Review

Genome Modification Technologies and Their Applications in Avian Species

Hong Jo Lee 1, Young Min Kim 1, Tamao Ono 2 and Jae Yong Han 1,3,*

1 Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea; cszjjang@naver.com (H.J.L.); ypoc01@snu.ac.kr (Y.M.K.)
2 Faculty of Agriculture, Shinshu University, 8304 Minamiminowa, Kamiina, Nagano 399-4598, Japan; tamaoon@shinshu-u.ac.jp
3 Institute for Biomedical Sciences, Shinshu University, 8304 Minamiminowa, Kamiina, Nagano 399-4598, Japan
* Correspondence: jaehan@snu.ac.kr; Tel.: +82-2-880-4810

Received: 21 September 2017; Accepted: 23 October 2017; Published: 26 October 2017

Abstract: The rapid development of genome modification technology has provided many great benefits in diverse areas of research and industry. Genome modification technologies have also been actively used in a variety of research areas and fields of industry in avian species. Transgenic technologies such as lentiviral systems and piggyBac transposition have been used to produce transgenic birds for diverse purposes. In recent years, newly developed programmable genome editing tools such as transcription activator-like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9) have also been successfully adopted in avian systems with primordial germ cell (PGC)-mediated genome modification. These genome modification technologies are expected to be applied to practical uses beyond system development itself. The technologies could be used to enhance economic traits in poultry such as acquiring a disease resistance or producing functional proteins in eggs. Furthermore, novel avian models of human diseases or embryonic development could also be established for research purposes. In this review, we discuss diverse genome modification technologies used in avian species, and future applications of avian biotechnology.

Keywords: genome modification; avian; transgenic technology; programmable genome editing; CRISPR/Cas9; primordial germ cells

1. Introduction

Genome modification in living organisms provides several advantages for research and industrial purposes. Transgenic technology, including lentiviral systems [1] and piggyBac transposition [2], has contributed to the development of transgenic animals for exogenous gene expression. Additionally, the development of revolutionary genome editing tools such as zinc finger nuclease (ZFN) [3], transcription activator-like effector nuclease (TALEN) [4], and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9) [5] have made it easy to precisely edit genome information. These tools are expected to contribute to the establishment of customized organisms for specific purposes through programmable genome editing. Applications of the genome editing technologies enable the exploration of unknown gene functions through targeted gene disruption, or they can be used for the development of therapies for genetic disorders through genome replacement. In agriculture, genome editing has been adopted as “precision breeding” using these technologies to recreate biological traits such as growth rate, disease resistance, and the production of bio-functional protein [6].
The development of new genome modification technologies is also creating a surge in the field of avian biotechnology. Due to their enormous potential in diverse disciplines, adaptations of genome editing technology to the avian genome are in high demand. Production of avian lines with disease resistance or the ability to produce functional egg proteins are the most highly desired results in avian biotechnology, as well as their use as an animal model for developmental studies [7]. As a result of long-term efforts in avian biotechnology, some success in avian genome modification has been achieved, and it will be further facilitated in the near future.

In this review, we cover the historical advances in genome modification technology and its applications to biotechnology, particularly in avian biotechnology. Future strategies in avian genome modification technology are also discussed.

2. Development of Transgenic Technologies and Application for Avian Genome Modification

Developing designed living organisms has many benefits for both research and industry. Genetically modified organisms, in particular, have tremendous benefits for the field of biological research. Transgenic technology has developed rapidly since the creation of the first genome-modified mouse by DNA virus injection into an early-stage mouse embryo was reported in 1974 [8]. In 1981, Gordon et al. produced the first transgenic mouse with germline transmission by injecting purified DNA into the pronucleus of a mouse embryo [9]. Although this method has limitations in germline transmission efficiency and random integration, it has been widely used to produce transgenic mice.

In addition to pronuclear injection, viral vector systems have also largely contributed to the development of transgenic animals. Viruses transport their viral genome into cells, and exogenous DNA is easily transduced into host genomes of infected cells using a modified viral genome. The development of several types of viral vector systems using retroviruses, adenoviruses (ADV), herpes simplex viruses (HSV), and adeno-associated viruses (AAV) facilitated the use of viral vectors as transgene over-expression systems for diverse purposes, including gene therapy as well as transgenesis [10].

The first report of viral DNA transduction was published in 1976, when a hybrid virus containing Simian virus 40 (SV40) and lambda phage DNA was transduced into cultured monkey cells [11]. After the first use of a viral vector for DNA delivery was reported, diverse viral vectors were developed for transgenic research. Notably, depending on the viral vector system, the maximum length of an exogenous DNA insert varies (8–10 kilobase pairs (kb) for retroviruses, more than 100 kb for HSV, 8 to 30 kb for ADV, and less than 5 kb for AAV), and the integration profiles are different [12]. With its relatively high packaging capacity and efficient integration profiles compared to ADV and AAV (episomal expression), viral vectors using retroviruses have been widely used in transgenic research [13]. In particular, lentiviruses, a subclass of retroviruses, have advantages for integration compared to other retroviruses. Most retroviruses can only integrate their viral genome into the genomes of dividing cells; however, lentiviruses can also integrate their viral genomes into the genomes of non-dividing cells [14]. This distinguishing characteristic of lentiviruses enables the use of this viral system for genome modification as well as exogenous gene over-expression.

As a result of a highly efficient genome modification capacity, these viral systems have also been used for avian transgenesis. After the first studies of transgenic animal production by retroviral infection in Eyal giladi and Kochav (EG&K) stage X chicken embryos, diverse transgenic birds have been successfully established for a variety of purposes, including functional protein expression and the development of model animals [15–21]. Specifically, mass production of functional proteins using retroviral systems in serum and egg whites of transgenic chickens suggests its practical use as a bioreactor system, although the silencing of transgenes was observed in the subsequent generation (~5.6 mg/mL) [22]. Moreover, oviduct-specific expression of therapeutic proteins in transgenic chickens was successfully achieved using a lentiviral system by delivering a transgene containing ovalbumin regulatory sequences [16]. The viral system has been shown to produce other transgenic birds in addition to chickens, including quail and zebra finches, demonstrating its versatility in the transgenesis of avian species [17,23,24].
Despite the high efficiency of genome modification in living organisms, viral infection systems have fundamental limitations, such as their use in food proteins for human consumption and random exogenous gene integration in host cells causing unexpected results with low germline transmission efficiency and silencing effects in transgenic animals [22,25,26]. To overcome these obstacles, a transgenic technology using transposons was developed. Transposons are mobile DNA elements that take up a large portion of the genome. These mobile elements move between genomes, and in certain cases, have important roles in genome function and evolution through the insertion of certain chromosomal regions. The characteristics of these elements make them useful tools for DNA integration into host genomes as well as in gene trap studies [27].

