

Precision genome engineering in the chicken: The gap between science and market place

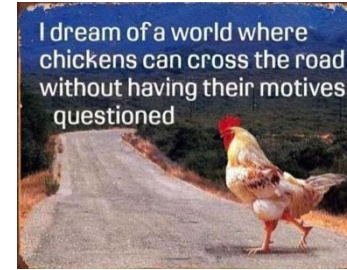
Mark Tizard, Arjun Challagulla, Kristie Jenkins, Anthony Keyburn,
David Cahill & Tim Doran

IWRAB-II, Brasilia, 18-21 August 2014

- Our animal and the industry
- The industry issues we are aiming to tackle
- The technology we are using
- The progress we have made
- A brain teaser – *GM or not GM, that is the question*
- Parting thoughts

Why the chicken?

- A model organism on the rise
- Land based production animal without peer
- 50 billion broilers per annum
- 70 million tonnes of egg products



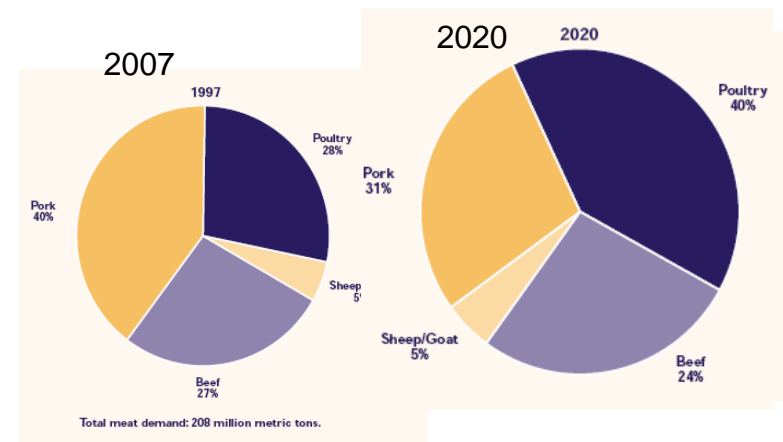
FCE 1.4



FCE 2.2



FCE 7.0



FAO Statistics 2007

- Produced off less land area and with less water input
- Easier to recover production system in cases of natural disaster (climate change impacts)

Production traits being targeted with PGE



Influenza virus resilient poultry: dual benefit

- Reduced production losses
- Prevention of zoonotic spread



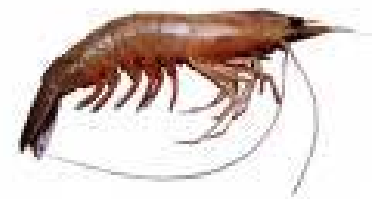
Sex manipulation or sex selection in poultry

- Redirecting sex differentiation
- Male for meat, female for eggs (!)
- Sex selection (for egg production)



Eggs for improved vaccine production

- Increased vaccine titre
- Removal of allergens



Modified traits in prawns

Major steps forward in chicken biotechnology

- Disabled retrovirus transformation – lentivirus proviral integration
 - McGrew *et al* 2004, EMBO Reports
 - Primordial Germ Cell (PGC) culture and transfection
 - Direct injection reintroduction of PCGs, GFP birds



- Tol2 mini-transposon – transposase mediated integration
 - Tyack *et al* 2013
 - Direct injection of DNA constructs
 - GFP birds



- Precision genome engineering with TALENs
 - J Y Han *et al* 2014, (embargoed – high IF journal)
 - PGC culture, single allele KO of the egg allergen ovalbumin



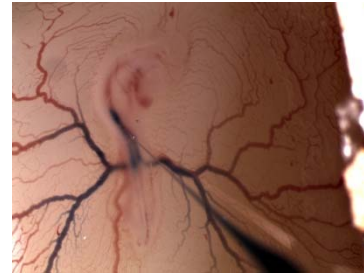
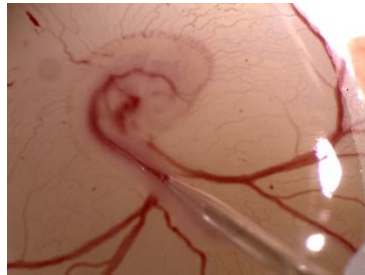
- Next up: *the CRISPR chicken...*



