"Kidding" Around: Making Spider Goats

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Introduction

Background

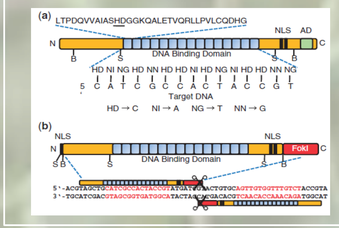
Spider silk has mechanical properties that surpass most man-made materials. These mechanical properties, which are due to a combination of strength and extensibility, can be tailored to a large variety of uses[1], ranging from tissue regeneration matrices to bulletproofing.

Because the orb weaving spider produces six types of silk and all of them are difficult to obtain in large quantities, it is necessary to develop artificial spider silk mimetics from recombinant spider silk proteins produced in other organisms to fully explore and harness the attractive properties of silk.[2] Some of the organisms that have been used for large scale silk production include *E. coli*, plants, and goats. Two of the major obstacles for producing silk in these hosts are the quantity and ease of extraction of silk.

To address these obstacles in a goat, a new system for creating transgenic goats is being used: Transcription Activator-Like Effector Nucleases (TALENs). TALENs are genetic constructs that code for proteins which produce DNA cuts at specifically targeted DNA sequences. A TALEN system is being designed that will facilitate multiple cuts in the goat genome to replace the coding region of one of the naturally-occurring milk proteins with the coding sequence for spider silk proteins. The spider silk insert also contains a His-Tag coding region that will be used to easily and efficiently extract the protein from the goat milk.

TALENs

TALENs consist of multiple Repeat-Variable Di-residues or RVDs – repetitive regions interrupted by small variable regions – and the DNA cleavage domain from the FokI endonuclease. The RVDs are designed to code for specific amino acids that recognize specific nucleotide binding sites within the desired genetic sequence, which allows for highly targeted cleaving of the DNA.[3]



Structure of TAL effector and TALEN. (a) Structure of a naturally occurring TAL effector. A consensus repeat sequence is shown with the RVD underlined. The sequence of RVDs determines the target nucleotide sequence. (b) Structure of a TALEN. Two monomeric TALENs are required to bind the target site to enable FokI to dimerize and cleave DNA. NLS, nuclear localization signal(s); AD, transcriptional activation domain; B, BamHI; S, SphI.[4]

Materials and Methods

TALEN Targeting and Assembly

TALEN cut sites were determined by using the TAL Effector Nucleotide Targeter 2.0[5] to analyze the α-casein sequence. The α-casein sequence was obtained from literature.[6] After determining the desired sequences, TALENs were created using the Golden Gate assembly protocol.[4]

pCDNA 3.1(-)

In order to express the TALENs in goats they were inserted into a mammalian expression vector, pCDNA3.1(-) (Invitrogen).

Nucleofection

Nucleofection, a high efficiency method for transfecting cells, was performed using a LONZA 4D Nucleofector™ System. This method of transfection has been shown to have up to 70% transfection efficiency while still maintaining cell functionality.[7]

Results

Completed TALENs

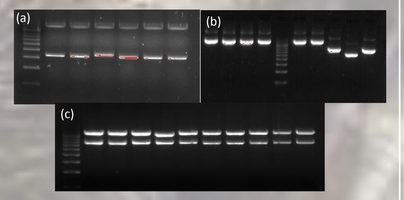
The proposed TALENs (below) have been created and confirmed using standard methodology:

Front End TALENs (RVDs):
NN HD HD NG NG NG NG NN NN HD HD NN NG NG NN
HD HD NG HD NI HD NG NN NN NI NN NN NI NN NI

Tail End TALENs (RVDs):
NI NI NN NI HD NI NI NI NG NN HD NG NI NG NG
NG HD NI NI NN NI NI NG HD NG NG NI NI NI NN

Digestion

Digestion checks were done using two unique restriction enzymes and analyzed on a 0.8% agarose gel. The presence of two clear bands indicates that the pTAL3 vector (~6kb) and the TALEN insert (~1.5kb) are both present. After insertion into pCDNA 3.1(-), the TALENs were checked for insertion orientation via digestion with appropriate restriction enzymes (c).