One of these elements, the piggyBac transposon isolated from the cabbage looper moth (Trichoplusia ni), has been used for genome modification in living organisms [28]. It recognizes TTAA sequences in the genome and inserts into such sites [29], therefore, diverse integration of transposons is possible. Transposon-mediated DNA introduction is very effective in mice and humans, and reports suggest that the germline transmission efficiency of transposons is higher than in viral systems [2,30]. Another transposon actively used for genome modification is Tol2, isolated from medaka fish (Oryzias latipes) [31]. The Tol2 transposons also have “cut and paste” mechanisms similar to other transposons, and they have been successfully used in diverse vertebrate cells from zebrafish, frogs, mice, and humans [32]. In addition, a synthetic DNA transposon, the Sleeping Beauty transposon, has been used as an exogenous gene delivery system. It was artificially synthesized from the genomes of salmonid fish [33]. Similar to other transposon systems, it can also recognize specific DNA sequences and integrate into the host genome.

Efficient transgenic technology has been successfully adapted for the production of transgenic chickens with PGC transplantation or direct injection in vivo [34–39]. Our previous studies reported that piggyBac transposition was effective in producing transgenic chickens mediated by transplantation of genome-modified PGCs (over 90% germline transmission efficiency) [38,39], and the system was used to develop a chicken model combined with site-specific recombination technology [36]. Not only piggyBac transposon-, but also Tol2 transposon-mediated transgenic chickens have been reported. The reports showed that Tol2 transposons integrated into the genomes of chicken cell lines, including the PGCs, in an efficient manner (45.2%) [37], and even direct in vivo injection of Tol2 transposons and transposase plasmids into the bloodstream of chicken embryos was successfully used to produce transgenic chickens, despite low germline transmission efficiency (<2%) [35,40].

3. Homologous Recombination Technology for Gene Targeting

Although transgenic technology provides opportunities for developing useful model organisms in a highly efficient manner, this technology also has fundamental limitations due to random integration. With precise genome editing technology under high demand, the first homologous recombination technology adapted to mouse embryonic stem cells (ESCs) was reported [41]. Homologous recombination is one of the natural DNA repair mechanisms in living organisms, along with non-homologous end joining (NHEJ), which occurs during meiosis, resulting in genetic diversity between parents and their offspring. To adopt this phenomenon to gene targeting technology, donor plasmids should contain DNA homology sequences for targeted loci with proper selective markers. DNA homology in the donor plasmid can induce targeted replacement between the host genome and donor plasmid; therefore, the length of a DNA homology arm is critical for recombination efficiency, although the efficiency could vary depending on the targeted locus [42]. Although the efficiency of homologous recombination technology was extremely low (0.001%) compared to transgenic technology for genome modification, attempts to enhance homologous recombination events by transient alteration of gene expression relating to DNA repair mechanism, specifically RAD52, have been performed [43]. Furthermore, the development of precise genome editing mediated by homologous recombination provides tremendous benefits for studying specific gene functions in living organisms.
The first knockout chicken was developed using this classical gene targeting technology [44]. The report showed that the joining (J) gene segment of the chicken immunoglobulin (Ig) heavy chain gene was targeted by homologous recombination in the genome of PGCs, and knockout chickens were produced by PGC transplantation. The efficiency of gene targeting of the Ig gene of PGCs was about one targeted clone per $10^7$ cells (0.00001%), and germline transmission of knockout clones was extremely low (0.005–0.2%). The results indicated that the combined gene targeting technologies of homologous recombination and chicken PGC culture systems was a promising method for producing gene-targeted chickens, but required improvement in terms of efficiency for further research.

4. Site-Specific Recombination

With homologous recombination technology, site-specific recombination using recombinases has contributed greatly to the field of genome editing technology. By inserting recombinase recognition target sites into donor plasmids, targeted regions were modified with recombinase treatment. The technology is called “conditional regulation”, which is a useful tool for studying genes that are critical for cell survival [45].

The most well-known site-specific recombinase is the Cre-loxP system. Cre recombinase, derived from the P1 bacteriophage [46], recognizes loxP sequences and modifies the sequences floxed by loxP sequences. When identical loxP sequences lay in the same orientation in the genome, Cre cleaves the floxed sequences (excision). When the same loxP sequences lay in different orientations, the floxed sequences are inverted (inversion). When two different loxP sequences lay in the same orientation, the floxed sequences are replaced when donor plasmids containing the same two different loxP sequences are used (recombinase-mediated gene cassette exchange; RMCE) [47]. Flipase/Flipase recognition target (Flp/FRT) system is an additional site-specific recombination technology. The system is derived from yeast (Saccharomyces cerevisiae) and can lead to genetic recombination [48]. The recombination depends on the FRT orientation, and the mechanism is similar to the Cre-loxP system. Two site-specific recombination systems have been used in diverse organisms, including mice and cows [49,50].

In avian species, site-specific recombination mediated by Cre-loxP was used to express monoclonal antibodies driven by the ovalbumin promoter in primary oviduct cells [51], and recently a transgenic chicken expressing Cre recombinase was produced for targeted excision of the loxP-floxed genome [52]. In both cases, Cre-loxP recombination was successfully adapted for genome rearrangement (excision), indicating its possible application for obtaining desired genotypes in avian species. Our group also demonstrated RMCE in transgenic-derived cells using the Flp/FRT recombination system [36]. The successful genome replacement on predetermined loci of a transgenic chicken genome suggests that it could be used to induce the expression of exogenous proteins at predictable levels in transgenic chicken lines.

Another well-known site-specific recombinase is PhiC31 integrase. It is derived from bacteria, and recognizes and modifies PhiC31-specific attP and attB sites. The recombinase recognizes, rotates, and pastes double strands of the floxed genome, and the attP and attB sites are converted to attL and attR that do not react with the PhiC31 integrase [53]. Therefore, unlike the Cre-loxP and Flp/FRT recombination systems, the recombination is irreversible. This recombination system is used as a cloning tool, called “gateway cloning” [54]. Site-specific recombination technology using PhiC31 integrase has also been used for transgene integration in avian species. Humanized antibody expression was induced by PhiC31 integrase with homologous recombination in the chicken B cell line, DT40 [55], and Cre-expressing transgenic chicken was mediated by adapting the technology to chicken PGCs [52]. The irreversible recombination of this technology may aid in establishing desired clones without further genome alteration.
5. Programmable Genome Editing Using Endonucleases

In biology, the emergence of programmable genome editing technology is considered the most remarkable event of the 21st century. Precise recognition and efficient cleavage of double strand DNA using programmable genome editing tools opened a new era of biotechnology. The tools not only induce loss-of-function of the gene, but also enhance homologous recombination by double-strand DNA breakage. Furthermore, the synthesis of these tools is becoming increasingly convenient, as thousands of customized vectors are now available.

The first generation of programmable genome editing tools was ZFN. ZFN is composed of zinc finger motifs that can bind to DNA nucleotides, and the FokI endonuclease, found in Flavobacterium okeanokoites, is involved in the double-strand breaks [3]. Zinc finger motifs can bind three nucleotides depending on amino acid composition, and pairs of ZFN motifs are more effective in DNA cleavage [56]. Furthermore, combinations of several ZFNs enable recognition and mutation of specific genomic loci [57]. ZFN has been widely used in genome editing in living organisms, including insects, plants, and even human cells [58].