Making transgenic chickens

- Steady progress for the “regulatability” of GM chickens
 - Disabled retro virus systems – efficient and safe but raise concerns
 - Transposon based systems – efficient and safe but random placement causes concerns
 - PGE systems – early days – efficient 10-15%, safe, precise, testable
- Transforming the germline
 - Primordial Germ Cell (PGC) culture
 - Transform the cells that will make ova and sperm in culture
 - Expensive and time consuming but efficient
 - Direct injection
 - Skilled but cheap and straightforward
 - Efficiency – in assessment

Direct *in vivo* transfection of PGCs – EGFP chicken



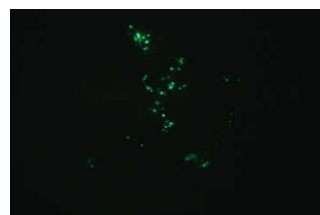
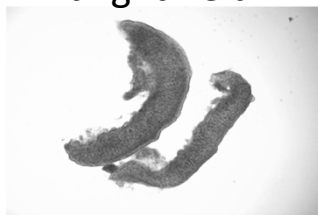
- Direct injection of DNA constructs into embryo ED 2.5 – seal and incubate
- Dissected gonads and whole embryos examined ED 7 and ED 14
- EGFP observed extensively throughout gonads of all embryos
- Chicken-Vasa antibody staining confirmed EGFP cells were PGCs
- EGFP expression in other cells throughout the embryo (e.g. heart and brain)

“Gonads”

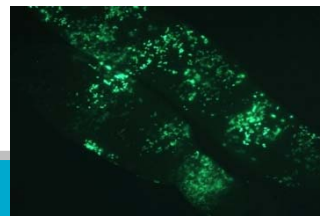
bright field

EGFP

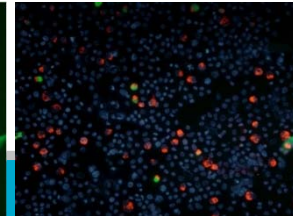
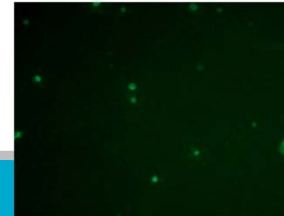
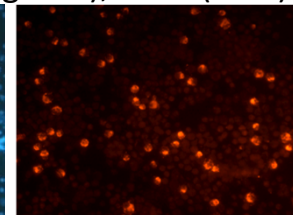
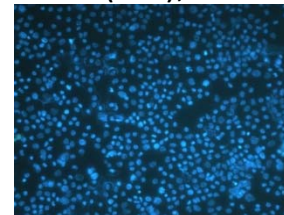
Day7



Day14



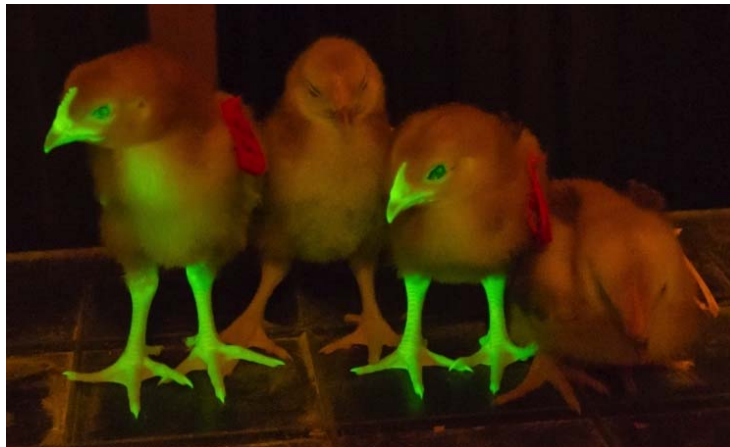
Dissociated gonad from ED 14
Vasa (red); EGFP (green); DAPI (blue)



