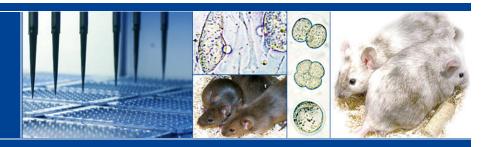# Cre-drivers

EUCOMMTOOLS

European Conditional Mouse Mutagenesis Program:

TOOLS for Functional Annotation of the Mouse Genome
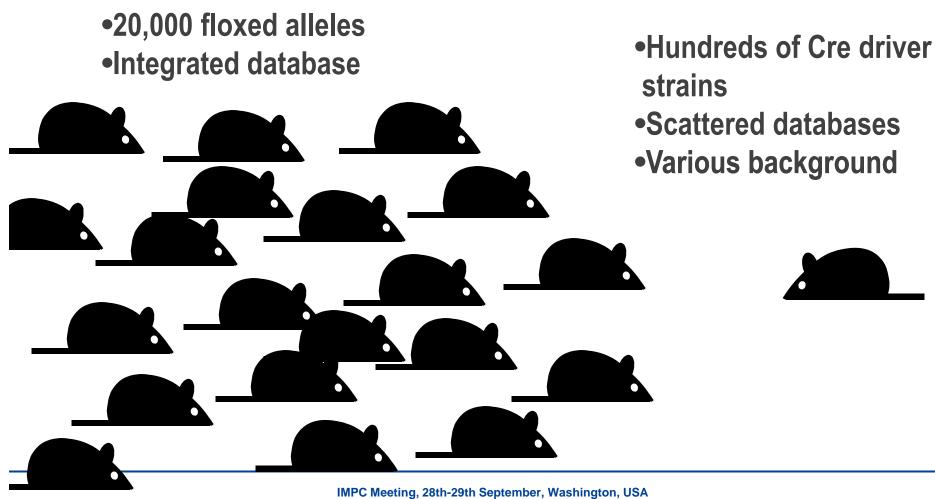
IMPC meeting