The second generation of programmable genome editing tools is TALEN. TALEN is composed of a TAL effector that is derived from pathogenic bacteria, Xanthomonas, and the same FokI endonuclease used in ZFN [4]. TALEN also recognizes and induces double-strand breaks of targeted nucleotides, and the TAL effector has a critical role in recognizing nucleotides. The DNA binding domain of the TAL effector contains 33–34 conserved amino acid sequences with a variable region at amino acid positions 12 and 13, called the repeat variable di-residue (RVD). Depending on the amino acids of the RVD, a TAL effector recognizes different nucleotides (HD binds to C, NG binds to T, NI binds to A, and NN binds to A or G) [4]. Unlike ZFN, each TAL effector containing one repeat domain can bind to one nucleotide, thus it has an advantage over ZFN in target site design [59]. Because TAL effectors can combine with activator domains, such as transcriptional activators and endonucleases, TALEN has been used widely for diverse purposes in diverse organisms [60]. With the development of the TALEN assembly kit using golden gate shuffling, customized TALEN is now easily synthesized [61–64].

We successfully used TALEN to produce chickens with a mutant ovalbumin gene. As a programmable genome-editing tool, TALEN breaks double-stranded genomic DNA and induces NHEJ [65]. NHEJ occurs more frequently than homology directed repair in nature; therefore, TALEN exhibits a higher efficiency in introducing indel mutations than homologous recombination-mediated genome editing. Our chicken ovalbumin gene knockout using TALEN was highly effective (~60% in DF-1 fibroblasts and ~36.7% in chicken PGCs), and the germline transmission efficiency also improved drastically (~53.2% germline transmission and ~10.4% genome-edited chicken production) compared to classical gene targeting technology using homologous recombination (0.005–0.2% efficiency) without genomic integration of exogenous DNA [44,66]. More recently, TALEN-mediated gene targeting was applied to chicken DDX4 gene disruption. The study showed that the DDX4 gene was successfully replaced by TALEN-mediated homologous recombination in PGCs (8.1%), and genome-edited chickens were produced by transplanting PGCs (~6%). Disruption of the DDX4 gene caused loss of PGCs in female chickens, but heterozygous male chickens remained fertile [67]. The enhanced genome editing efficiency compared with previous research suggests that the induction of double-strand breaks by endonucleases is an efficient tool for precise genome editing in avian species [44,67].

The third generation of programmable genome editing tools is CRISPR/Cas9. CRISPR and Cas9 are components of prokaryotic DNA that have important roles in the bacterial immune system. In nature, bacteria have clustered repeats called CRISPRs that are identical to viral genomes. When a virus invades a cell, a CRISPR binds to viral RNA and disrupts it with the Cas9 protein [68]. The CRISPR/Cas9 system also recognizes and induces double-strand breaks in targeted DNA sequences, similarly to ZFN and TALEN. To recognize and disrupt targeted nucleotides, the CRISPR/Cas9 system requires the Cas protein, CRISPR RNA (crRNA), and trans-activating CRISPR RNA (tracrRNA) [5]. Unlike ZFN and TALEN, CRISPR/Cas9 does not require paired units
to induce double strand breaks. Moreover, as synthesis of crRNA and tracrRNA is relatively simple, thousands of customized CRISPR/Cas9 systems for targeting genes have been constructed. Owing to the convenience of CRISPR/Cas9 vector construction, this programmable genome editing tool has been used in most living organisms [69].

As the most versatile tool for genome editing, the CRISPR/Cas9 system has also been successfully adopted in many avian species, including chicken and quail. CRISPR/Cas9 has been successfully used to modify genes with high efficiency in avian somatic cells, chicken ESCs, and spermatogonial stem cells (SSCs) [70–74]. Furthermore, introduction of CRISPR vectors into PGCs succeeded in producing genome edited chickens with indel mutations in the ovomucoid gene and replaced gene cassettes in the chicken immunoglobulin gene [75,76]. This study showed that targeted gene knockout mediated by CRISPR/Cas9 was very effective in chicken PGCs (~100%), and germline transmission was also relatively higher (~58%) compared with TALEN [66,76]. Germline transmission was more efficient with CRISPR-mediated knock-in (~48%) than TALEN-mediated knock-in [67,75]. These results indicate that this revolutionary tool is the most effective programmable genome editing technology for avian species to date, see Table 1.

### Table 1. Comparison of programmable genome editing technologies adopted in avian species.

| Methods                        | Efficiency of Genome Editing in Chicken PGCs | Efficiency of Germline Transmission (Genome-Edited Chickens) | References |
|--------------------------------|---------------------------------------------|---------------------------------------------------------------|------------|
| TALEN                          | 33.3%                                       | 22.3–53.2% (0.0–10.4%)                                       | [66]       |
| CRISPR/Cas9                    | 0–100%                                      | 67–79% (48–58%)                                              | [76]       |
| Homologous recombination       | 0.00001%                                    | 0.005–0.2% (NA)                                              | [44]       |
| TALEN + homologous recombination | 8.1%                                        | NA 1 (0–6%)                                                 | [67]       |
| CRISPR/Cas9 + homologous recombination | 20–33%                                    | 0–96% (0–48%)                                               | [75]       |

1 Not available.

In addition to the development of precise and efficient genome editing tools such as CRISPR/Cas9, new technologies for enhancing genome-editing capacity have been developed. CRISPR from Prevotella and Francisella 1 (Cpf1) is an example. It is also derived from the bacterial immune system, and has similar effects to the CRISPR/Cas9 system. The Cpf1 protein, which has RuvC domains that are responsible for cleaving DNA strands, works with Cpf1-specific single CRISPR guide RNA. In contrast to CRISPR/Cas9, protospacer adjacent motif (PAM) sequences of Cpf1 guide RNAs (TTN or TTTN depending on the bacterial strain) are different to CRISPR/Cas9 (NGG, NNAGAAW or NNNNGATT depending on the bacterial strain) [77,78], and the cleavage site is separate from the PAM sequence [78]. The cleavage pattern suggests that CRISPR/Cpf1 can be used to target different sites simultaneously using several gRNAs that cannot be modified by CRISPR/Cas9, and genome editing mediated by homologous recombination may be enhanced compared to the CRISPR/Cas9 system. Moreover, several studies using genome-wide analysis such as Digenome-seq have reported that CRISPR/Cpf1 has lower off-target effects—which is the main problem for genome editing in all species—than CRISPR/Cas9, thus it is considered more suitable for gene correction [79,80].

In addition to Cpf1, applications combined with CRISPR/Cas9 have also been reported. These studies introduced CRISPR-cytidine deaminase complexes, which convert cytidine to other nucleotides without indel mutations on targeted loci, called “base editing” [81,82]. The base editing system could be applied to gene correction without homologous recombination technology or indel mutations.