Direct *in vivo* transfection of PGCs – EGFP chicken

Table 1: Germline transmission and transgenic chick production

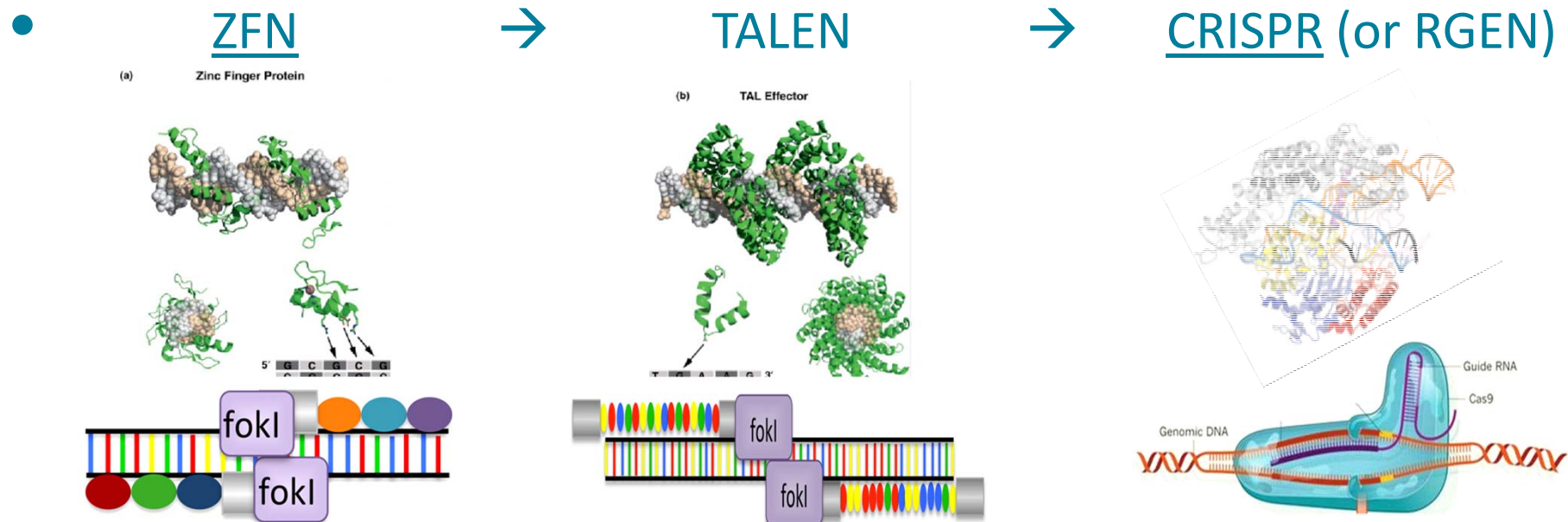
G0 Rooster #	Relative levels of miniTol in semen*	Offspring Hatched	Transgenic offspring	Percentage transgenesis		
1	0					
2	0.100					
3	0					
4	0.053					
5	0					
6	0					
7	0					
8	0.109	95				
9	0.145	131				
10	0					
11	0.221	193				



*The relative levels of integrated miniTol DNA in semen were calculated by comparing the mean Ct values from genomic and miniTol qPCR from two semen samples.

The Precision Genome Engineering toolkit

- “Meganulceases” – cuts DNA but has a large recognition sequence
- Systems for very tightly specific DNA sequence recognition
 - Recognising > 20bp sequences (sometimes 36-40 bp)
 - Perfect recognition should yield a single cut in a 3 billion bp genome



Our focus currently with CRISPR

- ZFN and TALEN – customised paired proteins that work together to seek the genome target
- CRISPR (RGEN) – a single common protein programmed by a short specific RNA