28th-29th September, 2011

Washington, USA

Wolfgang Wurst

Helmholtz Zentrum Munich

The Challenge

- 20,000 floxed alleles
- Integrated database

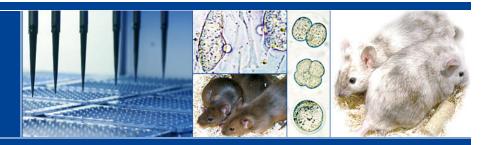- Hundreds of Cre driver strains
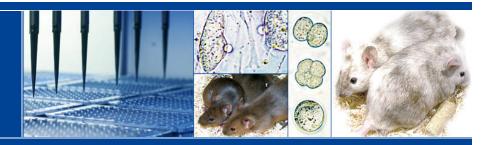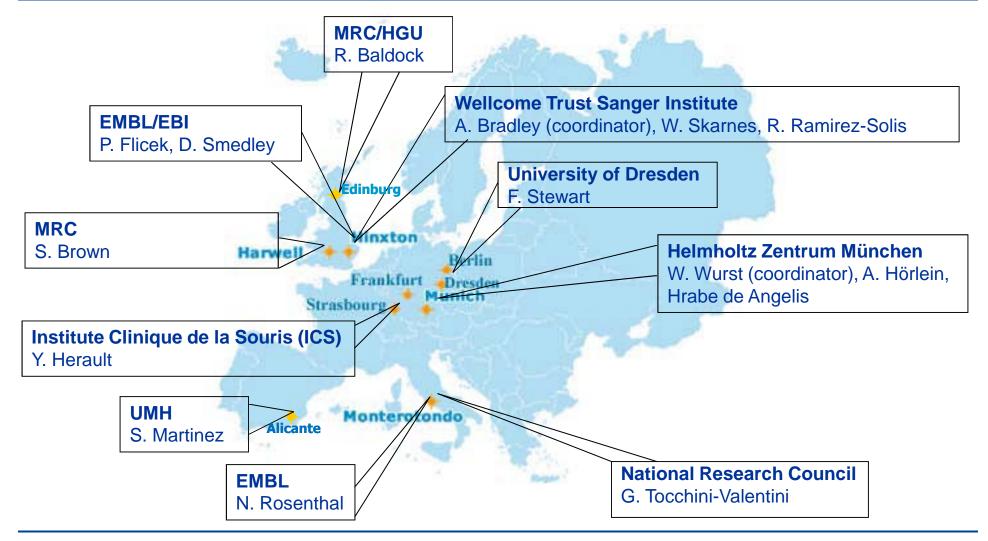- Scattered databases
- Various background
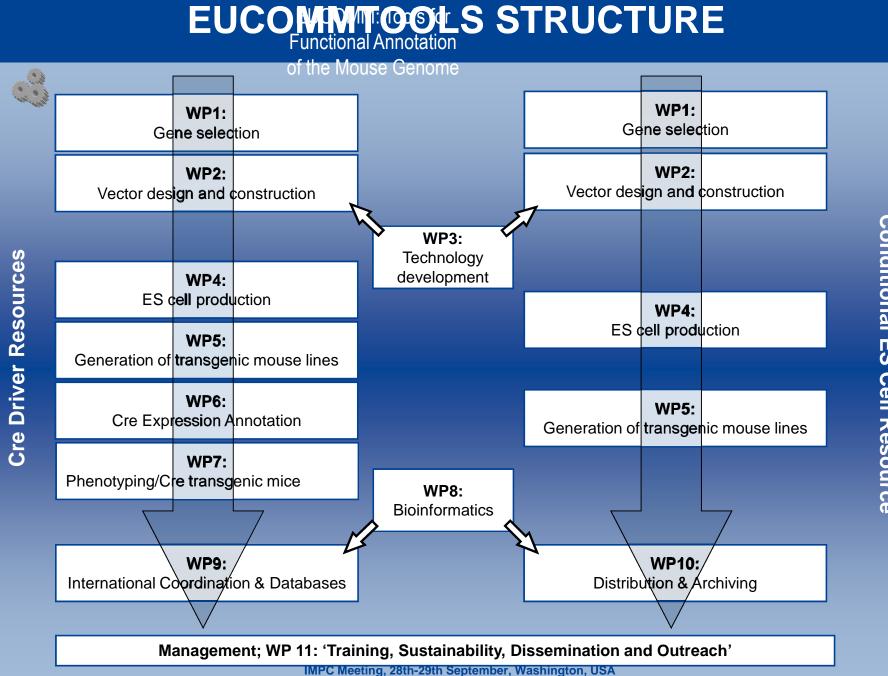
Objectives:

» Identification of **500** genes and their promoters suitable as Cre drivers covering all organs and major cell types

» Establishment of **500** BAC-CreERT2T2A-eGFP/BAC-Cre-T2A-eGFP (in the Rosa26 or Hprt locus) constructs and recombinant ES cells Cre driver transgenic mouse lines

» Establishment of **250** BAC-CreERT2T2A-eGFP/BAC-Cre-T2A-eGFP driver transgenic mouse lines

» Archiving and distribution by EuMMCR/EMMA

EUCOMM: Tools for Functional Annotation of the Mouse Genome

MRC/HGU
R. Baldock

Wellcome Trust Sanger Institute
A. Bradley (coordinator), W. Skarnes, R. Ramirez-Solis

EMBL/EBI
P. Flicek, D. Smedley

University of Dresden
F. Stewart

MRC
S. Brown

Helmholtz Zentrum München
W. Wurst (coordinator), A. Hörlein, Hrabe de Angelis

Institute Clinique de la Souris (ICS)
Y. Herault

UMH
S. Martinez

EMBL
N. Rosenthal

National Research Council
G. Tocchini-Valentini

Edinburg
Harwell
Hinxton
Berlin
Frankfurt
Dresden
Munich
Strasbourg
Alicante
Monterotondo

