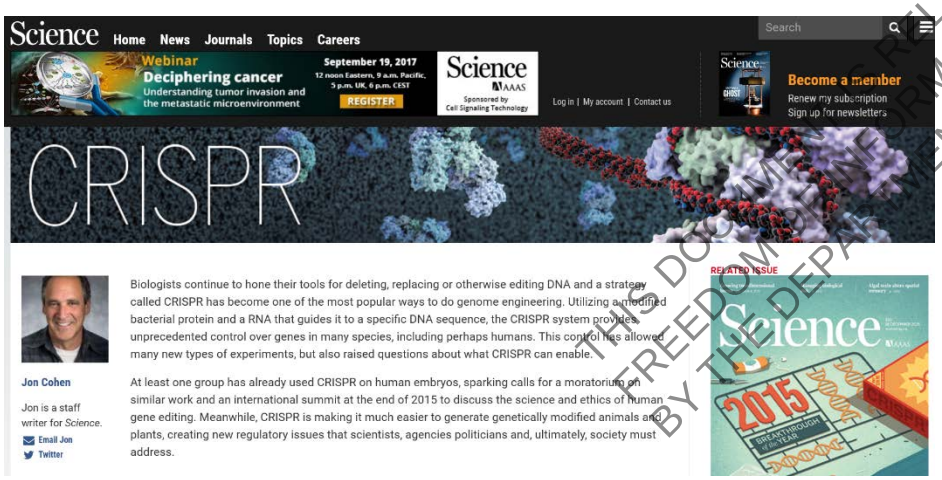
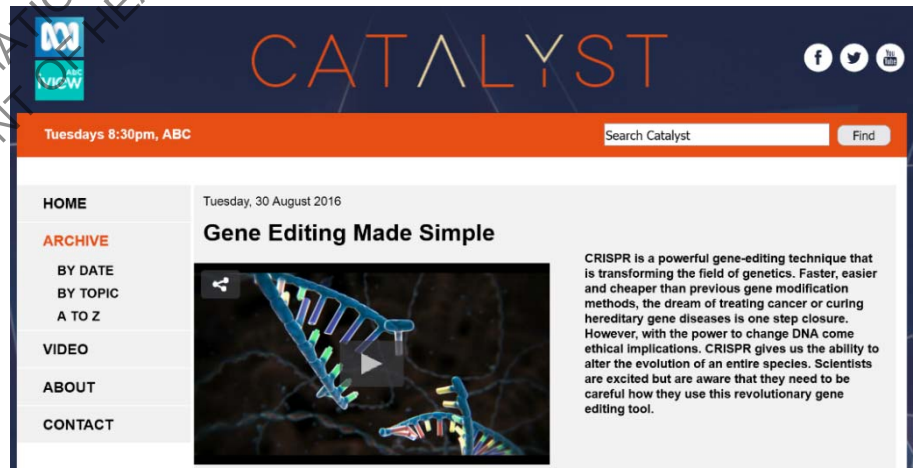
Australian National University

