

# **An Efficient, Comprehensive Platform for Genetic Target Validation, Liability Assessment and Characterization of Genetically Engineered Mouse Models**



**Rodent Breeding and Colony Management  
Seminar and Vendor Fair**

***August 9, 2011***

**Joan Krakowsky, Ph.D.**

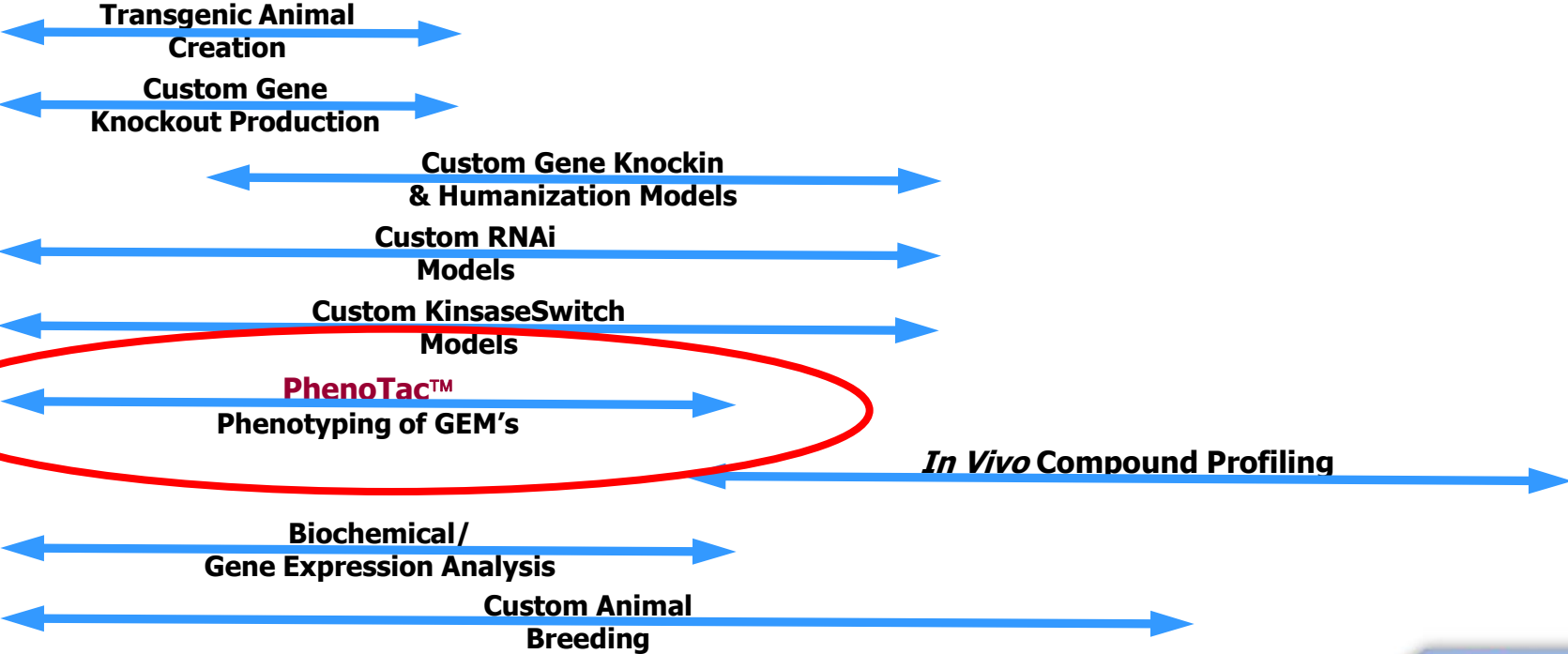
**Associate Director**

**Scientific Project Management and Sales**

# Overview: *In Vivo* Research Solutions



## Taconic's *In Vivo* Services



# Taconic: *In Vivo* Research Programs

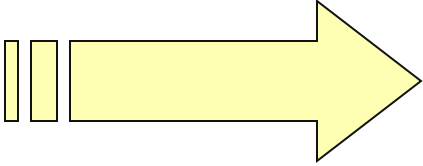


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**ANIMAL PRODUCTION /  
TRANSGENICS**

