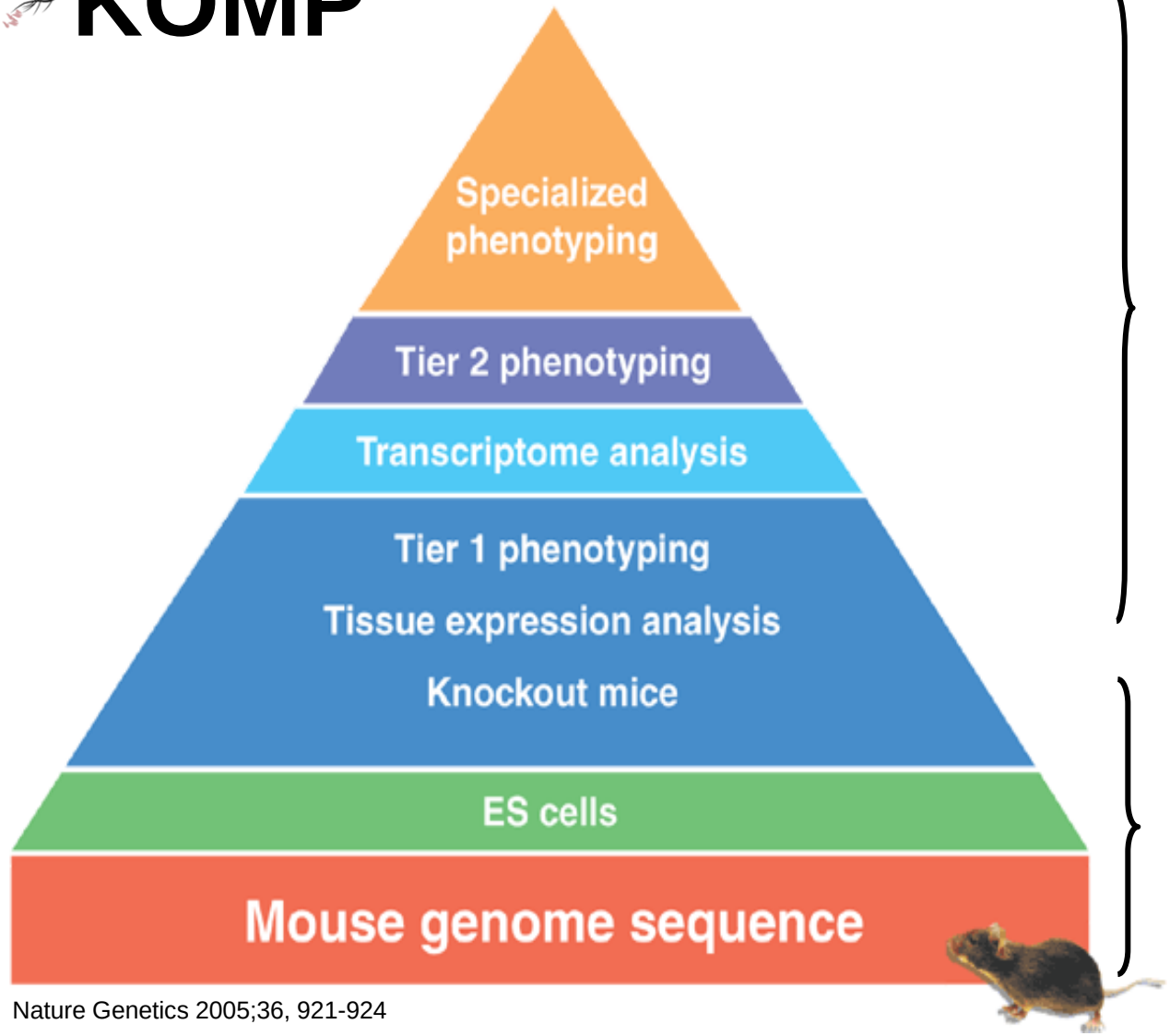
KOMP Phenotyping:
the users' perspective

Kent Lloyd
KOMP Repository, MMRRC-UC Davis

KOMP



2011 and beyond…

2006-2011

Nature Genetics 2005;36, 921-924

KOMP Repository

THE KNOCKOUT MOUSE PROJECT

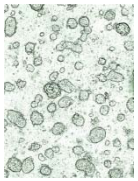
UCDAVIS

C·H·O·R·I
Children's Hospital Oakland Research Institute

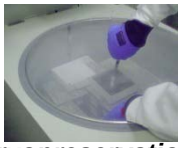
and

microinjection

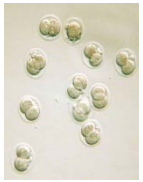
mice



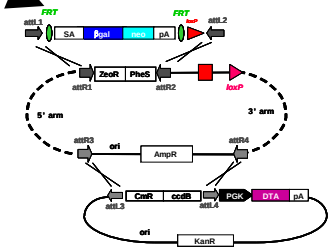
ES cells



cryopreservation/ cryorecovery



sperm & embryos



vectors

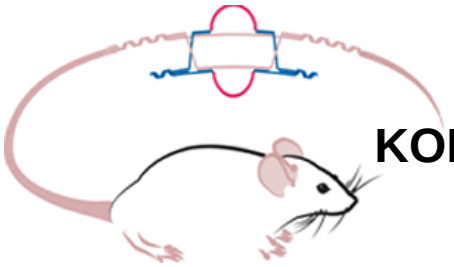
Sponsored by (U42):

United States Department of Health & Human Services

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

National Center for Research Resources
NATIONAL INSTITUTES OF HEALTH

IKMC (INTERNATIONAL KNOCKOUT MOUSE CONSORTIUM)



EUCOMM

NorCOMM

TIGM

17,000 genes
- **8,500 genes (KOMP)**
- **8,000 genes (EUCOMM)**
- **500 genes (NorCOMM)**

-C57BL6/N
-*lacZ*-tagged
-K/O alleles:
- **conditional null**
- **targeted trap**
- **deletion**

Mutant ES cell resource + **Vector resource**

250-500 mutant mice/yr

The IKMC (EUCOMM, KOMP, NorCOMM and TIGM) have produced over 8,000 KO ES cell lines

Welcome to the IKMC

The International Knockout Mouse Consortium (IKMC) aims to mutate all protein-coding genes in the mouse using gene trapping and gene targeting in C57BL/6 ES cells. Read more...