EUCOMMTOOLS STRUCTURE

EUCOMM: Tools for
Functional Annotation
of the Mouse Genome

Cre Driver Resources

Conditional ES Cell Resource

WP1:
Gene selection

WP2:
Vector design and construction

WP4:
ES cell production

WP5:
Generation of transgenic mouse lines

WP6:
Cre Expression Annotation

WP7:
Phenotyping/Cre transgenic mice

WP3:
Technology development

WP8:
Bioinformatics

WP9:
International Coordination & Databases

WP1:
Gene selection

WP2:
Vector design and construction

WP4:
ES cell production

WP5:
Generation of transgenic mouse lines

WP10:
Distribution & Archiving

Management; WP 11: 'Training, Sustainability, Dissemination and Outreach'

IMPC Meeting, 28th-29th September, Washington, USA

WP1 Gene/Promoter Selection:
(Damian Smedley, Nadia Rosenthal)

» **Create** – categorize existing Cre lines

» **Create survey** - collecting requests from the scientific community

» **Coordinate** production with e.g. NorCOMM2, CanEuCre, Pleiades, NIH Blueprint, Allen Brain Institute, Riken, Jax

» **Experts** opinions and suggestions

» **Identify specific expression** - Eurexpress (embryo), GXD (adult), Sanger LacZ

» **Remake** most popular cre lines not on C57BL6/N avaibale

» More than 250 genes have been selected
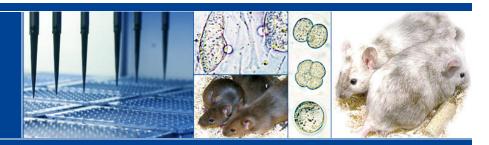
Overall summary of Cre driver selection

| ANALYSIS	DRIVERS
Suggested genes from survey	17
Suggested tissues from survey	18
Specific adult expression identified from Eurexpress	64
Specific adult expression identified from GXD	94
Specific adult expression identified from BioGPS	71
Sanger LacZ expression	11
Popular Cre lines to remake as inducible B6N	41
Tissues not covered by current Cre lines	7
Expert suggestions	26
TOTAL	**349**
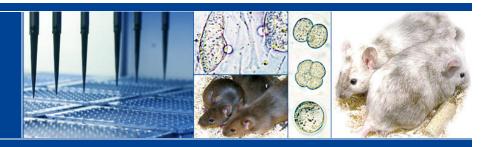
Selection status:

» Curated to a non-redundant list of 275 genes with official MGI symbols and IDs

» Excluded those with a known Cre line at www.creline.org

» Excluded those with a Cre line in the GENSAT, CDN or ICS projects

» Identify specific expression in normal adult samples at GXD, ArrayExpress and BioGPS (GNF microarray datasets) with possible backup from Eurexpress embryonic expression patterns
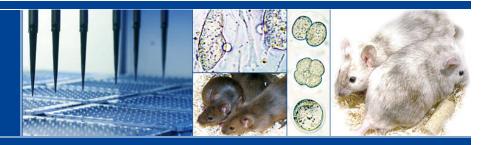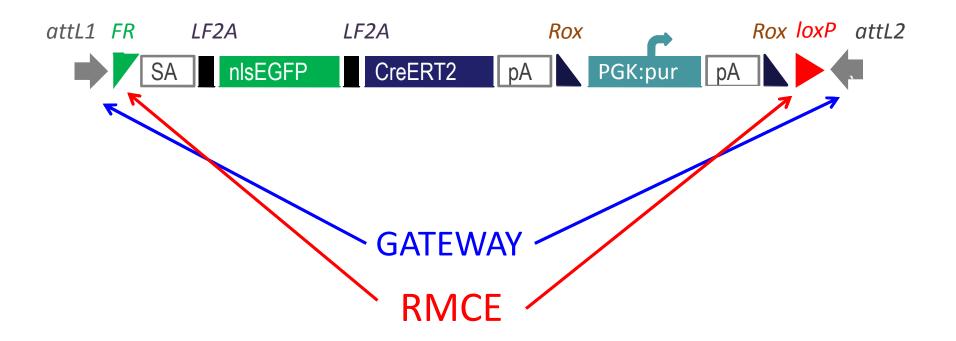
Strategy: Cre Knock-ins

» Exploit EUCOMM/KOMP Modular Vector Resources to rapidly build a set of Cre Knock-ins

