Genome Editing Opportunities for reduction?



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Molecular and Cellular Biology Generation of new GA lines and archiving

Breeding and Colony Management Phenotyping and *in vivo* experiments *Ex-vivo* testing and Pathology



Genome editing: Supporting the generation of mouse models for biomedical research









Genome editing: Capacity, Allele validation and versatility

- ► Deletions/Point mutation/Base insertion
- Mouse models with mutation thought to be causative of human disease, GWAS candidates, ...
- ▶ Integration of small cassettes (loxP, tag) in embryos
- ► Large locus integration (i.e. Humanisation)
- ► ES cell and one-cell embryo
- Any genetic background
- ► Increasingly versatile and efficient







CRISPR-induced mutants: an assembly line



Genome Editing capacity: 150 new lines/year

• Over 250 projects in the pipeline, 180 analysed:

	Indel	Deletion	PM and other HR
Percentage of mutated founders / F0 born	38.5	ND	24.6
Percentage of expected mutants / F0 born	28.5 15.7		6.8
Frequency of animal with desired mutation	1/3.5	1/6	1/15



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Deletions with CRISPR can yield many artefacts



Generation of a point mutation with CRISPR



- Mutation Cdh23^{753A} = synonymous SNP in exon 7 which causes inframe skipping of exon 7
 - ✓ SNP associated with Age-related Hearing Loss (AHL)





Mosaicism in F0 mice and illegitimate repair

Cdh23 target, F0 #30 ear clip genotyping results:



- Allele 1 = NHEJ repair \rightarrow 24 nt deletion
- Allele 2 = Illegitimate repair \rightarrow Correct repair at the target + 24 nt deletion

Cdh23 target, alleles found in F1s (#30 offspring):



- Allele 1 = NHEJ repair \rightarrow 24 nt deletion
- Allele 2 = Illegitimate repair \rightarrow Correct repair at the target + 24 nt deletion
- Allele 3 = WT
- Allele 4 = HDR \rightarrow Correctly repaired



Genotype complexity and mosaicism



Correcting inbred defects



Cdh23^{ahl/ahl} is the genotype of the background strain and is not regulated.

Cdh23^{ahl/755A>G} is currently regulated, although we can apply to have it removed from the act.



Genome Editing screening and validation



Small indel, Point Mutation

- **Sequencing of all F_0s**
- ▶ PCR products sub-cloning and sequencing for selected animals

Deletion

► PCR across deleted fragment

All allele types

- ▶ Whenever possible, PCR-based pre-screen
- ► Sequencing of all F₁s to be carried forward



Screening of F_0s - Genotyping of F_1s

Analysing the outcome of CRISPR-aided genome editing in embryos: Screening, genotyping and quality control

Joffrey Mianné, Gemma Codner, Adam Caulder, Rachel Fell, Marie Hutchison, Ruairidh King, Michelle E. Stewart, Sara Wells, Lydia Teboul

PII:S1046-2023(16)30270-5DOI:http://dx.doi.org/10.1016/j.ymeth.2017.03.016Reference:YMETH 4168

To appear in:

Methods





Phenotyping of F₀s?

Mamm Genome (2017) 28:377–382 DOI 10.1007/s00335-017-9711-x



Phenotyping first-generation genome editing mutants: a new standard?

Lydia Teboul¹ · Stephen A. Murray² · Patrick M. Nolan³

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CRISPR/cas9 aided mutagenesis: Lessons learned

- Repairs following CRISPR cut are error-prone: "illegitimate repairs", rearrangements. Quality control of the alleles obtained is essential and complex.
- ▶ F0s should be treated as mosaic.
- Phenotype data should be acquired from F1 onwards, once the alleles have been segregated and fully characterised (screens can be an exception).







Extending genome editing capacity



Genome editing: Fast evolving technology



Long single stranded DNA donors Conditional design





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Conditional Tm1c first results: Syt7

Η

• $F_0 #2 \rightarrow$ Homozygous for the repair?



3'-LoxP

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ddPCR screening for random insertion

- LoxP PCR informs of the presence/absence of 3' and 5' LoxP sites, but unable to indicate whether there are random integrations/large deletions present.
- TaqMan assay centred on CR through ddPCR







Analysis of Syt7 cKO – line 2

		1				
	Animal	PCR Result		ddPCR result		
	F ₀ #2	Correct mutant: Homozygous		2.78 (3 copies)		
			Bre	eedi	ing gave rise to 8 F1 mic	
A	nimal		PCR Result		ddPCR result	
#2	2 offspring 1		WT		1	
#2	2 offspring 2		WT		2	
#2	2 offspring 3		Correct mutant		3	
#2	2 offspring 4		WT		3	
#2	2 offspring 5		Correct mutant		2	
#2	2 offspring 6		Correct mutant		2	
#2	2 offspring 7		Correct mutant		3	
#2	2 offspring 8		WT		3	





Additional base changes



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Allele rearrangements









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New opportunities for mouse models







Genome Editing

- Humanised genes
- Modified existing models
- Modified backgrounds
- Controlled heterogeneity

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Breeding and Archiving

- Large cohort breeds
- Oligogenic breeds
- Archiving of intermediate generations

Phenotyping

- Automated platforms
- Data capture LIMS
- Analysis pipelines





THE	10					
The ten most studied genes of all time are described in more than 40,000 papers.						
1 TP53	8 479 citations					
2 TNF	5,314					
3 EGFR	4,583					
4 VEGFA	4,059					
5 APOE	3,977					
6 IL6	3,930					
7 TGFB1	3,715					
8 MTHFR	3,256					
9 ESR1	2,864					
10 AKT1	2,791					



Out of the 20,000 or so protein-coding genes in the human genome, just 100 account for more than one-quarter of the papers tagged by the NLM. Thousands go unstudied in any given year.



Mice and many many genes

- There are mouse models for less than 50% of genes
- There are many different models foe lots of the same genes
- There are many papers on selected models of mice
- (Apo E KO mice= over 6k papers)





This is just the start...

There's the rub: it takes a certain confluence of biology, societal pressure, business opportunity and medical need for any gene to become more studied than any other. But once it has made it to the upper echelons, there's a "level of conservatism with certain genes emerging as safe bets and then persisting until conditions change".





Wider use of mouse models





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Modelling Human Mutations



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Humanising the mouse

Genes, Physiology and Behaviour = same but not identical!!!

- Mice aren't little humans
- The differences are important





Can we close the gap?





Critique of the mouse as a model system

- Knockouts don't model human gene changes
- Mouse data doesn't translate to human drug trials







Humanising mouse genes



Kymouse[™] has full human antibody system





Controlled Heterogeneity



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The New Age of Genetically Altered (GA) mice (another one!)

Genome Editing (CRISPR/Cas9)

- Rapid establishment of genome editing Expansion of Molecular Biology teams
- Change in the type of models generated
 - More diverse
 - More refined
 - Potentially more unpredictable

What differences will we see in animal facilities??

- More complex crosses
- More different lines •
- More unpredictability







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Mousing human behaviour??



Should we be doing certain tests at night???

- Anxiety
- Cognition





Summary



- Animal numbers likely to increase as a result of genome editing
- More species



- Sophistication of mouse alleles
- Refinement towards human models
- Better translatable science that in the long term- will reduce animal numbers