### 6. Future Directions in Avian Biotechnology

Over the past few decades, the development of genome editing technologies has brought about many changes in the field of biology. In avian species, genome-editing technology has enabled precise
and efficient modification of the avian genome, and now, it is expected to maximize the value of the avian system for industrial and research purposes.

One of the most highly anticipated goals of avian technology is to establish valuable chicken lines with high growth and egg production rates through genome editing technology, called “precision breeding” [6]. Because the chicken is a highly productive protein resource for humans, enhancing its economic traits through genome editing will be helpful in providing a low-cost food source. Not only could the amount of protein be increased, but the quality of chicken protein could also be improved through genome editing. By modifying egg protein genes such as ovalbumin and ovotransferrin, as well as other egg proteins, diverse properties such as nutrient levels, allergenicity, or the production of bio-functional materials in eggs could be regulated. Furthermore, mass production of valuable functional proteins would be possible through precise gene insertion into endogenous egg protein coding sequences with genome editing technology [7]. These new breeding approaches could yield great benefits to human society.

Another expected outcome for avian genome editing technology is the development of disease resistant avian lines. Pandemic diseases such as avian influenza (AI) and avian leukosis virus (ALV) have caused enormous damage to the poultry industry and to the general population. Previously reported research has suggested that overexpression of small hairpin RNA specifically regulating viral RNA polymerase expression may prevent AI transmission [83], suggesting that genome editing is the one of most promising strategies for disease resistance in avian species. Alternatively, genome editing of host receptors could be applied for disease resistance. Fortunately, the host receptors of AI and ALV have been well described, and our previous results suggest genome mutations on host receptor alter disease resistant in chicken cell line [84–90]. Furthermore, the strategy was proven in human immunodeficiency virus (HIV) by genetic mutations in the CCR5 host receptor gene [91]. With genome editing of these receptors, modified organisms could be easily evaluated in vitro and in vivo, and such studies could contribute to the establishment of disease-resistant chickens (Figure 1).

**Figure 1.** (a) CRISPR/Cas9 system for programmable genome editing. Guide RNA specifically binds to genomic DNA, and then Cas9 enzyme breaks double strand of DNA adjacent to protospacer adjacent motif (PAM) sequence; (b) Genome modification of egg protein coding genes for production of bio-functional materials; (c) Precise genome modification of host receptor for preventing viral infections, T arrow indicates blockade of virus-host binding; (d) CRISPR/Cas9-mediated knock-in of fluorescent protein gene to germ cell specific genes (tagging) for establishment of germ cell tracing model following embryogenesis (dashed arrow).
Despite the rapid development of genome editing technology, there is still much room for improvement. Germline transmission in avian species largely depends on PGC transplantation methods, and long-term in vitro culture of germline-competent cells is a prerequisite for precise genome editing in avian species [92]. Although there have been many successful reports on long-term culturing of PGCs following genome editing, the methods are time-consuming and require great skill [93,94]. To establish highly germline-competent cell lines, in-depth studies on the cells, involving signaling pathways, gene expression, and the development of new in vitro culture systems, should be performed. Alternatively, direct gene injection into the embryonic bloodstream or testis is a potential method for inducing germline transmission [40]. Transplantation of genome-edited testicular cells or SSCs into adult chicken testis may reduce the length of time needed to establish genome-edited chickens [95,96]. Furthermore, a recent study reporting sperm transfection-assisted gene editing (STAGE) suggested the utilization of bird sperm as a mediator for genome modification in avian species [97].

7. Conclusions

The advent of genome editing technology heralds a new era in avian biotechnology. Because avian species have a great deal of merit in diverse fields due to their unique biological characteristics, the variety of research involving genome editing in avian species using these genome editing technologies is expected to increase. The development of avian biotechnology will contribute to the development of diverse research disciplines and fields of industry benefitting human society.

Acknowledgments: This work was supported by a National Research Foundation of Korea (NRF) grant, funded by the Korean government (MSIP; No. 2015R1A3A203826). This research was also supported by the International Research & Development Program of the National Research Foundation of Korea (NRF) funded by Ministry of Science, Information and Communication Technology (ICT) and Future Planning of Korea (NRF-2016K1A3A1A21005676).

Author Contributions: Hong Jo Lee wrote first draft of the manuscript. Young Min Kim and Tamao Ono revised the whole manuscript. Jae Yong Han wrote and revised the whole manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| TALEN | Transcription activator like effector nuclease |
| CRISPR/Cas9 | Clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 |
| ADV | Adenoviruses |
| HSV | Herpes simplex viruses |
| AAV | Adeno-associated viruses |
| SV40 | Simian virus 40 |
| kb | Kilobase pairs |
| EG&K | Eyal Giladi and Kochav |
| PGC | Primordial germ cell |
| ZFN | Zinc finger nuclease |
| ESC | Embryonic stem cell |
| NHEJ | Non-homologous end joining |
| RMCE | Recombinase mediated gene cassette exchange |
| Flp/FRT | Flipase/Flipase recognition target |
| RVD | Repeat Variable Di-residue |
| crRNA | CRISPR RNA |
| tracrRNA | Trans-activating CRISPR RNA |
| PAM | Protospacer adjacent motif |
| AI | Avian influenza |
| ALV | Avian leukemia virus |
| Cpf1 | CRISPR from Prevotella and Francisella |
GUIDE-seq  Genome-wide, unbiased identification of double strand breaks enabled by sequencing
SSCs  Spermatogonial stem cells
HIV  Human immunodeficiency virus
STAGE  Sperm transfection-assisted gene editing

