

EUCOMM Progress Report

Overview

- EUCOMM
 - Objectives
 - Alleles
 - Production Summary
 - Distribution
 - Validation in mice
- EUCOMMTOOLS
 - Objectives



EUCOMM Partners



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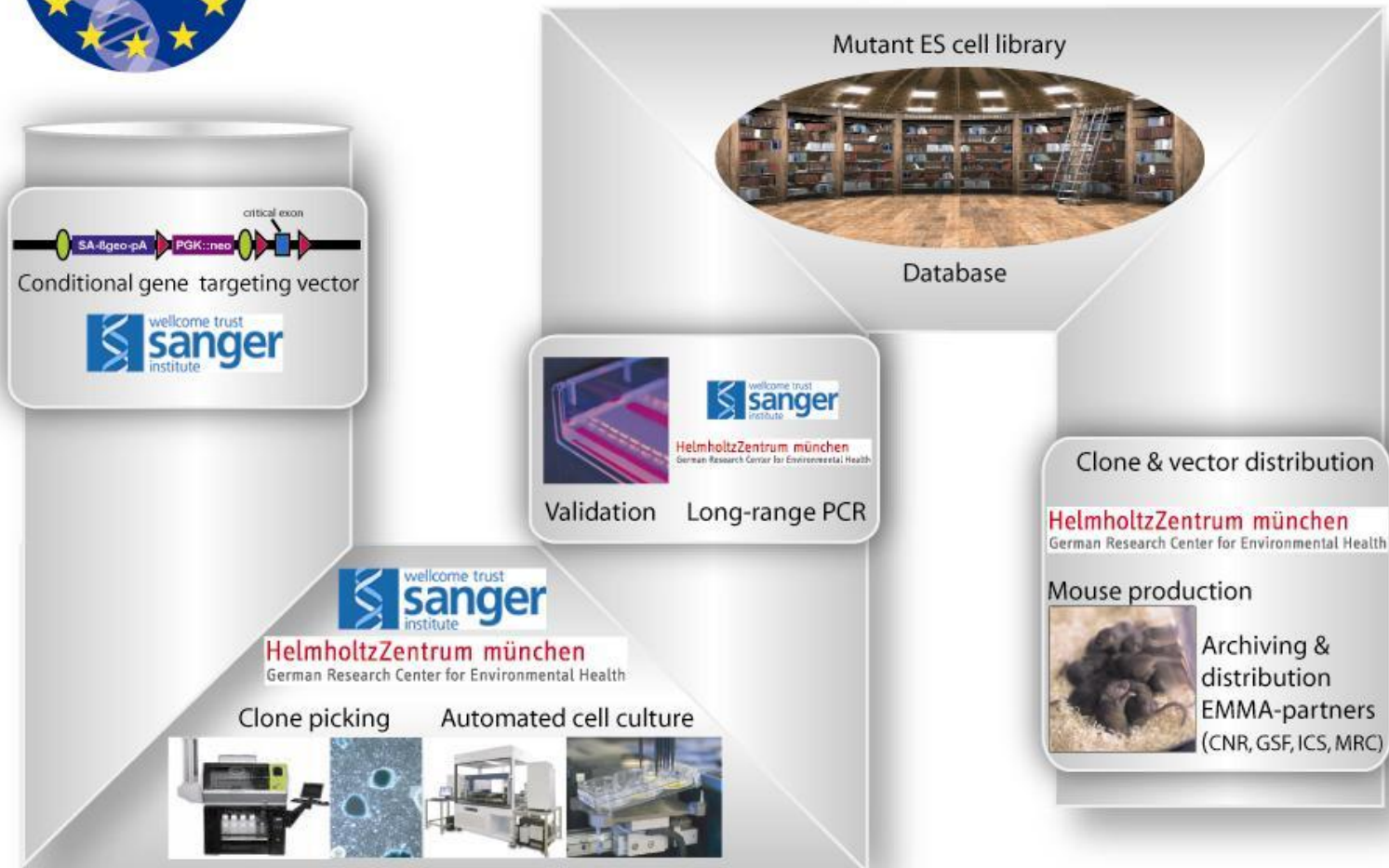
EUComm Objectives

- Generation of up to 12,000 (**5,000**) conditional gene trap mutations
- Generation of up to **8,000** targeted conditional mutations (Skarnes et al., *Nature* 2011)
- Establishment of up to **320** mutant mouse lines
- Establishment of 20 (**10**) ligand-inducible Cre-recombinase expressing transgenic mouse lines
- Archiving and distribution of vectors, ES cells and mutant mice
- Training in EUComm technology
- Integration of EUComm into IKMC web portal

