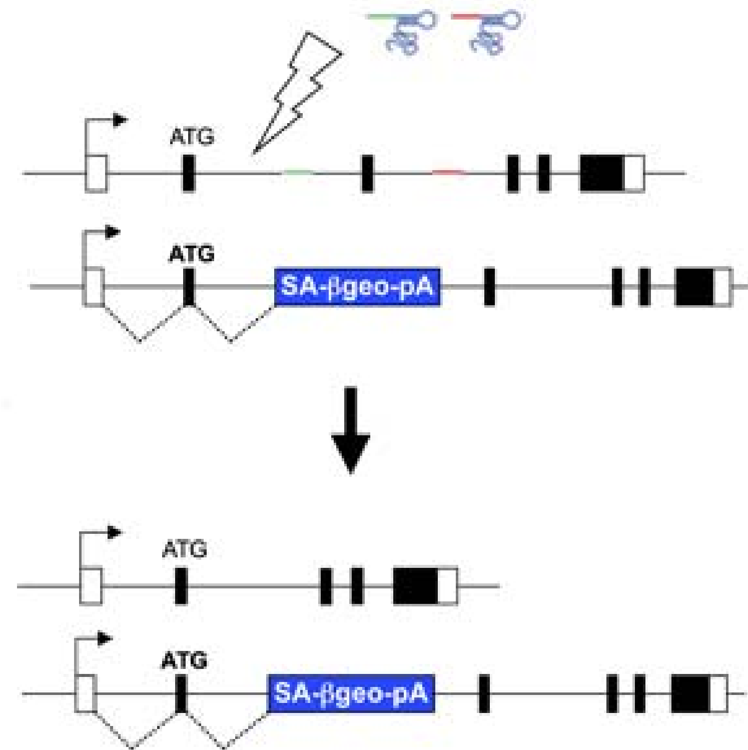
- **Injecting**

Conditional KO Phactr1

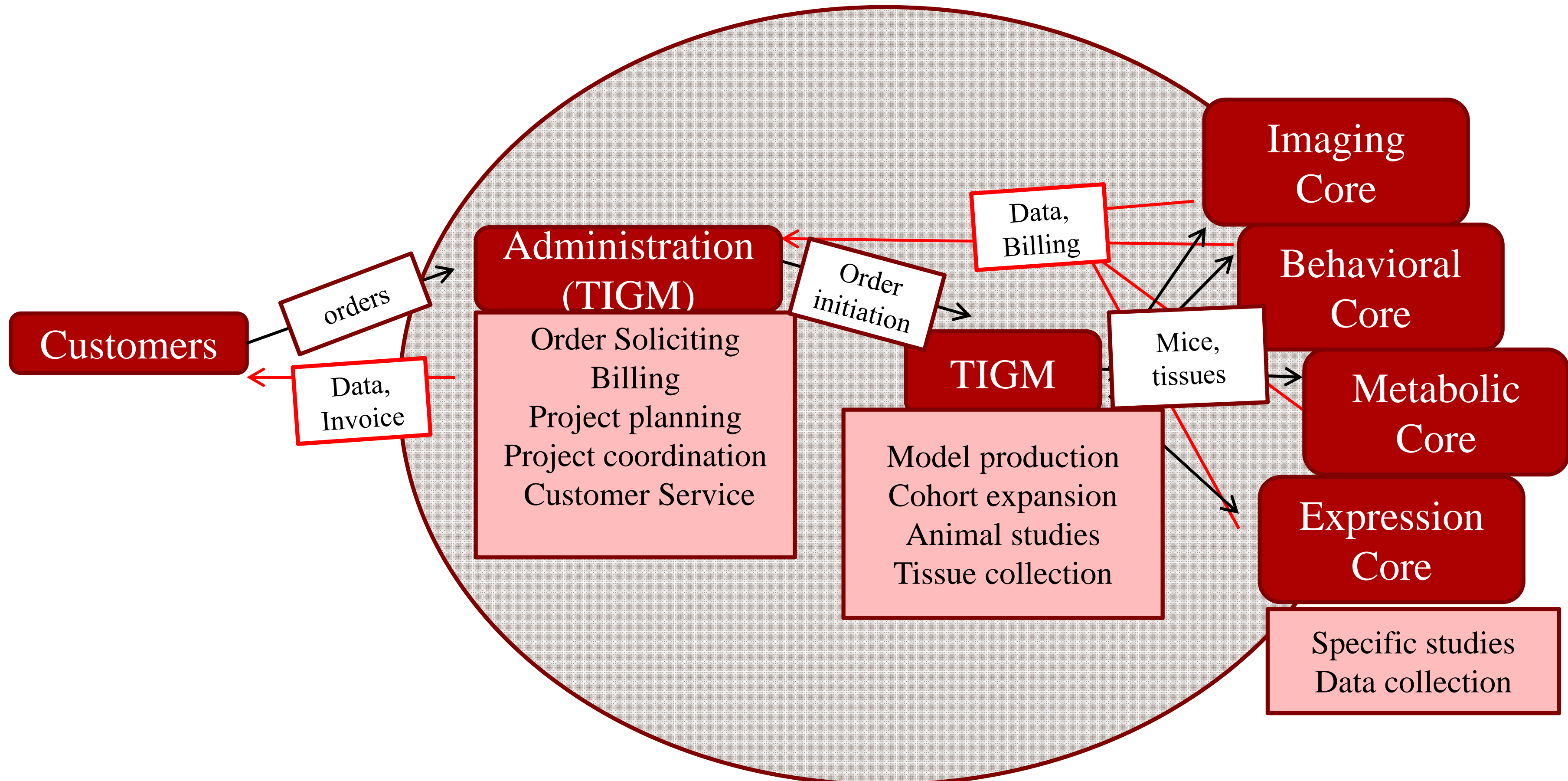
- **Preparing for injections**

# Future Plans

NHEJ-mediated deletion of the second allele in heterozygous gene trapped ES cells



# TAMU Mouse Model Production and Analysis Center





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