References
1. Uchida, N.; Sutton, R.E.; Friera, A.M.; He, D.; Reitsma, M.J.; Chang, W.C.; Scollay, R.; Weissman, I.L. HIV, but not murine leukemia virus, vectors mediate high efficiency gene transfer into freshly isolated G0/G1 human hematopoietic stem cells. *Proc. Natl. Acad. Sci. USA* 1998, 95, 11939–11944. [CrossRef] [PubMed]
2. Ding, S.; Wu, X.; Li, G.; Han, M.; Zhuang, Y.; Xu, T. Efficient transposition of the piggybac (pb) transposon in mammalian cells and mice. *Cell* 2005, 122, 473–483. [CrossRef] [PubMed]
3. Kim, Y.G.; Cha, J.; Chandrasegaran, S. Hybrid restriction enzymes: Zinc finger fusions to fok I cleavage domain. *Proc. Natl. Acad. Sci. USA* 1996, 93, 1156–1160. [CrossRef] [PubMed]
4. Boch, J.; Scholze, H.; Schornack, S.; Landgraf, A.; Hahn, S.; Kay, S.; Lahaye, T.; Nickstadt, A.; Bonas, U. Breaking the code of DNA binding specificity of TAL-type III effectors. *Science* 2009, 326, 1509–1512. [CrossRef] [PubMed]
5. Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J.A.; Charpentier, E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012, 337, 816–821. [CrossRef] [PubMed]
6. Tizard, M.; Hallerman, E.; Fahrenkrug, S.; Newell-McGlashan, M.; Gibson, J.; de Loos, F.; Wagner, S.; Laible, G.; Han, J.Y.; D’Occhio, M.; et al. Strategies to enable the adoption of animal biotechnology to sustainably global food safety and security. *Transgenic Res.* 2016, 25, 575–595. [CrossRef] [PubMed]
7. Lee, H.J.; Lee, H.C.; Hauer, M.; Doudna, J.A.; Charpentier, E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012, 337, 816–821. [CrossRef] [PubMed]
8. Jaenisch, R.; Mintz, B. Simian virus 40 DNA sequences in DNA of healthy adult mice derived from preimplantation blastocysts injected with viral DNA. *Proc. Natl. Acad. Sci. USA* 1974, 71, 1250–1254. [CrossRef] [PubMed]
9. Gordon, J.W.; Ruddle, F.H. Integration and stable germ line transmission of genes injected into mouse pronuclei. *Science* 1981, 214, 1244–1246. [CrossRef] [PubMed]
10. Bouard, D.; Alazard-Dany, N.; Cosset, F.L. Viral vectors: From virology to transgene expression. *Br. J. Pharmacol.* 2009, 157, 153–165. [CrossRef] [PubMed]
11. Goff, S.P.; Berg, P. Construction of hybrid viruses containing sv40 and lambda phage DNA segments and their propagation in cultured monkey cells. *Cell* 1976, 9, 695–705. [CrossRef]
12. Thomas, C.E.; Ehrhardt, A.; Kay, M.A. Progress and problems with the use of viral vectors for gene therapy. *Nat. Rev. Genet.* 2003, 4, 346–358. [CrossRef] [PubMed]
13. Brown, P.O. Integration of retroviral DNA. *Curr. Top. Microbiol. Immunol.* 1990, 157, 19–48. [PubMed]
14. Yamashita, M.; Emerman, M. Retroviral infection of non-dividing cells: Old and new perspectives. *Virology* 2006, 344, 88–93. [CrossRef] [PubMed]
15. Salter, D.W.; Smith, E.J.; Hughes, S.H.; Wright, S.E.; Fadly, A.M.; Witter, R.L.; Crittenden, L.B. Gene insertion into the chicken germ line by retroviruses. *Poulit. Sci.* 1986, 65, 1445–1458. [CrossRef] [PubMed]
16. Lillico, S.G.; Sherman, A.; McGrew, M.J.; Robertson, C.D.; Smith, J.; Haslam, C.; Barnard, P.; Radcliffe, P.A.; Mitrophanous, K.A.; Elliot, E.A.; et al. Oviduct-specific expression of two therapeutic proteins in transgenic hens. *Proc. Natl. Acad. Sci. USA* 2007, 104, 1771–1776. [CrossRef] [PubMed]
17. Shin, S.S.; Kim, T.M.; Kim, S.Y.; Kim, T.W.; Lee, S.K.; Kwon, S.C.; Lee, G.S.; Kim, H.; Lim, J.M.; et al. Generation of transgenic quail through germ cell-mediated germine transmission. *FASEB J.* 2008, 22, 2435–2444. [CrossRef] [PubMed]
18. Kamihira, M.; Kawabe, Y.; Shindo, T.; Ono, K.; Esaka, K.; Yamashita, T.; Nishijima, K.; Iijima, S. Production of chimeric monoclonal antibodies by genetically manipulated chickens. *J. Biotechnol.* 2009, 141, 18–25. [CrossRef] [PubMed]
19. Velho, T.A.; Lois, C. Generation of transgenic zebra finches with replication-deficient lentiviruses. *Cold Spring Harb. Protoc.* 2014, 2014, 1284–1289. [CrossRef] [PubMed]
20. Huss, D.; Benazeraf, B.; Wallingford, A.; Filla, M.; Yang, J.; Fraser, S.E.; Lansford, R. A transgenic quail model that enables dynamic imaging of amniote embryogenesis. Development 2015, 142, 2850–2859. [CrossRef] [PubMed]

21. June Byun, S.; Yuk, S.S.; Jang, Y.J.; Choi, H.; Jeon, M.H.; Erdene-Ochir, T.O.; Kwon, J.H.; Noh, J.Y.; Sun Kim, J.; Gyu Yoo, J.; et al. Transgenic chickens expressing the 3D8 single chain variable fragment protein suppress avian influenza transmission. Sci. Rep. 2017, 7, 5938. [CrossRef] [PubMed]

22. Kamihira, M.; Ono, K.; Esaka, K.; Nishijima, K.; Kigaku, R.; Komatsu, H.; Yamashita, T.; Kyogoku, K.; Iijima, S. High-level expression of single-chain Fv-Fc fusion protein in serum and egg white of genetically manipulated chickens by using a retroviral vector. J. Virol. 2005, 79, 10864–10874. [CrossRef] [PubMed]

23. Agate, R.J.; Scott, B.B.; Haripal, B.; Lois, C.; Nottebohm, F. Transgenic songbirds offer an opportunity to develop a genetic model for vocal learning. Proc. Natl. Acad. Sci. USA 2009, 106, 17963–17967. [CrossRef] [PubMed]

24. Kwon, S.C.; Choi, J.W.; Jang, H.J.; Shin, S.S.; Lee, S.K.; Park, T.S.; Choi, I.Y.; Lee, G.S.; Song, G.; Han, J.Y. Production of biofunctional recombinant human interleukin 1 receptor antagonist (rhIL1Rn) from transgenic quail egg white. Bioll. Reprod. 2010, 82, 1057–1064. [CrossRef] [PubMed]

25. Mizuarai, S.; Ono, K.; Yamaguchi, K.; Nishijima, K.; Kamihira, M.; Iijima, S. Production of transgenic quails with high frequency of germ-line transmission using vsv-g pseudotyped retroviral vector. Biochem. Biophys. Res. Commun. 2001, 286, 456–463. [CrossRef] [PubMed]

26. Ellis, J. Silencing and variegation of gammaretrovirus and lentivirus vectors. Hum. Gene Ther. 2005, 16, 1241–1246. [CrossRef] [PubMed]

27. Bucher, E.; Reinders, J.; Mirouze, E. Epigenetic control of transposon transcription and mobility in arabidopsis. Curr. Opin. Plant Biol. 2012, 15, 503–510. [CrossRef] [PubMed]

28. Cary, L.C.; Goebel, M.; Corsaro, B.G.; Wang, H.G.; Rosen, E.; Fraser, M.J. Transposon mutagenesis of baculoviruses: Analysis of trichoplusia ni transposon IFP2 insertions within the FP-locus of nuclear polyhedrosis viruses. Virology 1989, 172, 156–169. [CrossRef]

29. Bauser, C.A.; Elick, T.A.; Fraser, M.J. Proteins from nuclear extracts of two lepidopteran cell lines recognize ttaa-specific transposons piggybac and tagalong. Insect Mol. Biol. 1999, 8, 223–230. [CrossRef] [PubMed]