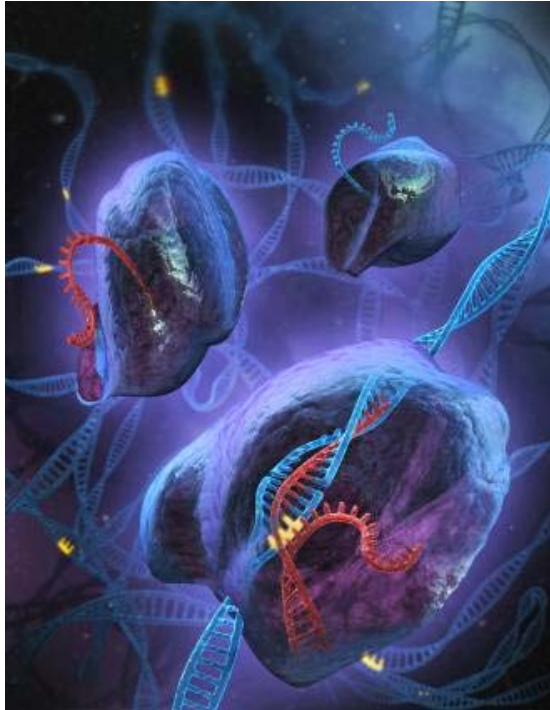


Image: Stephen Dixon, MIT News website

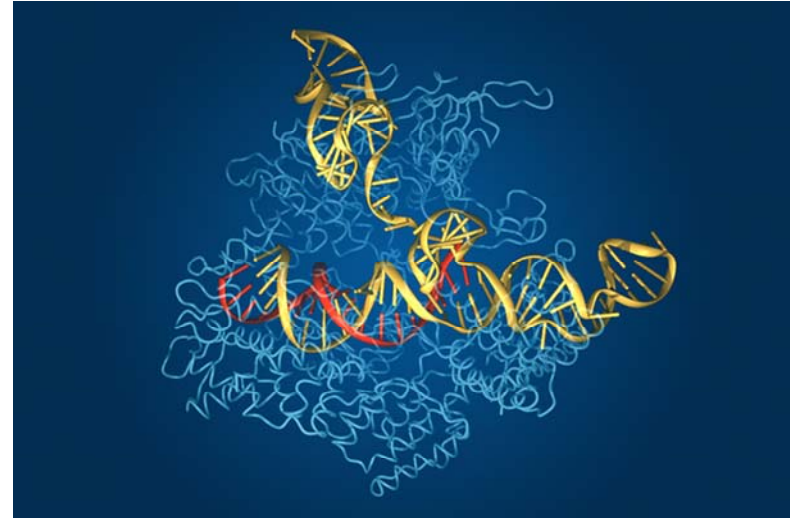
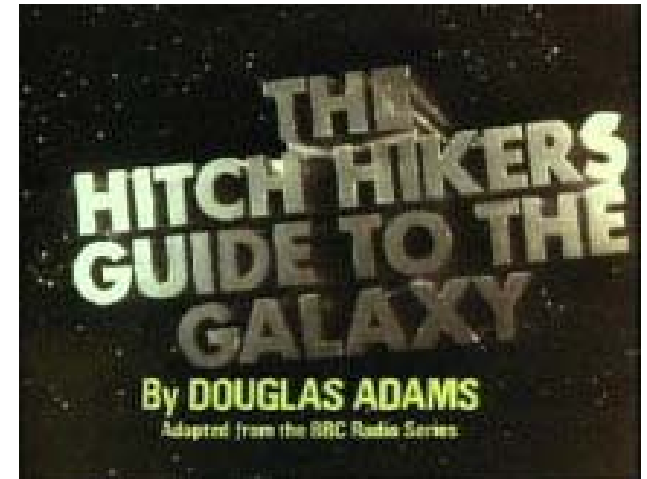


Image: Bang Wong, MIT News website

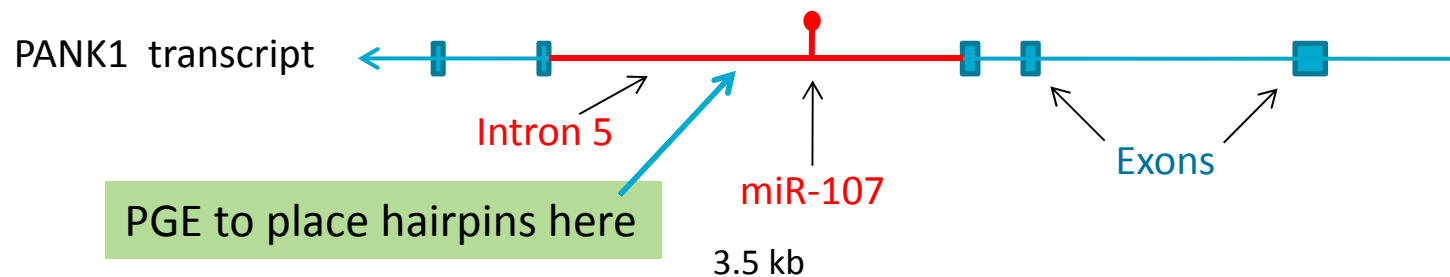
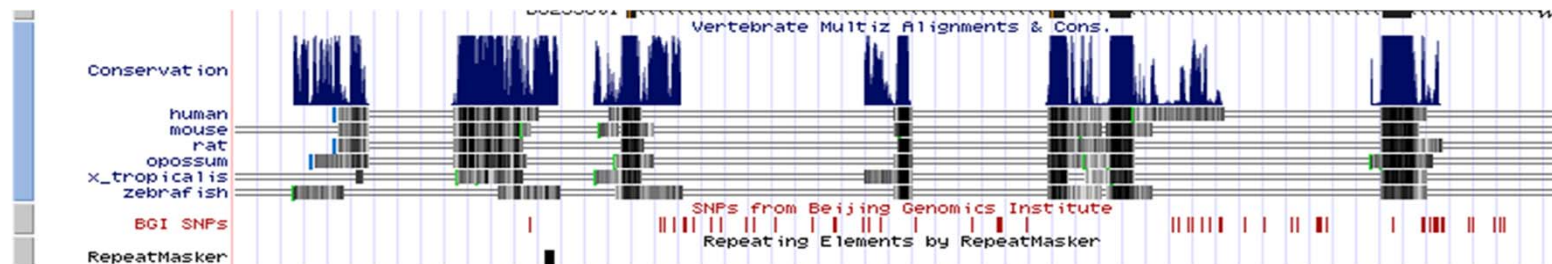
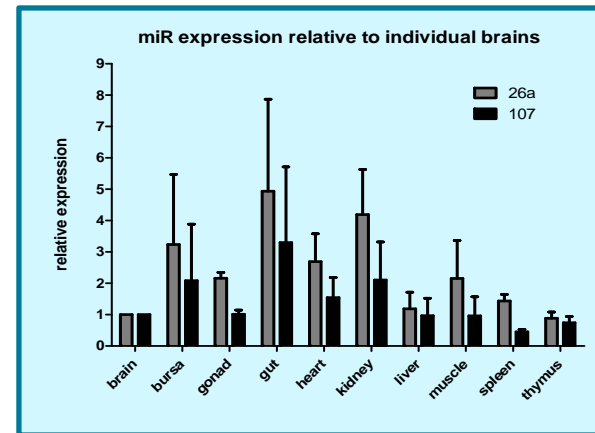
- First patent has been awarded to Broad Institute, MIT, Feng Zhang, WO2014093661A2 - Dec 2012
- UC Berkley, Jennifer Doudna, WO2013176772 A1 - May 2012