**ANIMAL PRODUCTION /  
GENE KNOCKOUT/KNOCKINS**

**Functional Analysis /  
Target Validation**



**PHENOTAC™  
PHENOTYPING**

**THERAPEUTIC AREA-FOCUSED  
PHENOTYPING**

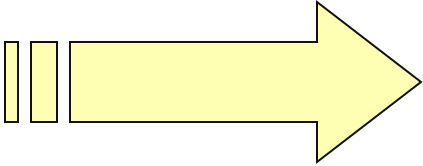
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**LIMITED  
TOXICITY STUDY**

**and/or**

**LIMITED  
PK STUDY**

**Efficacy**



**Side Effects**

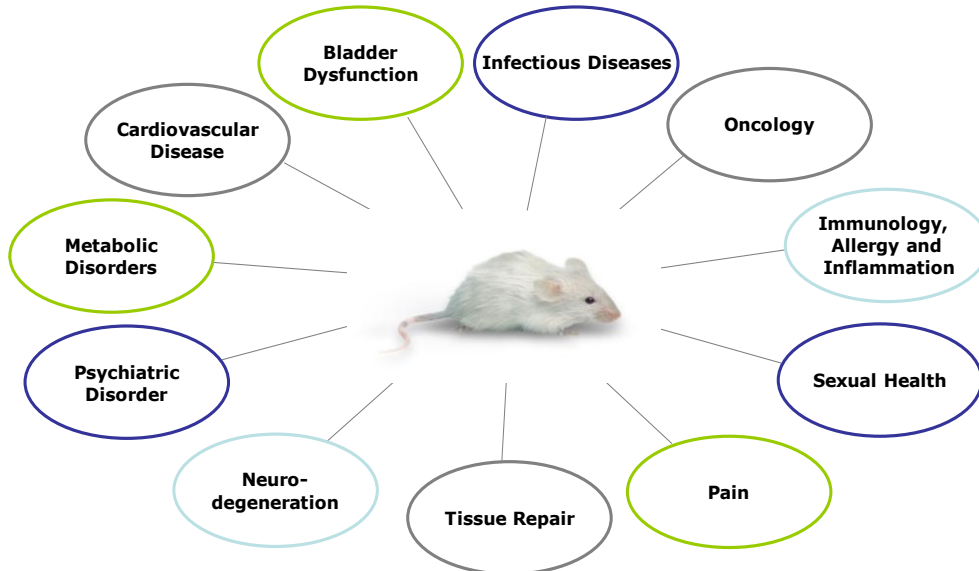
***IN VIVO* COMPOUND PROFILING =  
INDICATIONS DISCOVERY**

**CNS  
ONCOLOGY  
METABOLIC DISEASES  
IMMUNOLOGY / INFLAMMATION**



## Use of phenotyping in pharma R&D

- **Validate a drug target in knockout mice**
- **Evaluate potential target-related liabilities (unwanted phenotypes)**
- **Phenotyping of genetically-humanized mice**
- **Analyze gene function and disease mechanisms**
- ***In vivo* characterization of compounds in genetically engineered mice**
- **Disease model characterization**



- **Platform Co-developed with pharma partner in a 2 year effort**
- **Validated on >200 KO lines**
- **More than 100 assays measuring ~450 biologic read outs**
- **Serial setup: multiple assays run on the same animal (less animals in use)**

# PhenoTac™: Complete List of Assays

## Therapeutic Depth...



### Immunology & Inflammation

#### Rheumatoid Arthritis

- Antigen-induced Arthritis (AIA)
- Collagen-Induced Arthritis (CIA)
- KRN-serum Induced Arthritis
- mAb-induced Arthritis

#### Multiple Sclerosis

- Exp Autoimmune Encephalitis (EAE)

#### Septic Shock

- Lethal Endotoxemia

#### Inflammatory Bowel Disease

- Crohn's Disease: TNBS-induced Colitis
- Ulcerative Colitis: DSS-induced Colitis

#### Pulmonary Inflammation

- AI Pulmonary Inflammation (asthma)
- LPS-induced Airway Inflammation

#### Others

- Allergic Dermatitis

#### Other *In vivo* Bioassays

- Non-lethal Endotoxemia
- Ag-mediated *In Vivo* T-cell Proliferation
- Ag-stimulated *In Vivo* Antibody Production
- Delayed Type Hypersensitivity
- Thioglycollate-induced Inflammation

#### *Ex vivo* Bioassay

- Serum and Tissue Cytokine Profiling
- Multi-color FACS Analysis
- Cytokine Production *In Vitro*
- T and B cell Proliferation *In Vitro*

