Genome-edited organisms: options for traceability

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1. Introduction

- Genome editing (GEd) provides more efficient ways to introduce targeted changes into the genomes of both plants and animals.
- Available to a wider variety of stakeholders for different applications (Lema, 2019) in comparison to GM technology.
- Regulators globally now examining their frameworks to assess their applicability to this new technology and products.
- GEd crops and animals now getting approval for commercialisation with most recent being GEd tomato, sea bream and tiger puffer fish in Japan and beef cattle in the US.
- Techniques currently used for detection of inserted transgenes in GM products include DNA and protein-based methods (Miraglia, 2004).
- Questions remain whether GEd products will be subjected to the same traceability requirements as GM products or whether new ones will be put in place.

2. Objectives

1. Design of editing reagents targeting the exon10 region of the ovine PRLR gene
2. Assessment of screening methodologies i.e. PCR-Restriction Fragment Length Polymorphism (RFLP) and PCR-Sanger sequencing for detection of HDR edits in samples

3. Materials and methods

- Scanning of sheep genomic sequence for location of prolactin receptor gene (PRLR)
- Design of guide RNAs and HDR templates incorporating a DNA footprint in the exon10 region of the ovine PRLR gene
- Transfection with two GEd techniques, Ribonucleoprotein (RNP) and plasmid transfection in sheep embryonic fibroblasts(SEF) cell line
- Isolation of single cell clones from edited population
- Screening for HDR events among single cell clones using PCR-RFLP and PCR-Sanger sequencing

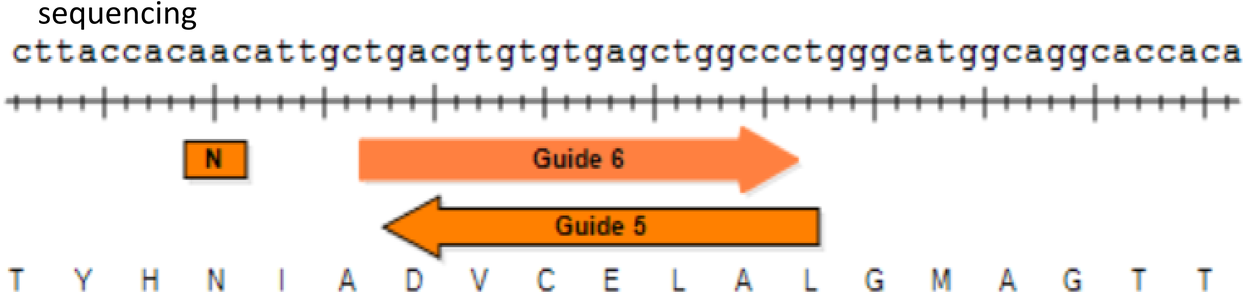


Figure 1: Sheep exon 10 partial sequence before changes introduced

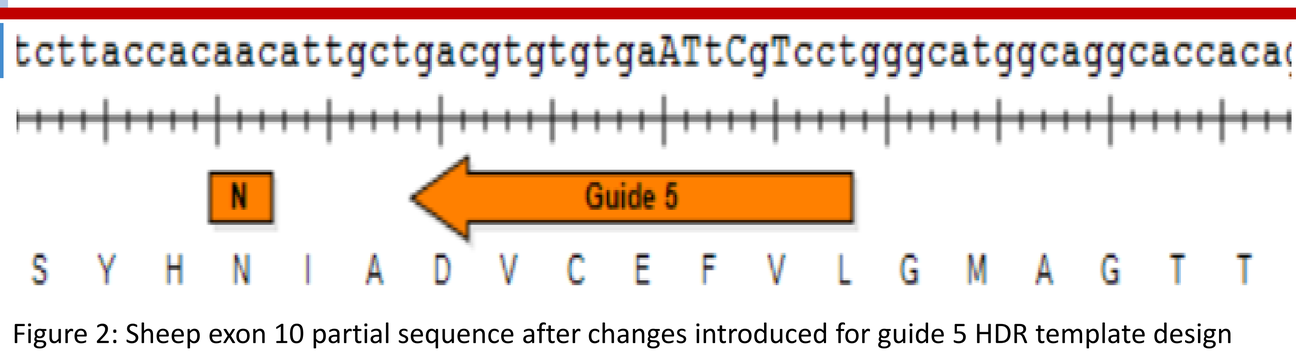


Figure 2: Sheep exon 10 partial sequence after changes introduced for guide 5 HDR template design