“Hitch-hiker” development

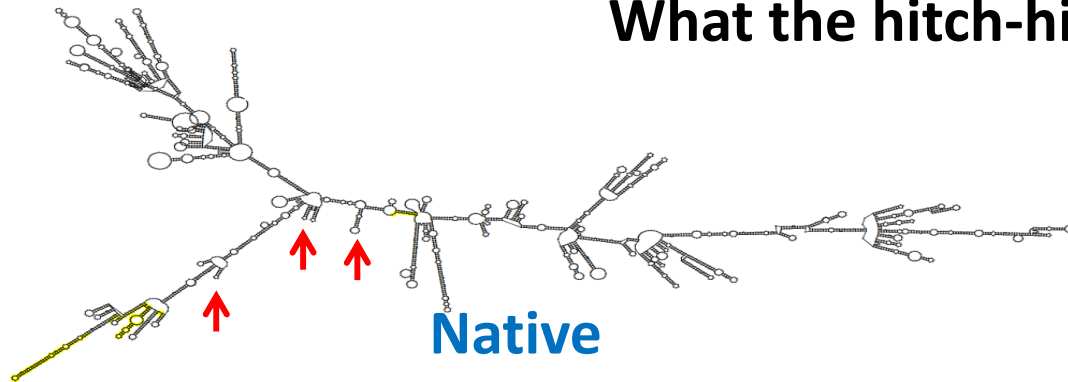
- The concept
 - Use RNA interference (RNAi) to control influenza virus infection
 - Couple anti-viral hairpin RNA (RNAi) to natural miRNA expression
 - Parallel processing – hitch-hiking
- PGE to achieve the goal
 - Placing single or multiple hairpins precisely in the genome
 - Use of ZFN
 - Generation of functional cell lines with engineered genomes
 - *Great work by an excellent PhD student*



