Genome editing in laboratory animals, ethical use and welfare challenge.

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The Mary Lyon Centre – MRC Harwell Institute

- Over 20 years of experience in genome engineering
- Generation of new mutants (>150 new models/year)
- Breeding (>50,000 mice housed)
- Archiving and distribution of mice
- I am a user (and developer) of genome editing, not a philosopher. There are more questions than answers.

Use of animals in research

Premises of this talk:

1. We are using research on animals to improve human health

2. We ought to use research on animals to improve human health

Outline

- Genome Editing, a disruptive technology
- 3Rs
- The 4th R
- The conversation

The special case of Genome Editing

- How is genome editing a disruptive technology?
- How does genome editing change how we implement the ethical imperatives of the 3Rs?

New considerations

- **The bespoke (mouse) model of a rare disease:**

  -> patient perspective ("their mutation", Acceptable/Wished for that research is undertaken 'In their name")? Counselling?

  -> when is sufficient evidence obtained to justify the creation of an animal model?

- **The increased sophistication of the animal model and the animal house**

- **The mouse as a pre-clinical research tool for exploring the safety and efficacy of human assisted reproductive technologies**

-> The new genome editing technologies trigger new ethical debates for our societies.

The special case of Genome Editing

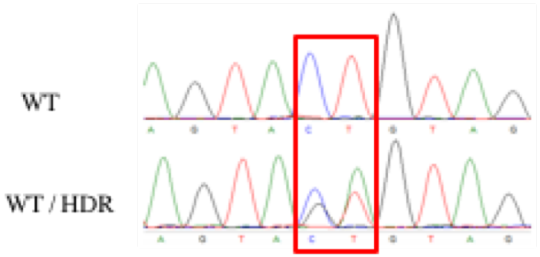
- How is genome editing a disruptive technology?
- How does genome editing change how we implement the ethical imperatives of the 3Rs?

3Rs

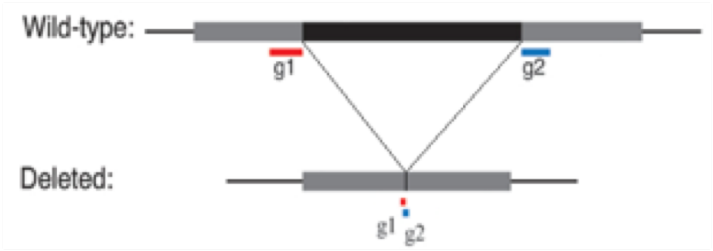
- **Reduction: Distinguishing between per-experiment reduction and overall reduction**

- **Indel(s) / Knock-out(s)** ✔

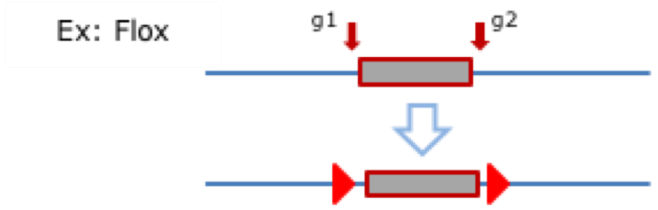
- **Point mutation(s)** ✔



- **Tailored deletion(s)** ✔



- **Gene targeting** ✔



Genome Editing capacity: 150 new lines/year

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- Over 250 projects in the pipeline, 180 analysed:

	Indel	Deletion	PM
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**
Completed projects so far	**22**	**56**	**32**
Positive founder produced	**8**	**31**	**14**

