KOMP$^2$: the *BaSH* consortium

Monica Justice and Rich Paylor
Baylor College of Medicine
Houston, TX
BaSH: experience translates into throughput

Baylor College of Medicine:
ES cell technology
Phenotyping technology
Unparalleled mouse space

Sanger:
KOMP ES cells
ES cell technology
Phenotyping technology
Throughput

Harwell:
EUMODIC
Phenotyping experience
and technology
Throughput
**BaSH:** Mouse Production linked with Phenotyping for KOMP²

- Microinjection
- Germline Transmission
- LacZ staining
- Lethality/Fertility
- Cryopreservation

**KOMP, EUCOMM ES cells**

- **Cohort Breeding**
- **Phenotyping**
- **Data Upload**

- **Tracking Analysis Display**

**Cost savings**
- no rederivations
- efficiency in cohort breeding for lacZ, fertility, fecundity, phenotyping
- Time savings

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**Microinjection**

**Germline Transmission**

**LacZ staining**

**Lethality/Fertility**

**Cryopreservation**

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**Cohort Breeding**

**Phenotyping**

**Data Upload**
Scientific Leadership and impact

• **BaSH** is comprised of **THREE** leading institutions
• ES cell and gene targeting technology has a strong foundation at Baylor
  – Unparalleled mouse infrastructure
  – Embedded within a medical school
  – Clinical and translational applications
• Sanger has brought genomics to mouse genetics
• Harwell has > 60 year history of mouse genetics
• KOMP and EUCOMM mice now constitute majority [80%] of alleles distributed
Coordination/Cooperation

NIH

PI Committee
- U42 Monica Justice
  - Bill Skarnes
  - Steve Brown
- U54 Monica Justice
  - Richard Paylor
  - Allan Bradley
  - Steve Brown

Advisory Board

BCM Financial Management

Operations Committee
- U42 Bill Skarnes
  - Franco DeMayo
  - Tom Weaver
- U54 Richard Paylor
  - Allan Bradley
  - Tom Weaver
  - Steve Brown, Corey Reynolds, Ramiro Ramirez-Solis
  - Jacqui White, Mary Dickinson
  - Ann-Marie Mallon, Bin Liu

Allan Bradley, Austin Cooney, Cindy Buckmaster
Ramiro Ramirez-Solis, Sarah Wells, Martin Fray,
Ann-Marie Mallon, Bin Liu
Sanger Institute: A record of production

- Allan Bradley, Bill Skarnes, Ramiro Ramirez-Solis
- Major player in KOMP\textsuperscript{1}
  - 5,000 conditional alleles + targeting vectors
- Major player in EUCOMM
  - 5,346 alleles to date, towards a total of 8,500
- MirKO, microRNA resource
- Vector production > 12,800 vectors
- 400+ mouse lines
Sanger Institute: ES cell resources

- KOMP and EU resource centers are a risk factor for KOMP²
- Distribution centers overwhelmed by demand
  - Backlog can affect throughput
- Sanger holds original archival copies of:
  - Majority of clones produced: CSD, EUCOMM, MirKO
  - All conditional targeting vectors and intermediates
  - All data supporting vector and allele construction
- Sanger will implement stringent allele QC
Sanger Institute: Technology developer

- High throughput recombineering
- Computational allele design
- JM8 and JM8 Agouti cell lines
- Anitrack – internal mouse tracking data base and electronic “health record” of more than 400,000 mouse citizens
- C57BL/6N – Cre and Flp deletor lines
- IT
  - KERMITS, iMITS
MRC Harwell: National Centre of Excellence in Mouse Genetics from 1950

• MRC Mission

  Improve human health through *world-class medical research*

International Renown in Mammalian Genetics
- Impact of Radiation on Genomes
- X Chromosome Inactivation & Imprinting
- Frozen Embryo Bank
- Mouse Models of Disease
- Systematic Mutagenesis & Phenotyping

Mammalian Genetics Unit (MGU)
- Genetics and functional genomics research into a wide variety of disease models
- 10 Research Programs
- Steve Brown, Director

Mary Lyon Centre
- National Infrastructure for Mouse Genetics
- Vivarium; 4,000 m2; 14,000 IVC Cages, max capacity 52,000 mice
- National Biorepository & Distribution Centre
- Tom Weaver, Director
MRC-MLC Harwell

1. Scientific Leadership
   Provide Expertise
   Influence National Funding Programs

2. Operational Engine
   Infrastructure to Deliver Capability and Quality

3. Resource Portal To Mouse Community
   Open Access to Mice, Data, Analysis Tools
European Mouse Programmes

- **EUCOMM - European Conditional Mouse Mutagenesis**
  - Developing mouse mutants for most of the genes in the mouse genome

- **EUMORPHIA - European Mouse Phenotyping**
  - Development and standardisation of mouse phenotyping platforms

