

*Dominant white  
deleted, that's the  
cuckoo chicken on  
the right*

# GENE EDITED CHICKENS

## Cooking soup analogy outdated

**You might have heard of Crispr, simply said: gene transplantation. Take a gene and put it in the genome of the embryo. The analogy of cooking soup when referring to breeding chicken colours as in picking the ingredients and stir well, is reality meanwhile, although not in our kitchens. Not that this example is exactly right, it is a bit more sophisticated. Welcome to the future of chicken breeding.**

Dec 2022 - text: Sigrid van Dort, photos: Roslin Institute

### **Editing is putting only the gene you want in a chicken**

First mammals were edited, birds are put together differently, eggs etc and not a fertilised egg which can be injected with modified sperm, or the other way around modified, in a petri dish and subsequently planted into a womb.

### **How do you get a single gene into a chicken?**

You know about recessive white do you? It blocks the juice necessary for the formation of black and red pigment. Long ago, there was a chicken infected with a virus and the virus jumped into the dna of this chicken and it messed up the colour juice. Because the virus plus its function (blocking feather colour) was embedded in the dna, it was passed on to the offspring of this

chicken. That is how recessive white came about. Now, this happened numerous times during the evolution of species. It is called a viral insertion. Not all viral insertions are a success story as is recessive white. If there are deleterious harmful (side) effects (pleiotrophy) the organism dies and all is over. It is a risky enterprise. However...

### **Virus hike or not?**

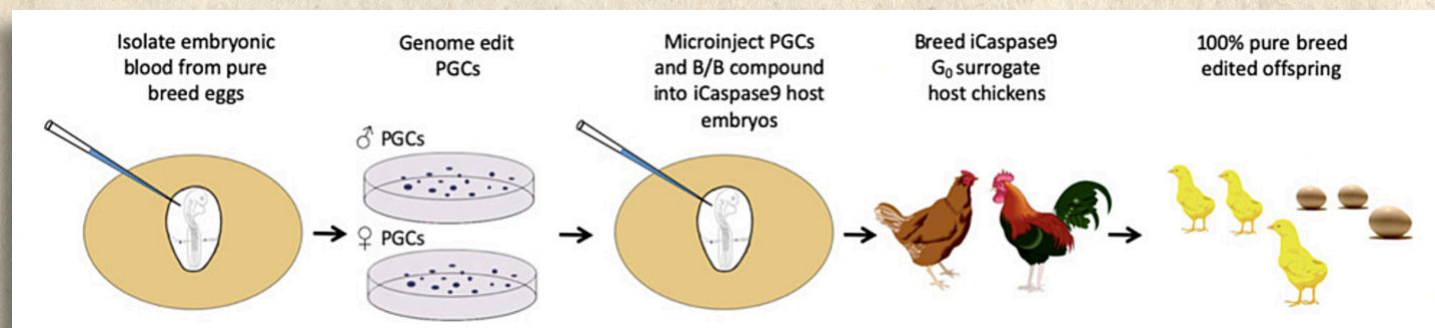
What nature can do, humans can do too. Ho, stop, it is not that easy. Even if you have a harmless transport-virus 'loaded' with a gene of which the effect is predictable (which is a major step already to be able to do such a thing), then still you get all the other stuff of the parents (new genotype made of 1+1). This is similar to our soup cooking albeit a bit 'cleaner'. Still the chickens have to be bred to

get the chickens all to carry the new gene, the same as we do. Next to that, the size of the gene which was meant to implement was limited, due to the virus this way and it was also not very accurate genewise.

### **Editing in practice**

The scientists developed another way. Thanks to Crispr it is possible to get an F1 generation from two modified 'by birth' parents. Two whatever colour chickens will give you only purple, or pink, or yellow offspring. Depending on the colour they put into the parents' egg and sperm producing cells. How do they do that you might ask. Because the parents don't have the gene they are going to give to their chicks. Magic? No, sophisticated, yes. Read on.