Genome Engineering





David Tscharke, John Curtin School of Medical Research, ANU

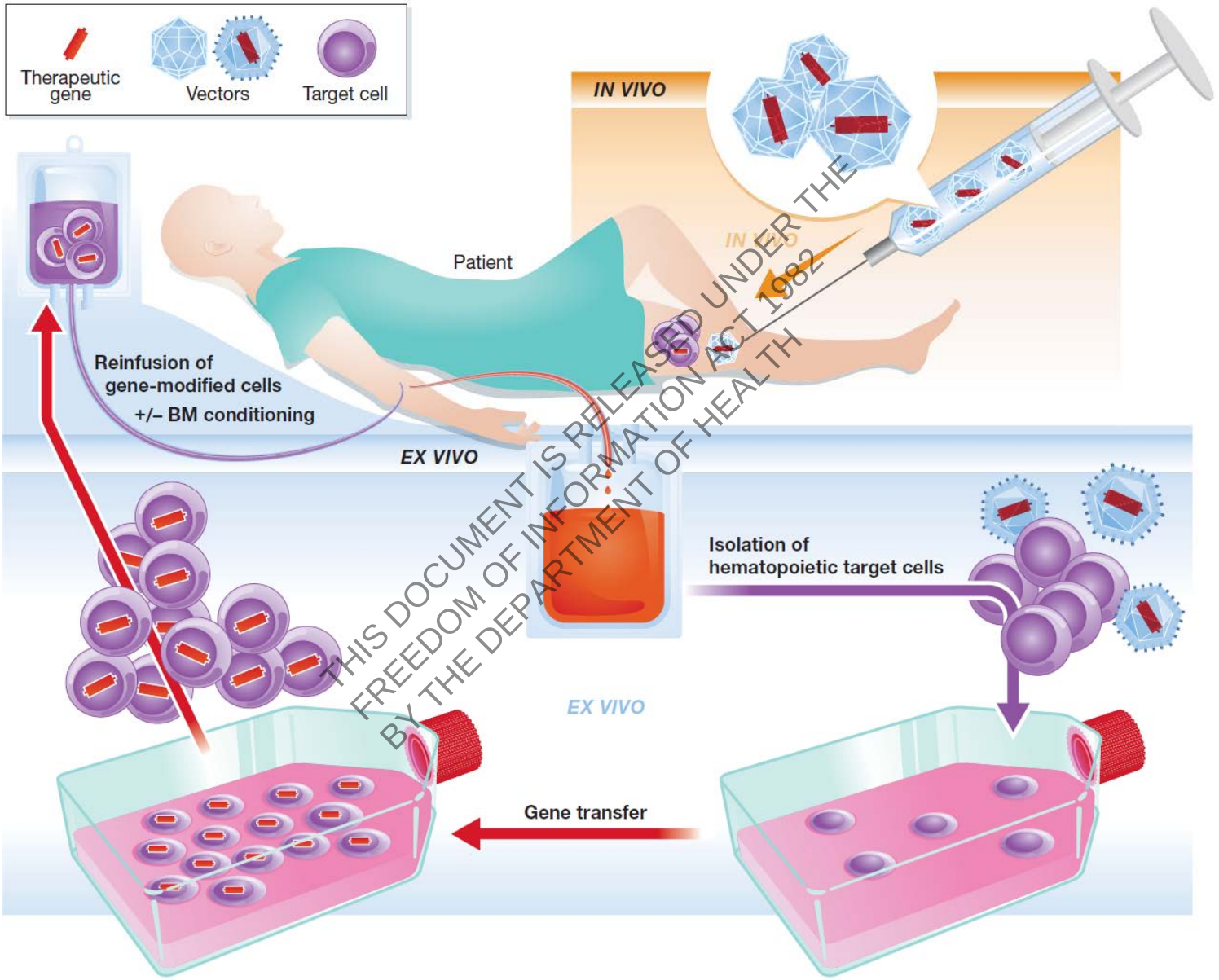
A Brief History of Genetic Modification

- **The first GMO was made in 1973**
  - **Bacteria carrying DNA of another species**
- **Most manipulation of DNA has been done using short fragments in test tubes and bacterial systems**
- **This DNA can be transferred to human / animal cells to add something new to their genome**
  - **Naked DNA or packaged into a virus 'vector' for delivery**
  - **Insertion is random → unexpected consequences**
  - **Could be inserted into fertilized ovum to make an animal**
- **First targeted transgenic animal (mouse) made in 1987**
  - **Genes could be removed or changed in an animal**

A brief history of genetic modification II

- Polymerase chain reaction (PCR) and chemical synthesis of DNA made things faster
- Methods like polymerase chain reaction (PCR) and chemical synthesis of DNA → synthetic biology
  - Still a long way off making mammals
- RNAi allowed genes to be turned down
  - Has to be a constantly present
- **Achieving a desired genetic change - even in cells in tissue culture remained practically impossible**
  - This is the first step towards therapeutic genome modification

Gene therapy – could only add new DNA



Kaufmann KB *et al*, EMBO Mol Med, 2014

Key limitations

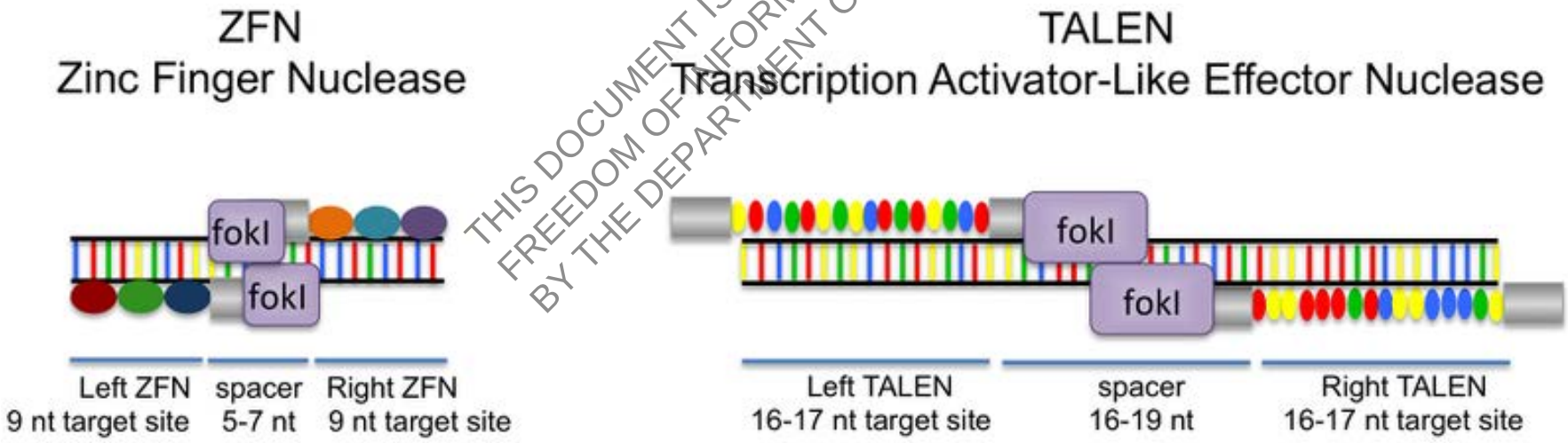
- **Most cutting and modification done *in vitro* not in cells**
  - **Have to get DNA out of cells and back in again**
  - **Limits the length that can be easily modified**
- **The enzymes used to cut had defined recognition sites**
  - **E.g. GAATTC**
  - **They rarely cut exactly where you want**
  - **They cut at many other places**
- **PCR and synthetic DNA methods were helping**
  - **But did not overcome these basic problems**

- **We all wanted a DNA cutting enzyme (a nuclease) that:**
  - **Cuts at a sequence of our choosing**
  - **Would work inside living cells**

Enter targetted nucleases

Zinc finger nucleases and TALENs

- A new protein required for each site to be cut
- Proprietary technology
- Expensive
- Efficiency limited