EUCOMM Pipeline



GENE TARGETING PIPELINE

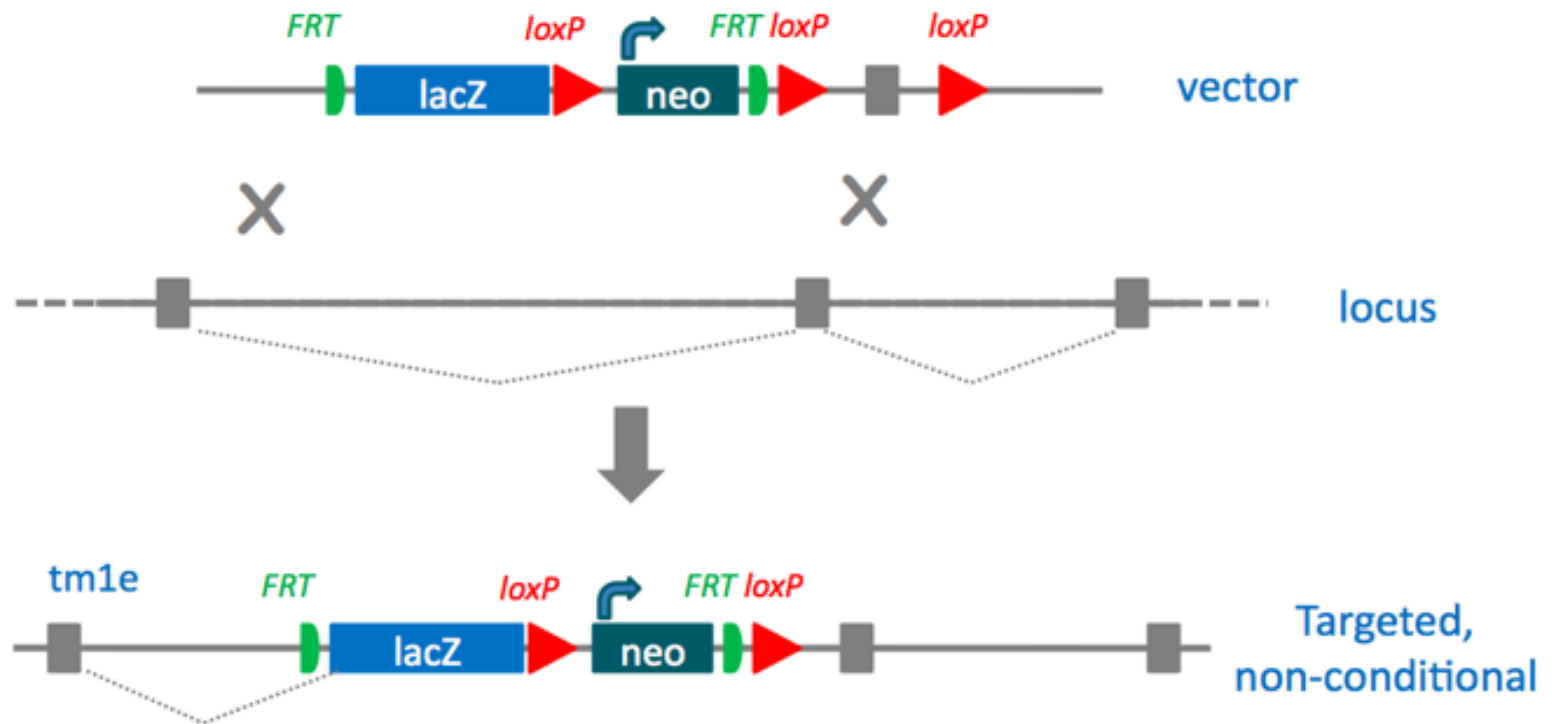




Knockout-first EUComm allele

Targeted, non-conditional allele

40% clones



Pipeline summary



Cumulative totals of pipeline products

Total Generated Products	EUComm
Mice ¹	573
ES Cells ²	6209
Vectors ³	7886
Designs	9429

¹ Sanger (264 strains), Helmholtz (45 strains), ICS (112 strains), Harwell (100 strains), Monterotondo (69 strains)

² Sanger (3445 genes), Helmholtz (2982 genes), average 8 clones/gene

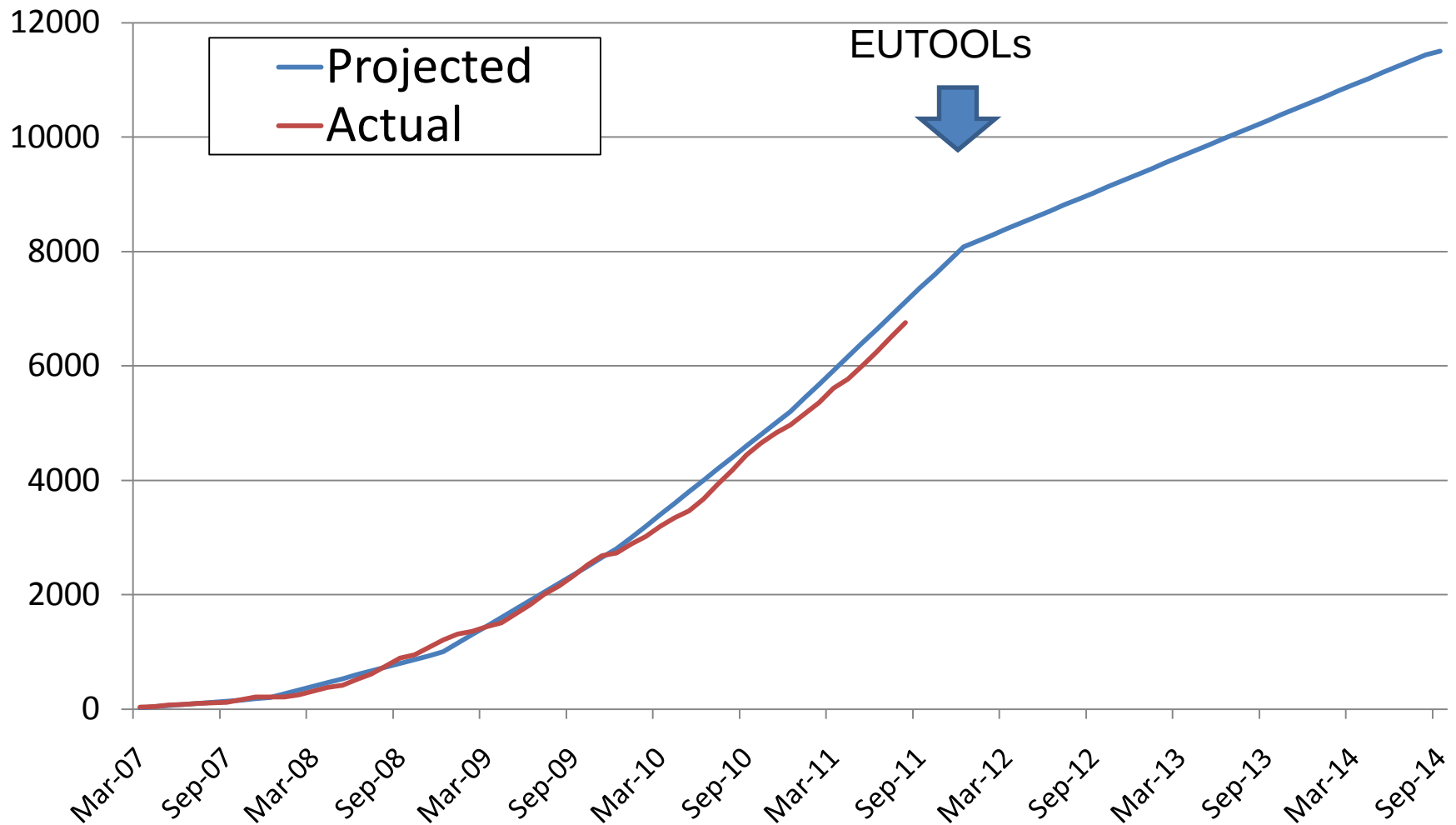
³ promoter –driven (7255 genes), promoterless (1317 genes)

Targeted ES cell production



Period	ES Cell Electroporations	Genes Electroporated	Colonies screened	Correctly Targeted Clones	Number of Genes Screened	Correctly Targeted Genes	Success Rate (%)	Targeted genes (Cumm)	Project goal (Cumm)
2007	704	500	10074	2965	366	234	47%	210	200
2008	2057	1721	46888	7790	1448	1014	59%	1207	1000
2009	2859	2217	95491	13211	2169	1595	72%	2730	2800
2010	3633	3020	111459	22385	2893	2274	75%	4970	5200
Feb-11	318	308	9960	1432	288	196	64%		
Mar-11	335	323	10304	2120	296	219	68%		
Apr-11	331	330	10096	1831	315	225	68%		
May-11	335	335	10496	1910	320	233	70%		
Jun-11	341	341	10480	1772	329	242	71%		
Jul-11	353	343	10568	1018	204	144	68%		
Aug-11	356	353	11496	299	85	50	57%		
Sep-11	325	315	856	73	23	13	57%		
2011	2708	2338	74624	10702	1732	1319	74%	6760*	7120
to date*	11914	7518	336803	57406	6955	6209	88%		