4. Results

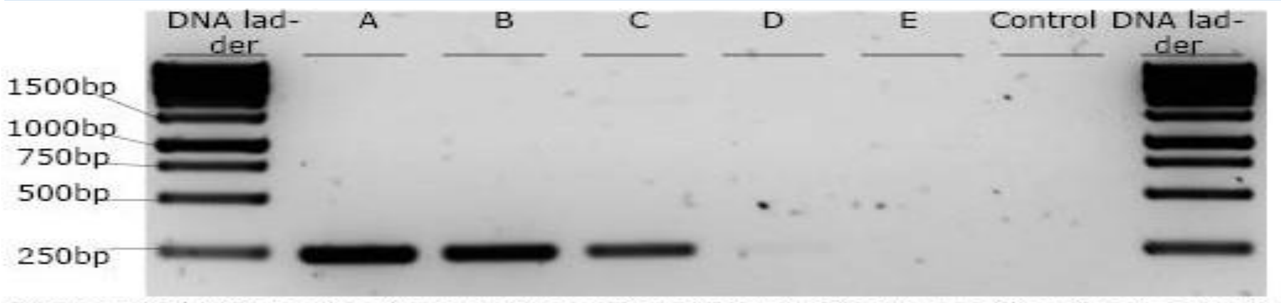


Figure 3:Gel picture showing amplicons after PCR with EcoR1 specific primers and Homozygous(HM) and Wild type(WT) clones' genomic DNA in varying dilutions. Lane 1 is the DNA ladder, Lane 2 (A) shows the band for 100% HM clone appearing at 250bp marker, Lane 3 (B) shows the band for 50%HM/50%WT dilution, Lane 4 (C) shows the band for 10%HM/90%WT dilution, LANE 5 (D) shows the band for 1%HM/99%WT dilution, Lane 6 (E) represents 100% WT clone which did not have the edits hence no band is showing, Lane 7(E) is the negative control while Lane 8 is the DNA ladder.