Sequencing

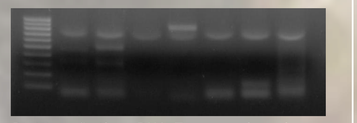
Sequencing of TALENs confirmed successful creation (green segments are RVDs):
ctatcgccagc**aacaat**ggcggcaagcaagcgctcgaaacggtgcagcggctgttgccggtgctgtgccaggaccatggcctgactcc
ggaccaagtggtggctatcgccagc**cacgat**ggcggcaagcaagcgctcgaaacggtgcagcggc

...

ggaccatggcctgaccccggaccaagtggtggctatcgccagc**aacggt**ggcggcaagcaagcgctcgaaacggtgcagcggctgtt
gccggtgctgtgccaggaccatggcctgaccccggaccaagtggtggctatcgccagc**aacggt**ggcggcaagcaagcgctcgaaac

Nucleofection

Digestion by appropriate enzymes within the TALEN spacer region indicated whether the TALENs were successful or not. An unsuccessful digestion indicated that the TALENs had worked as desired. It appears that the front end TALEN pair worked successfully in goat fibroblast cells. However, the published sequence used to create the TALENs was not from a European milk goat, so we believe this is the reason that the TALENs are not working correctly in the Saanen goats used in our research.



Post-transfection gDNA. The α-casein region was amplified by PCR, then digested. 1.2% agarose gel run at 70V for 50 mins. Row 1: 100 bp ladder; rows 2-3: front end TALENs; rows 4-5: back end TALENs; rows 6-7: all TALENs; row 8: control.

Final α–casein Sequence

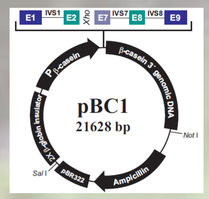
After the proposed TALEN cuts, the sequence coding for α-casein in the goat genome will have been excised between base pairs 88 and 700 and a sequence coding for spider silk proteins will have been incorporated; only 5' and 3' control regions will remain from the original sequence (green sequence indicates TALEN recognition site):

GGACTACTTGTCTTCCTTTTAGGAAGCAAGGACCAAGTAAACATGAAGTTCTTCATTTTTACCT**GCCTTTT**
GGCCGTTGCCCTTGCA^{A}^{88} ---Spider Silk Gene plus His Tag---^{700}**T**GAGGTAC**CTTTAAGATTCTTGA**A
TTAACTGCTTCTACCTGGTTATGGTTGGACTGGAAAATCTATCTTCTACAGTTTCAGATCTACCACTTTACT
TCATACAACCAGCATGTTTGGGGTGGCGCATCAATAAGACAAATCGCAGAGTATTTCCTGAATTATTTATA
CTCTCCTGGTTAGTTTATATTGAATATACTGGGTTTATATGGTGGTTTCATACAAACTTAGCTGATGATTATT
TAAAATCCTTTCCCTAATCTTTCTGAGTTATAGAAATATTTTTCTTTCCCTTTG

Other Work

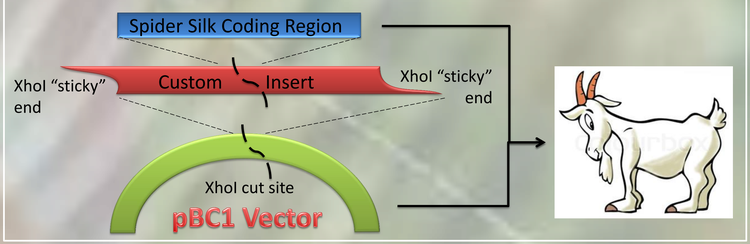
pBC1

Although combining TALENs with nucleofection appears to be a very promising method to produce high yields of silk proteins with optimal properties, another short-term method is being employed to create transgenic goats: the pBC1 vector. This method, which has been proven successful in the past, takes advantage of random integration. Three different sequences for spider silk production will be expressed. Although this method is less likely to produce yields as high as expected using TALENs, we will still be able to establish a His-Tag, which will improve the method of protein extraction.



In(sert)ception

In order to incorporate silk protein coding regions into the pBC1 vector, it was necessary to create an insert with certain components for proper expression and vector integration. The silk construct has been successfully incorporated into this specially designed insert, which is being cloned into the pBC1 vector. The final construct will then be put into a goat via somatic cell nuclear transfer.



Acknowledgements

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References

1. Holland et al 2008 Biomacromolecules 9, 651-657
2. Widhe et al 2011 Bioplymers 97, 6, 468-478
3. http://en.wikipedia.org/wiki/Transcription_Activator-Like_Effector_Nuclease
4. Cermak et al 2011 Nucleic Acids Research 39, 12, 1-11
5. http://boglabx.plp.iastate.edu/TALENT/
6. Bouniol 1993 Gene 125, 235-236
7. http://www.lonza.com/products-services/bio-research/transfection/nucleofector-devices/4d-nucleofector-system.aspx
8. Background Image: http://phys.org/news194539934.html
9. Goat clip-art: http://www.colourbox.com/vector/cute-cartoon-sea-creatures-vector-3461059
10. Spider image: http://www.istockphoto.com/stock-illustration-5193881-spider-hanging-cartoon.php