3Rs

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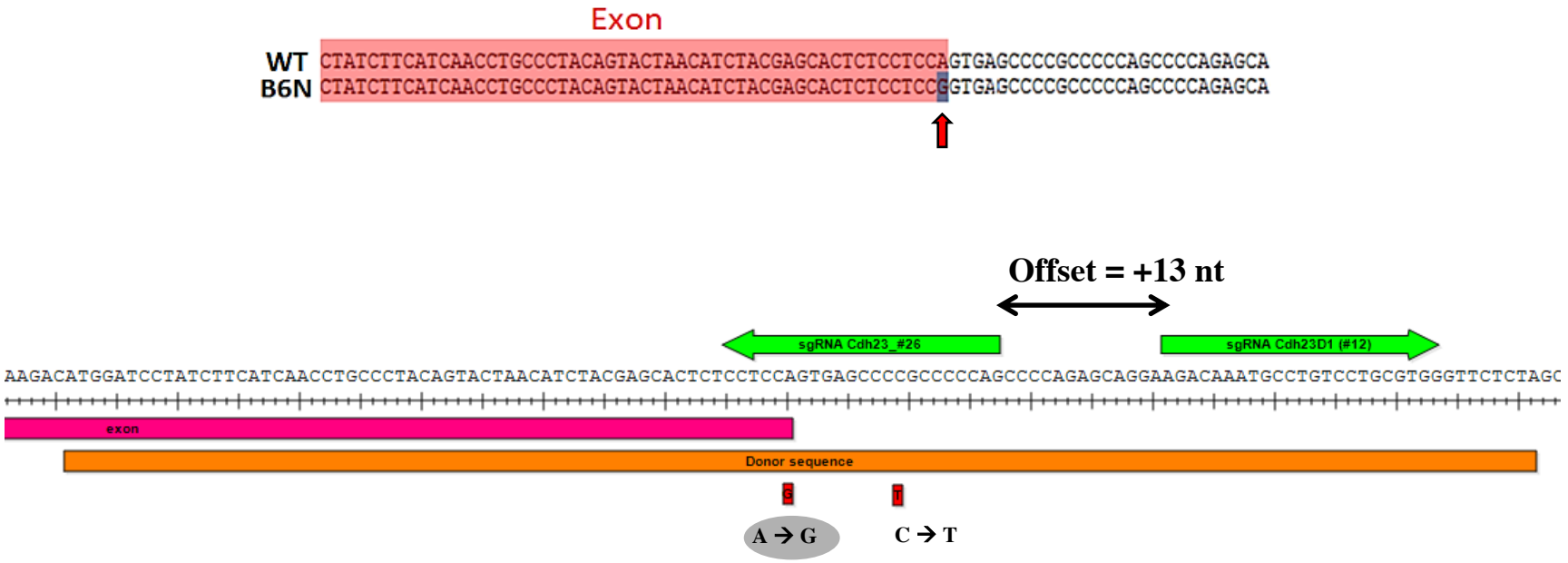
- **Refinement: Know your model's limitations.**

Example of phenotyping of G_0s

"the CRISPR/Cas system allows the one-step generation of animals carrying mutations in multiple genes, an approach that will greatly accelerate the in vivo study of functionally redundant genes and of epistatic gene interactions."
(Wang et al. 2013, *Cell).*

Generation of a point mutation with CRISPR

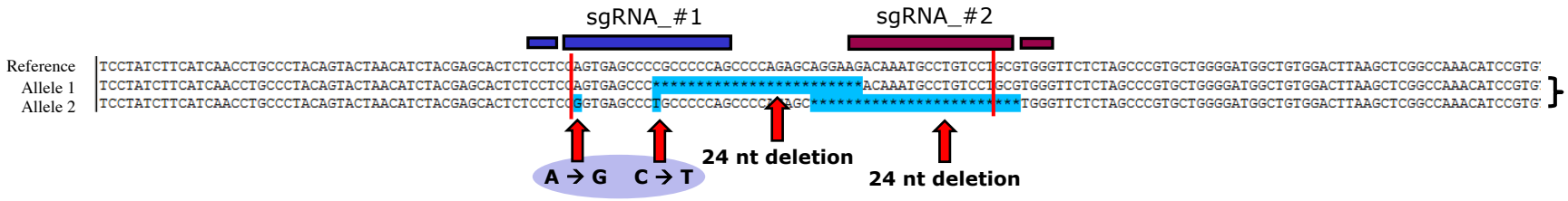
- Mutation repair of *Cdh23*^{753A} SNP associated with Age-related Hearing Loss (AHL)



Mianné et al., 2016, Genome Medicine.

