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KRISPR-KRINGLEi Utilized for Leptin Supression in Elves (*Denisova hominin*).

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ABSTRACT

The ability to make precise modification of complex genomes has been a long-time goal in the fields of applied genetics, biotechnology, drug development, gene therapy, and synthetic biology. Recent discoveries in TALEs (transcription activator-like effectors) and CRISPR (clusters of regularly interspaced short palindromic repeats) have enabled rapid genome engineering in a variety of cell types and different model organisms at a high efficiency and specificity. CRISPRi technology uses an RNA-guided dCas9 (dead Cas9- with inactive nuclease domains) from a Type II CRISPR system to efficiently repress expression of targeted genes with no detectable off target effects (Qi, 2013). This study briefly summarizes the use of a novel CRISPRi, KRISPR-KRINGLEi, developed by Santa with the help of DNA2.0, in the first successful gene therapy trial performed at the University of NorthPole. Results display the ability to use the KRISPR-KRINGLE system to repress expression of the obesity-linked Leptin gene with no detectable off-target effects in the subset of the North Pole *Denisova hominin* population, commonly referred to as elves.

INTRODUCTION

After millennia of stealth existence, a small bone fragment from the fifth finger of a juvenile elf was uncovered in 2008 at the Denisova cave in the Altai mountains in Siberia (home to *Pseudotsuga menziesii*, commonly known as the Douglas Fir tree, and selected by over 56% of households for their holiday interior arboreal activities (Fezziwig, 2007)). Svante Pääbo and co-workers sequenced DNA extracted from the sample (Krause, Fu *et al*. 2010) and showed it to belong to that of elves (Snowden 2013). Genomic information of elves can now accordingly be utilized for re-engineering of specific elfen features.

A study released in 2010 by the NorthPole Institute of Health (NPIH) (Scrooge, Cratchit *et al*. 2010) indicated that obesity in the elf population has reached record highs (Fig 1). The study found that average Body Mass Index (BMI) in the full North Pole elf cohort had risen to an average of 35 in 2010. This is in stark contrast to documents from 18th century (Blommer 1850) verifying that elves had healthy BMIs at that time.

The NPIH study identified a strong correlation (R^2 CV = 0.72) between high levels of Leptin and elf obesity. Leptin is an extensively studied 16-kDa peptide hormone that plays a critical role in regulating energy intake and expenditure including: appetite and hunger, metabolism, and behavior. Leptin is expressed, synthesized and secreted by adipose tissue in proportion to its mass. It circulates in blood and acts on the hypothalamus via the AGRP neuron to stimulate voracious food intake and consumption behavior (Snap, Crackle *et al*. 1996; Betley, Cao *et al*. 2013). As elves are well known to enjoy the culinary prowess of Mrs. Claus, and to spend far too much time watching holiday movies on Netflix (Crosby, Kaye *et al*. 1954). This increase in elf obesity has been previously observed (aka Elves Presleum) and validated by Johan Elf himself, and is thus, not entirely surprising.

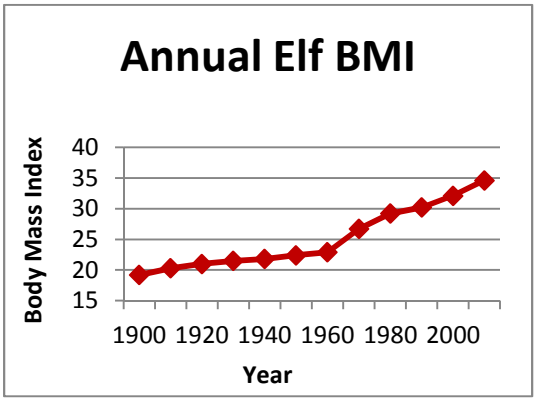

Fig 1. Average Elf Body Mass Index (BMI) by decade. Note the dramatic increase in elf BMI following the introduction of Rice Crispr Treats in the 1950s.

The ongoing studies and media attention on the effect of obesity in disease development in conjunction with decreasing diameter of modern chimney shafts has created a demand for the development of a therapy. Santa Claes gifted a grant to fund collaboration of Laura Menorah's Lab at the University of the NorthPole and DNA2.0, for the development of a therapy to treat obesity in elves.

