## Modification of the SLCO1B3 gene in order to establish gene editing in chicken

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Recent developments of highly specific designer nucleases allow the precise genetic modification of vertebrate genomes. Here, we aim to establish a designer nuclease protocol for the genetic modification of chicken primordial germ cells (PGC). A first target is a provirus (EAV-HP) insertion in the 5 flanking region of the SLCO1B3 gene in Araucana chicken, which is supposed to be causative for the blue eggshell color in this breed. For this reason we will perform knock-out experiment using the CRISPR/Cas9 designer nuclease system to cut out the retroviral sequence. In addition, we also pursue a knock-in approach in White Leghorn breed using the Sleeping Beauty transposon system. The integration or removal of the EAV-HP sequence will be performed in vitro in PGCs, the precursors of germ cells. After the genetic modifications in vitro, the PGCs will be injected into a blood vessel of 2.5 day old chicken embryos to obtain chimeric offspring. The male germ line chimera will be raised to sexual maturity and mated to produce transgenic hens whereas the gonads of female chimeric chicks are inspected to assess the germline colonization by injected PGCs. The eggs of the adult transgenic chickens will be checked for the desired color.

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