### Metabolism & Obesity

#### Body Weight and Body Composition

- Longitudinal Body Weight Gain
- Body Composition by DEXA
  - Fat mass, % Fat, Lean Mass, % Lean
  - Total Tissue Mass, Tissue Area
  - Bone Mineral Density, Mineral Content, Area
- Assessment of Regional Adipose Depots

#### Energy Homeostasis

- Food Intake
- Metabolic Parameter and Activity in CCMS
  - Oxygen Consumption
  - Carbon Dioxide Production
  - Heat Production
  - Respiratory Exchange Ratio (RER)
  - Total Horizontal Activity
  - Ambulatory Horizontal Activity
  - Non-Ambulatory Horizontal Activity
  - Vertical Activity
- Licking Frequency
- Glucose Homeostasis
  - Glucose and Insulin Levels in OGT Test
  - Gastric Emptying
  - Insulin Tolerance Test
  - Tissue Glycogen Levels
  - Pancreatic Insulin Content
  - Glucagon Levels
  - Serum Adiponectin Levels

#### Specialized Blood Chemistries

- Serum Corticosterone, Leptin, Ketone
- Serum TGs, FAs, Cholesterol VLDL, LDL, HDL)

### Cardiovascular Function

#### Hypertension

- Non-invasive BP and Heart Rate
- High Salt or High Fat Dietary Challenge
- L-NMA Induced Hypertension
- Renin-Angiotensin-Aldosterone System

#### Thrombosis

- Activated Partial Thrombosis Time (aPTT)
- Collagen-Induced Platelet Aggregation
- Prothrombin Time (PT)
- Thrombin-Anti-thrombin III Complex (TAT)
- Tail Bleeding Time

### Oncology

#### Xenograft Models

- Subcutaneous models
- Orthotopic models (IVIS®)
- Metastatic models (IVIS®)
  - Prostate Cancer
  - Lung Cancer
  - Breast Cancer
  - Ovarian Cancer
  - Pancreatic Cancer

#### *In Development*

- Bone cancer-related pain

# PhenoTac™: Complete List of Assays

## Therapeutic Depth...



### CNS/Behavior

#### Anxiety & Depression

- Elevated Plus Maze for Anxiety
- Novel Environment-Induced Feeding Suppression for Anxiety
- Open Field for Locomotion and Anxiety
- Tail Suspension Test for Depression

#### Learning & Memory

- Novel Object Recognition for Learning and Memory

#### Motor Strength & Coordination

- Rotarod Assay for Motor Coordination

#### Schizophrenia

- Startle Amplitude and Pre-Pulse Inhibition for Schizophrenia

#### General CNS / Behavior

- Pentylentetrazole-(PTZ-) induced Seizures
- Irwin Observational Battery
- Long Term Monitoring for Activity
- Feeding Behavior (Food Intake)
- Micturition (Urinary Bladder Function)

### Pain

#### Thermal Pain

- Hot-Plate Test
- Tail Flick Test

#### Mechanical Pain

- Automated von Frey Test
- Manual von Frey Test

#### Inflammatory Pain

- Acetic Acid-induced Writhing Test
- Formalin Footpad Test
- Carrageenan-induced Inflammatory Pain
- CFA-induced Inflammatory pain

#### Neuropathic Pain Models

- Partial Sciatic Nerve Ligation (PNL)
- Chronic Constrictive Injury (CCI)
- Tibial Nerve Transection (TNT) Model

#### Pain Associated with Arthritis (CIA)

- Thermal Hyperalgesia (Radiant Heat Test)
- Mech Hyperalgesia (Manual von Frey Test)

#### In Development

- Bone Cancer-related pain

### In Vitro Neurodegeneration Assays

- Ceramide-Induced Apoptosis
- Glutamate-NDMA Excitotoxicity
- In Vivo Kainic Acid Challenge
- Lactacystin-Induced Apoptosis
- Staurosporine-Induced Apoptosis
- Vitamin K-Induced Necrosis