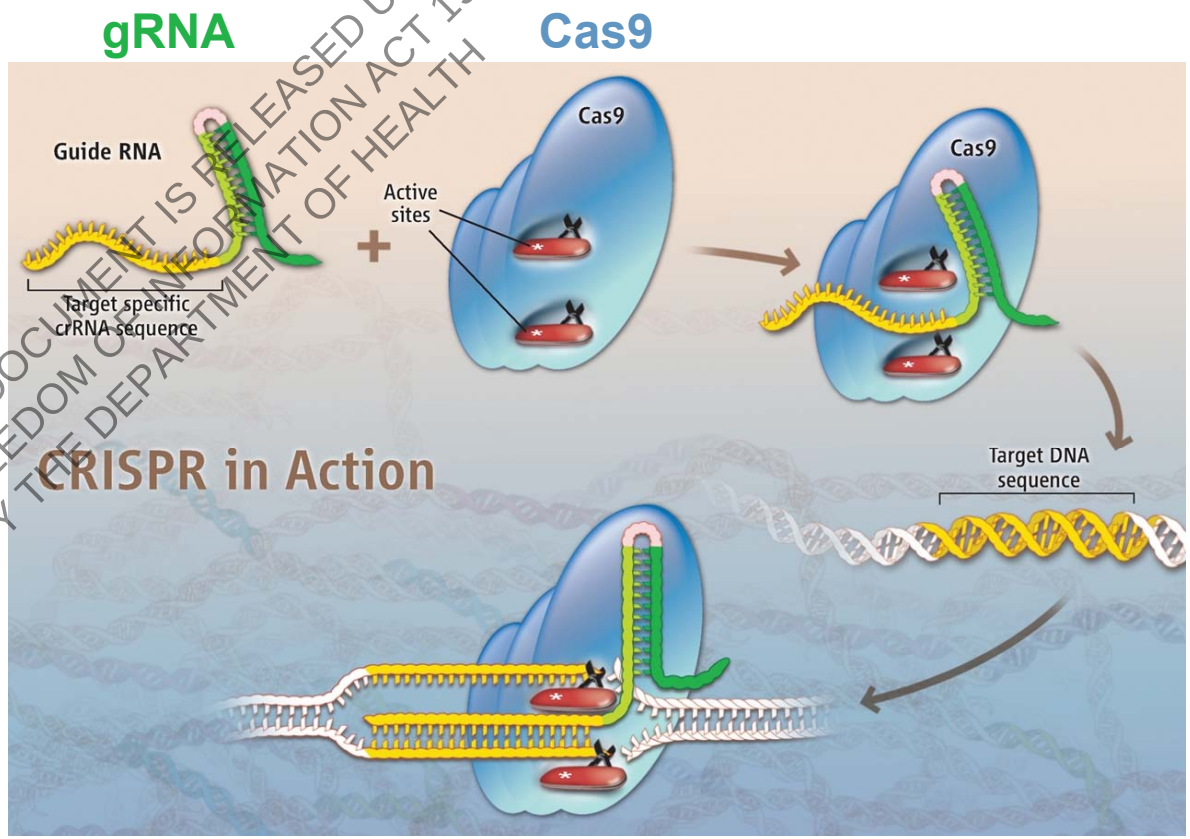


Walker-Daniels J, Mater. Methods, 2013

CRISPR/Cas9 (and variants)

Lives up to the hype!

- Open source (for research)
- Cheap
- Highly efficient



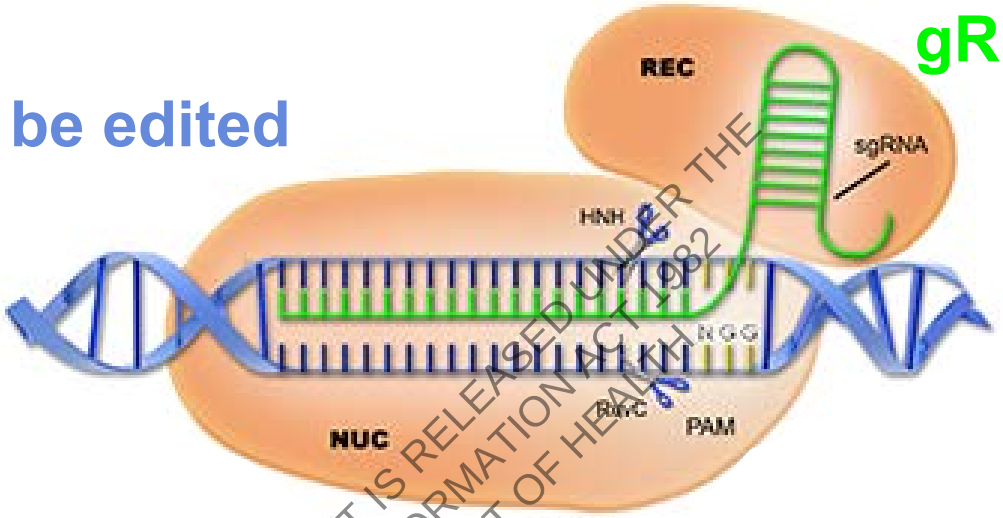
Clustered Regularly Interspaced Short Palidromic Repeats