Download the IKMC Gene List
View targeting strategies
View all allele types

Search or Browse

Search IKMC database help

Enter gene symbols, gene IDs or genome location

Search

e.g., Adam19, Pax, ENSMUSG00000020681, Chr13:22210730-22311689

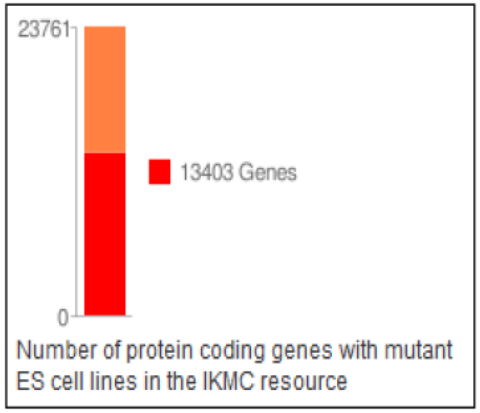
Browse IKMC database help

Use the following links to browse genes

- Browse by Gene Symbol
- Browse by Chromosome

Status

ES Cell Lines Progress



Number of protein coding genes with mutant ES cell lines in the IKMC resource

IKMC Gene Progress Summary

Total Genes	KOMP		EUCOMM	NorCOMM	TIGM
	CSD	Regeneron			
Project goal	5000	3500	8000	500	-
Vectors generated	5111	3327	4521	312	-
Vectors available	4842	2296	4521	312	-
ES cells generated	2674	1846	2443	45	-
ES cells available	1942	1136	2443	45	10699
Mutant mice generated	168	160	279	0	-
Mutant mice available	168	71	279	0	-

View details about this table View details about the acronyms used

Efforts to solicit input:

--online surveys

--RFI

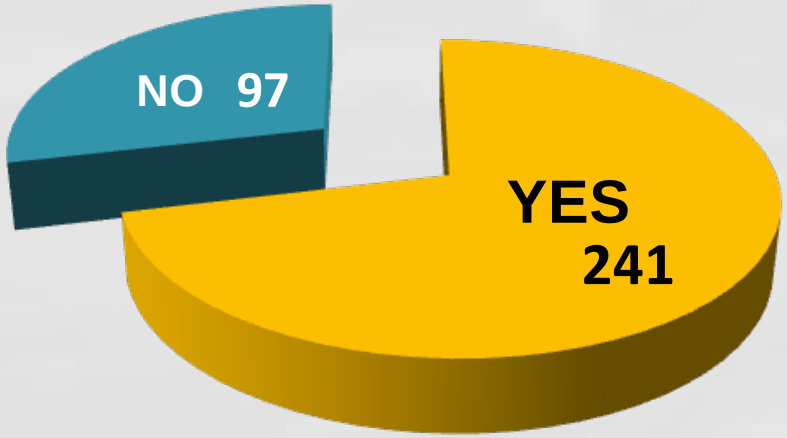
--conferences

KOMP Phenotyping Survey

September 8, 2009 - October 15, 2009

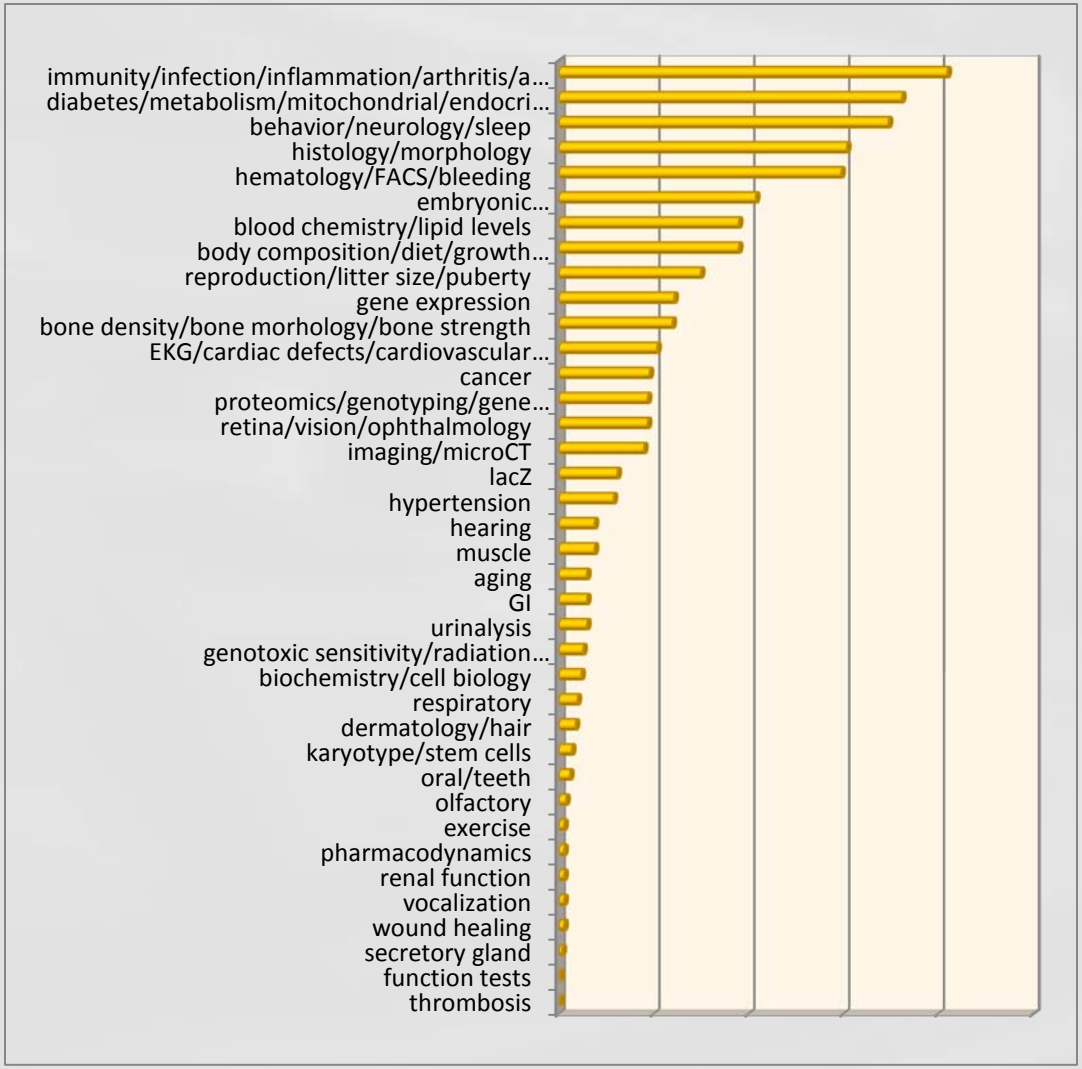
~300 respondents out of 2517

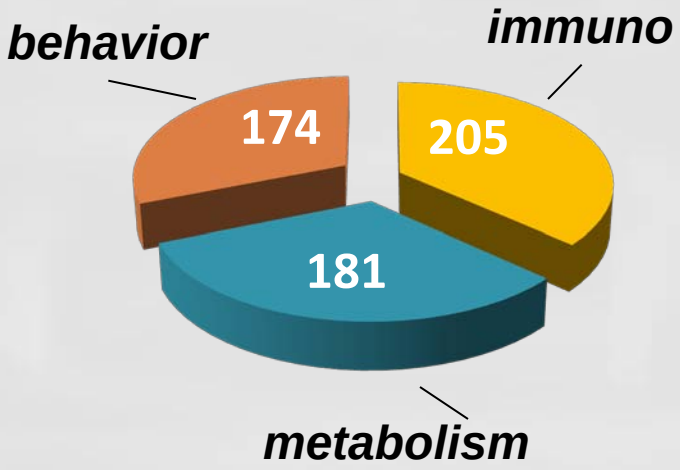
To ascertain what researchers would want to see from a primary phenotype screen.



Survey Summary Report

Question #2: Thinking beyond your laboratory, what do you see as the 3 essential tests, analyses, and/or examinations that would most likely reveal the utility of a mutant mouse line in your field? Two caveats: the numbers of mice used per test are limited to 5-10 and the tests must be high throughput (100's/y).

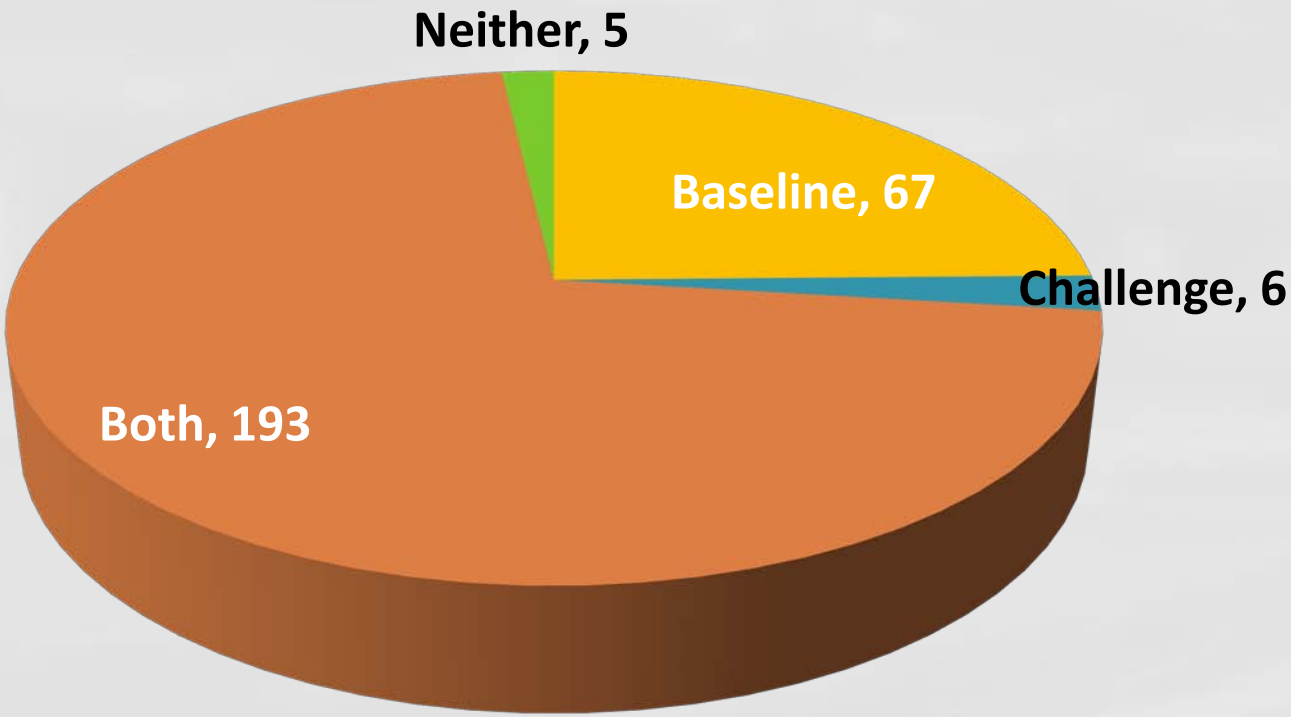




Survey Summary Report

Question #3: In general, should tests be administered under naïve and/or challenge conditions?

Response: 271 responses; 182 (67%) left comments



KOMP Phenotyping RFI

September 24, 2009 - October 15, 2009

Issued by NCRR, NHGRI, & NICHD

~25 respondents (anonymous)

To seek input on large scale, high-throughput phenotyping of KOMP mice.

RFI Summary Report

Question #6: How would you value an NIH-subsidized program of standardized phenotypes in a high throughput manner, at a lower cost per gene and encompassing more fields than individual labs could perform?

Response: 23 responses (92% of RFI respondents)…***all AGREED on value***

Would the availability of data (regardless of the outcome of the experiment) be of benefit to the larger community?

Response: 23 responses (92% of RFI respondents)…***all AGREED on benefit***

Would this effort help individual PIs focus on custom phenotyping of fewer but very relevant genes?