Targeted ES cell production



EUCOMM targeting statistics



11,202 electroporations

7148 genes electroporated

336,803 colonies screened

6435 genes targeted (90% success rate)

Average targeting efficiency:	40%
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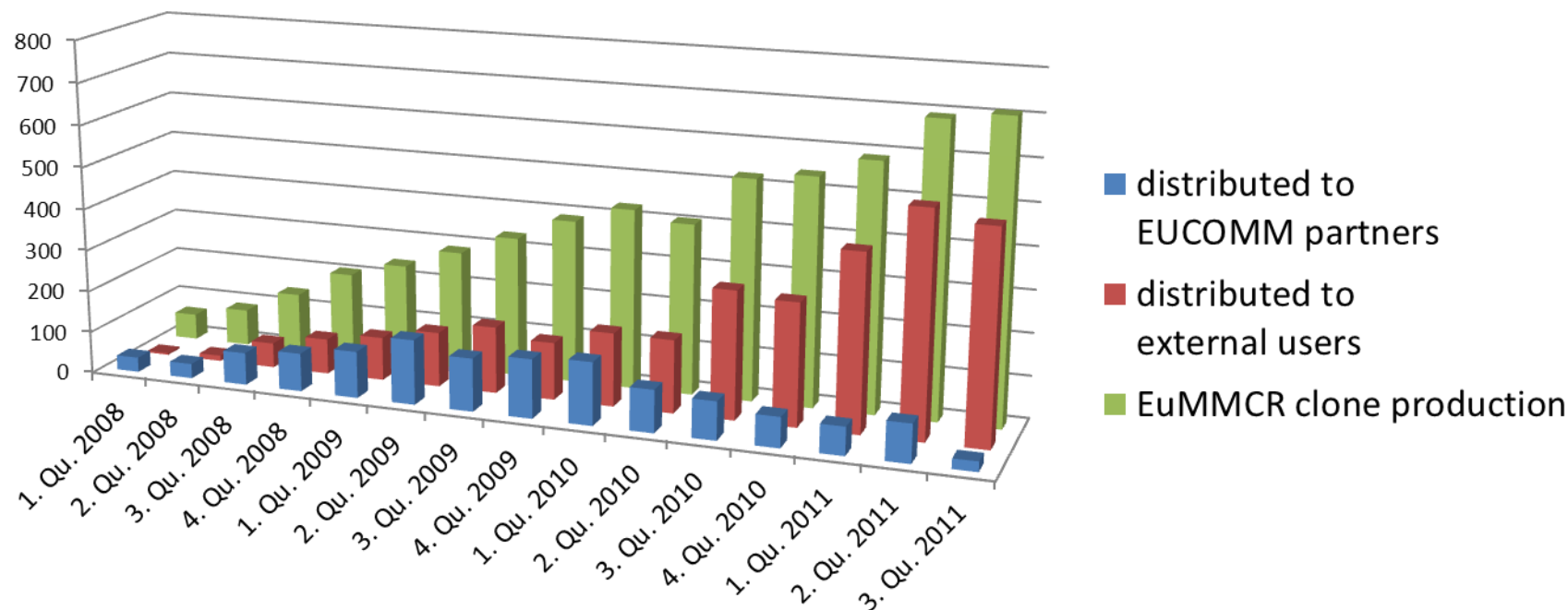
Average # clones screened:	32
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Average # targeted clones:	13
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Average # KO-first clones:	8
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Median # KO-first clones:	7
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EUCOMM ES clone distribution

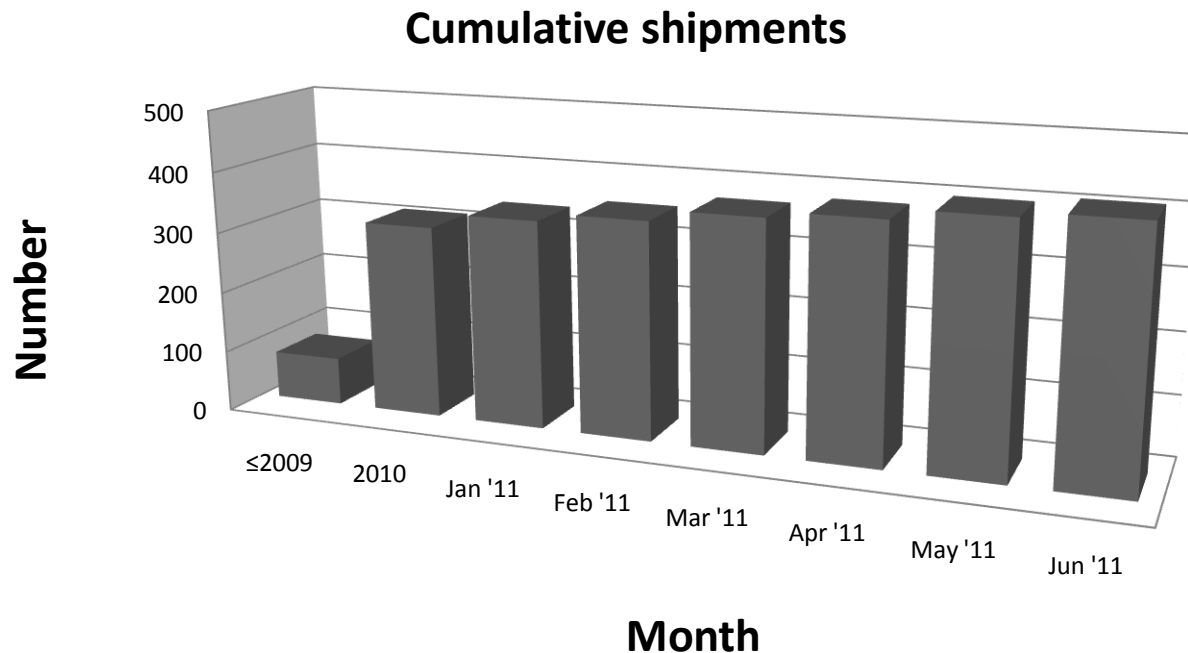


	2008				2009				2010				2011			Total
	1. Qu.	2. Qu.	3. Qu.	4. Qu.	1. Qu.	2. Qu.	3. Qu.	4. Qu.	1. Qu.	2. Qu.	3. Qu.	4. Qu.	1. Qu.	2. Qu.	3. Qu.	
EUCOMM partners	35	35	77	91	111	154	126	140	149	101	91	72	67	91	24	1364
External users	4	13	59	84	103	130	158	136	174	173	303	290	418	528	500	3073
EuMMCR production	62	87	141	205	240	286	334	388	428	406	524	541	588	692	709	5631