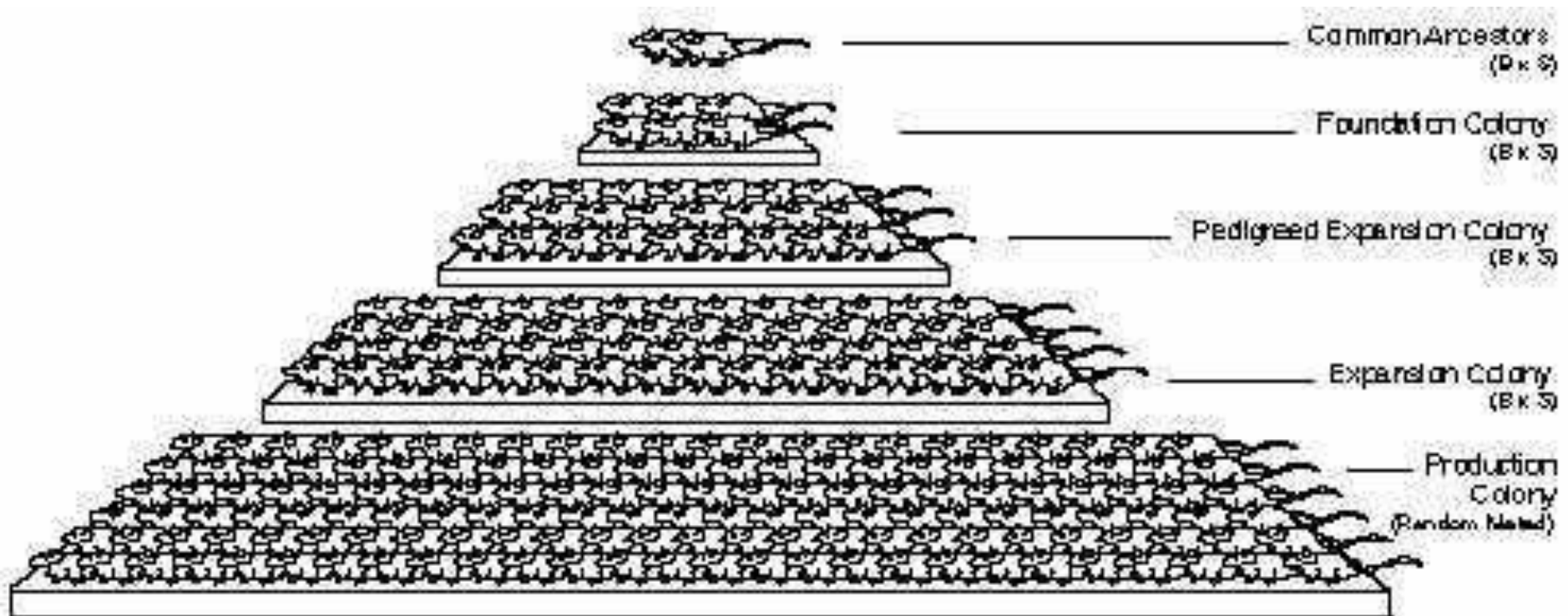
### Sexual Health

- Forced Erection Test
- Male Contact Sexual Behavior
- Female Contact Sexual Behavior
- Male Fertility
- Serum Testosterone Levels