30. Wang, W.; Lin, C.; Lu, D.; Ning, Z.; Cox, T.; Melvin, D.; Wang, X.; Bradley, A.; Liu, P. Chromosomal transposition of piggybac in mouse embryonic stem cells. Proc. Natl. Acad. Sci. USA 2008, 105, 9290–9295. [CrossRef] [PubMed]

31. Koga, A.; Suzuki, M.; Inagaki, H.; Bessho, Y.; Hori, H. Transposable element in fish. Nature 1996, 383, 30. [CrossRef] [PubMed]

32. Kawakami, K. Tol2: A versatile gene transfer vector in vertebrates. Genome Biol. 2007, 8, S7. [CrossRef] [PubMed]

33. Ivics, Z.; Hackett, P.B.; Plasterk, R.H.; Izsvak, Z. Molecular reconstruction of sleeping beauty, a tcl-like transposon from fish, and its transposition in human cells. Cell 1997, 91, 501–510. [CrossRef]

34. Glover, J.D.; Taylor, L.; Sherman, A.; Zeiger-Poli, C.; Sang, H.M.; McGrew, M.J. A novel piggybac transposon inducible expression system identifies a role for akt signalling in primordial germ cell migration. PLoS ONE 2013, 8, e77222. [CrossRef] [PubMed]

35. Lambeth, L.S.; Morris, K.R.; Wise, T.G.; Cummins, D.M.; O’Neil, T.E.; Cao, Y.; Sinclair, A.H.; Doran, T.J.; Smith, C.A. Transgenic chickens overexpressing aromatase have high estrogen levels but maintain a predominantly male phenotype. Endocrinology 2016, 157, 83–90. [CrossRef] [PubMed]

36. Lee, H.J.; Lee, H.C.; Kim, Y.M.; Hwang, Y.S.; Park, Y.H.; Park, T.S.; Han, J.Y. Site-specific recombination in the chicken genome using flipase recombinase-mediated cassette exchange. FASEB J. 2016, 30, 555–563. [CrossRef] [PubMed]

37. Macdonald, J.; Taylor, L.; Sherman, A.; Kawakami, K.; Takahashi, Y.; Sang, H.M.; McGrew, M.J. Efficient genetic modification and germ-line transmission of primordial germ cells using piggybac and tol2 transposons. Proc. Natl. Acad. Sci. USA 2012, 109, E1466–E1472. [CrossRef] [PubMed]

38. Park, T.S.; Han, J.Y. Piggybac transposition into primordial germ cells is an efficient tool for transgenesis in chickens. Proc. Natl. Acad. Sci. USA 2012, 109, 9337–9341. [CrossRef] [PubMed]
39. Park, T.S.; Lee, H.G.; Moon, J.K.; Lee, H.J.; Yoon, J.W.; Yun, B.N.; Kang, S.C.; Kim, J.; Kim, H.; Han, J.Y.; et al. Deposition of bioactive human epidermal growth factor in the egg white of transgenic hens using an oviduct-specific minisynthetic promoter. *FASEB J.* **2015**, *29*, 2386–2396. [CrossRef] [PubMed]

40. Tyack, S.G.; Jenkins, K.A.; O’Neil, T.E.; Wise, T.G.; Morris, K.R.; Bruce, M.P.; McLeod, S.; Wade, A.J.; McKay, J.; Moore, R.J.; et al. A new method for producing transgenic birds via direct in vivo transfection of primordial germ cells. *Transgenic Res.* **2013**, *22*, 1257–1264. [CrossRef] [PubMed]

41. Thomas, K.R.; Capecechi, M.R. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. *Cell* **1987**, *51*, 503–512. [CrossRef]

42. Inbar, O.; Liefshitz, B.; Bitan, G.; Kupiec, M. The relationship between homology length and crossing over during the repair of a broken chromosome. *J. Biol. Chem.* **2000**, *275*, 30833–30838. [CrossRef] [PubMed]

43. Vasquez, K.M.; Marburger, K.; Intody, Z.; Wilson, J.H. Manipulating the mammalian genome by homologous recombination. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 8403–8410. [CrossRef] [PubMed]

44. Schusser, B.; Collarini, E.J.; Yi, H.; Izquierdo, S.M.; Fesler, J.; Pedersen, D.; Klasing, K.C.; Kaspers, B.; Harriman, W.D.; van de Lavoir, M.C.; et al. Immunoglobulin knockout chickens via efficient homologous recombination in primordial germ cells. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 20170–20175. [CrossRef] [PubMed]

45. Skarnes, W.C.; Rosen, B.; West, A.P.; Koutoutrakis, M.; Bushell, W.; Iyer, V.; Mujica, A.O.; Thomas, M.; Harrow, J.; Cox, T.; et al. A conditional knockout resource for the genome-wide study of mouse gene function. *Nature* **2011**, *474*, 337–342. [CrossRef] [PubMed]

46. Abremksi, K.; Hoess, R. Bacteriophage P1 site-specific recombination. Purification and properties of the cre recombinase protein. *J. Biol. Chem.* **1984**, *259*, 1509–1514. [PubMed]

47. Nagy, A. Cre recombinase: The universal reagent for genome tailoring. *Genesis* **2000**, *26*, 99–109. [CrossRef]

48. Schlake, T.; Bode, J. Use of mutated FLP recognition target (frt) sites for the exchange of expression cassettes at defined chromosomal loci. *Biochemistry* **1994**, *33*, 12746–12751. [CrossRef] [PubMed]

49. Graham, C.; Cole, S.; Laible, G. Site-specific modification of the bovine genome using cre recombinase-mediated gene targeting. *Biotechnol. J.* **2009**, *4*, 108–118. [CrossRef] [PubMed]

50. Ohtsuka, M. Development of pronuclear injection-based targeted transgenesis in mice through cre-loxP site-specific recombination. *Methods Mol. Biol.* **2014**, *1194*, 3–19. [PubMed]

51. Oishi, I.; Kim, S.; Yoshii, K.; Esteban, C.R.; Izpisua Belmonte, J.C. Cre-loxP-regulated expression of monoclonal antibodies driven by an ovalbumin promoter in primary oviduct cells. *BMC Biotechnol.* **2011**, *11*, 5. [CrossRef] [PubMed]

52. Leighton, P.A.; Pedersen, D.; Ching, K.; Collarini, E.J.; Izquierdo, S.; Jacob, R.; van de Lavoir, M.C. Generation of chickens expressing cre recombinase. *Transgenic Res.* **2016**, *25*, 609–616. [CrossRef] [PubMed]

53. Bateman, J.R.; Lee, A.M.; Wu, C.T. Site-specific transformation of drosophila via phiC31 integrase-mediated cassette exchange. *Genetics* **2006**, *173*, 769–777. [CrossRef] [PubMed]

54. Hartley, J.L.; Temple, G.F.; Brasch, M.A. DNA cloning using in vitro site-specific recombination. *Genome Res.* **2000**, *10*, 1788–1795. [CrossRef] [PubMed]