- **EUMODIC - European Mouse Disease Clinic**
  - Undertake a major pilot programme to utilise standardised phenotyping platforms for the analysis of mutants from EUCOMM

- **EMMA - European Mouse Mutant Archive**
  - Archiving and dissemination of mice

- **InfraFrontier - European Infrastructure Network**
  - Preparing European Infrastructure for phenotyping and archiving
Baylor College of Medicine

- Monica Justice, Franco DeMayo, Richard Paylor
- Top medical school
- Genetics department ranked in top 10
- Unparalleled mouse facilities
  - Operated by Center for Comparative Medicine
  - 115,000 cages, 4.4 acres
  - New TMFT facility free of all pathogens
    - Helicobacter and MNV
  - Techniplast Green line ventilated cages
  - Techniplast robotic cage washers
- IT Infrastructure, Mouse ES cell and GEM Cores, Mouse Phenotyping and Behavior Cores
History of collaborative interactions

• Numerous EU projects
  – EMMA, EUCOMM, EUMODIC, EUCOMMTools

• KOMP CSD [CHORI-Sanger-UC Davis]

• Strong interaction among PIs
  – Baylor/Sanger : Justice-Bradley
  – Sanger/Harwell : Brown-Weaver-Bradley-Skarnes
  – Harwell/Baylor : Brown-Justice

• KOMP$^2$ preparation
Benefits of **BaSH** consortium

• International cooperation and coordination
  – Ongoing, proven to work in EUMODIC
  – Required for success of IMPC
  – Paradigm for global science initiatives

• Multi-member composition
  – Elasticity
    • One member can increase capacity
    • Reduced risk
  – Cross-referencing strain values for QC

• Technology transfer
  – Genotyping platform
  – IT developments
  – Phenotyping assays
  – Encourages innovation
Work Distribution

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Consortium will allow ramp up
BCM will produce over ½ of mutant lines
Role of consortium will end by year 5
Preparation for next phase
Knockout First allele

Cryopreserve after Germline transmission

Cryopreserve after critical exon deletion

Intercross tm1b allele
- Determine lethal status
- Mate for infertile status
- lacZ analysis
- Homozygous or heterozygous
  Cohorts/littermates to phenotyping
BaSH Consortium: Broad Based Phenotyping

Overall Goals:
- Assess gene function in multiple systems
- Communicate observations and interpretations
- Develop technologies and platforms

Domains
- Nervous system
- Cardiac
- Integumentary
- Metabolism
- Sensory
- Respiratory
- Skeletal
- Immune & Blood

Assay selection
- moderate/high-throughput
- reliable & reproducible
- opportunities for ‘challenge’ assessments
- transferable technology
- ‘Easy’ statistical analysis
Pipeline Development & Implementation

**Expertise**
BaSH consortium better than sum of parts
Leaders in field are part of BaSH
Training: transferring protocols

**Capacity**
throughput, housing, etc

**Assay sensitivity and variability**
Behavior well known for challenge

**Control inclusion**
B6N not sufficient, need WT littermates

**Analysis**
Easily imported, and simplest ‘stats’ possible
Core Phenotyping Tests

Weight measures

- Hair follicle cycling
- Open Field Activity
- Motor Coordination
- Grip Strength
- Pain test
- Dysmorphology
- PPI
- IP-GTT
- X-Ray & DEXA
- ABR
- Eye Screen
- Terminal bleed & Necropsy
- Lymphoid phenotypes
- Clinical Chemistry
- Chromosome Instability
- Hematology

Week

4

6

7

8

9

10

11

12

13

14

15

16

Ex vivo

Tests in Development

- Improved Motor Coordination
- Respiratory Challenge
- Ultrasound & ECG
- Metabolites: Mass Spec
- Eye OCT Imaging
- Neuro Imaging
- Embryo Imaging

Key:
- Cardio
- Respiratory
- Metabolism
- Immune & Blood
- Neuro
- Skin
- Dysmorphology/skeleton/bone
BCM Mouse Behavioral Testing
Rich Paylor, Director

TMF (transgenic mouse facility)
13 testing rooms
30-35 different assays available
12 laboratories - 35 investigators
8000 hrs of use

* (most of the users have moved to NRI)
Perfect timing to access state-of-the-art facility
BCM Mouse Phenotyping Core

- 3400 sq.ft state-of-the art Phenotyping facility
- 2 Full time staff members
- Corey Reynolds, Director- 8 years experience
Mouse Phenotyping Core

- Vevo 770 Ultrasounds
- Bruker 7.0T MRI (body and head imaging)
- Gamma Medica CT/SPECT
- Kodak X-Ray/Fluorescence Imager
- Piximus Bone Densitometer
- Oxymax Indirect Calorimetry (chambers/treadmill)
- Unrestrained Whole Body Plethysmography
- Metabolic Cages
- DSI Blood Pressure and ECG telemetry
- Non-Invasive Blood Pressure
- ECGenie
- Treadmills
- Mini Mitter running wheel
- Slit lamp microscope
- Full Surgical suite with several procedures
BCM CCM Pathology Laboratory