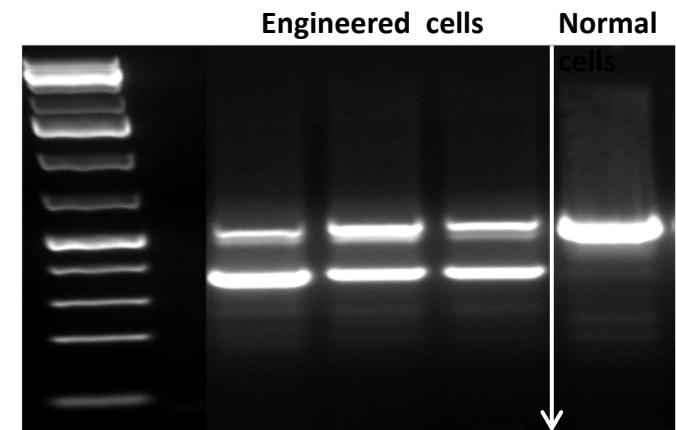
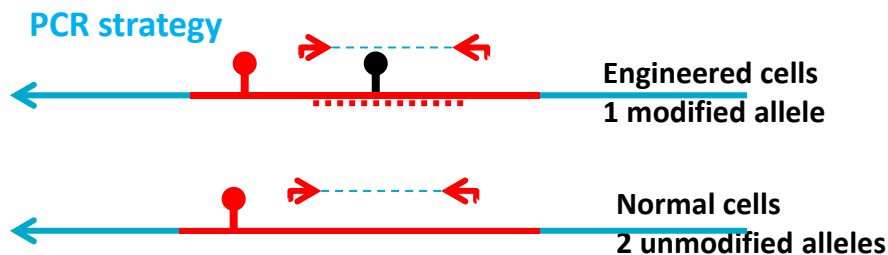
miR-107 and pantothenate kinase 1 (CoA biosynthesis)



What the hitch-hiked intron looks like

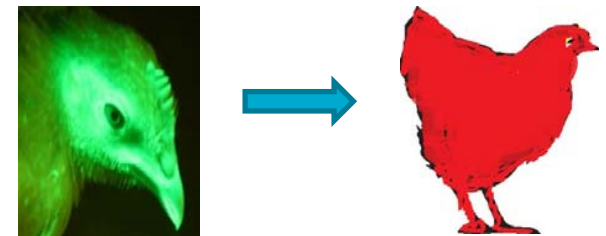
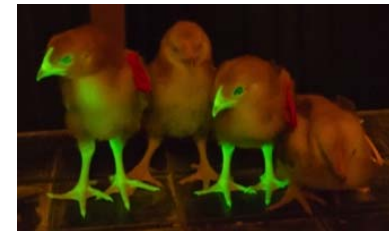
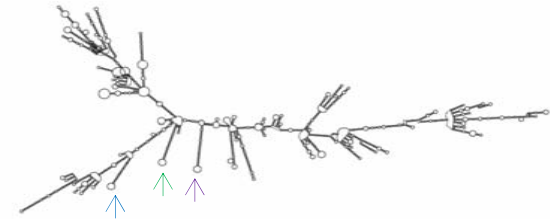


- Retain whole of intron secondary structure (retain native activity – keep it natural)
- It works in cells – can it be translated to a living animal?



Summary of PGE progress:

- Successful engineering of the chicken genome with ZFN
- Successful transformation of PGCs by Direct Injection to generate transgenic chicken (resulting in GFP chicken)
- Adoption of CRISPR – confirmed functional knockout of GFP in chicken cell line
- CRISPR combined with Direct Injection to knock-out GFP and replace with Red Fluorescent Protein...



PGE current ideas and future activities:

Knock-in, knock-out, knock-about

- Knock-in

- RNAi transgenes e.g. Hitch-hiker
- Protein coding genes
- Recombinant (hybrid) live virus vaccine manufacture

- Knock-out

- Real “model” of disease systems and development (IL-6)
- Improved vaccine growth in eggs (interferon receptor) (NIH grant)

- Knock-about

- Gene editing e.g. allergen modification
- Virus receptor modification
- Rational SNP integration