Response: 23 responses (92% of RFI respondents)…***all AGREED would help focus***

Approximately what range of costs does your lab invest in phenotyping, with or without challenges (please include all personnel and overhead cost, as well as mouse production)?

Response: 12 responses (46% of RFI respondents)

Costs per line: $15K - $175K *(5 responses)*
Costs per lab: $100K - $3M *(7 responses)*

KOMP Phenotyping Conference

October 29-30, 2009

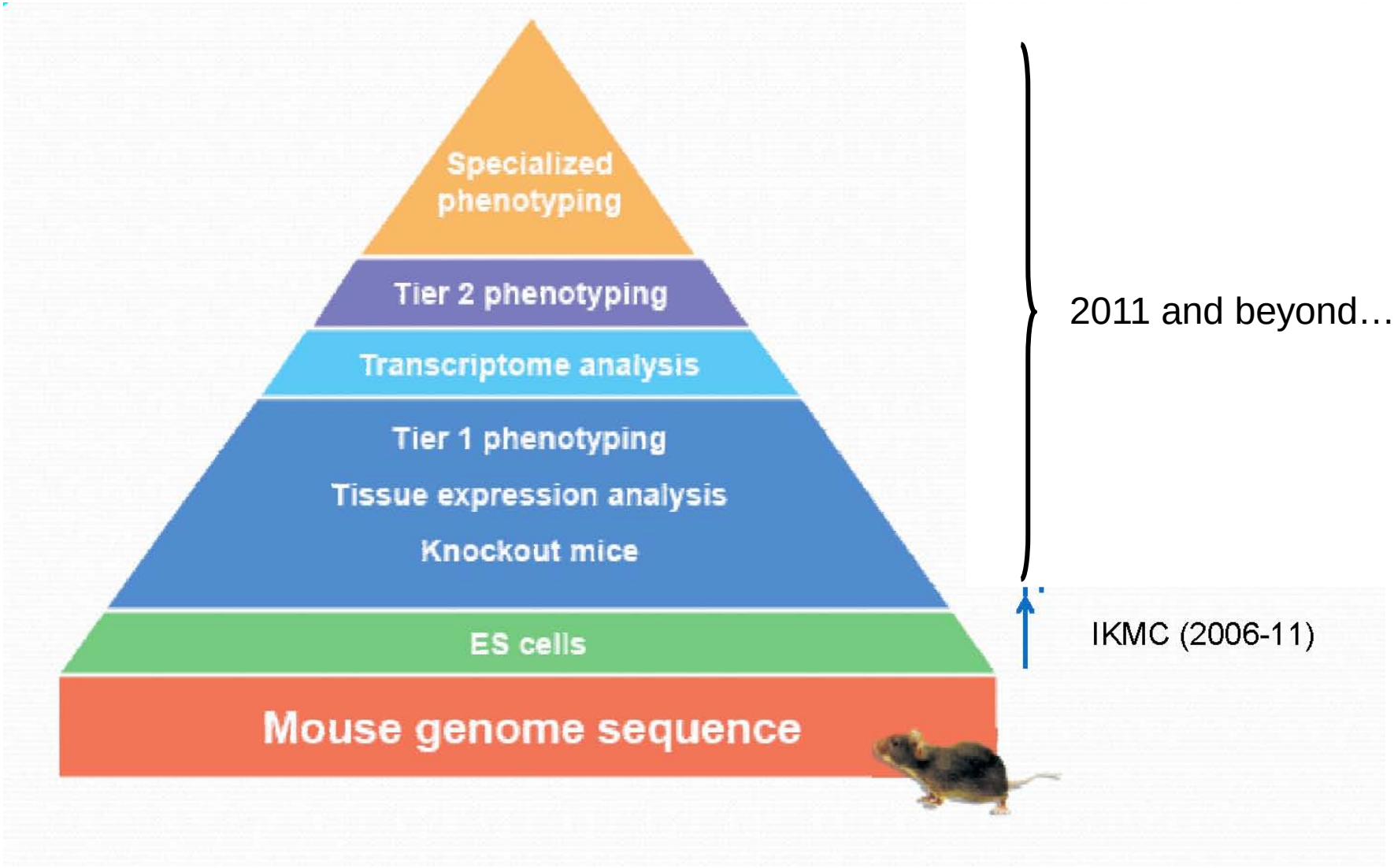
Hyatt Regency Hotel, Bethesda, Maryland

IKMC PI's, invited researchers, NIH staff

KOMP Phenotyping Conference

Consensus:

1) Gene selection (directed, not “encyclopedic”)
2) Include unannotated genes
3) Mouse production (convert ESC to mice, cost)
4) High throughput tests (baseline, informative)
5) Data access and availability (free, real-time)
6) Coordinate with international efforts