Distribution of EUCOMM mice



- 411 shipments in total have been sent out (Jun '11)
- EUCOMM lines account for ~50% of the EMMA exports



Analysis of *Aspa*^{tm1a} mice

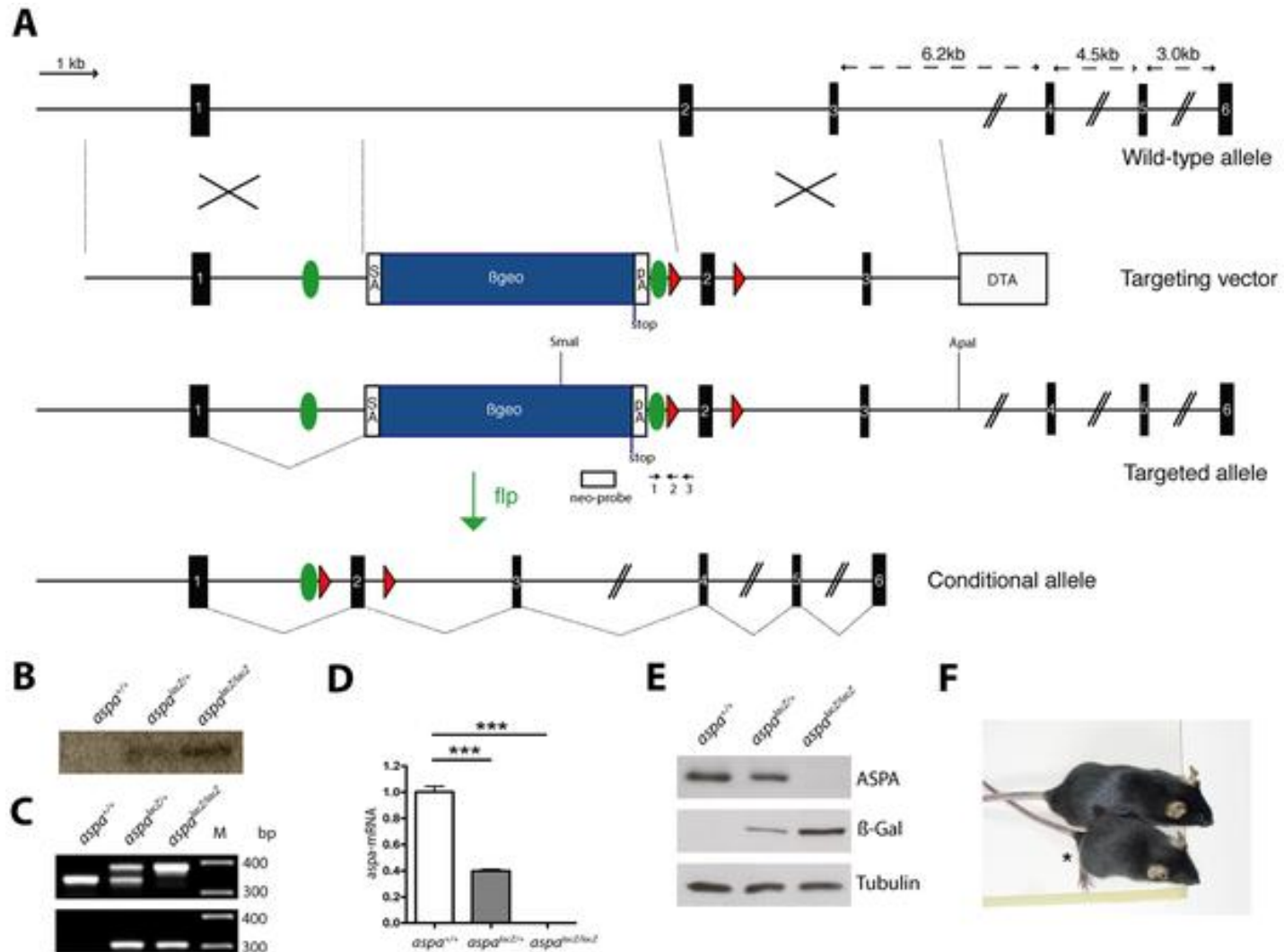
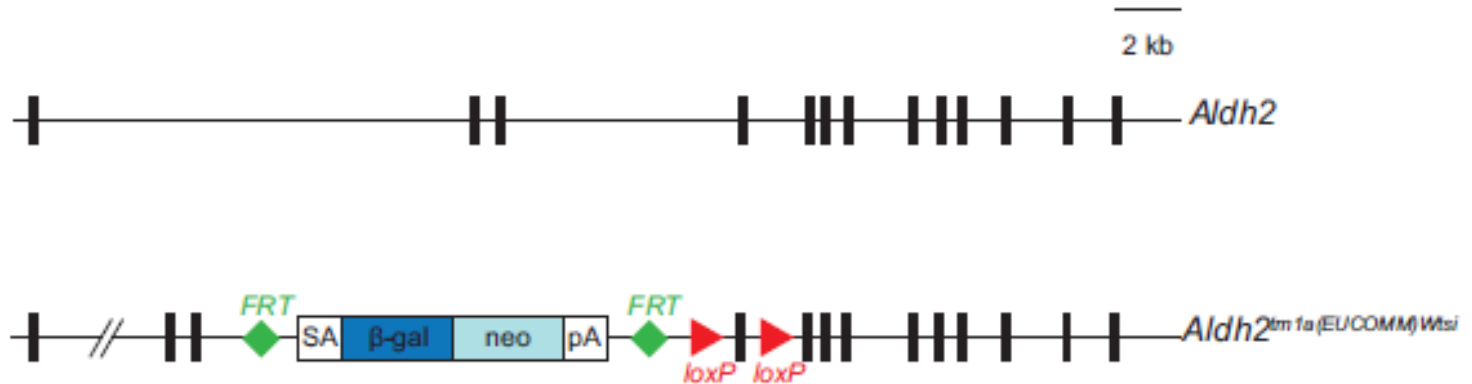