  » Mixture of re-targeting and RMCE

» Using Cre and inducible CreERT2

» CreERT2 Knock-in vector design

  » Vector includes nlsEGFP reporter

  » T2A or LF2A for signal sequence containing genes

» Comparison of Targeted Knock-ins versus BAC RMCE

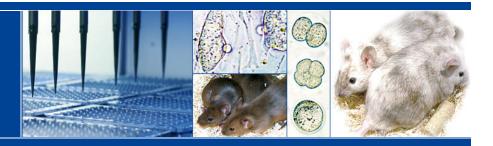 » Coordination with Haydn Prosser from Bradley laboratory
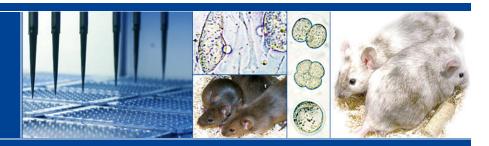
» **CreERT2 Gateway/RMCE Cassette:**

attL1	FR	LF2A		LF2A		Rox		Rox	loxP	attL2
	SA	nlsEGFP	CreERT2	pA	PGK:pur	pA				

GATEWAY

RMCE

Barry Rosen, Bill Skarnes

Considerations of EUCOMMTools Cre Knock-ins

» Simple to make with existing technology and resources

» **Pro's of Cre-Knock-ins**

- Re-utilization of previously validated rate

  – No new technology needed, only CreERT2 vector cassette

- Knocked-in Cre should faithfully recapitulate endogenous expression

» **Con's of Cre Knock-ins**

- Difficult to breed animals to homozygosity in most cases

- Potential for haplo-insufficient phenotypes
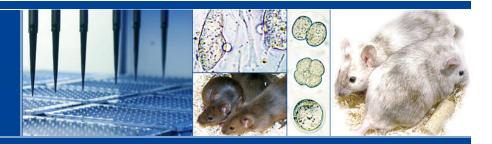
Alternative Cre Alleles

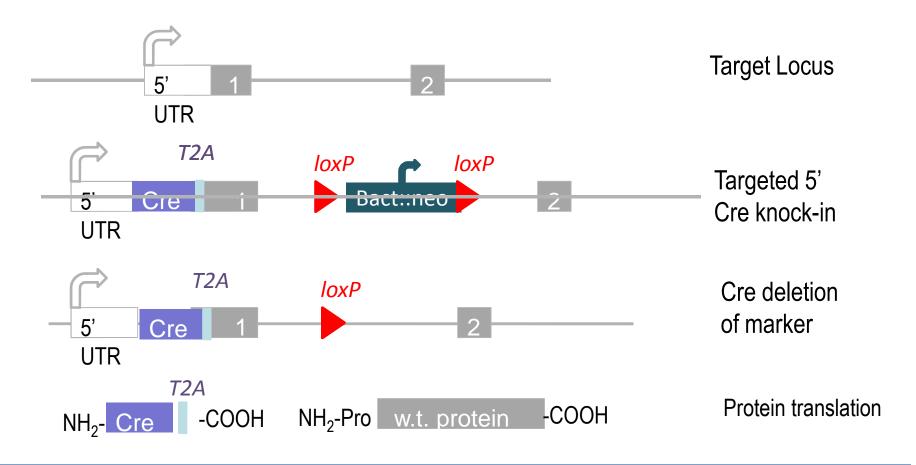## 3'UTR IRES Cre Insertion
Leaves endogenous protein intact

2A Mediated Cre 3' Insertion
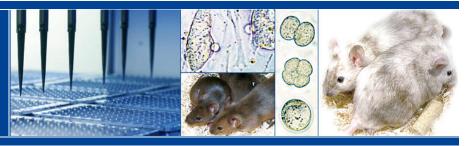N-terminal fusion, adds COOH extension to endogenous protein