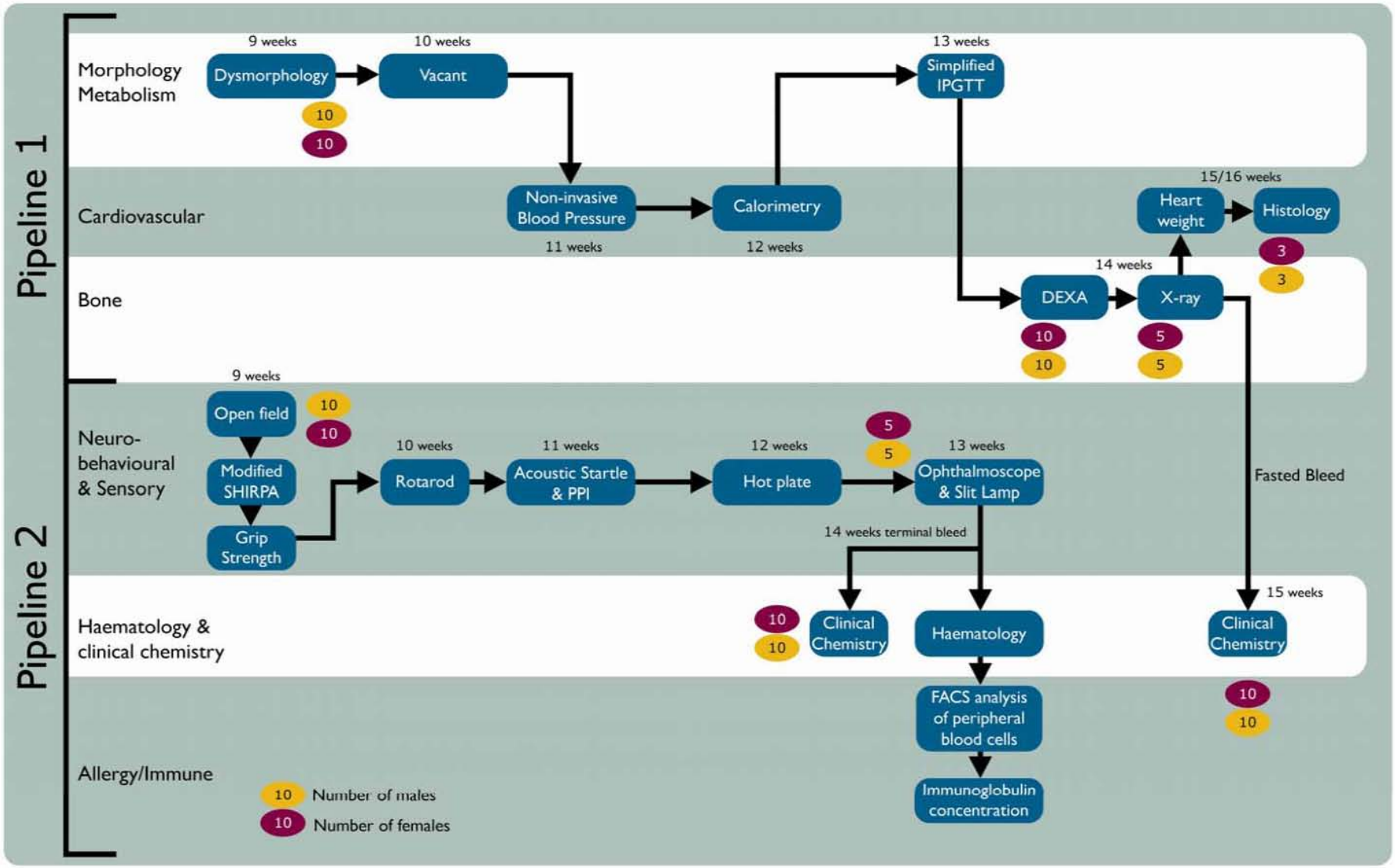
IMPC

(INTERNATIONAL MOUSE PHENOTYPING CONSORTIUM)

Why the IMPC

- Build a resource of KO mice and associated
  of gene functions
- Free thousands of researchers from tool generation
- This resource will be revolutionize research for the next 20-30 years
- Novel genes will be brought to light that would otherwise be ignored
- Potential for breakthrough discoveries

EMPReSSslim Primary Phenotyping Pipelines



IMPC Phenotyping Conference

April 7-9, 2010

c

Toronto Center for Phenogenomics, Canada

IMPC participants, international funders, research/phenotyping scientists

IMPC Phenotyping Conference

Highlights:

1) Imaging
2) Embryonic lethality
3) Planning informatics infrastructure
4) Industry perspectives

IMPC Phenotyping Conference

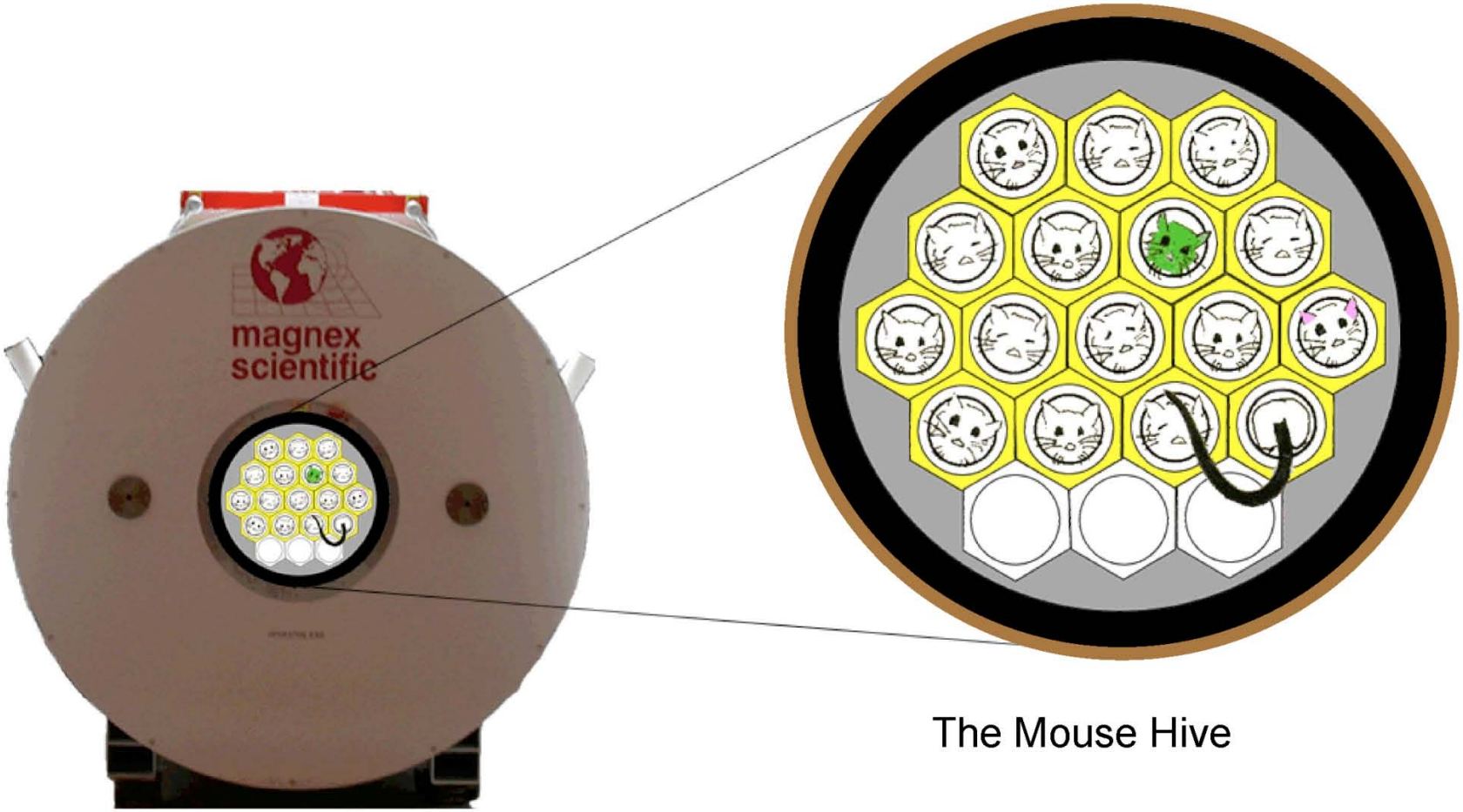
IMAGING

Take home messages:

important, affordable, “right thing to do”
data, functional, non-invasive, non-lethal
quick conceptual analysis of complex structures (cf histology)
MRI (brain & embryos…other tissues ?)
CT (embryos; KI soaking ↑ resolution, ↓ scan time)
OPT (for young adults)

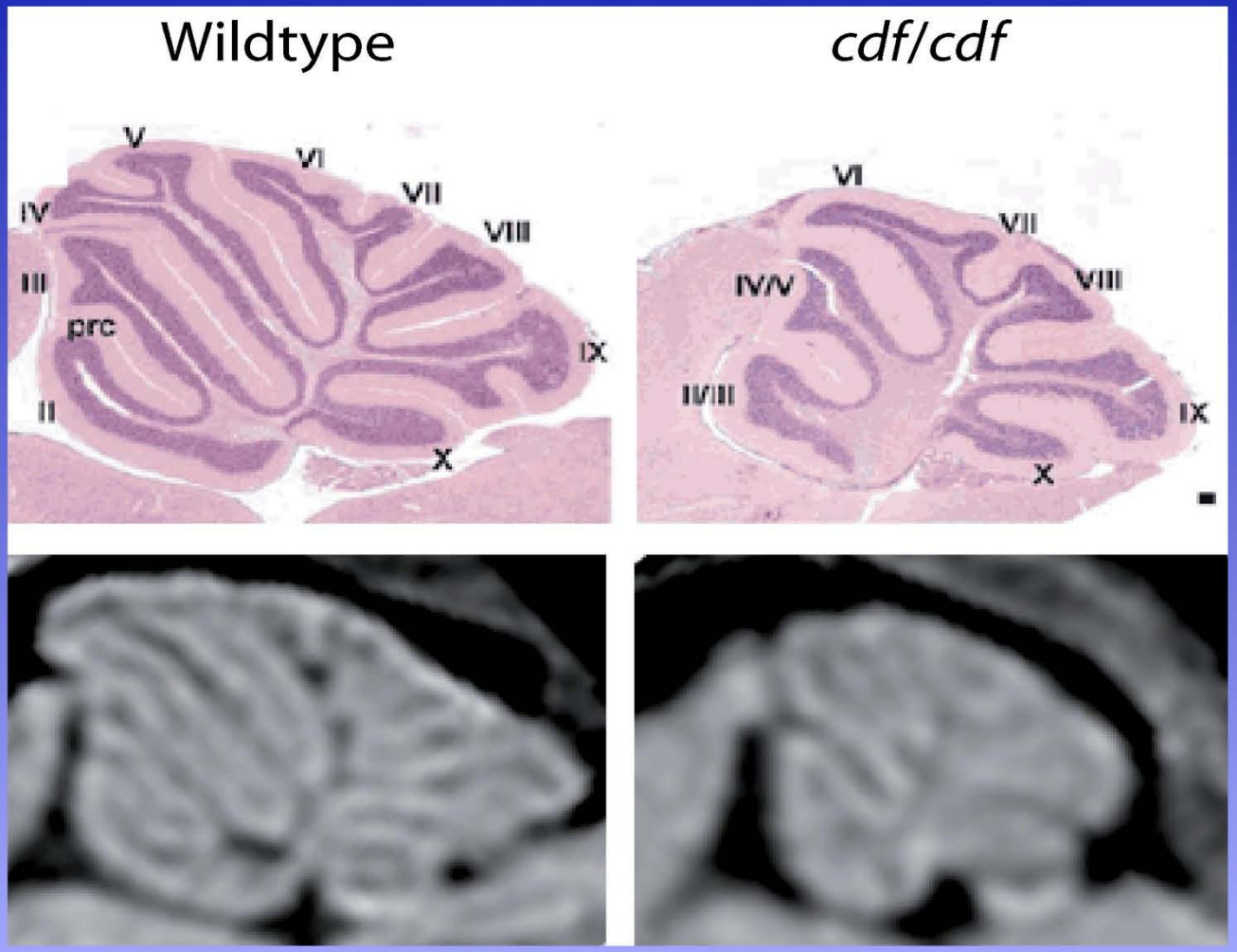
Mark Henkelman, The Hospital for Sick Children
Jason Lerch, The Hospital for Sick Children