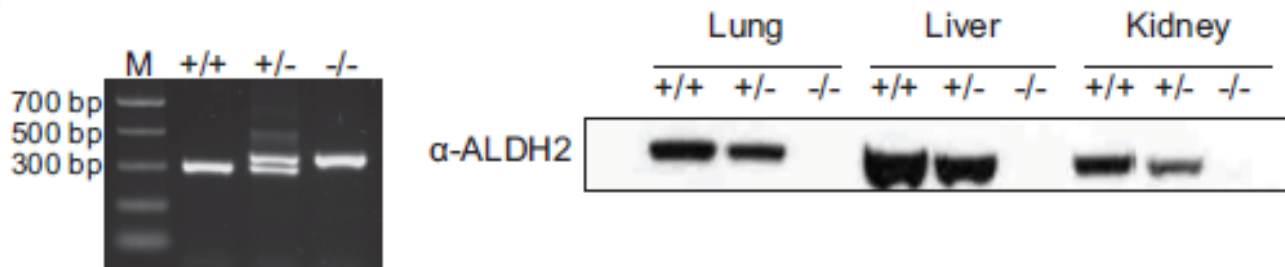
Fig 1 from Mersman et al., PLoS One. 2011

Analysis of $Aldh2^{tm1a}$ mice

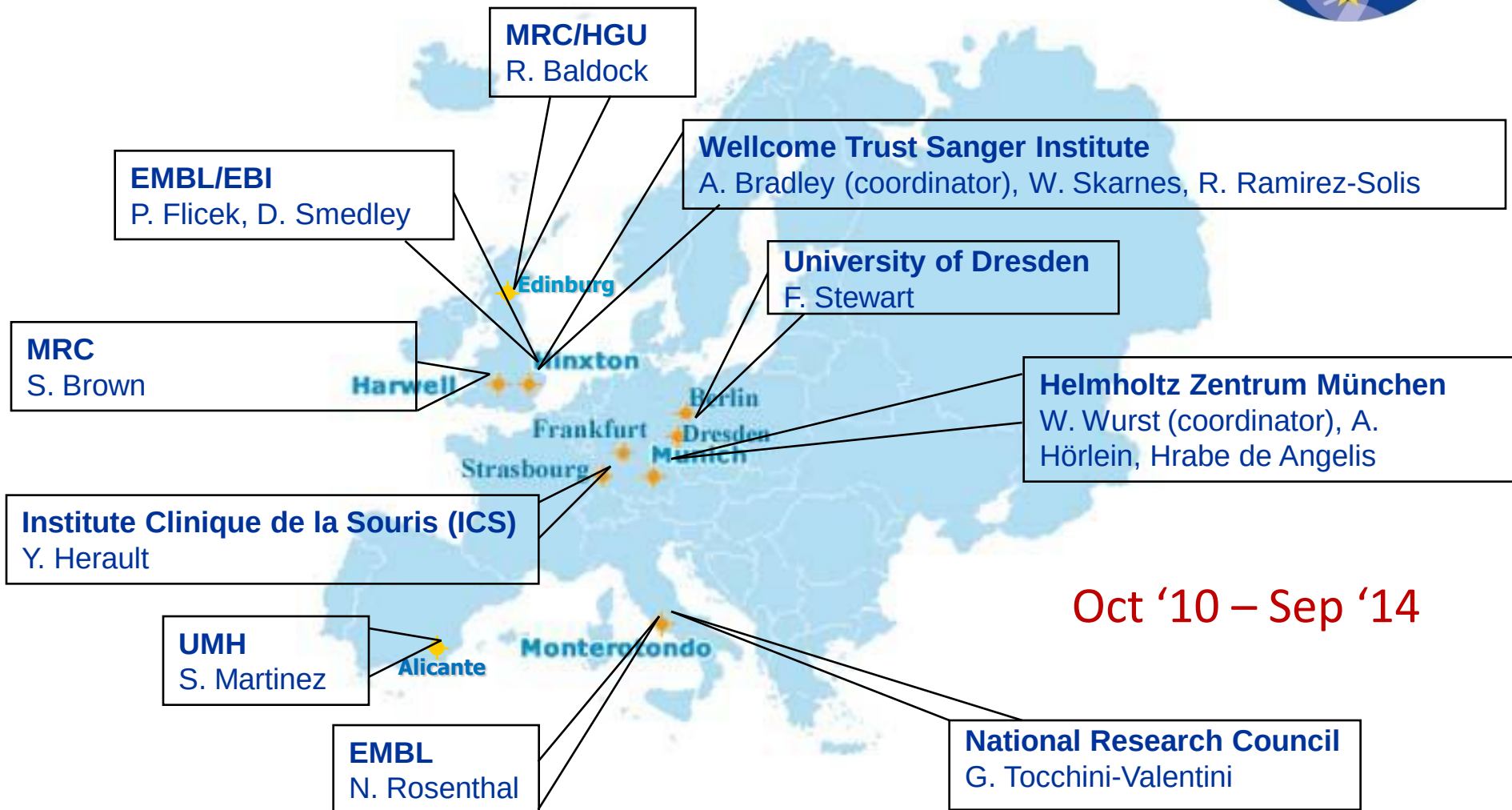
a



b



EUCOMMTOOLS Partners



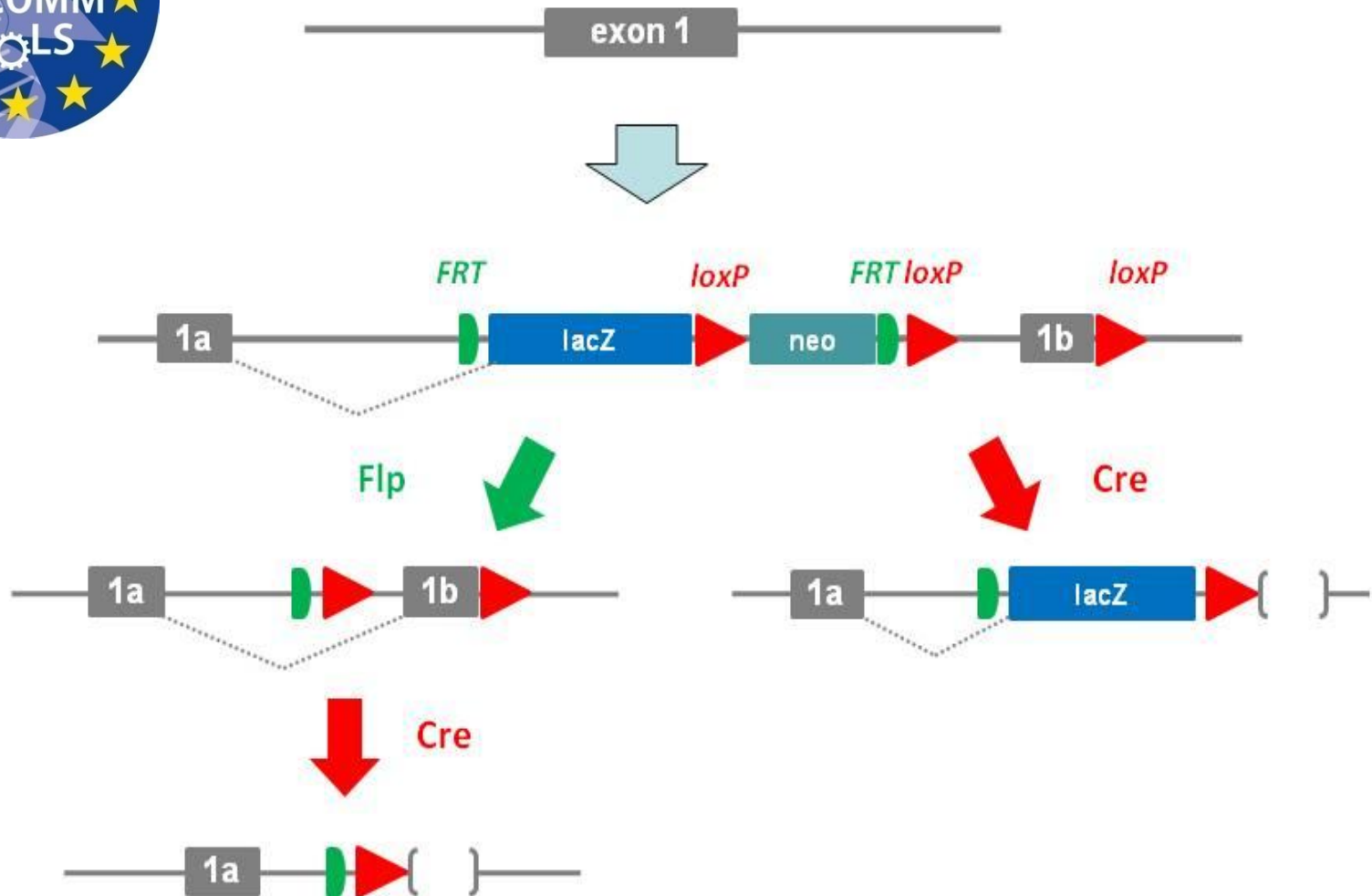
Oct '10 – Sep '14



EUCommTOOLS Objectives

- Improvement of KO technologies (Toolbox)
- Additional **3,500** conditional targeted ES cell lines to complete IKMC resource
- Mutant ES cells for **500 miRNA loci** (Prosser et al., *Nat Biotechnol.* 2011)
- Identification of **500** genes suitable as Cre drivers covering all organs and major cell types
- Establishment of **500** BAC-eGFP-T2A-CreERT2 ES cell lines (in the Rosa26 or Hprt locus)