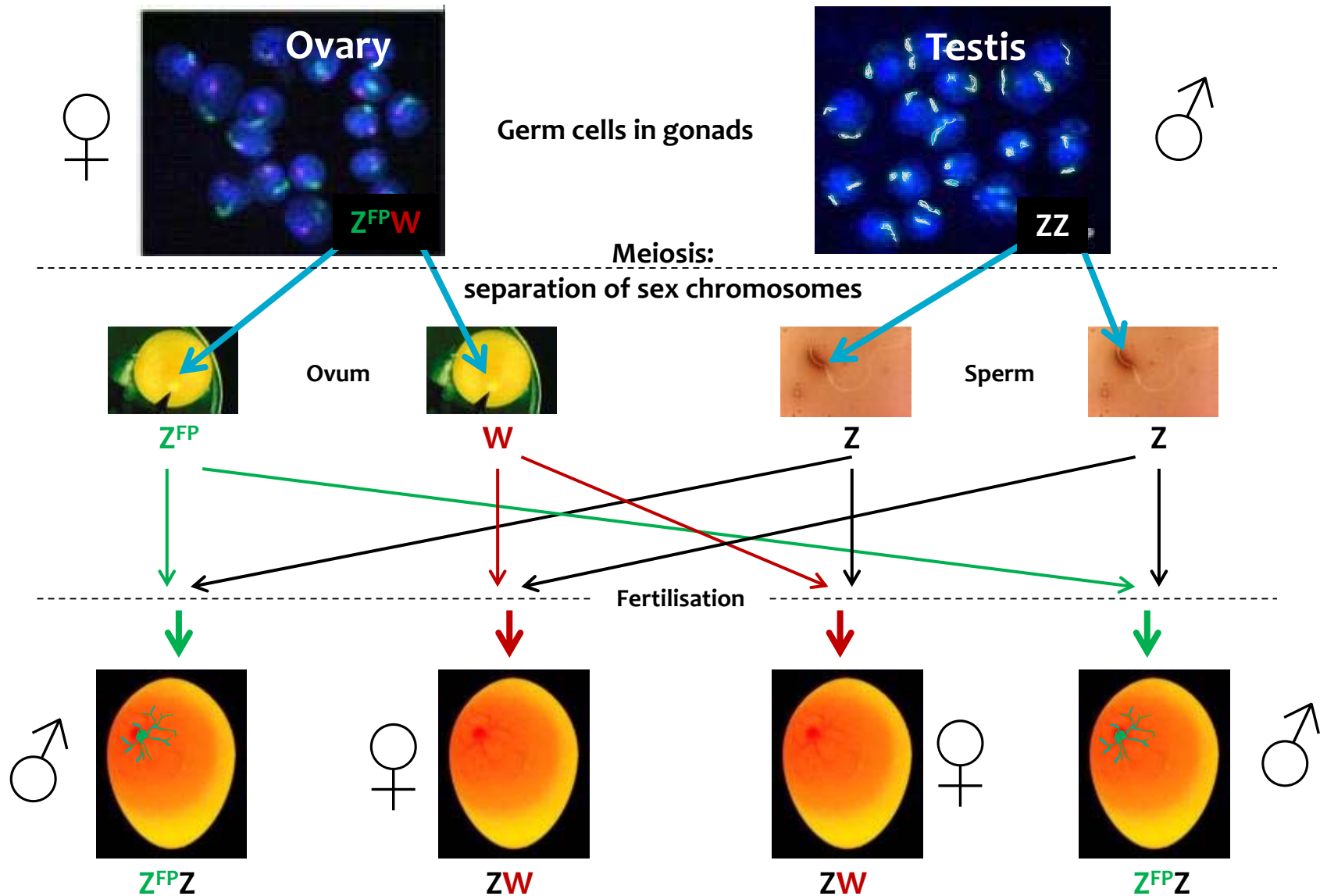
The brain teaser

- To a scientist and a regulator this may be very clear...
but with fully opposing conclusions
- The conundrum, the dilemma
- When is a GMO not a GMO? or Can a GMO produce a non-GMO?