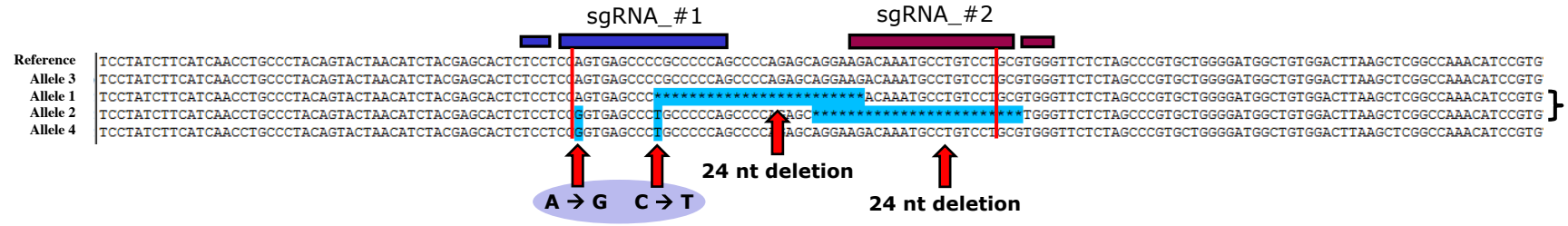
Unpredictable repair and mosaicism in G_0 mice

Cdh23 target, F0 #30 ear clip genotyping results:



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

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



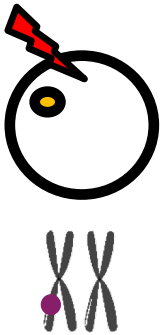
- Allele 1 = NHEJ repair → 24 nt deletion
- Allele 2 = Illegitimate repair → Correct repair at the target + 24 nt deletion
- Allele 3 = WT
- Allele 4 = HDR → Correctly repaired

Genotype complexity and mosaicism

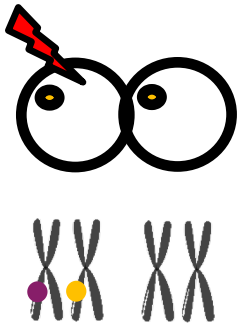
- Cas9 mRNA
- sgRNA(s)
- DNA donor template

Pronuclear injection in 1 cell stage embryos

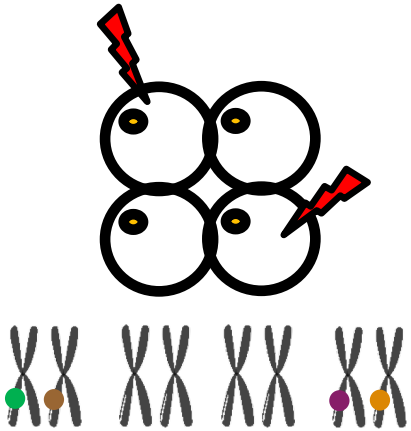
= Mutagenesis event



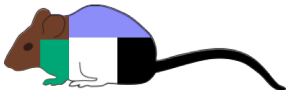
Mutant heterozygous
(2 alleles)