- Establishment and characterization of **250** BAC-eGFP-T2A-CreERT2 driver transgenic mouse lines

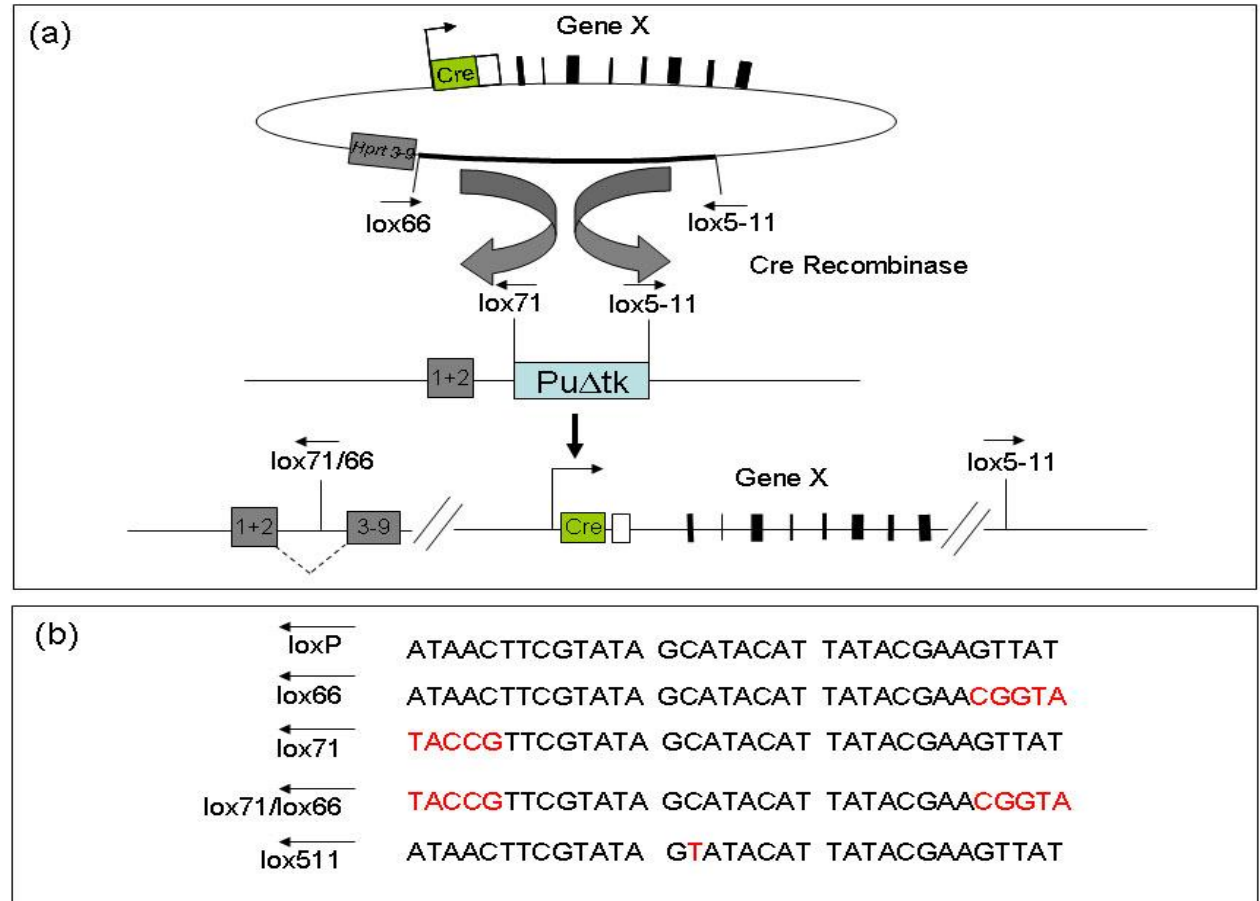
Conditional targeting strategy for single exon genes





Cre alleles

RMCE strategy for introducing eGFP-T2A-CreERT2 transgenes into the ES cell genome



Hayden Prosser, Allan Bradley

Common web portal for KO resources

www.knockoutmouse.org



International Knockout Mouse Consortium

[Home](#) [About IKMC](#) [Download](#) [BioMart](#) [Prototypes](#) [Nominate gene](#) [FAQ](#) [Order Products](#) [Contact IKMC](#)

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

e.g., *Adam19*, *Pax*, *ENSMUSG00000020681*, *Chr13:22210730-22311689*
(coordinates from NCBI mouse genome assembly 37)