Pennisi E, *Science*, 2013

Two ways to edit DNA with CRISPR/Cas9

Genome to be edited

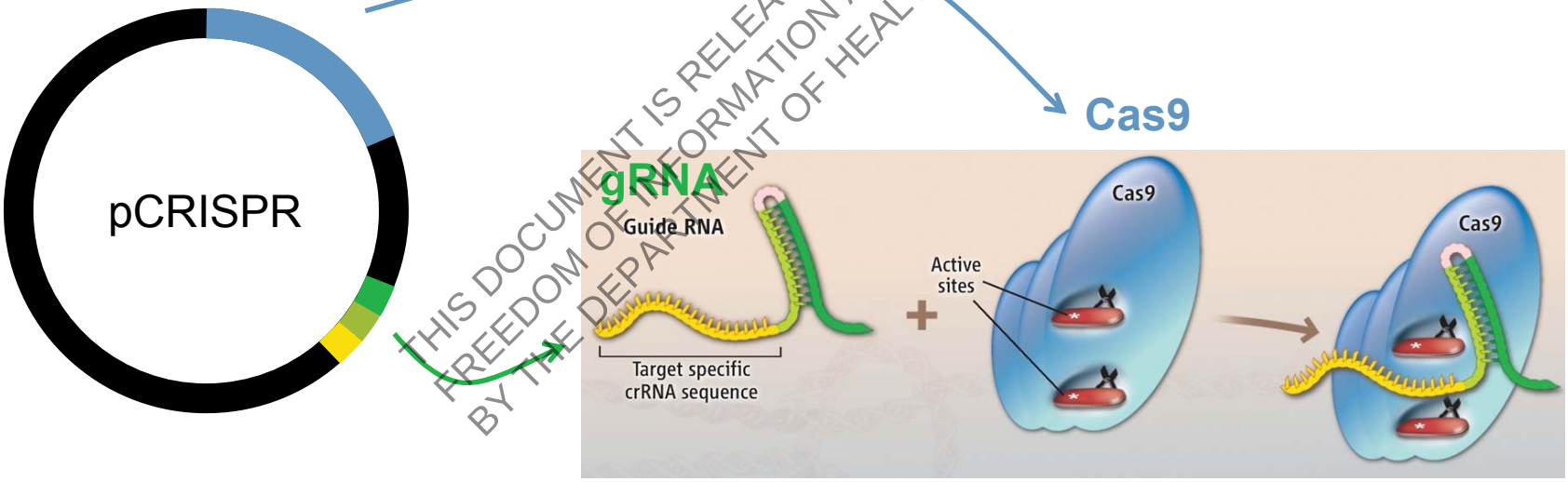
gRNA/Cas9



http://www.aati-us.com/images/crispr-dual-editing-method.png

Why is it so easy?

- There are genetic constructs that make Cas9 and have a site to add DNA for any gRNA you like
  - https://www.addgene.org/CRISPR/
- If this is put into cells, it just goes to work…



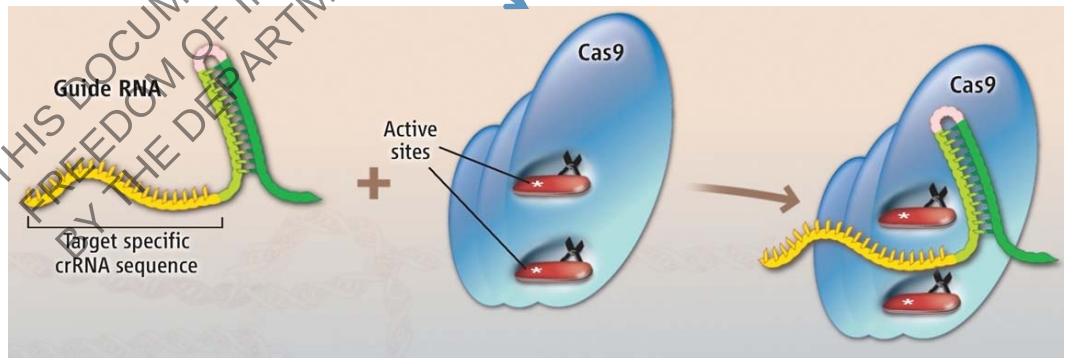
Pennisi E, *Science*, 2013

So very easy

- You can buy Cas9 protein and any gRNA you like
- Mix them in a tube
- 'Transfect' them into a cell
  - with or without a repair DNA
- If there is no DNA, is it a GMO?