First, they take blood from an embryo in a very early stage. They sort out the stem cells that will later give eggs and sperm. These cells are edited, a gene is added, the cells are cultured in a petri dish. Next to this the scientists developed sterile chicken embryo hosts (this is new). They can't use normal embryos otherwise all the other stuff comes with it again and it is even possible the genes you want, are lost in action. The host embryos are sterilised with a chemical compound which is 'microinjected' into their blood. At the same time the host embryo is injected with the edited stem cells that will develop the organs giving eggs and sperm which will contain the edited gene. Twenty-one days fast forward.

#### F1 and ready

These embryos become chickens - cocks and hens - which have the new gene in their eggs and sperm. When breeding them, they immediately give edited chicks in F1. Think of doing this with a colour gene, or blue egg gene. A home kit would help greatly to reduce the amount chickens which have to be bred and tested. In general

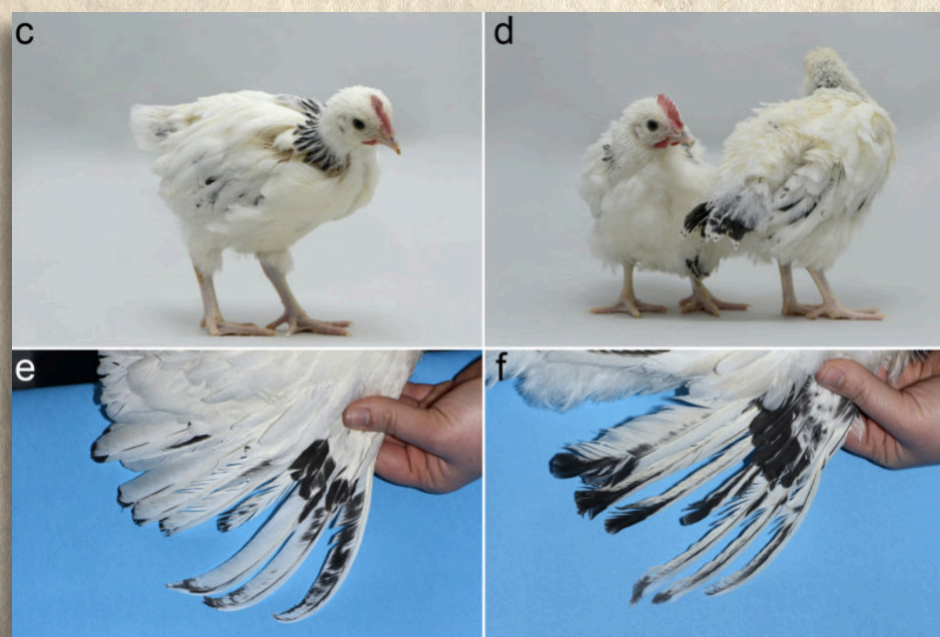
you need 2 to 4 years to get homozygous traits, if you can see them. Now you can create pure breeding chickens in one year. Think of the possibilities. Joking aside, this technique can be used to create bird flu or Marek's disease resistant chickens for example. Or other beneficial traits can be implemented really fast. It would greatly improve animal welfare, and you breed the edited chickens as usual, offspring will always have the gene. You can line breed if you have unrelated edited chickens to keep some variety. These chickens aren't clones.

#### Who is doing this?

The Roslin Institute, from sheep Dolly and steer Herman. Their department 'NARF' has a special disease free facility to create gene-edited (transgenic) chickens. These chickens are used to figure out how exactly their immune system responds to certain infections. Immunity is made of genes, you know this because you breed for disease resistance too. We talk about the innate (by birth) immune system and the immune

system they acquire themselves. If the scientists know what genes are necessary, effective vaccines can be made or don't have to be used anymore. Think of immunity against bird flu or Marek's disease.