### Cross Therapeutic Assays

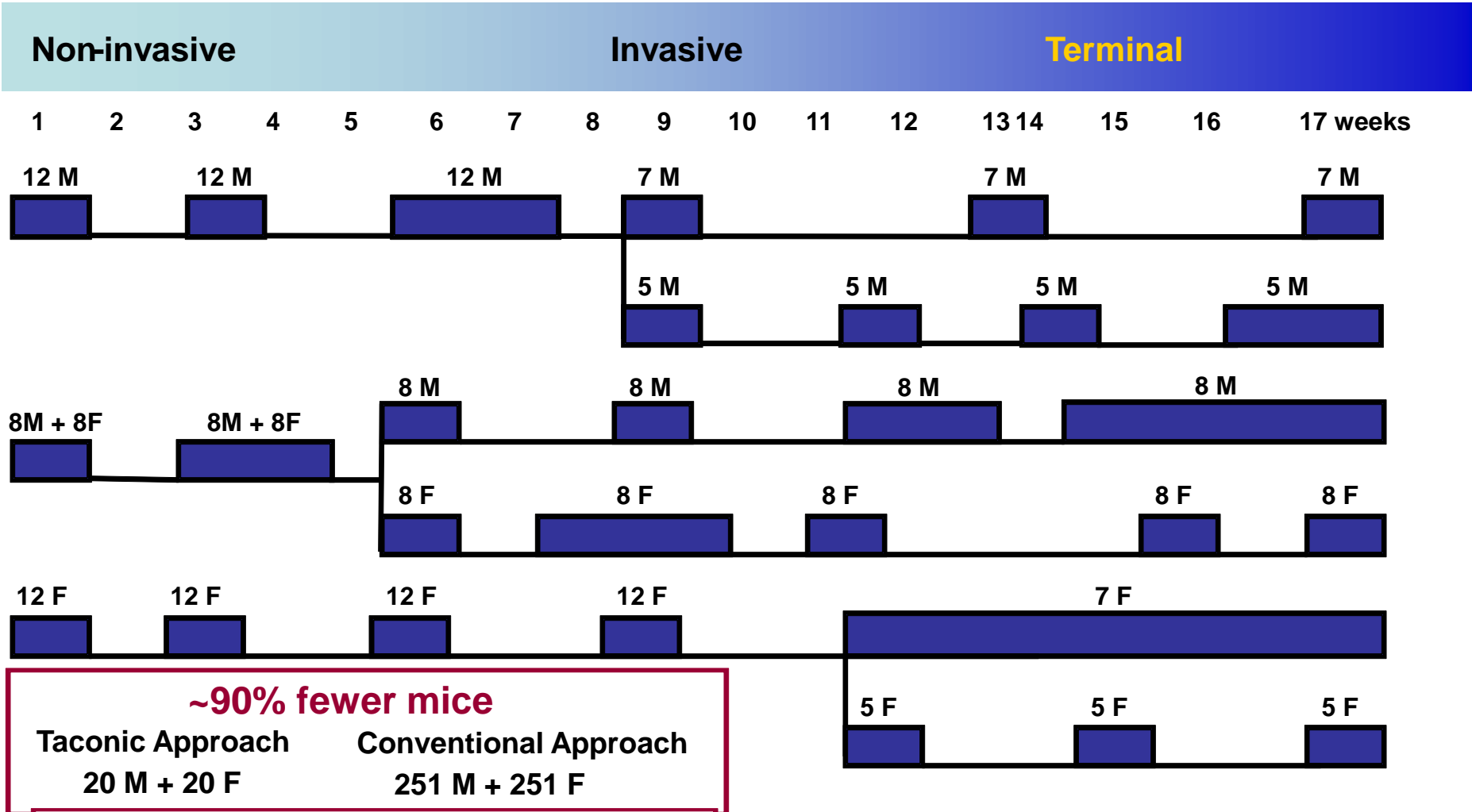
- Automated Blood Chemistry Panel (ACE)
- Urine Chemistry
- Hematology 5 parts Differential Cell Count
- Body Weight Measurements
- Organ Weights
- Histopathology

# Management of Breeding Colonies



***The pyramid structure is designed to avoid accidental crossbreeding, minimize genetic drift and prevent the development of genetic sublines. (B x S indicates Brother and Sister mating.)***

# How Does PhenoTac™ Work?



**~90% fewer mice**  
**Taconic Approach**      **Conventional Approach**  
 20 M + 20 F              251 M + 251 F

**Maximal Information per Mouse**

# Validation of Individual Assays and Their Combinations



- **Most assays (especially challenge assays) have been validated pharmacologically**
- **Groups of assays were compiled into protocol branches – from non-invasive to invasive and then terminal ; several versions of each branch were created**
- **Assay series (branch) validation: WT mouse performance in each assay from each branch was compared with the performance of experimentally naïve sex and age matched WT mice in the same assay**
  - Experimental branches with assay orders that did not show statistically significant differences as compared to the results from experimentally naïve mice were selected

# Criteria for Assay Selection



- **Relevance to human disease**
- **Translational value**
- **Assays that are key decision drivers for the therapeutic area teams**
- **Robustness**
- **Amenability to multiplexing**