[Advanced Search](#)

Browse IKMC database

[help](#)

Use the following links to browse genes

- [Browse by Gene Symbol](#)
- [Browse by Chromosome](#)



IKMC Gene Pages

Gene Information

Gdpc2

ES Cell Clones With Conditional Potential

Express inte
Gene Name:
Synonyms:
Gene Locati

Show Other

IKMC K

CSD
Project: 79

Regeneron
Project:
VG15938

NorCOMM
Project: 71063

ES Cell Clones (Cond

5' arm (2888 bp)

F3

UipCR

Note: These mutations can be made

Genome Browsers: [Ensembl](#) [Southern](#)

Tools:

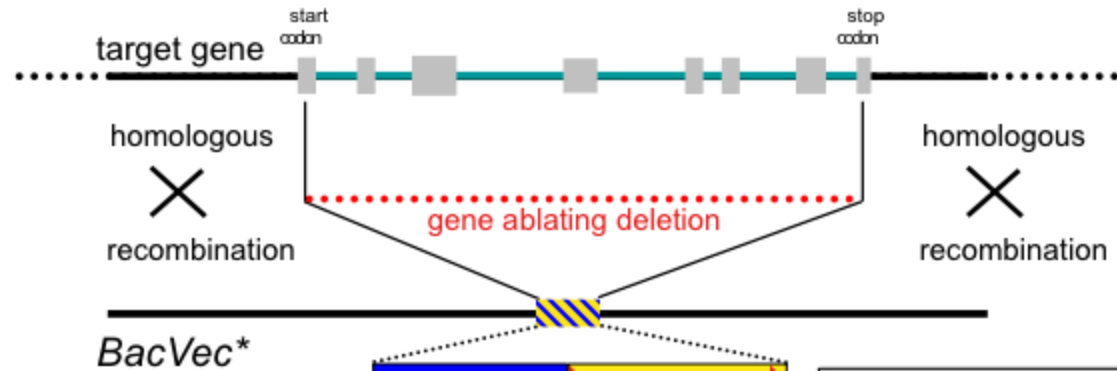
ES Cell Clone

order N02076P1_C_237W_B2

Pre Pipeline

Gdpc2^{tm1(KOMP)Vlcg} **D**

VelociGene's KOMP Definitive Null Allele Design



Cassettes:

ZEN-UB1	lacZ-p(A)	hUbCpro-neo ^r -p(A)
ZEN-UB1-rev	lacZ-p(A)	(neo ^r -p(A)-hUbCpro)
ZEN-UB1-hyg	lacZ-p(A)	hUbCpro-hyg ^r -p(A)
TM-ZEN-UB1	TM-lacZ-p(A)	hUbCpro-neo ^r -p(A)

 expression-selection cassette

 exon

 intron

 loxP

*large BAC-based targeting vector

lacZ β-galactosidase coding sequence from the *E. coli lacZ* gene

TM-*lacZ* a transmembrane sequence fused to β-galactosidase

neo^r or hyg^r coding sequences for neomycin or hygromycin phosphotransferases

hUbCpro promoter from the human ubiquitin C gene

p(A) polyadenylation signal

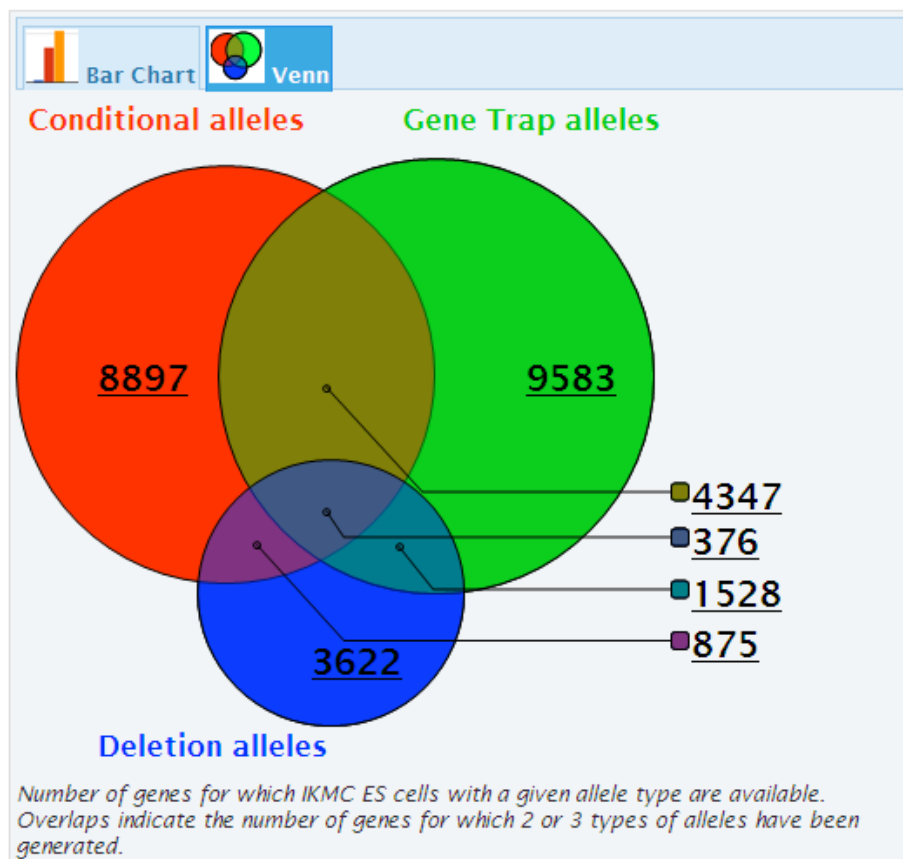
reported
(out)

Allele
Details
D

Allele
Details
D/C

IKMC Summary Page

IKMC Gene Progress Summary



Targeted alleles

Total Genes	KOMP		EUComm/ EUCommTools	NorComm
	CSD	Regeneron		
Vectors available	6604	4733	7911	839
ES cells available	4789	3424	6479	569
Mutant mice available	332	273	573	4

[View details and project goals](#) [View details about the acronyms used](#)

Gene trap alleles

Total Genes	TIGM	EUComm	NorComm
ES cells available	9394	4421	4588
Mutant mice available	81		