On a side-note, they still need to figure out what determines the sex of a chicken - cock/hen. It is, how strange it may seem, still not known how sex differentiation works in chickens. If they know that, then there are no male chickens anymore in the egg laying production lines. Sex determination is now done by a machine after incubation before hatch, still they need to be incubated and they are removed before they go in the hatching rooms at day 18. Scientists also made a production layer strain with frizzled feathers for hot tropical climates to improve welfare. When you cross a frizzled chicken into a carefully selected layer line, you will mess up their genetics. A set-back from decades probably. Better add only the frizzled gene, which they did, see photos below.



Putting frizzled in a layer strain to cope with heat. Genome edit to introduce the FRZ (frizzled) allele into the LSX chicken breed. Photos C, E: Control 6-week-old offspring and wing (18 weeks) from LSX birds. n=10/10 female birds. Photos: D, F: FRZ heterozygote LSX G1 offspring (6 weeks) and wing (18 weeks) displaying crumpled flight feathers, n=5 of 5 female birds. All photographs of live birds at The Roslin Institute.

#### a DOW allele (PMEL17<sup>WAP</sup>)

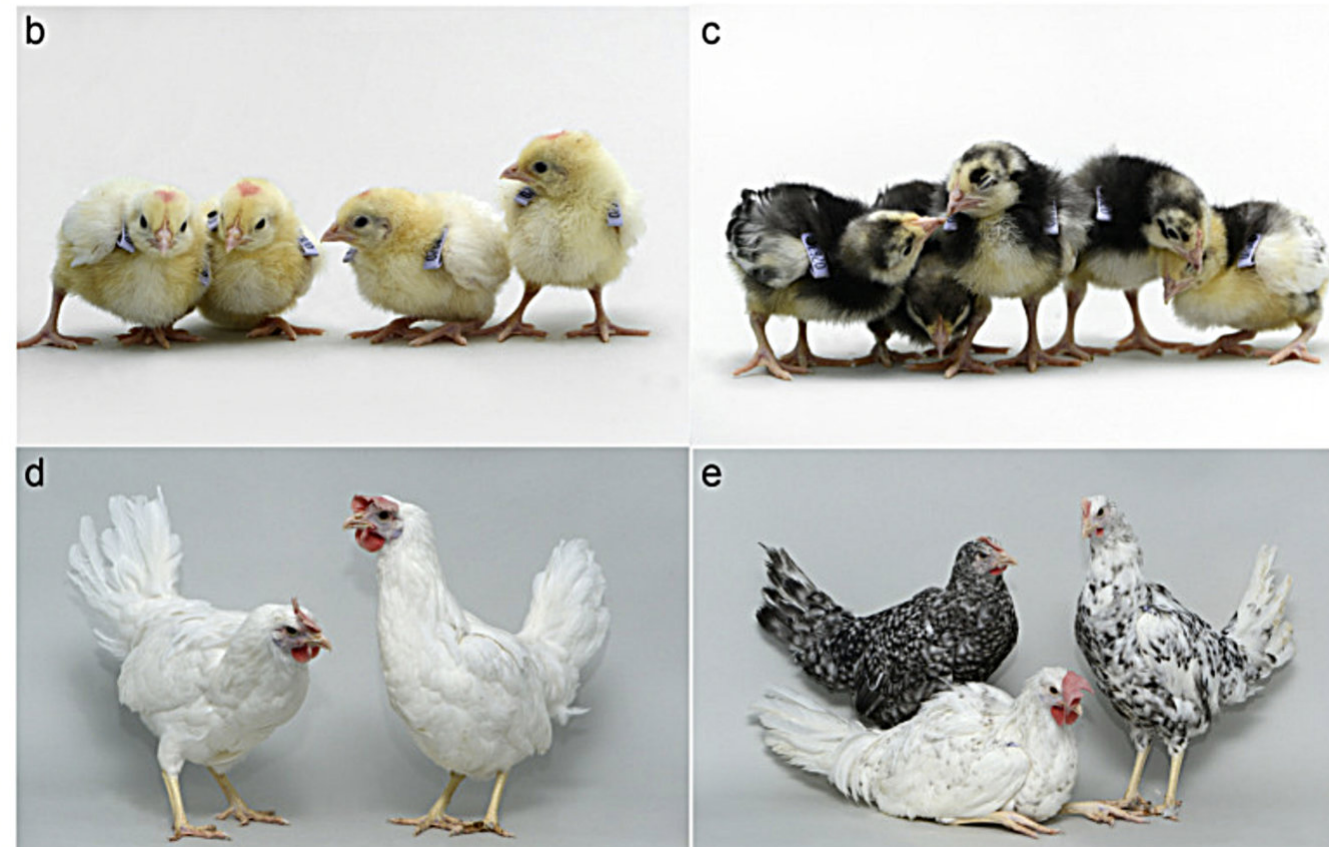
G A S P A A S G T T L T V G L L W A P L I A A A L G T A A Y T Y R  
GGA GCA TCC CCA GCC GCC AGT GGC ACC ACC CTC ACC GTG GGG CTG CTC TGG GCA CCG CTC ATC GCC GCT GCT CTG GGC ACC GCT GCC TAC ACC TAC CGG TGAGCGG  
CCA CGT AGG GGT CGG CGG TCA CCG TGG TGC GAG TGG CAC CCC GAC GAG ACC CGT GGC GAG TAG CGG CGA CGA GAC CGG TGG CGA CGG TGT GGA ATG GCC ACTCGCC