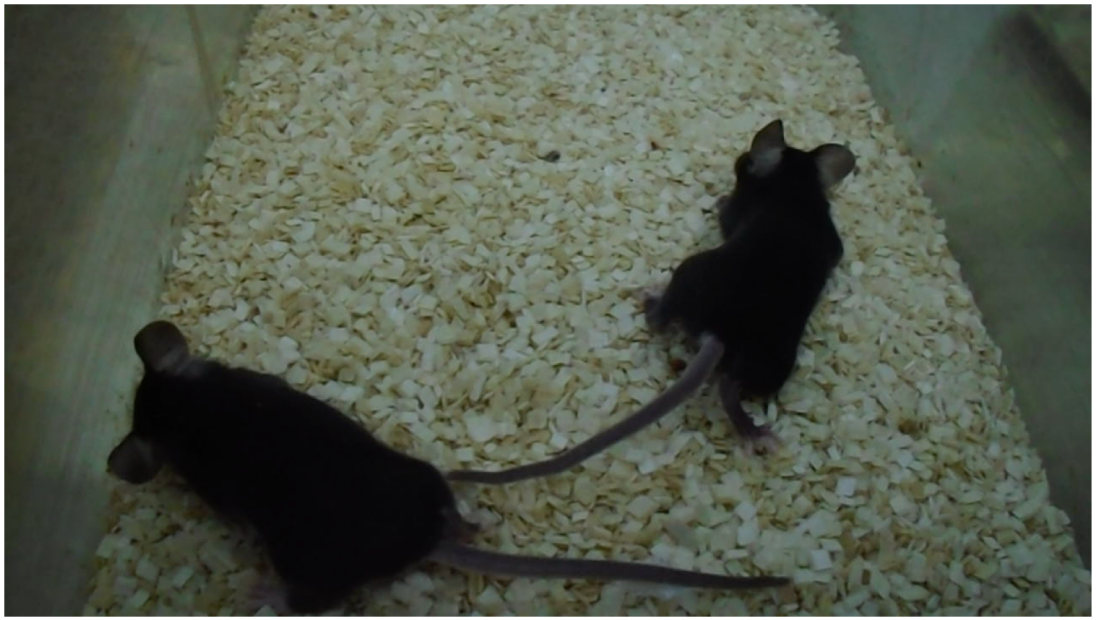
Mutant mosaic
(3 alleles)

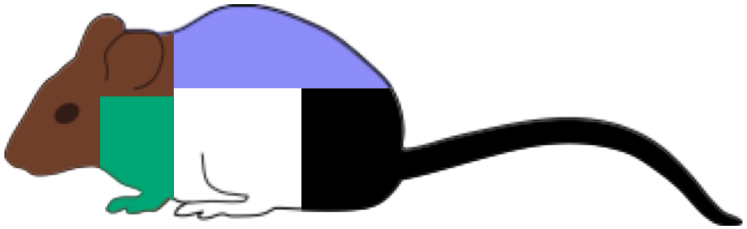


Mutant mosaic
(5 alleles)



Founder animals can display welfare issue





3Rs

- **Replacement:**

Increased accessibility of other models, including human and other species – places a greater onus on scientists to justify use of a particular animal model.

A better model could be a more evolved organism.

