Editing of the coatomer protein complex subunit alpha gene in bovine blastocysts using CRISPR/Cas9

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Conclusion

Microinjection of CRISPR plasmid and protein in presumptive zygotes is possible and mosaic editing can result from this procedure.

Future work – Since coatomer protein complex subunit alpha (COPA) acts as protein carrier, it is possible that homozygous knock-out causes developmental arrest in embryos. Homology-directed repair should ensure proper missense mutation of the gene in future founder animals.

Background

- CRISPR studies in cattle have focused on cell editing and subsequent cloning (SCNT), attempting founder animal production.

- COPA is involved in ER-Golgi transport and its mutation is associated with coat color variation in cattle (Dorshorst et al., 2015), but also in lung diseases and arthritis in humans (Watkin et al., 2015) and increased proliferation, migration and invasion of cancer cells *in vitro* (Peng et al., 2018).

Aim

We investigated the feasibility of CRISPR-Cas9 gene editing via micro-injection in a conventional IVF protocol to derive edited blastocysts.

Methods

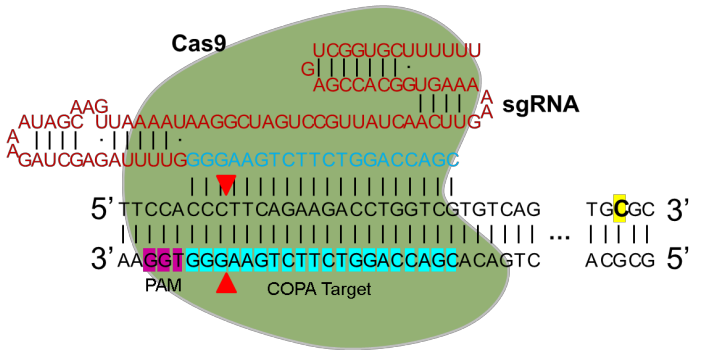

Figure 1 – Schematic representation of the COPA sgRNA target. The missense mutation associated with coat color in cattle is highlighted in yellow.

Micro-injection *Selection*

Presumptive zygotes → PX-458 plasmid / Ribonucleoprotein (RNP) ATTO 550 → Day 8 GFP+ / ~ 24h post-injection

Editing validation

Nested PCR → T7E1 assay → Clonal sequencing

Results

Table I – Metrics of the micro-injection procedures

Injection type	Conc. [ng/µL]	n	Survival (%)	Day 8 Blast. (%)	GFP+ Blast.	Edited Blast.
Plasmid	25	246	69.5	38.0	-	1
Plasmid	25	339	77.5	27.7	7	2
RNP	20	314	79.5	23.4	-	4
RNP	50	133	60.2	27.5	-	1
	100	132	62.9	20.5	-	-
	200	126	50.0	28.6	-	-

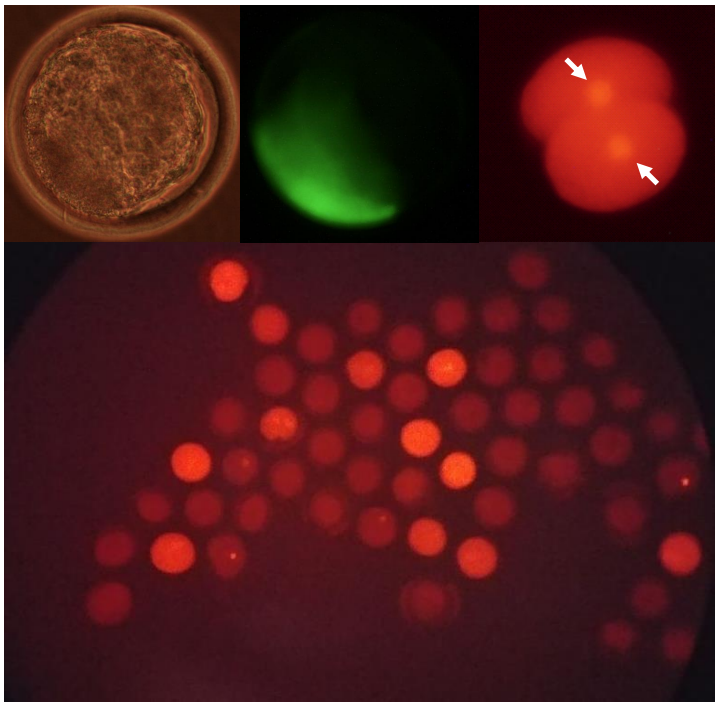

Figure 2 – Fluorescence selection of CRISPR injected presumptive zygotes. Arrows indicate the localization of the RNP effector complex in the nucleus.

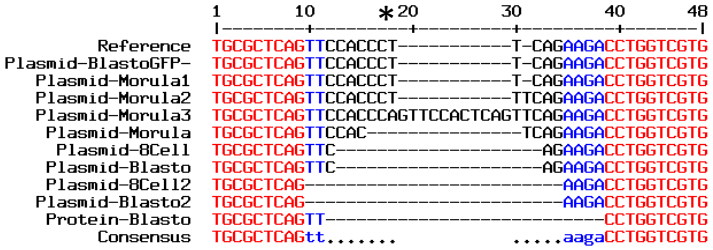

Figure 3 – Characterization of editing events in plasmid and protein injected embryos. * represents the sgRNA guided restriction site.

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