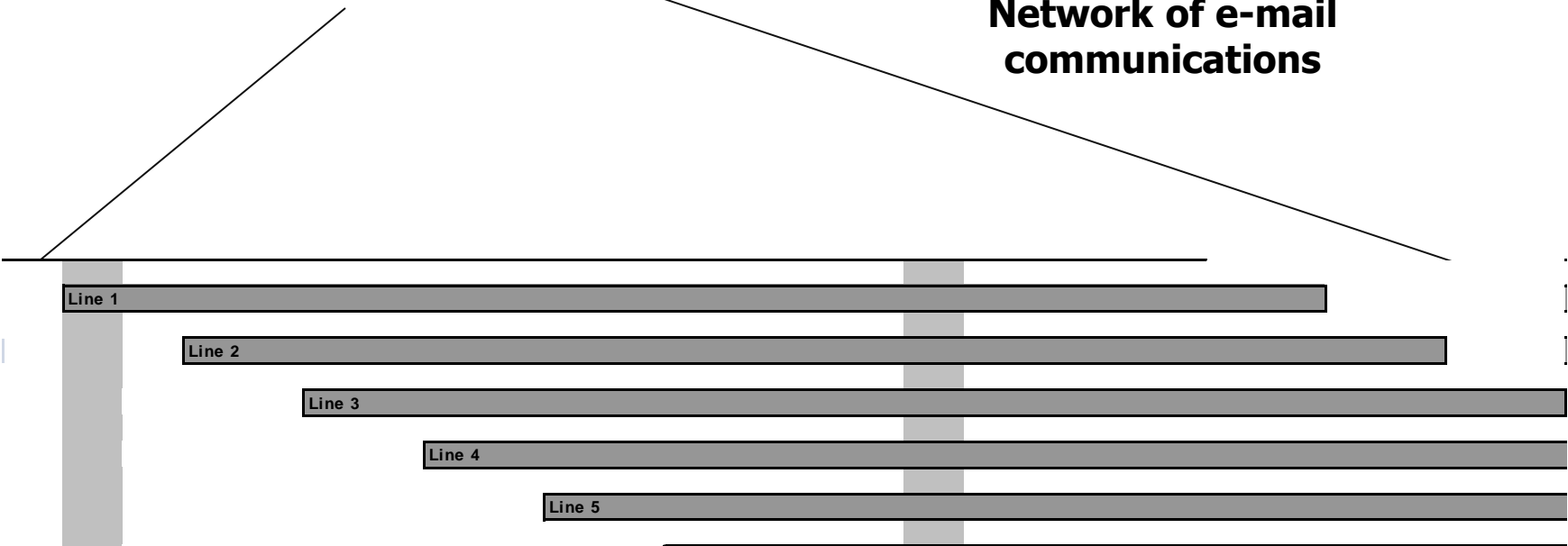
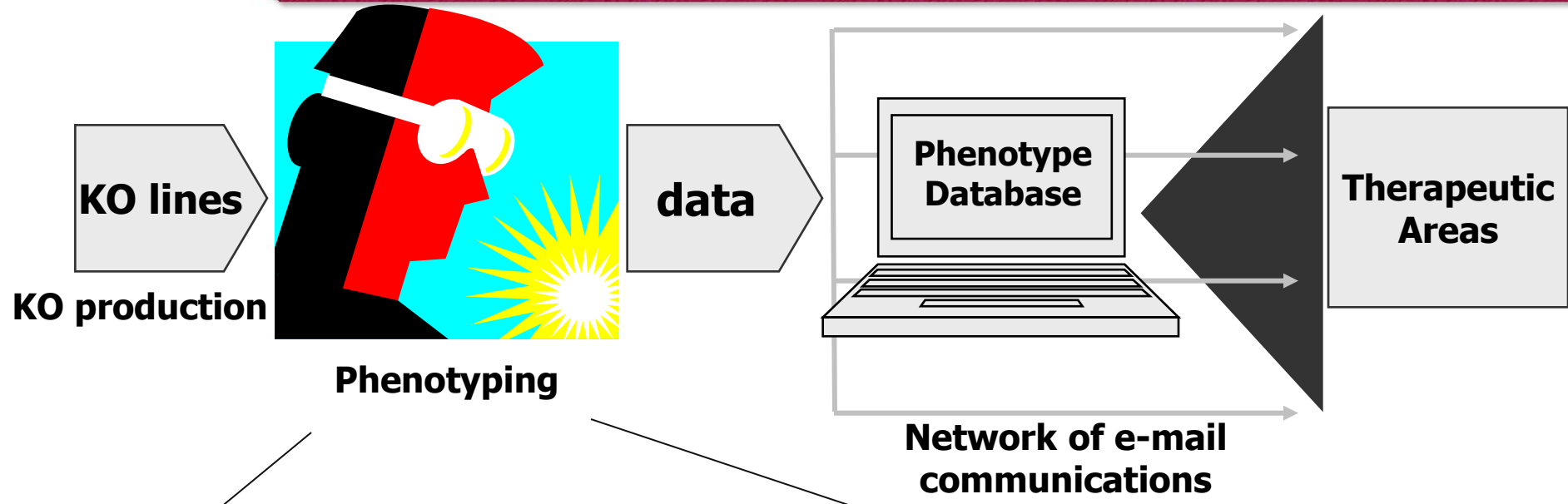