Alternative Cre alleles: 5' Cre 2A-mediated insertion

Target Locus

Targeted 5' Cre knock-in

Cre deletion of marker

Protein translation

Pros and Cons of Different Alternative Cre Alleles

Pro

Con

Universal LF2ACre Knock-in

Pro:
- Applicable to all genes
- Exploits existing IKMC resources

Con:
- Still deletes an allele

3'UTR IRES Cre Insertion

Pro:
- Applicable to all genes
- Endogenous protein intact
- Validated in literature
- HT vector construction

Con:
- Reduced Cre from IRES'
- new vector, no RMCE

2A Mediated Cre 3' Insertion

Pro:
- Should express high levels of Cre

Con:
- Adds 3' aa's to endogenous protein
- Additional aa's with LF2A
- new vector, no RMCE

5' Cre 2A-mediated insertion

Pro:
- Minimal perturbation of locus
- Promoter specific applications

Con:
- Initiating ATG must be defined carefully
- new vector, no RMCE

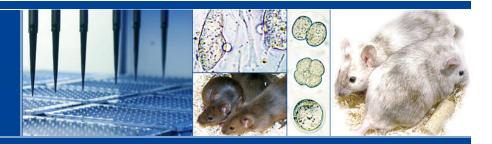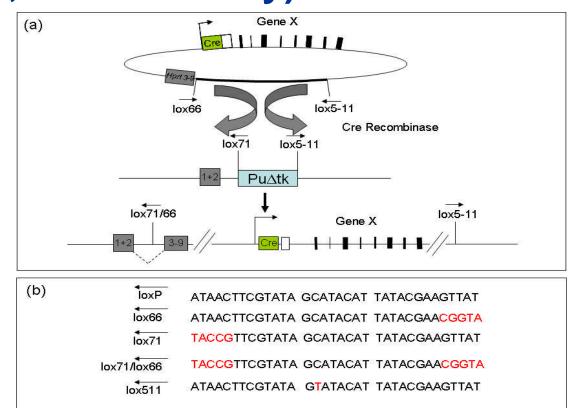# CreERT2 Knock-ins: Progress Re-Targeting

» 49 Intermediate Vectors Identified

» Criteria: Targeted ES Cells Status in EUCOMM/KOMP

» Avoid signal sequence/secreted for moment(50% of genes)
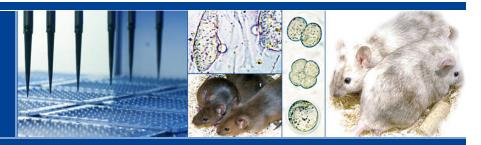
EUCOMMTOOLS – BAC knock-in Technology (Hayden Prosser, Allan Bradley)

RMCE strategy for introducing BAC eGFPT2ACreERT2/Cre-T2A-eGFP transgenes into the ES cell genome

Cre-BAC transgenesis by site specific integration
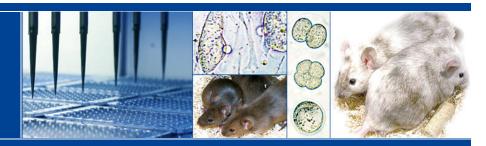
Pros:

» Can use Human or Mouse BACs
» Avoids disruption of endogenous alleles as would occur in targeted Cre knock-in to "Driver" gene
» Avoids variation that might occur with random integration
» Cassette exchange removes extraneous prokaryotic vector DNA
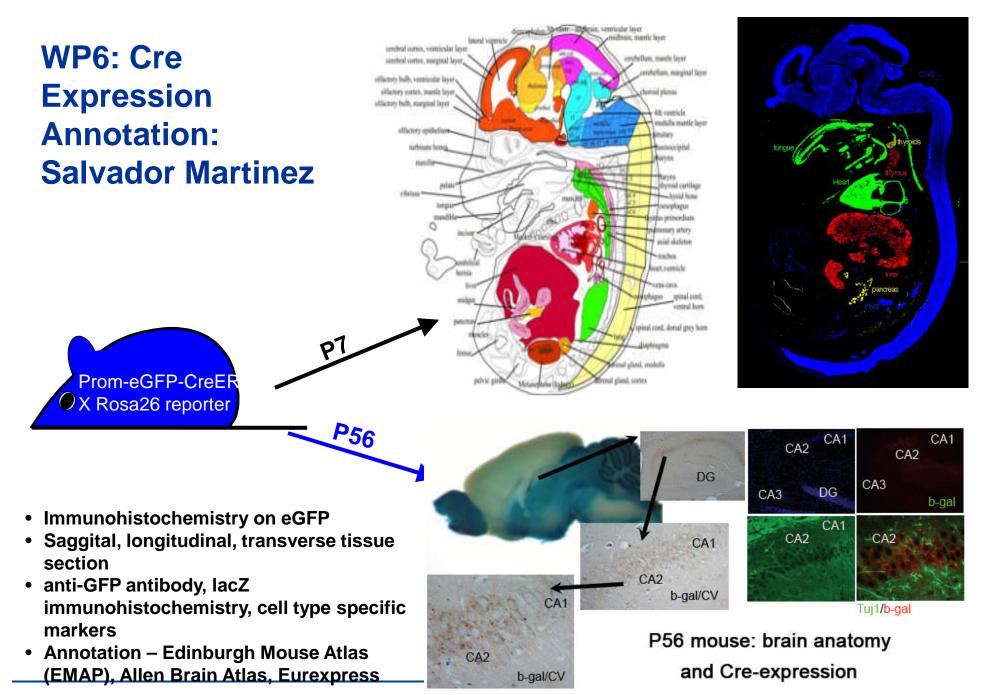
Cons:

» Integrated BACs do exhibit deletions in a proportion of cases
» "Passenger" genes may be associated with Cre driver transgene
» Gene size limits
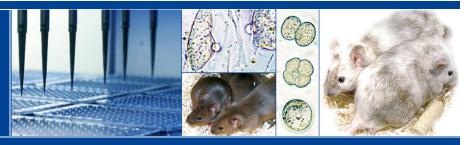
» Currently:

» Direct comparison of Knock-in versus Bac-knock-in strategy!

WP6: Cre Expression Annotation: Salvador Martinez

Prom-eGFP-CreER X Rosa26 reporter

P7

P56

- Immunohistochemistry on eGFP
- Saggital, longitudinal, transverse tissue section
- anti-GFP antibody, lacZ immunohistochemistry, cell type specific markers
- Annotation – Edinburgh Mouse Atlas (EMAP), Allen Brain Atlas, Eurexpress

P56 mouse: brain anatomy and Cre-expression

WP6: Cre Expression Annotation

P7 mouse:
9 sections sagittal plane (10 series) +
20 sections transversal plane (10 series)

90 sections/mouse
250genesx3samples: 750x90sections: 67.700 sections-slides
Public images and reports: 250genes x 9sections=2.250 images

EUCOMM: Tools for Functional Annotation of the Mouse Genome

DESCIBE GENE EPRESSION IN RELATION TO ANATOMY (CELLS IN THE SPACE)

Cells (neurons+glia) in the space: BRAIN

E14.5

P
AV
AD

Ep7

Hi
DG   Hb   PT
MOTIV
CONC   DM   rf
                DM   DP
        AM   VM
        PTh
LIMBIC   MOTOR

H

EUCOMM
Tools
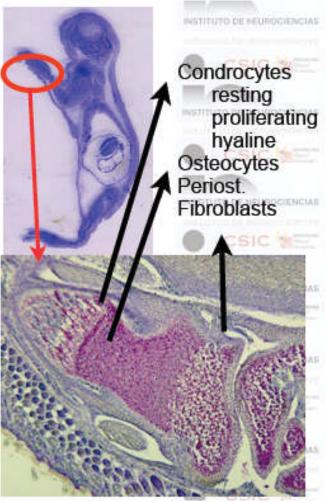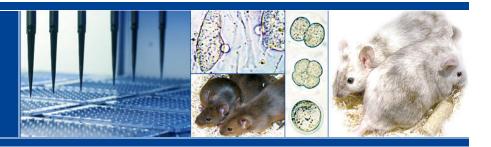
P7 mouse: 15 sections sagittal plane
NEW BODY ORGANS ONTOLOGY