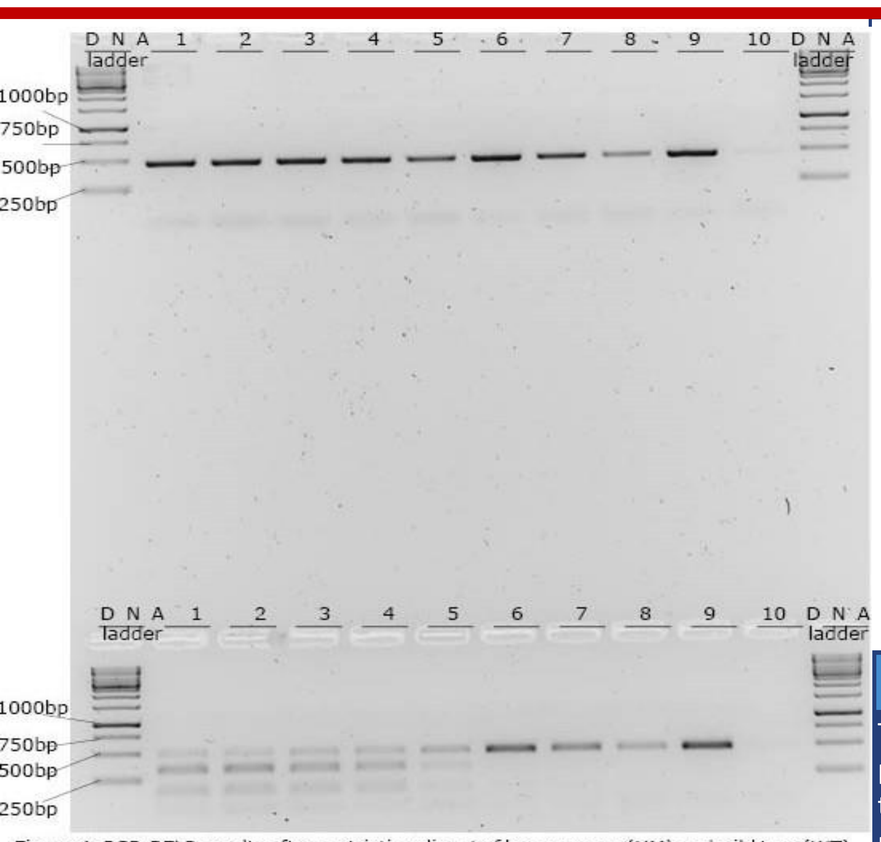
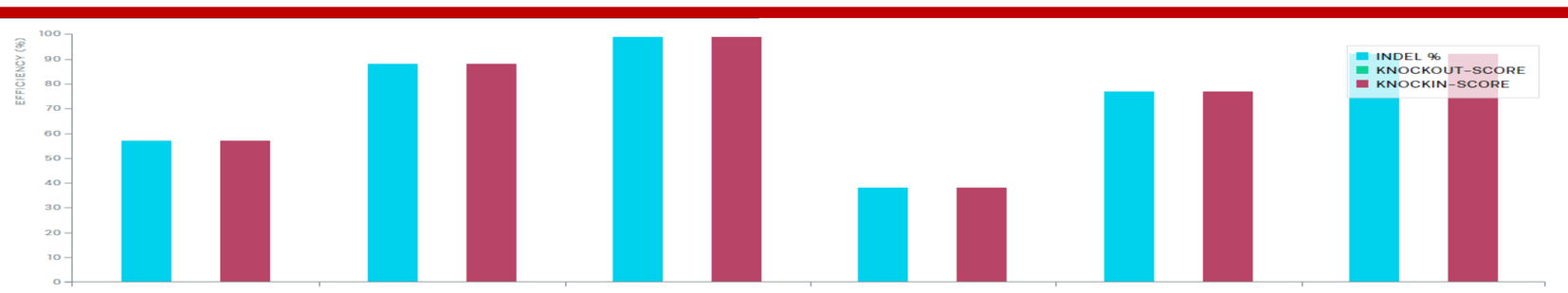


Figure 4: PCR-RFLP results after restriction digest of homozygous(HM) and wild type(WT) clones genomic DNA in varying dilutions with EcoRI enzyme. Upper gel section shows uncut PCR products of the HM and WT genomic DNA dilutions in lanes labelled 1-9. Bottom section of gel shows digested PCR products: lanes 1&12 are DNA ladder; lanes 2-10 show banding pattern for HM/WT dilutions at 100%, 99%,95%,90%,50%,10%,5%,1% and 0%.



SUCCESSFULLY ANALYZED SAMPLES

SAMPLE	GUIDE TARGET	PAM SEQUENCE	INDEL %	MODEL FIT (R^2)	KN... SC...	KN... SC...	
SampleI	GACGTGTGTGAGCTGGCCCT	GGG	57	1		57	View Details
SampleA	GACGTGTGTGAGCTGGCCCT	GGG	88	1		88	View Details
SampleB	GACGTGTGTGAGCTGGCCCT	GGG	99	1		99	View Details
SampleE	GACGTGTGTGAGCTGGCCCT	GGG	38	1		38	View Details
SampleD	GACGTGTGTGAGCTGGCCCT	GGG	77	1		77	View Details
SampleC	GACGTGTGTGAGCTGGCCCT	GGG	92	1		92	View Details

Figure 5: ICE analysis results after Sanger sequencing showing proportion of edits present in different samples

5. Discussion

Target-specific PCR confirms whether your edit of interest is present or not, PCR-RFLP confirms presence of edits in absence of target-specific primers and PCR-Sanger sequencing gives slightly more details in terms of specific changes introduced to the genome or product of interest.

6. Conclusions

- Current transgene detection methods can be modified to detect GEd organisms however challenge remains to differentiate between GEd, conventional breeding and/or natural mutation.
- Consensus is needed with regard to traceability of GEd organisms, considering efficiency and availability of infrastructure in both high-income and low-income settings.

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