- Roger Price, DVM, Director
- Cobas Mira Chemical chemistry analyzer
- Advia Veterinary Hematology analyzer
- Training platform for Veterinary Pathology
- Necropsies/pathology
Under Development: Cardiac Challenge

Isoproterenol or Dobutamine

• Mimics the effects of exercise (treadmill stress test in humans)

• Stimulates $\beta$-adrenergic receptors $\rightarrow$ Increase in HR in a healthy animal

• Baseline echo $\rightarrow$ IP injection of the drug $\rightarrow$ Post-Injection echo (data points taken every minute for 5 minutes)

• Duration (6-8 min/mouse)
Transmit Frequency: 30 MHz
Under Development: Respiratory Screen

- Challenge: Methacholine or Ozone
  - assesses airway hyper-responsiveness
- Control Group → Aerosolized isotonic saline
- Experimental Group → Aerosolized Methacholine (mg/ml) of increasing concentrations (4)
- Baseline Collection → Administer drug for 2 min → 5 min data collection → 10 min rest period → repeat
- Duration (1hr 30min/group of 8 mice)
Technology Development
Imaging
Studying embryonic development

Live embryonic imaging is critically important

Confocal microscopy of vital fluorescent markers is a powerful tool
- Barriers: skin/fur, uterine wall

ε-globin-GFP
Flk1-H2B::EYFP
Flk1-myr::mCherry

Mary Dickinson
Irina Larina
Baylor College of Medicine

Kirill Larin
University of Houston
Optical Coherence Tomography

Images produced by backscatter from an interferometer
Uses light instead of sound

Image depth = 2 - 3 mm
Confocal and 2-photon = 300 - 500 um

High resolution
2 - 5 um
“Image pathology”

**Swept-Source OCT system**
scanning rate - 16 kHz
central wavelength - 1325 nm
spectral width – 100 nm
output power - 12 mW
Using OCT to image the adult eye

Human:
D190N mutation in RHO causes retinitis pigmentosa: imaged using OCT

Mouse:
D190N mutation in B6 imaged using OCT
Live OCT imaging of mouse embryos
In utero embryonic imaging

1. Live embryo imaging in sacrificed females (terminal surgeries)
   • Pregnant CD-1 females were sacrificed at 12.5 to 18.5 dpc.
   • The animal remains on heating pad during the procedure to keep the embryos alive.
   • Abdominal wall was cut to expose the uterus and covered with clear plastic wrap to prevent dehydration.
   • Live OCT imaging was performed through the uterine wall.

2. Following development in the same embryos (survival surgeries)
   • Pregnant females were anesthetized with isoflurane and kept on the heating pad during the whole procedure.
   • The uterine horn was exposed for imaging through an abdominal incision.
   • After imaging, the incision was stitched back.
   • The procedure was repeated after 48 and 96 hours. The animal was sacrificed after the third imaging session.
Imaging embryo morphology at different stages of development

1-head
2-forelimb
3-hindlimb
4-pinna of ear

5-eye
6-yolk sac
7-uterine wall
8-follicles of vibrissae
Imaging of embryonic limb development

1-cartilage primordium of distal phalangeal bone

2-cartilage primordium of phalangeal bone

3-cartilage primordium of metacarpal bones

4-follicles of vibrissae
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Longitudinal studies
New Technology Evaluation
High-Resolution Episcopic Microscopy (HREM)

- Shoumo Bhattacharya (Ox), Tim Mohun (NIMR)

- Automated setup
  - Resin embedded samples
  - Fluorescent dyes (acridine + eosin) - stain tissue & provide fluorescent background
  - E8.5 - 17.5
  - Section every 2u
  - Block face serially imaged
  - 24 hours per sample

- Allows resolution to 2 u / voxel

- Can be done after MRI imaging

Weninger et al Nat Genetics 2001; Pieles et al J Anat 2007

HREM Allows visualisation of coronary vasculature, myocardial anatomy

3D Rendering & Analysis
High Throughput Phenotyping Using MRI

Shoumo Bhattacharya, Jurgen Schneider

- Magnetic resonance imaging
  - 32 embryos at E15.5 embedded in agarose
  - Imaged overnight on 11.7 T system

Schneider et al BMC Dev Biol 2004
Ultimate Goal:

Providing for the local, national, and international scientific community
Thank You

The BaSH Consortium

BCM: Monica Justice, Rich Paylor, Franco DeMayo, John Sharp, Corey Reynolds
Sanger: Allan Bradley, Bill Skarnes, Ramiro Ramirez-Solis
Harwell: Steve Brown, Tom Weaver

And our collaborators
Mary Dickinson, Kirill Larin, Shuomo Battychara, Tim Mohun, Stephen Wang