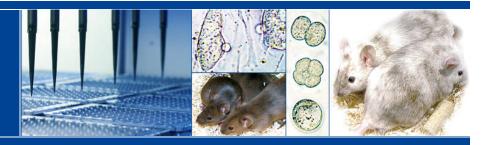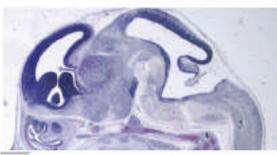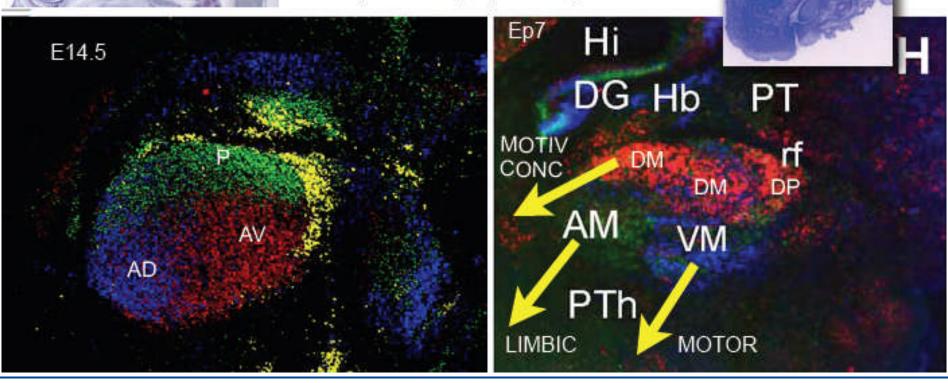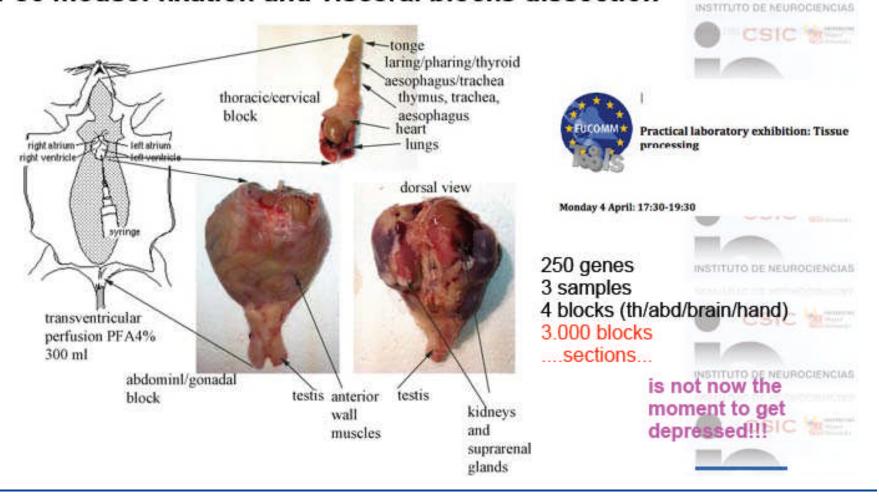| system/organ	structure	tissues	cell
cardiovascular system			
	arterial system		
	carotid artery		
	aorta		
	principal artery to forelimb		
	pulmonary artery		
	iliac artery		
	principal artery to hindlimb		
heart			
	pericardium		
	atrium		
		interatrial septum	
		left atrium	
		right atrium	
	valve		
		aortic valve	
		mitral valve	
		pulmonary valve	
		tricuspid valve	
	ventricle		
		interventricular septum	
		left ventricle	
		right ventricle	

536 terms⊣

STEPS TO DEVELOP FOR THE DESCRIPTIVE WORK: ANNOTATION OF CRE EXPRESSION IN RELATION TO THE GENE EXPRESSION.