Multiple Mouse MRI



The Mouse Hive

The *cdf/cdf* Phenotype on MRI

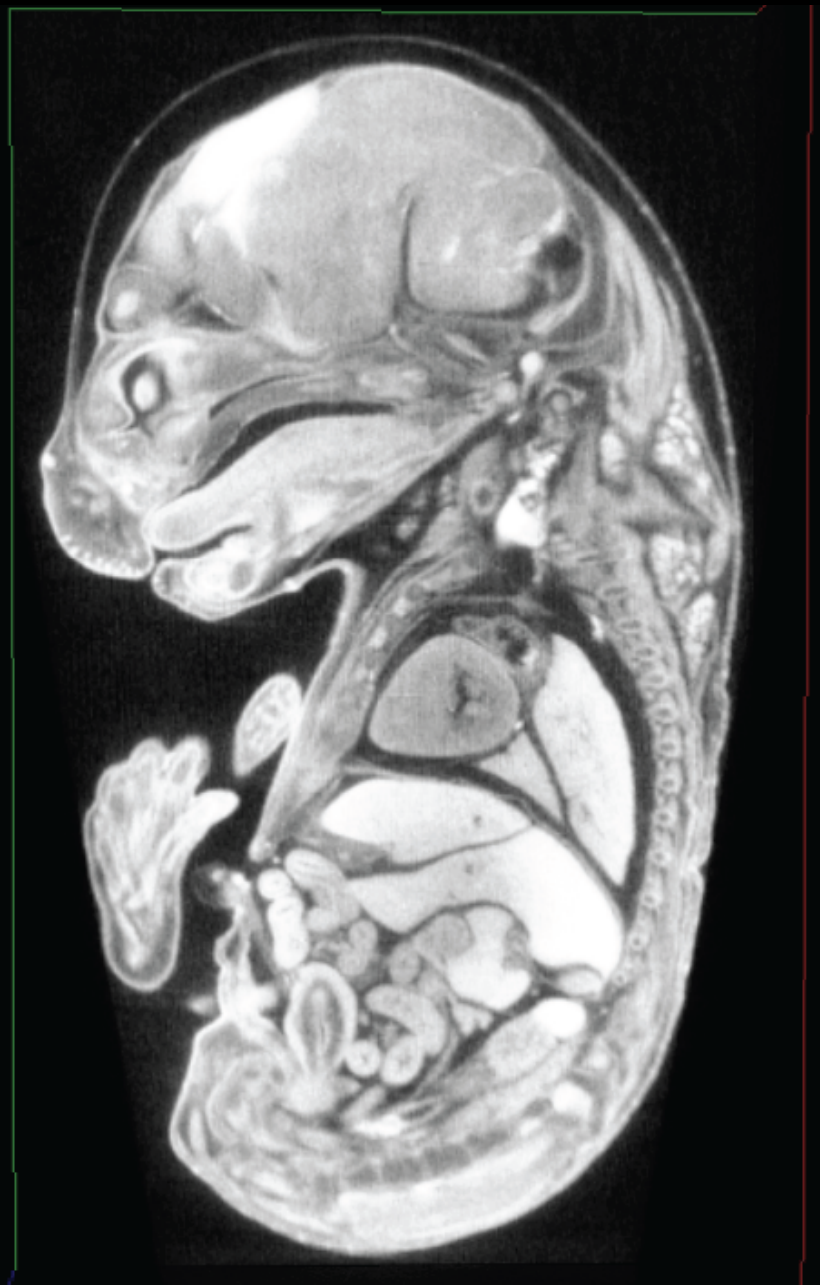


Sue Ackerman JAX

CT

Embryo

E15.5



IMPC Phenotyping Conference

EMBRYONIC LETHALITY

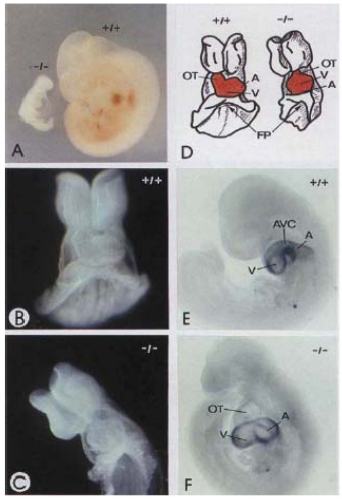
Take home messages:

identify first by lack of homos in het crosses
screen must consider placental & CVS phenotype
cause of death related to gestational time of death
homozygous lethal points to heterozygous pheno
insight into human disease pathways
identify druggable (human) targets
imaging: non-invasive *in utero* echocardiography

Janet Rossant, The Hospital for Sick Children
Cecilia Lo, University of Pittsburgh ("bench to bassinette")

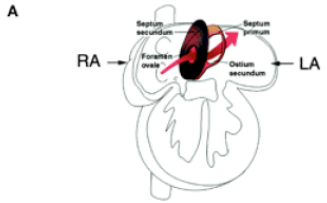
Homozygous knockout to human haploinsufficiency and back to mouse heterozygous phenotype- the case of Nkx2.5

null

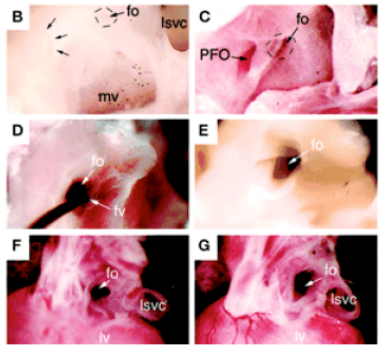


Nkx2.5 is tinman ortholog, required for early heart looping.
Lyons et al, 1995, Genes Dev. 9, 1654-1666

NKX2.5 haploinsufficiency underlies atrial septal and conduction defects in humans
Schott et al. 1998. Science, 218, 108-111