$200

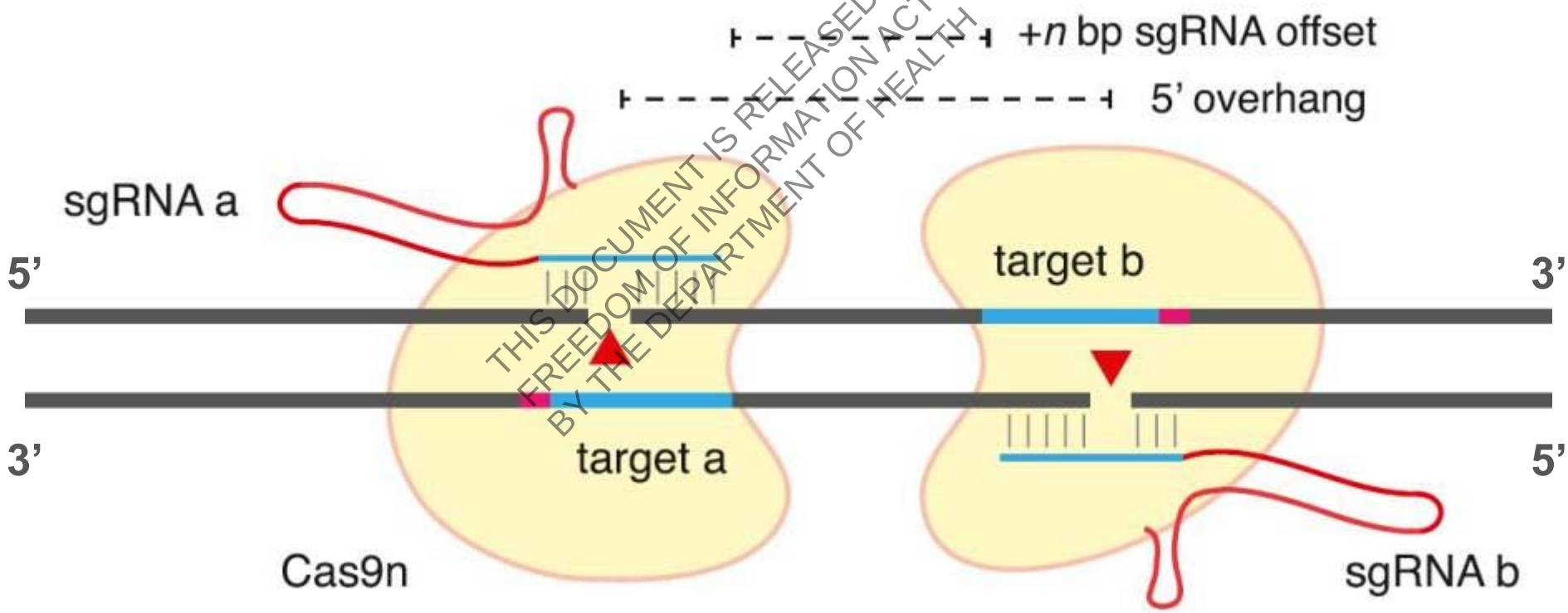
$600



Pennisi E, *Science*, 2013

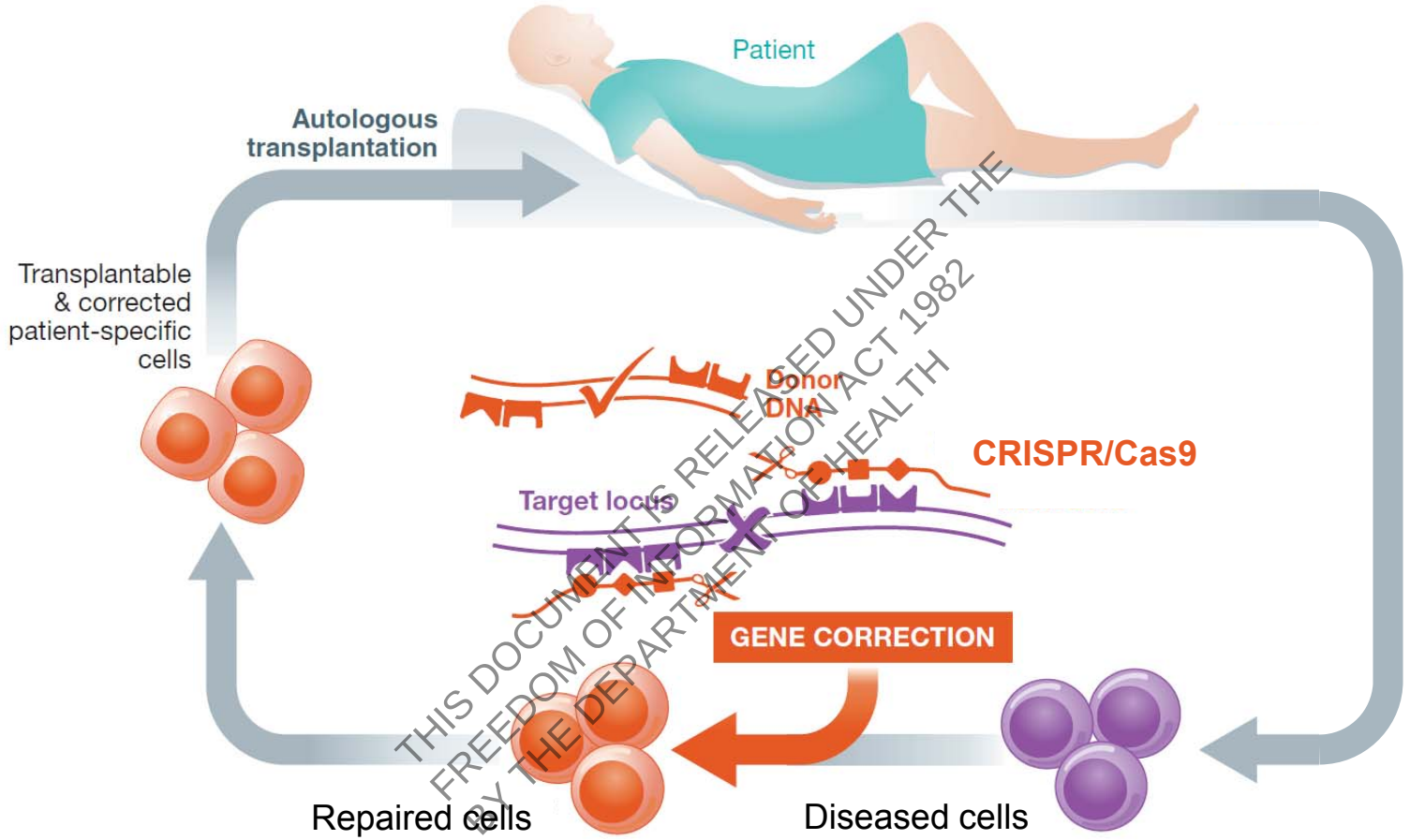
The catch: Specificity and 'off-target effects'

- **Off-target effects are considered the main limitation**
  - **Cutting at undesired locations**
  - **Partly addressed by combining two Cas9 'nickases'**



http://www.genecopoeia.com/resource/crispr-cas9-specificity-taming-off-target-mutagenesis/

Gene therapy can now include gene correction



Targetting the CRISPR/Cas9 is still a major hurdle for in vivo gene therapy

‘CRISPR’ clinical trials on the US NIH database

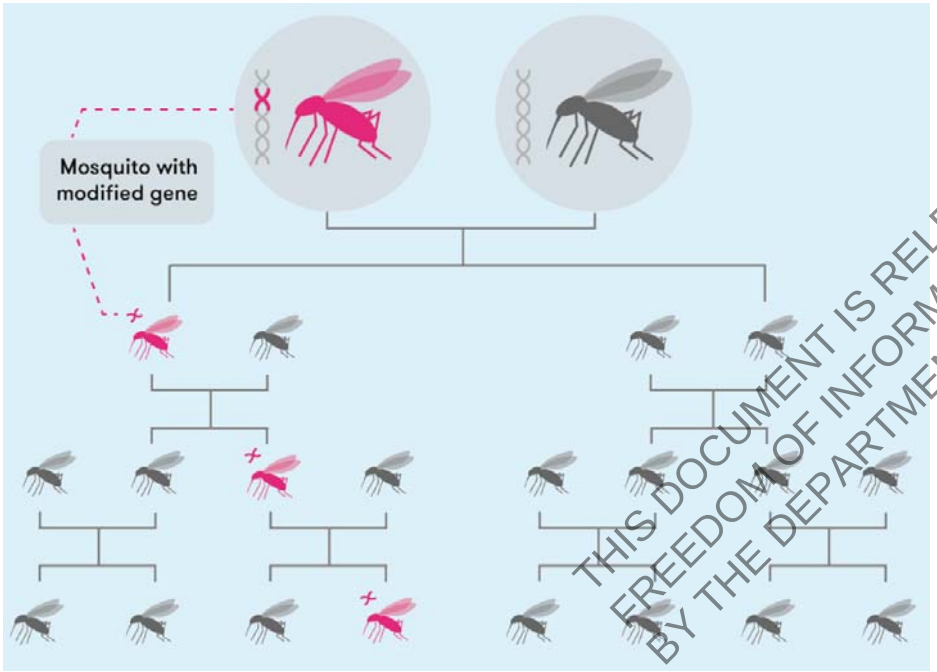
ClinicalTrials.gov Search Results 09/18/2017