WE NEED AN ONTOLOGY

Condrocytes
resting
proliferating
hyaline
Osteocytes
Periost.
Fibroblasts
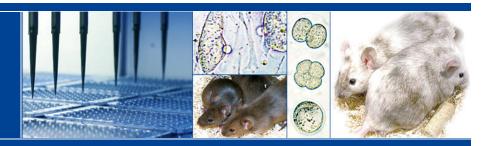
P56 mouse: fixation and visceral blocks dissection

thoracic/cervical block
- tonge
- laring/pharing/thyroid
- aesophagus/trachea
- thymus, trachea, aesophagus
- heart
- lungs

right atrium
right ventricle
left atrium
left ventricle

syringe

transventricular perfusion PFA4% 300 ml

abdominl/gonadal block

testis anterior wall muscles

dorsal view

testis

kidneys and suprarenal glands

Practical laboratory exhibition: Tissue processing

Monday 4 April: 17:30-19:30

250 genes
3 samples
4 blocks (th/abd/brain/hand)
3.000 blocks
....sections...

is not now the moment to get depressed!!!

INSTITUTO DE NEUROCIENCIAS
CSIC

EUCOMM: Tools for Functional Annotation of the Mouse Genome

THORACIC/CERVICAL REGION	522 sections/ gene
ABDOMINAL REGION	3600 sections/gene
BRAINS, transversal sections	396 sections/gene
BRAINS, sagittal sections	255 sections/gene
TOTAL	**4783** sections/gene

WORK TO DO

WORK TO SHOW

In practical terms:
29 sections THX
200 sections ABD
22 (Brain T)
10 (Brain S)
261 SECTIONS/GENE

?......show each single one section with a large annotation ontology

.....only selected illustrtative sections and the annotation report

P56 mouse: brain anatomy
and Cre-expression

Richard Baldock
MRC Human Genetics Unit
Edinburgh

COLOURED CAPITAL BOLD --> direct annotation
Lower case black --> propagated annotation.
Lower case grey --> not annotated
Annotation Colour Codes:

Gene Symbol:	Tpm1	Assay ID:	euxassay_009503
Gene Description:	tropomyosin 1, alpha	Template ID:	T36871
Embryo Age:	14.5 dpc	Sanger Viewer:	N/A
Syn Expression:	N/A	Annotation Summary:	strong regional (300 annotations on 31 structures)

Assay Information Gene Information Expression Chart Similar Assays Expression Links

cerebellum
  cerebellar cortex
    molec. layerular
      neurons
        BC
        SC
      glia
        Olig
        Astro

  PC Layer
    neurons
      PC
      LC
    glia
      BergC

  Granular layer
    neurons
      GranC
      GolC
      LugC
      Brush(
    glia
      Olig
      Astro

Annotation Tools
Annotation report
Public page

P7: 2.250 images
P56: 4.000 images

specificity

GFAP/b-gal

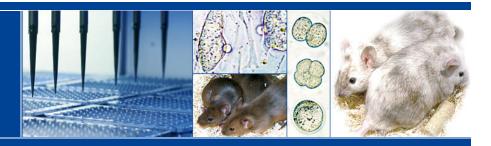**IMPC Meeting, 28th-29th September, Washington, USA**

Distribution of EUCOMMTOOLS Material

Dissemination of
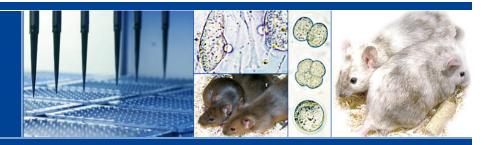- **mutant ES cell lines**
  http//:www.eummcr.org

- **mouse lines**
  http//:www.emma.org

Dissemination asap as QC confirmed
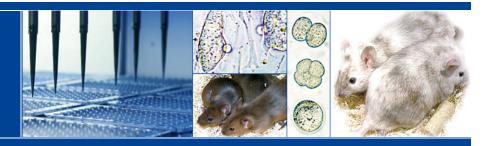
EUCOMMTOOLS integration in IKMC portal (www.knockoutmouse.org)

"one stop shop" portal

Long term **SUSTAINABILITY**

EUCOMM: Tools for Functional Annotation of the Mouse Genome

» **Outlook:**

» **Technology and reagent development**

  » **IKMC toolkit extension**

  » **New ligand/SSR-LBD pairs**

  » **New conditional switches**

  » **Additional optogenetic tools, markers, cell survival**

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