#### DOW edited allele (PMEL17<sup>WT</sup>)

G A S P A A S G T T L T V G L L L L I A A A L G T A A Y T Y R  
GGA GCA TCC CCA GCC GCC AGT GGC ACC ACC CTC ACC GTG GGG CTG CTC CTC ATC GCC GCT GCT CTG GGC ACC GCT GCC TAC ACC TAC CGG TGAGCGG  
CCA CGT AGG GGT CGG CGG TCA CCG TGG TGC GAG TGG CAC CCC GAC GAG GAG TAG CGG CGA CGA GAC CGG TGG CGA CGG TGT GGA ATG GCC ACTCGCC

#### Dun 'like' edited allele (PMEL17<sup>DEL</sup>)

G A S P A A S G T T L W A P L I A A A L G T A A Y T Y R  
GGA GCA TCC CCA GCC GCC AGT GGC ACC ACC CTC TGG GCA CCG CTC ATC GCC GCT GCT CTG GGC ACC GCT GCC TAC ACC TAC CGG TGAGCGG  
CCA CGT AGG GGT CGG CGG TCA CCG TGG TGC GAG ACC CGT GGC GAG TAG CGG CGA CGA GAC CGG TGG CGA CGG ATG TGG ATG GCC ACTCGCC



#### Genome edit to remove the dominant white allele from a White leghorn chicken breed.

Fig. a PMEL17 locus containing the dominant white allele (WAP insertion) within the 10th exon which encodes for the PMEL17 transmembrane domain (amino acids in black). Genome-edited PMEL17 locus removing the WAP (highlighted green). Genome-edited second allele creating a five amino acid Dun-like deletion (deleted amino acids highlighted yellow). The five amino acids deleted in the Dun allele are highlighted in blue.

Photos: b, d Wild-type WL Line 6 chicks and adults.

Photos: c, e PMEL17 edited chicks and adults; females (standing) were barred and speckled feathers, males (sitting) were white with black dots. All photographs of live birds at The Roslin Institute.

#### The future is now

Probably much more 'smart genes' can now be introduced in highly specialised chicken lines without messing everything up. You can now pull an ingredient off the shelf and use it in your soup, it is feasible. Wait for the homekits.

More cool stuff:  
<https://www.ed.ac.uk/roslin/national-avian-research-facility>

Source of frizzled and dominant white deleting: Direct allele introgression into pure chicken breeds using Sire Dam Surrogate (SDS) mating, 2021