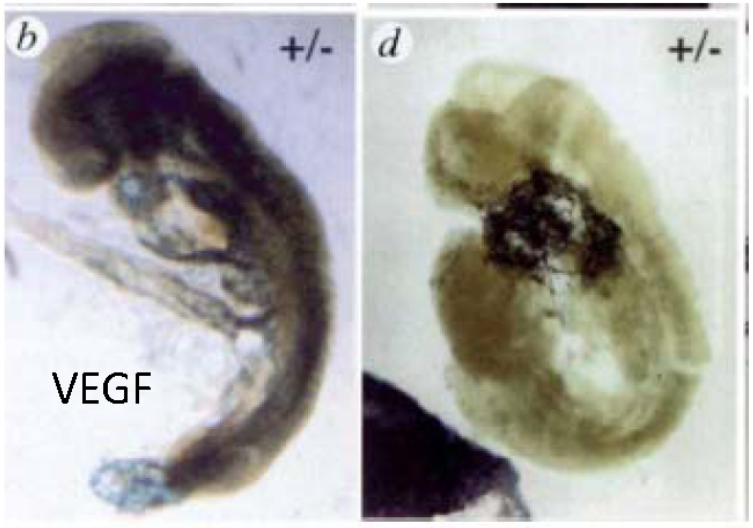


het

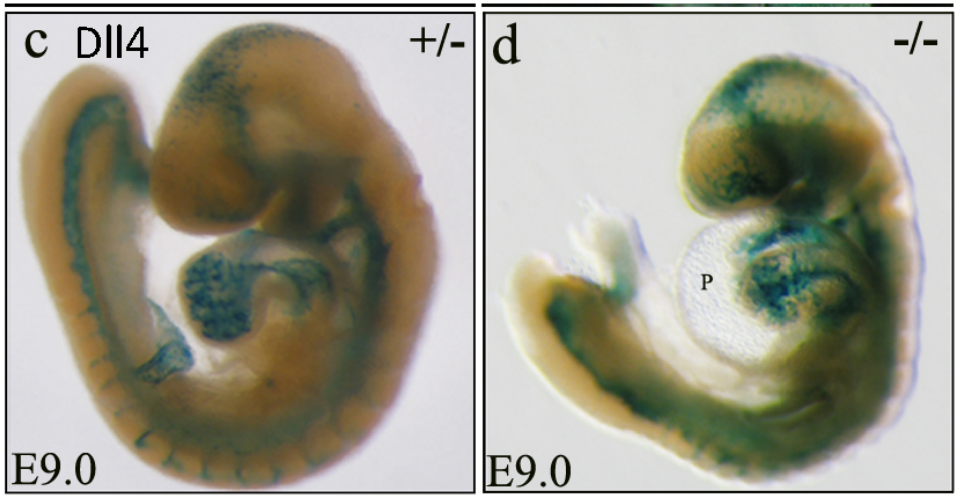


Reexamination of heterozygous mouse mutations reveals adult cardiac defects similar to human
Biben et al, 2000. Circ. Res 87, 888-895

Dosage sensitive pathways identify potential druggable targets



- VEGF inhibitors block tumour angiogenesis by blocking vessel formation (Avastin-Genentech)



- Dll4 inhibitors block tumour angiogenesis by promoting non-productive vessel development (Regeneron/Genentech)

IMPC Phenotyping Conference

PLANNING INFORMATICS INFRASTRUCTURE

Phenotyping effort will generate large amts data
2 components to informatics effort:
- data collection and export at production sites
- data center depot and portal at informatics hub

Shared governance (phenotypers, funders)
Issues and challenges
- consensus on data presentation
- data curation and qc
- images and other large data sets
- evaluation and continuing maintenance
- budget

IMPC Phenotyping Conference

INDUSTRY PERSPECTIVES

Nick Gale (Regeneron,Inc)

- lacZ expression analysis informs phenotype
- molecular phenotyping (microarray)
- part of KOMP mutagenesis project

Andy Peterson (Genentech, Inc)

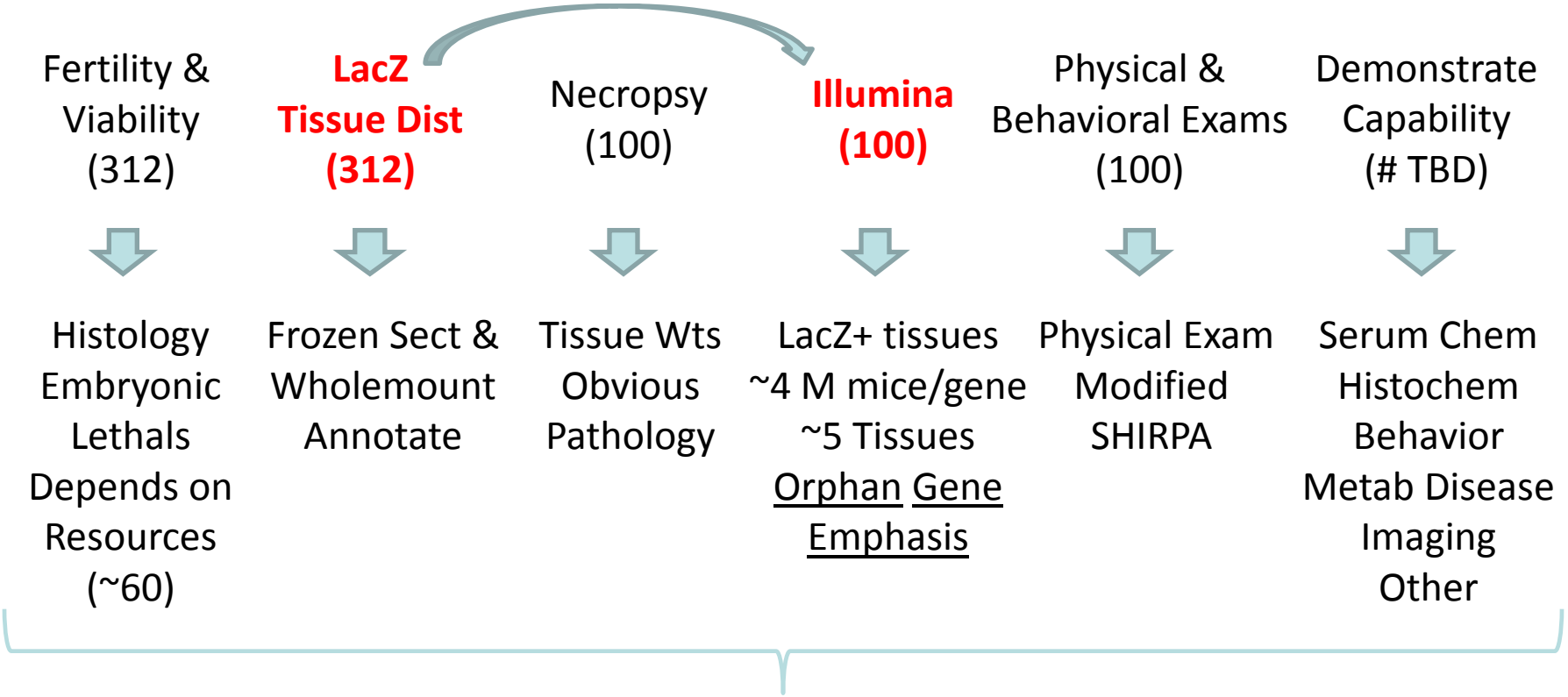
- immunological, metabolic phenotypes common
- phenotype & expression pattern incongruent (may be due to extracellular proteins)
- deposited 496 mutant lines & data in MMRRC

Activities at UC Davis:

KOMP mutagenesis (CSD Consortium)

KOMP repository (with CHORI)

KOMP phenotyping *pilot* (ARRA funding):



Celebrating 10 YEARS
Mouse Biology Program
www.mousebiologyprogram.org

www.mousebiology.org

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