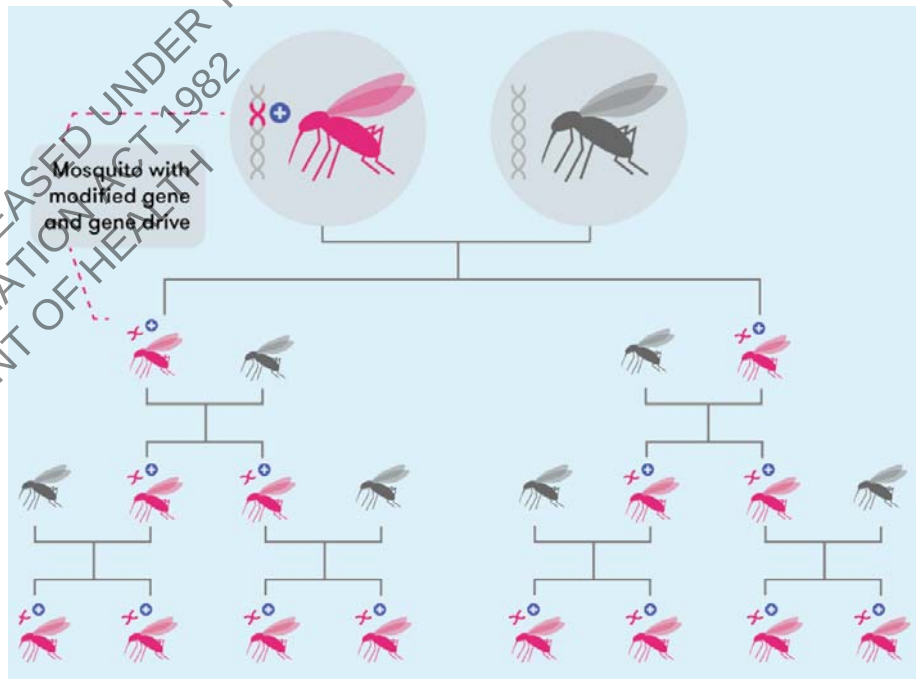
	Title	Recruitment	Study Results	Conditions	Interventions
1	A Safety and Efficacy Study of TALEN and CRISPR/Cas9 in the Treatment of HPV-related Cervical Intraepithelial Neoplasia#	Not yet recruiting	No Results Available	•Human Papillomavirus-Related Malignant Neoplasm	•Biological: TALEN •Biological: CRISPR/Cas9
2	Safety of Transplantation of CRISPR CCR5 Modified CD34+ Cells in HIV-infected Subjects With Hematological Malignances	Recruiting	No Results Available	•HIV-1-infection	•Genetic: CCR5 gene modification
3	Examining the Knowledge, Attitudes, and Beliefs of Sickle Cell Disease Patients, Parents of Patients With Sickle Cell Disease, and Providers Towards the Integration of CRISPR in Clinical Care	Not yet recruiting	No Results Available	•Sickle Cell Disease	
4	A Study Evaluating UCART019 in Patients With Relapsed or Refractory CD19+ Leukemia and Lymphoma	Recruiting	No Results Available	•B Cell Leukemia •B Cell Lymphoma	•Biological: UCART019
5	PD-1 Knockout Engineered T Cells for Advanced Esophageal Cancer	Recruiting	No Results Available	•Esophageal Cancer	•Drug: Cyclophosphamide •Drug: Interleukin-2 •Other: PD-1 Knockout T Cells
6	PD-1 Knockout Engineered T Cells for Muscle-invasive Bladder Cancer	Not yet recruiting	No Results Available	•Invasive Bladder Cancer Stage IV	•Biological: PD-1 Knockout T Cells •Drug: Cyclophosphamide •Drug: IL-2
7	PD-1 Knockout Engineered T Cells for Castration Resistant Prostate Cancer	Not yet recruiting	No Results Available	•Hormone Refractory Prostate Cancer	•Biological: PD-1 Knockout T Cells •Drug: Cyclophosphamide •Drug: IL-2
8	PD-1 Knockout Engineered T Cells for Metastatic Renal Cell Carcinoma.	Not yet recruiting	No Results Available	•Metastatic Renal Cell Carcinoma	•Biological: PD-1 Knockout T Cells •Drug: Cyclophosphamide •Drug: IL-2

