# IMPROVED ANNOTATION OF THE EQUINE ELASTINE GENE

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## Introduction

Chronic progressive lymphedema (CPL) is an incurable disorder affecting the lower limbs of the Belgian Draught horse. Skin and perilymphatic elastin alterations were shown in CPL-susceptible breeds, suggestive of a hampered elastic functions. Therefore elastin (*ELN*) was proposed as a possible candidate gene.

## Aim

To verify the completeness of annotated *ELN* sequence as missing information might hinder the localization or impede the correct interpretation of *ELN* polymorphisms found in Belgian Draught Horse.

## Material

The annotated *ELN* reference (ENSECAG 00000011106) is located on ECA13 at position 11,564,768-11,588,410 with a length of 23,643bp and 21 exons.

Sanger sequencing was performed to sequence the full *ELN* reference as well as exploratory sequencing of a region 3' of *ELN* (similar to human exon 31) in 3 Belgian Draught Horses with severe CPL. Flemish Horse, a non-susceptible related Belgian Draught Horse, and Thoroughbred, were used as controls in case new polymorphisms were found.

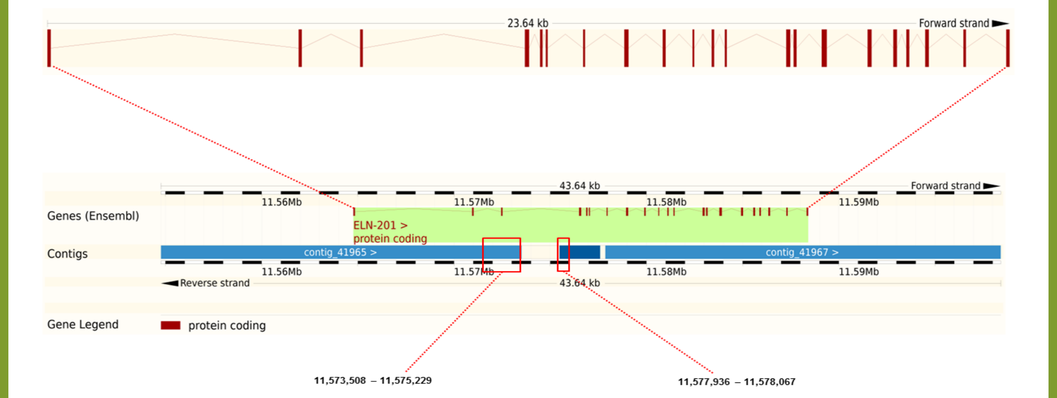


Figure 1: Position of the *de novo* annotated regions in equine ELN

## Results

***In silico:***

After alignment of the equine reference gene with cDNA sequences of different species we retained regions with >80% identical nucleotides. This was our "estimated total" equine *ELN* cDNA.

1. After comparison with annotated *ELN* sequence, 2 unknown regions were found on positions 11,573,508 and 11,578,192, with estimate sizes of 1,977bp and 278bp (Figure 1).
2. RNA hybridization in other species showed estimated mRNA sizes of 2.4kb, much more than the estimated equine mRNA of 1.5kb mRNA.
3. Similar cDNA regions in different species stretched further than last reference exon.

***Sequencing:***

1. The 2 formerly unknown regions were determined with lengths 1,721bp and 131bp, giving a total length of 23,240bp for the reference sequence (Figure 1).
2. Exploratory sequencing revealed 81% identical nucleotides to human exon 31, suggesting longer equine *ELN* coding region.
3. We found 49 new polymorphisms, comprising 48 SNPs and 1 CA>YG transition. None of these polymorphisms were shown to be unique for CPL-affected Belgian Draught Horses.

## Conclusion

1. This research reported 2 formerly unknown regions.
2. None of the polymorphisms were unique in CPL-affected horses, so until now *ELN* cannot be confirmed as a causative gene.
3. Some information is missing which might impede the correct interpretation of *ELN* polymorphisms found. Multiple species alignment demonstrated a high conformity with cDNA sequences, suggestive for the presence of additional exons 3' of the reference, this was consolidated by sequencing.

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