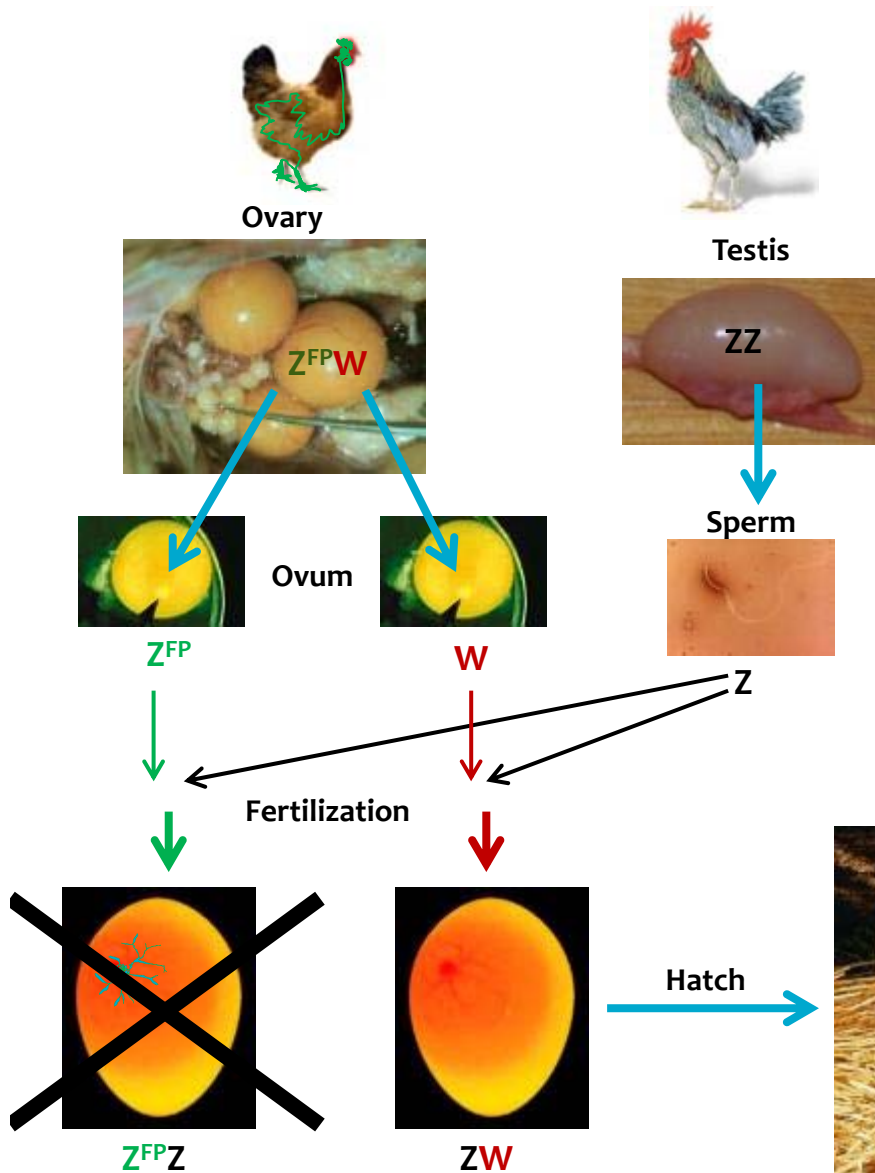
Background - the industry issue:

- Egg production – only females lay eggs (duh)
- But the genetics of the quality traits are so precise that males from layer-lines of birds are poor producers of meat – therefore culled
- Expensive process and inhibits uptake of in ovo vaccine technologies – attracts negative public perceptions
- Objective - remove males before they hatch

PGE: For sex selection of egg laying chicken



PGE for sex selection of egg laying chicken



- Parent-line of females – transgenic for fluorescent protein on the Z chromosome
- Normal male parent (no transgene)
- Cross to produce the eggs that will go on to become egg layers
- All sperm from normal male parent are normal Z
- Meiosis segregates the transgenic FP Z from the female parent separates from the non-transgenic W
- Fertilization means that all females are normal Z normal W

- Female only population
- Females have NO transgene
- Are these eggs a GM food ?

Summary: the impact of our PGE in poultry

- Presents a conundrum - sex selection: is the animal GM? is the food product GM?
- Our first GMO regulation challenge may be via PGE to introduce 300 bp precisely placed change (hitch-hiker RNAi viral RNAi)
 - no protein
 - no regulatory sequence
 - natural processing for biological effect
- Use of PGE paves the way for single nucleotide changes
- What (if any) benefit does PGE bring in terms of how these animals and their products will be regulated and adopted?

Acknowledgements

CSIRO Biosecurity Flagship Australian Animal Health Laboratory

- Tim Doran
- Kristie Jenkins
- Terri O'Neil
- Terry Wise
- Kirsten Morris
- Matthew Bruce
- Robert Moore
- Anthony Keyburn
- John Lowenthal
- Sandy Matheson
- Susanne Wilson
- Mark Ford

Dept of Microbiology and Immunology College of Veterinary Medicine Cornell University, USA

- Prof Karel A. Schat

Murdoch Children's Research Institute, The University of Melbourne

- Prof Andrew Sinclair
- Craig Smith
- Luke Lambeth


Seoul National University, Korea

- Prof Jae Han
- Tae Min Kim
- Jin G. Jung




Thank you

www.csiro.au



Removing barriers to the uptake of GM animals as a sustainable solution to food security and safety




Date:
Thursday 9 October 2014 to
Friday 10 October 2014

Time:
Commences 9:00am

Location:
AAHL, Corner of Port Arlington and
Boundary Roads, East Geelong

RSVP before:
Monday 29 September 2014



BETTER POLICIES FOR BETTER LIVES

OECD - 9-10th October

PGE - 16-17th October

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