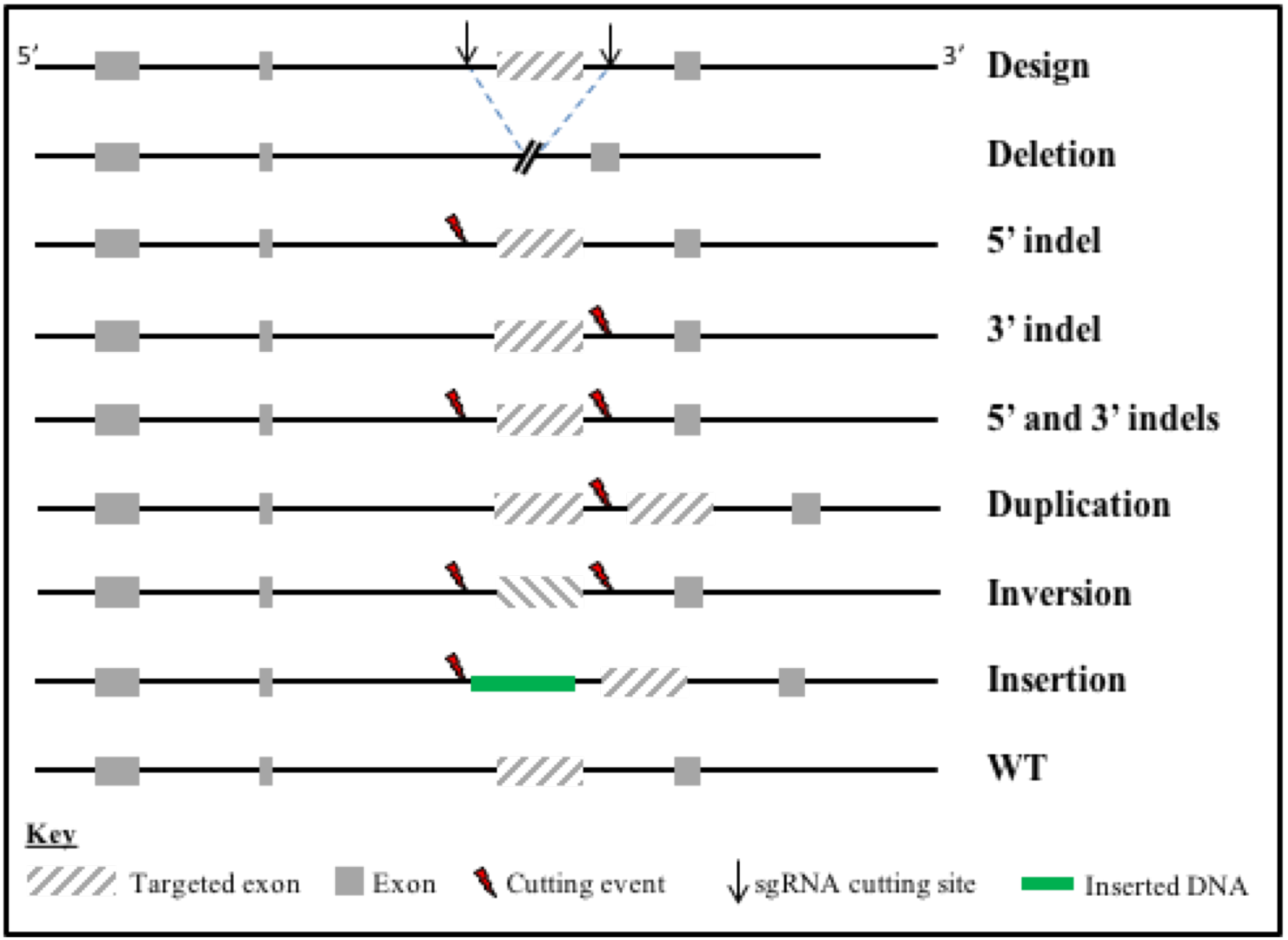
The 4th R: Reproducibility

- **Reproducibility:**

There can not be any justification for the use of animals in research if the research is not reproducible.

Genome editing tools just cut DNA, they do not control the sequence of the new mutations.