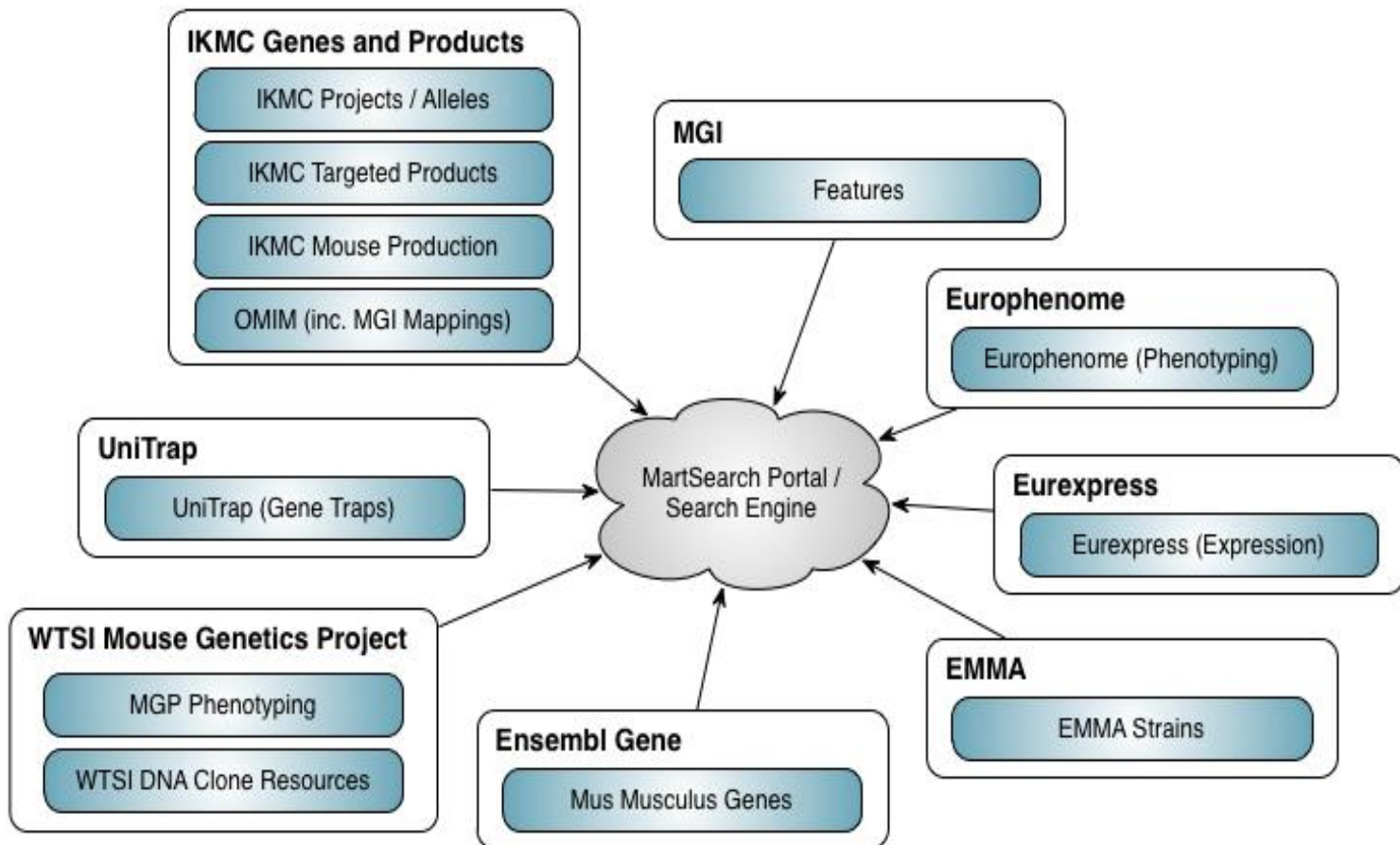
NOTE: Not all gene traps are in C57BL/6 ES cells. [View strain information](#)

MartSearch interface



International Knockout Mouse Consortium - MartSearch

A- A A+



MartSearch interface

MartSearch  HOME BROWSE HELP ABOUT

Available Products

Mammalian Phenotype

adipose tissue	(27)
behavior/neurological	(125)
cardiovascular system	(32)
cellular	(0)
craniofacial	(4)
digestive/alimentary	(1)
embryogenesis	(0)
endocrine/exocrine gland	(0)
growth/size	(95)
hearing/vestibular/ear	(0)
hematopoietic system	(116)
homeostasis/metabolism	(167)
immune system	205

Data Browsing

This page allows you to browse the data within this portal. Expand one of the browse controls to the left (by clicking on them), and then select the category for which you would like to see data. The numbers in parenthesis indicate the number of genes that match (and will be returned by) the given browse category.

Browse by...

- **Available Products** - browse for genes that have products produced by IKMC projects, which are available to order
- **Mammalian Phenotype** - browse for genes that when knocked-out have been annotated as causing a distinct phenotype (as classified by the mammalian phenotype ontology)
- **Chromosome** - browse for genes that are found on a given chromosome
- **Marker Symbol** - browse for genes that start with a given letter/number

MartSearch interface

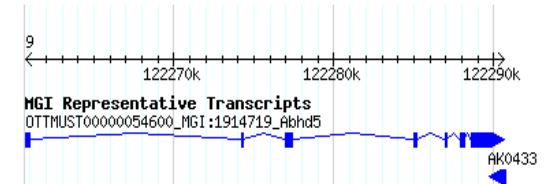
MartSearch

Abhd5

Datasets: [Gene Details](#) [Europhenome](#) [Eurexpress](#) [Mice](#) [IKMC Vectors and ES Cells](#) [IKMC Trapped Products](#) [Other Mutants/Resources](#)

Gene Details

Marker Name(s):	abhydrolase domain containing 5 view this gene in MGI
Marker Type:	protein coding gene
Synonyms:	1300003D03Rik, 2010002J10Rik, CGI-58, IECN5, NCIE2
Location:	Chr9:122260726-122290642(+)



Related Human Cor

- Chanarin-Dorfman

More Informa

Data provided by Mouse

	Parameter	P Value	Effect Size	MP Annotation	
	Fat Mass	3.79e-05	-2.10e+00	decreased total body fat amount	graph

[original data](#)

Europhenome

	adipose tissue	behavior/neurological	cardiovascular system	cellular	craniofacial	digestive/alimentary	embryogenesis	endocrine/exocrine gland	growth/size	hearing/vestibular/ear	hematopoietic system	homeostasis/metabolism	immune system	lethality-postnatal	lethality-prenatal/perinatal	life span-post-weaning/aging	limbs/digits/tail	liver/biliary system	muscle	nervous system	no phenotypic analysis	normal	other	pigmentation	renal/urinary system	reproductive system	respiratory system	skeleton	skin/coat/nails	taste/olfaction	touch/vibrissae	tumorigenesis	vision/eye
Abhd5 (Hom)																																	

Parameter	P Value	Effect Size	MP Annotation	
Fat Mass	3.79e-05	-2.10e+00	decreased total body fat amount	 graph

Data provided by Europhenome

Significant Annotation Present

[view original data](#)

Eurexpress