- **Four lines of KO mice partially characterized elsewhere were run through the PhenoTac protocol**
- **Results from the overlapping assays were compared to the data generated by other groups**



- **Statistical power analysis has been performed for each assay**
- **The size of the group was selected to enable detection of therapeutically relevant phenotypes (i.e. to be able to detect a difference of pre-determined magnitude between the KO and concurrent control)**
- **The magnitude of therapeutically relevant phenotypes is assay dependent**

# Industrialized Process





- **Eleven years experience with respect to industrialized comprehensive phenotyping**
- **Characterized over 200 genetic targets (in an average of over 50 assays each)**
- **Appreciation of the Importance of “Challenge” Assays**
- **Importance of working with academic and industrial scientific leaders in specific therapeutic areas**
- **Expertise in statistical analysis and IT is important**

# Evolution of Industrial Applications of Comprehensive Phenotyping



**Identification of new genetic targets for drug development**



**Liability Assessment of Genetic Targets**

**Validation of genetically humanized mice (in application to preclinical compound evaluation)**



## **Target Validation, Target Liability Assessment, Characterization of Genetically Engineered Mouse Models**



# Cross-Therapeutic Evaluation of the PCSK9 Gene KO Mouse

## Case Study

# Phenotypic Characterization of Proprotein Convertase Subtilisin/Kexin 9 (PCSK9) KO Mice



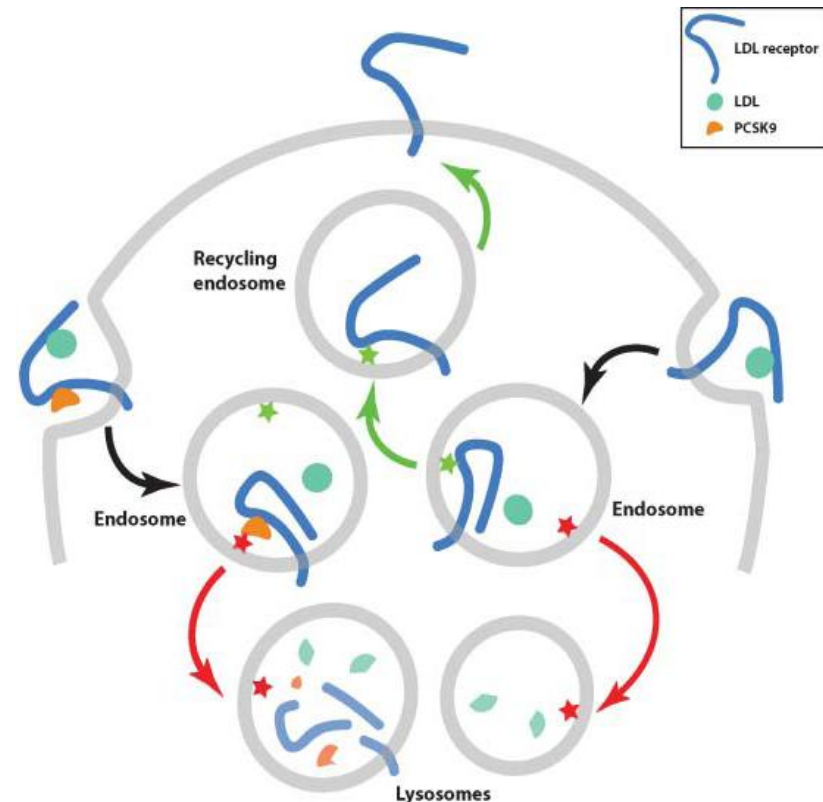
□ **72 kd Serine protease originally cloned from brain (also known as Neural Apoptosis-Regulated Convertase-1)**

□ **Three domains:**

- **N-terminal pro-domain**
- **Catalytic domain**
- **Carboxy-terminal domain**

□ **Autocatalytic clipping of pro-domain is required for secretion**

□ **Inhibition of PCSK9 increases LDL receptor (LDLR) levels in hepatic and extra-hepatic tissues by altering recycling of LDLR from the endosomal to the lysosomal compartment**