Use od 2 sgRNAs can yield many artefacts

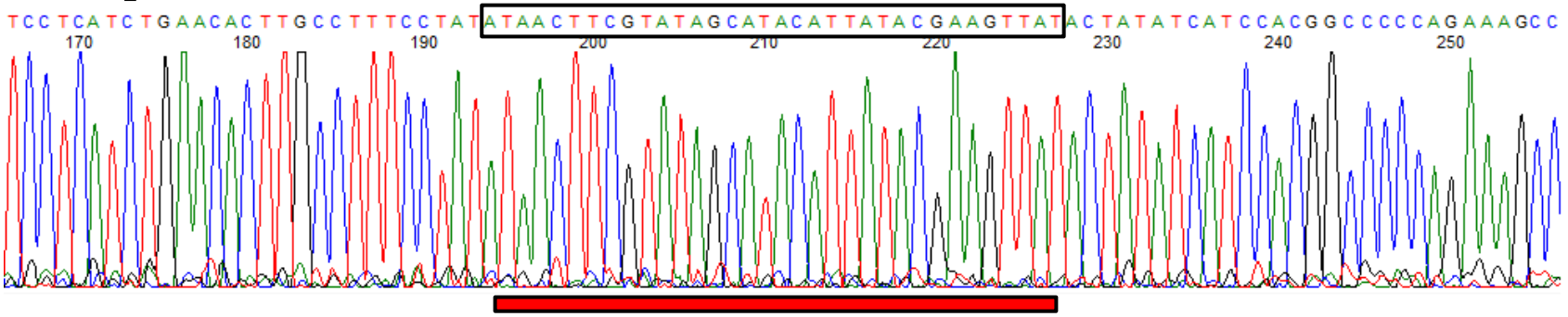


Mianné *et al*., 2017, Methods.

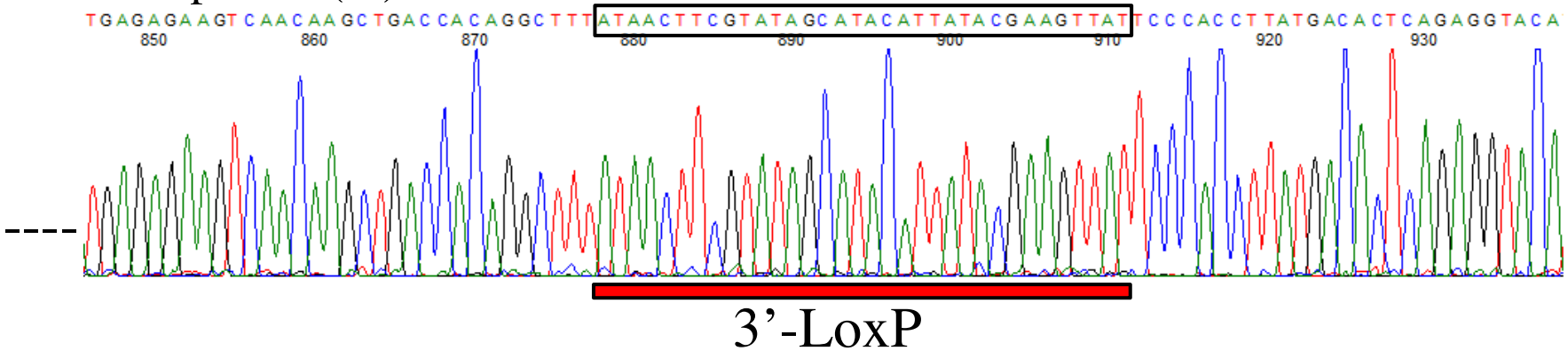
Conditional allele: Full validation is a complex exercise

- G_0 #2 → Homozygous for the repair?

Forward primer (5')



Reverse primer (3')



Critical region all correct!