Gene drives

Gene drives

- **Genes that are inherited at greater than Medelian rates**

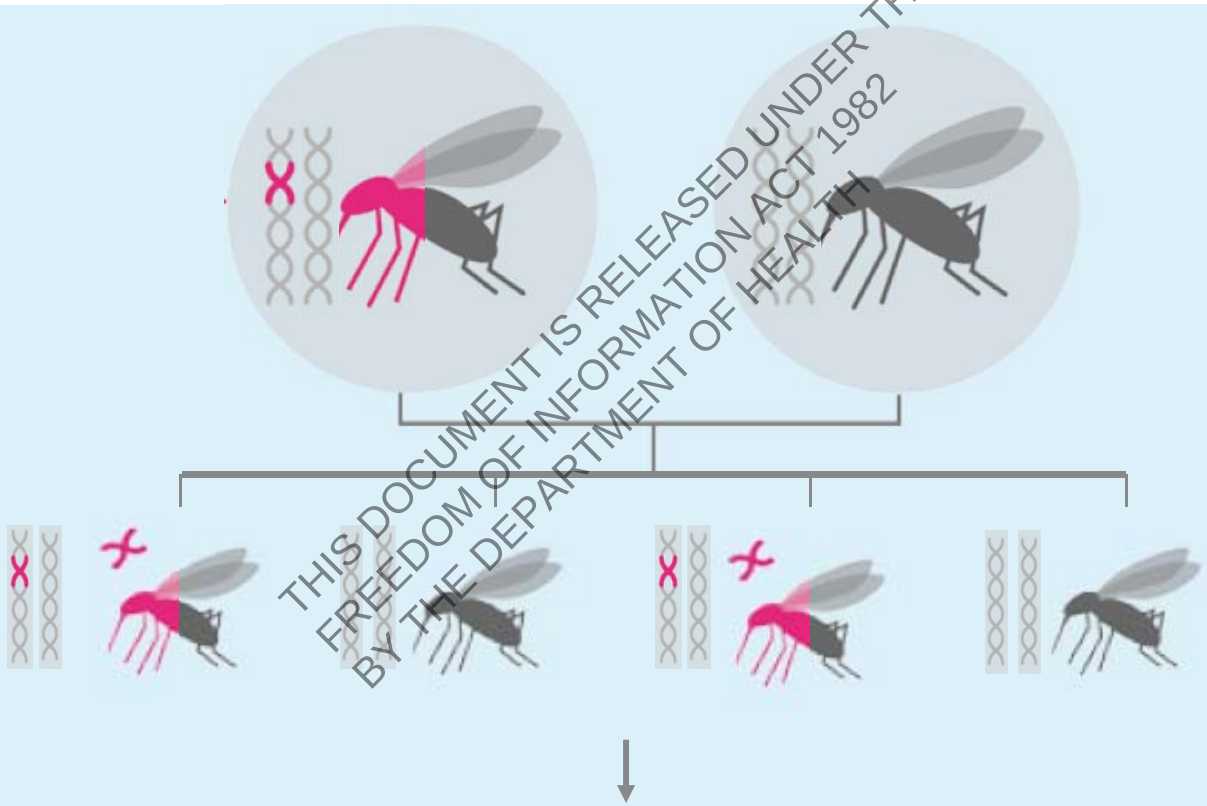




SYNTHETIC GENE DRIVES IN AUSTRALIA:
IMPLICATIONS OF EMERGING TECHNOLOGIES
AUSTRALIAN ACADEMY OF SCIENCE

Normal (Mendelian) inheritance

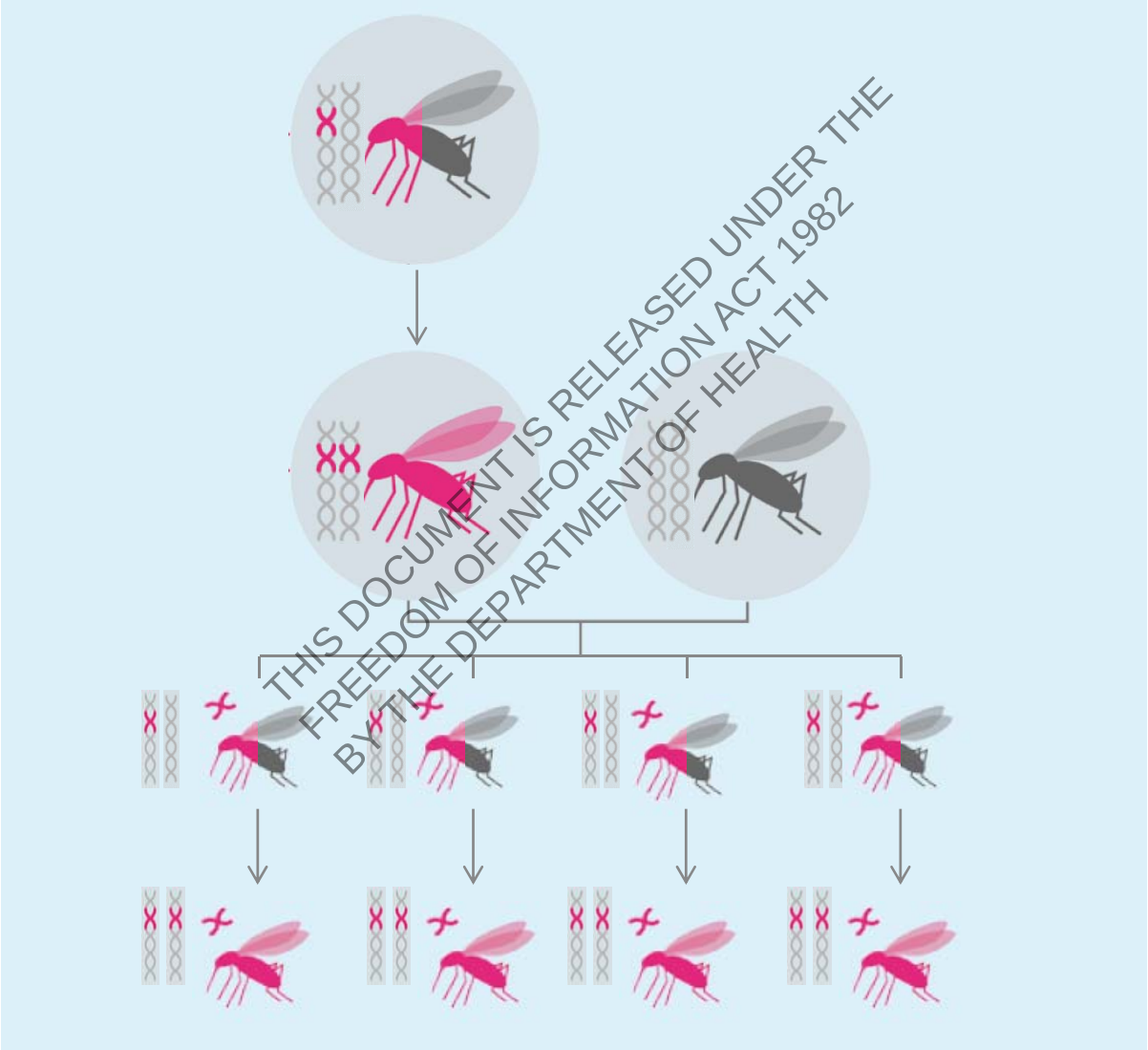
- There are two copies of the genome
- One copy of each gene comes from each parent