55. Schusser, B.; Yi, H.; Collarini, E.J.; Izquierdo, S.M.; Harriman, W.D.; Etches, R.J.; Leighton, P.A. Harnessing gene conversion in chicken b cells to create a human antibody sequence repertoire. *PLoS ONE* **2013**, *8*, e80108. [CrossRef] [PubMed]

56. Smith, J.; Bibikova, M.; Whitby, F.G.; Reddy, A.R.; Chandrasegaram, S.; Carroll, D. Requirements for double-strand cleavage by chimeric restriction enzymes with zinc finger DNA-recognition domains. *Nucleic Acids Res.* **2000**, *28*, 3361–3369. [CrossRef] [PubMed]

57. Porteous, M.H.; Carroll, D. Gene targeting using zinc finger nucleases. *Nat. Biotechnol.* **2005**, *23*, 967–973. [CrossRef] [PubMed]

58. Urnov, F.D.; Rebar, E.J.; Holmes, M.C.; Zhang, H.S.; Gregory, P.D. Genome editing with engineered zinc finger nucleases. *Nat. Rev. Genet.* **2010**, *11*, 636–646. [CrossRef] [PubMed]

59. Miller, J.C.; Tan, S.; Qiao, G.; Barlow, K.A.; Wang, J.; Xia, D.F.; Meng, X.; Paschon, D.E.; Leung, E.; Hinkley, S.J.; et al. A tale nuclease architecture for efficient genome editing. *Nat. Biotechnol.* **2011**, *29*, 143–148. [CrossRef] [PubMed]

60. Mussolino, C.; Cathomen, T. Tale nucleases: Tailored genome engineering made easy. *Curr. Opin. Biotechnol.* **2012**, *23*, 644–650. [CrossRef] [PubMed]
81. Komor, A.C.; Kim, Y.B.; Packer, M.S.; Zuris, J.A.; Liu, D.R. Programmable editing of a target base in genomic DNA. *PLoS ONE* 2009, 4, e5553. [CrossRef] [PubMed]

82. Nishida, K.; Arazoe, T.; Yachie, N.; Banno, S.; Kakimoto, M.; Tabata, M.; Mochizuki, M.; Miyabe, A.; Araki, M.; Hara, K.Y.; et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. *Science* 2016, 353, aaf8729. [CrossRef] [PubMed]

83. Sanjana, N.E.; Cong, L.; Zhou, Y.; Cunniff, M.M.; Feng, G.; Zhang, F. A transcription activator-like effector toolbox for genome engineering. *Nat. Protoc.* 2012, 7, 171–192. [CrossRef] [PubMed]

84. Cermak, T.; Doyle, E.L.; Christian, M.; Wang, L.; Zhang, Y.; Schmidt, C.; Baller, J.A.; Somia, N.V.; Bogdanove, A.J.; Voytas, D.F. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Res.* 2011, 39, e82. [CrossRef] [PubMed]

85. Christian, M.; Cermak, T.; Doyle, E.L.; Schmidt, C.; Zhang, F.; Hummel, A.; Bogdanove, A.J.; Voytas, D.F. Targeting DNA double-strand breaks with tal effector nucleases. *Genetics* 2010, 186, 757–761. [CrossRef]

86. Park, T.S.; Lee, H.J.; Kim, K.H.; Kim, J.S.; Han, J.Y. Targeted gene knockout in chickens mediated by talens. *Proc. Natl. Acad. Sci. USA* 2014, 111, 12716–12721. [CrossRef] [PubMed]

87. Taylor, L.; Carlson, D.F.; Nandi, S.; Sherman, A.; Fahrenkrug, S.C.; McGrew, M.J. Efficient talen-mediated targeting of chicken primordial germ cells. *Development* 2017, 144, 928–934. [CrossRef] [PubMed]

88. Barrangou, R.; Fremaux, C.; Deveau, H.; Richards, M.; Boyaval, P.; Moineau, S.; Romero, D.A.; Horvath, P. CRISPR provides acquired resistance against viruses in prokaryotes. *Science* 2007, 315, 1709–1712. [CrossRef] [PubMed]

89. Reardon, S. Welcome to the CRISPR zoo. *Nature* 2016, 531, 160–163. [CrossRef] [PubMed]

90. Abu-Bonsrah, K.D.; Zhang, D.; Newgreen, D.F. CRISPR/Cas9 targets chicken embryonic somatic cells in vitro and in vivo and generates phenotypic abnormalities. *Sci. Rep.* 2016, 6, 34524. [CrossRef] [PubMed]

91. Ahn, J.; Lee, J.; Park, J.Y.; Oh, K.B.; Hwang, S.; Lee, C.W.; Lee, K. Targeted genome editing in a quail cell line using a customized CRISPR/cas9 system. *Poult. Sci.* 2017, 96, 1445–1450. [CrossRef] [PubMed]

92. Veron, N.; Qu, Z.; Kipen, P.A.; Hirst, C.E.; Marcelle, C. CRISPR mediated somatic cell genome engineering in the chicken. *Dev. Biol.* 2015, 407, 68–74. [CrossRef] [PubMed]

93. Zhang, Y.; Wang, Y.; Zuo, Q.; Li, D.; Zhang, W.; Wang, F.; Ji, Y.; Jin, J.; Lu, Z.; Wang, M.; et al. CRISPR/cas9 mediated chicken Stra8 gene knockout and inhibition of male germ cell differentiation. *PLoS ONE* 2017, 12, e017207. [CrossRef] [PubMed]

94. Zuo, Q.; Jin, K.; Wang, Y.; Song, J.; Zhang, Y.; Li, B. CRISPR/cas9-mediated deletion of c1eis inhibits chicken embryonic stem cell differentiation into male germ cells (gallus gallus). *J. Cell. Biochem.* 2017, 118, 2380–2386. [CrossRef] [PubMed]

95. Dimitrov, L.; Pedersen, D.; Ching, K.H.; Yi, H.; Collarini, E.J.; Izquierdo, S.; van de Lavoir, M.C.; Leighton, P.A. Germline gene editing in chickens by efficient CRISPR-mediated homologous recombination in primordial germ cells. *PLoS ONE* 2016, 11, e0154303. [CrossRef] [PubMed]

96. Oishi, I.; Yoshii, K.; Miyahara, D.; Kagami, H.; Tagami, T. Targeted mutagenesis in chicken using CRISPR/cas9 system. *Sci. Rep.* 2016, 6, 23980. [CrossRef] [PubMed]

97. Esvelt, K.M.; Mali, P.; Braff, J.L.; Moosburner, M.; Yang, S.J.; Church, G.M. Orthogonal Cas9 proteins for RNA-guided gene regulation and editing. *Nat. Methods* 2013, 10, 1116–1123. [CrossRef] [PubMed]

98. Zetsche, B.; Gootenberg, J.S.; Abudayyeh, O.O.; Slaymaker, I.M.; Makarova, K.S.; Essletzbichler, P.; Volz, S.E.; Joung, J.; van der Oost, J.; Regev, A.; et al. Cpf1 is a single rna-guided endonuclease of a class 2 crispr-cas system. *Cell* 2015, 163, 759–771. [CrossRef] [PubMed]