Beware of additional sequence changes

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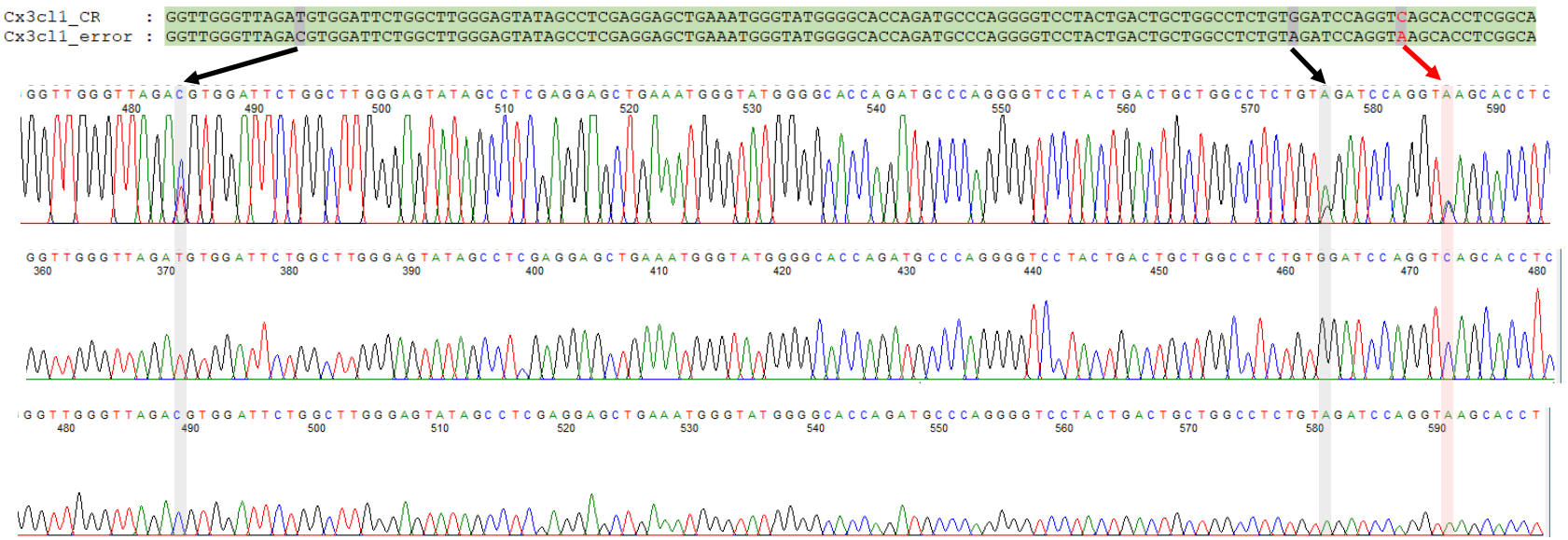
b



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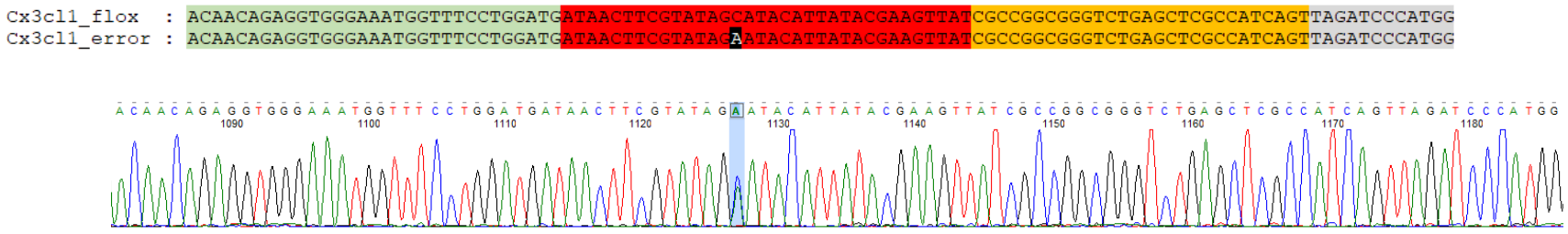

Analysis of a new cKO line

Animal	PCR Result	ddPCR result
F_0 #2	Correct mutant: Homozygous	2.78 (3 copies)

Breeding gave rise to 8 F1 mice

Animal	PCR Result	ddPCR result
#2 offspring 1	WT	1
#2 offspring 2	WT	2
#2 offspring 3	Correct mutant	3
#2 offspring 4	WT	3
#2 offspring 5	Correct mutant	2
#2 offspring 6	Correct mutant	2
#2 offspring 7	Correct mutant	3
#2 offspring 8	WT	3

Evidence of additional random integration of the donor.