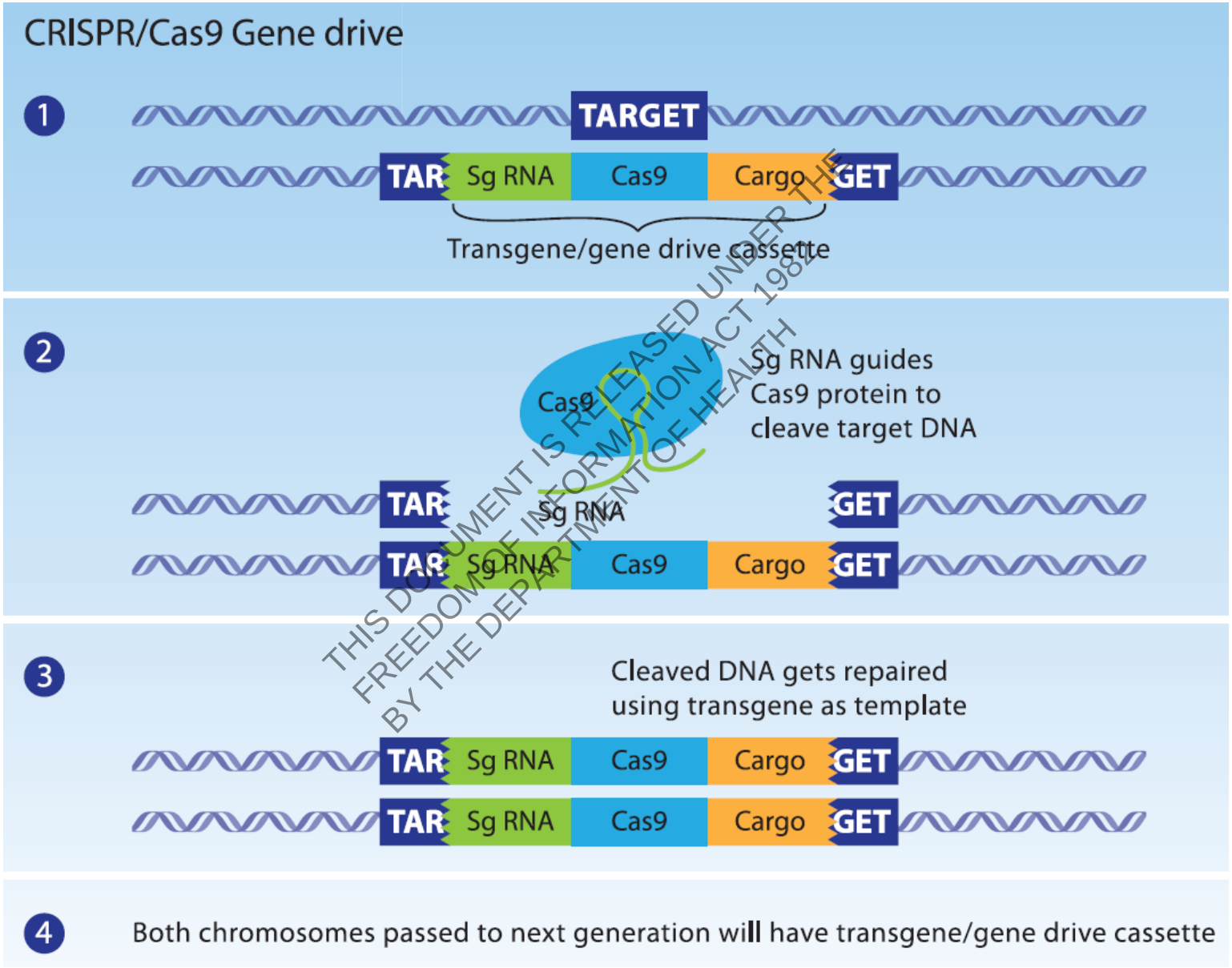
The gene will be in ¼ of next generation (assuming most are grey)

Gene drives

- **A gene drive can cause a gene to be duplicated**



What does CRISPR have to do with gene drives?



Applications of gene drives

- **Inhibiting / controlling insect vectors of disease**
  - **Health benefits, especially for developing nations**
- **Pest animal control**
  - **Potential environmental benefit**
- **Controlling pests of agriculture**
  - **Commercial benefit**
  - **Improved food security**
- **Any release requires enourmous care and consideration**
- **Public scrutiny required**
  - **Very substantial dread**

Strategic issues

- How does Australia ensure we are active participants and not bystanders?
- How will we decide when genome engineering is considered safe for human medicine?
  - **What will be the quality benchmarks for certification?**
- How will we balance potential health benefits against environmental concern (e.g. gene drives)?
- How will we ensure that policy and the public are informed by science?
- How will we respond to international regulatory moves (e.g. licencing or moratoria)