Peterson et al., 2008

# Phenotypic Characterization of Proprotein Convertase Subtilisin/Kexin 9 (PCSK9) KO Mice



- The phenotypic data corresponds to a mutant line of mice having a functional KO (missense mutation in the prodomain of PCSK9 protein coding sequence at Y119)
- Some assays included the PCSK9 heterozygote group of mice, in addition to WT and KO mice

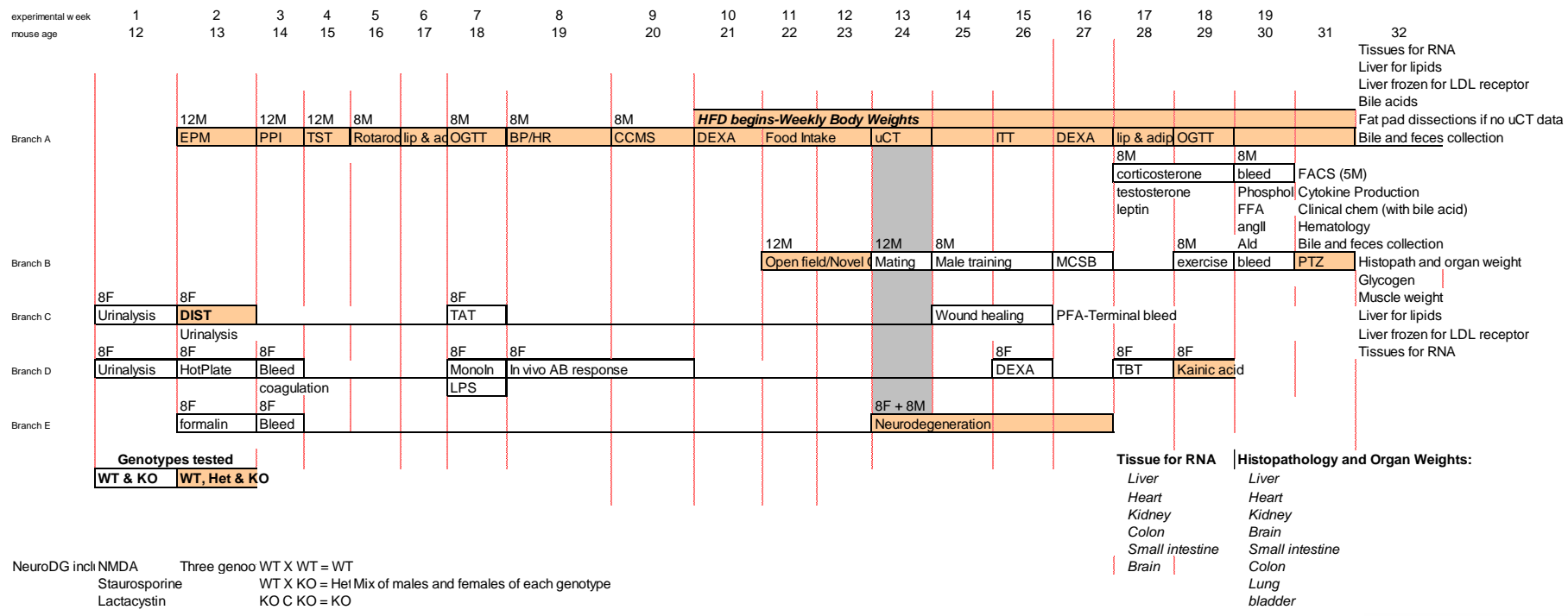
# Application of PhenoTac™ to Comprehensive Evaluation of PCSK9 Mutant Mice



■ 51 different bioassays conducted on cohort of wt, het-, homo-Y119X

■ Validated assay design and sequence of tests

- Use of automation, imaging instrumentation
- M or F depending on assay; chow + high fat diet where relevant



# Specialized Clinical Chemistries



- **Total Cholesterol and LDL-C reduced**
- **Serum phospholipids B lower in KO.** May be related to LDL-C/Triglyceride effect.
- **Serum aldosterone levels lower for KO**
  - However, no difference in systolic BP,
- **or heart rate**
  - No decrease in serum Na or Cl
  - No difference in corticosterone
  - No difference in angiotensin II
- **Serum thyroxine**
  - Avg 25% higher for KO ( $P = 0.0449$ )
  - Have not assayed TSH levels