Genome editing versatility

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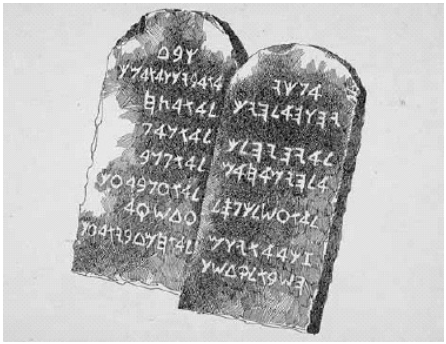
	Syt7	*Ikzf2*	*USP45*	*Syt4*	*Rapgef5*
ssDNA size (nt)	1149	1107	914	1567	899
Nb of pups	17	17	19	67	30
Nb of mutants	10	5	6	32	12
Mutant with exon deletion	3	3	2	3	5
Animals with 2 sides cut	29%	24%	11%	9%	20%
Mutant with floxed allele	**2**	**1**	**0**	**3**	**1**
GLT	Yes	Yes		Yes	Founder dead

= a greater burden on allele validation

Standards for allele validation

- G_0s should be treated as mosaic and can be screened for the presence of a mutation.
- New alleles should be fully sequenced in G_1s.
- Model validation should also include
  - copy counting of deleted segments,
  - copy counting of donor sequences,
  - Sequencing of off-target sites linked to locus of interest.



The challenge of genome editing is becoming **validation**.

The conversations

Society

- Importance of engaging stakeholders and “public”

Professionals

- Researchers, medics and animal technologists

Importance of engaging stakeholders & 'publics'

- Important because political legitimisation is essential.
- Does involve offering an ethical justification.
- Requires a lot of engagement from scientists on this issue.
- Literature on the ethics of animal experimentation (speciesism, utilitarianism, animal rights etc) all still applies.
- Acknowledge the technology underpins working in close relation with the clinic (and therefore the patients!).

Importance of training of researchers, medics and animal technologists

- Expertise: This is still a new technology, we are all learning about it.
- Need to agree on standard for best practice: ie case of archiving, how do we control genetic integrity of new models.
- Not all about regulation, character and culture (Greenfield, 2017, Mammalian Genome).

Character and culture

"We acknowledge that the UK has the most detailed legislative framework regarding animal research in the world. But…regulation can act as an emotional screen between the researcher and an animal, possibly encouraging researchers to believe that *simply to conform to regulations is to act in a moral way*. It is therefore crucial to promote best practice more actively and to **improve the culture of care** in establishments licensed to conduct experiments using animals."

NCOB, 2005 – "The Ethics of Research Involving Animals"

Summary

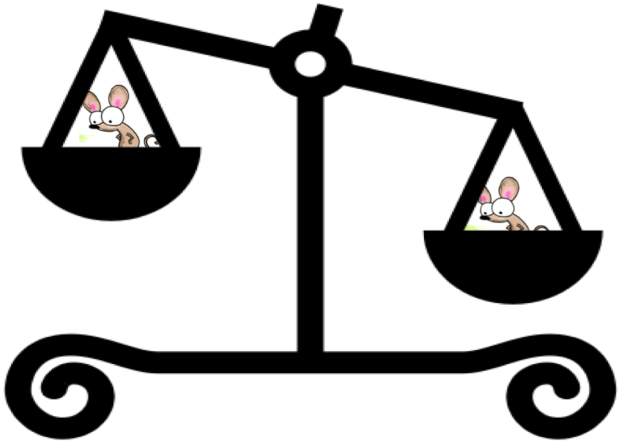
- New technology brings new questions: ethics, 3Rs application, but also thinking of research reproducibility.
- Facing important debates both in society and in scientific community.

Cost

Animal suffering

Research Resources

Public Opinion



Benefits

Health

Fundamental Knowledge

Engagement in Research

Many thanks!

MCB group at Mary Lyon Centre

Gemma Codner

Joffrey Mianné

Colleagues in genome engineering field

Andy Greenfield (Harwell)

MRC Harwell

The Mary Lyon Centre

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