99. Hur, J.K.; Kim, K.; Been, K.W.; Baek, G.; Ye, S.; Hur, J.W.; Ryu, S.M.; Lee, Y.S.; Kim, J.S. Targeted mutagenesis in mice by electroporation of cpf1 ribonucleoproteins. *Nat. Biotechnol.* 2016, 34, 807–808. [CrossRef] [PubMed]

100. Kim, D.; Kim, J.; Hur, J.K.; Been, K.W.; Yoon, S.H.; Kim, J.S. Genome-wide analysis reveals specificities of cpf1 endonucleases in human cells. *Nat. Biotechnol.* 2016, 34, 863–868. [CrossRef] [PubMed]

101. Komor, A.C.; Kim, Y.B.; Packer, M.S.; Zuris, J.A.; Liu, D.R. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 2016, 533, 420–424. [CrossRef] [PubMed]

102. Nishida, K.; Arazoe, T.; Yachie, N.; Banno, S.; Kakimoto, M.; Tabata, M.; Mochizuki, M.; Miyabe, A.; Araki, M.; Har, K.Y.; et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. *Science* 2016, 353, aaf8729. [CrossRef] [PubMed]

103. Sanjana, N.E.; Cong, L.; Zhou, Y.; Cunniff, M.M.; Feng, G.; Zhang, F. A transcription activator-like effector toolbox for genome engineering. *Nat. Protoc.* 2012, 7, 171–192. [CrossRef] [PubMed]

104. Zhang, F.; Cong, L.; Lodato, S.; Kosuri, S.; Church, G.M.; Arlotta, P. Efficient construction of sequence-specific tal effectors for modulating mammalian transcription. *Nat. Biotechnol.* 2011, 29, 149–153. [CrossRef] [PubMed]
83. Lyall, J.; Irvine, R.M.; Sherman, A.; McKinley, T.J.; Nunez, A.; Purdie, A.; Outtrim, L.; Brown, I.H.; Rolleston-Smith, G.; Sang, H.; et al. Suppression of avian influenza transmission in genetically modified chickens. Science 2011, 331, 223–226. [CrossRef] [PubMed]

84. Brojatsch, J.; Naughton, J.; Rolls, M.M.; Zingler, K.; Young, J.A. Car1, a tnfr-related protein, is a cellular receptor for cytopathic avian leukemia-sarcoma viruses and mediates apoptosis. Cell 1996, 87, 845–855. [CrossRef]

85. Klucking, S.; Adkins, H.B.; Young, J.A. Resistance to infection by subgroups b, d, and e avian sarcoma and leukosis viruses is explained by a premature stop codon within a resistance allele of the tvb receptor gene. J. Virol. 2002, 76, 7918–7921. [CrossRef] [PubMed]

86. Elleder, D.; Stepanets, V.; Melder, D.C.; Senigl, F.; Geryk, J.; Pajer, P.; Plachy, J.; Hejnar, J.; Svoboda, J.; Federspiel, M.J. The receptor for the subgroup c avian sarcoma and leukosis viruses, tvc, is related to mammalian butyrophilins, members of the immunoglobulin superfamily. J. Virol. 2005, 79, 10408–10419. [CrossRef] [PubMed]

87. Chai, N.; Bates, P. Na+/h+ exchanger type 1 is a receptor for pathogenic subgroup j avian leukosis virus. Proc. Natl. Acad. Sci. USA 2006, 103, 5531–5536. [CrossRef] [PubMed]

88. Varki, A.; Gagneux, P. Multifarious roles of sialic acids in immunity. Ann. N. Y. Acad. Sci. 2012, 1253, 16–36. [CrossRef] [PubMed]

89. Lee, H.J.; Lee, K.Y.; Park, Y.H.; Choi, H.J.; Yao, Y.; Nair, V.; Han, J.Y. Acquisition of resistance to avian leukosis virus subgroup B through mutations on tvb cysteine-rich domains in DF-1 chicken fibroblasts. Vet. Res. 2017, 48. [CrossRef] [PubMed]

90. Lee, H.J.; Lee, K.Y.; Jung, K.M.; Park, K.J.; Lee, K.O.; Suh, J.Y.; Yao, Y.; Nair, V.; Han, J.Y. Precise gene editing of chicken Na+/H+ exchange type 1 (chNHE1) confers resistance to avian leukosis virus subgroup J (ALV-J). Dev. Comp. Immunol. 2017, 77, 340–349. [CrossRef] [PubMed]

91. Liu, R.; Paxton, W.A.; Choe, S.; Ceradini, D.; Martin, S.R.; Horuk, R.; MacDonald, M.E.; Stuhlmann, H.; Koup, R.A.; Landau, N.R. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 1996, 86, 367–377. [CrossRef] [PubMed]

92. Van de Lavoir, M.C.; Diamond, J.H.; Leighton, P.A.; Mather-Love, C.; Heyer, B.S.; Bradshaw, R.; Kerchner, A.; Hooi, L.T.; Swanberg, S.E.; et al. Germline transmission of genetically modified primordial germ cells. Nature 2006, 441, 766–769. [CrossRef] [PubMed]

93. Choi, J.W.; Kim, S.; Kim, T.M.; Park, T.S.; Jeong, J.W.; Song, G.; Han, J.Y. Basic fibroblast growth factor activates mek/erk cell signaling pathway and stimulates the proliferation of chicken primordial germ cells. PlaS ONE 2010, 5, e12968. [CrossRef] [PubMed]

94. Macdonald, J.; Glover, J.D.; Taylor, L.; Sang, H.M.; McGrew, M.J. Characterisation and germline transmission of cultured avian primordial germ cells. PlaS ONE 2010, 5, e15518. [CrossRef] [PubMed]

95. Lee, Y.M.; Jung, J.G.; Kim, J.N.; Park, T.S.; Kim, T.M.; Shin, S.S.; Kang, D.K.; Lim, J.M.; Han, J.Y. A testis-mediated germline chimera production based on transfer of chicken testicular cells directly into heterologous testes. Biol. Reprod. 2006, 75, 380–386. [CrossRef] [PubMed]

96. Pramod, R.K.; Lee, B.R.; Kim, Y.M.; Lee, H.J.; Park, Y.H.; Ono, T.; Lim, J.M.; Han, J.Y. Isolation, characterization, and in vitro culturing of spermatogonial stem cells in japanese quail (Coturnix japonica). Stem Cells Dev. 2017, 26, 60–70. [CrossRef] [PubMed]

97. Cooper, C.A.; Challagulla, A.; Jenkins, K.A.; Wise, T.G.; O’Neil, T.E.; Morris, K.R.; Tizard, M.L.; Doran, T.J. Generation of gene edited birds in one generation using sperm transfection assisted gene editing (STAGE). Transgenic Res. 2017, 26, 331–347. [CrossRef] [PubMed]