KRISPR-KRINGLEi Technology

The KRISPR-KRINGLEi technology developed by DNA2.0 is based on the CRISPR immune system discovered in prokaryote genomes more than 30 years ago (Horvath and Barrangou 2010). CRISPR associated genes (Cas genes) form the elemental components of the CRISPR defense pathway, which utilizes crRNA molecules to target and destroy the DNAs of invading viruses and plasmids. The DNA2.0 KRISPR-KRINGLEi technology utilizes a GeneGPS® codon-optimized Cas9, directed by a guide RNA (gRNA) sequence to suppress gene expression by recruiting cas9 to bind to the leptin promoter. Cas9 is driven by a GAPDH promoter, a weak promoter in mammalian systems, to avoid the effects of over-suppression to allow minimal Leptin expression to allow for its essential properties. We were able to utilize the DNA2.0 gRNA computational design tool to

generate gRNA sequences against the first exon in the Leptin gene promoter to suppress the expression of Leptin in preliminary *in vitro* experiments (data not shown). A total of three gRNAs were selected based on their score, generated by the DNA2.0 algorithm, to minimize the probability of off-target effects. The gRNAs were Electra cloned into a pDAUGHTER vector with a GeneGPS codon-optimized cas9 followed by Santa's favorite new spicy PaprikaRFP (IP-Free, DNA2.0).

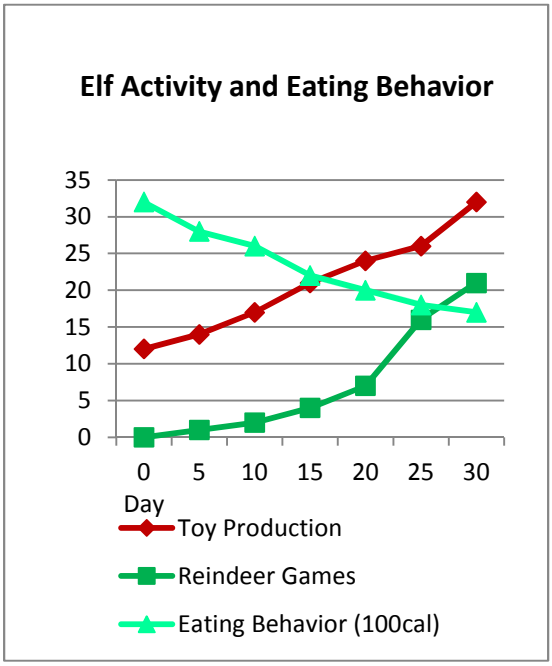

Fig 2. Activity and Eating Behaviour in elves following KRISPR-KRINGLEi treatment. Red diamonds denotes toys produced per day per elf in Santa's workshop. Green squares denotes hours per week per elf playing reindeer games. Green triangles denotes calories consumed per day per elf.

DNA2.0 developed the KRISPR-KRINGLE vectors in their Electra Cloning System to ensure easy, fast, and efficient cloning for this study. The platform utilizes the SapI restriction enzyme, which was first discovered by the University of the NorthPole. It has long been hypothesized that the existence of restriction enzyme SapI is evidence of the almighty Gene Designer - a universal tool that designs all genetic information (Villalobos, Ness *et al*. 2006). Not only is SapI a typeIIS restriction enzyme (digesting the DNA away from the recognition sequence), but also leaves a three nucleotide overhang (consistent with the universal ATG start codon of every ORF), and is fully active under the same conditions as T4 DNA ligase. It is generally agreed that such a perfect cloning tool is unlikely to be the result of evolution (Whitman, Gore *et al*. 2013) but instead bears consistent hallmarks of an almighty Gene Designer.

Fig 3. Photo of elf patient zero prior to and 30 days post gene therapy treatment with KRISPR-KRINGLEi.

To effectively and safely deliver the KRISPR-KRINGLE complex, special treats coined Rice KRISPRs were used. The treats incorporated cas9-2a-PaprikaRFP in the construct (Mrs Claus, see supplement A), and were provided to the elf population during their daily afternoon snack. After consuming treats, the elf population was invited to Santa Claus's Annual North Pole gift-wrapping party where he implemented a UV disco-ball (gifted by the Dr. Hermey T. Elf D.D.S. Technology Grant) to fluoresce the subjects. 99% of the treated subjects fluoresced red, as a result of the PaprikaRFP reporter; thus confirming successful delivery of the KRISPR construct.

To effectively analyze the effects of KRISPR-KRINGLE in the elf population, several biological and behavioral parameters

were assayed. The elves daily caloric consumption and activity levels were tracked and recorded. Figure 2 displays a significant decrease in appetite and eating behaviors. In addition, Santa noted an increased efficiency of toy production in the workshop as well as increased energy for participation in Rudolph's organized after-work activities. Decreased cookie consumption was also noted by Mrs. Claus.

APPENDIX A: Mrs. Claus' Rice KRISPR Treats reagents and protocol

160g (1400 ml by volume) *Oryza sativa*, toasted and popped (Kellogg)

284g *Althaea officinalis* (Jet Puffed)

42.5g *Bovinae bos taurus* (preferably Holstein) churned organic lactation product (Berkeley Farms)

Protocol: Place churned bovine product into large, heat-resistant, metal container and place over Bunsen burner at low flame. When butter is melted, add marshmallows and stir until completely melted. Remove from heat. Add popped rice cereal. Stir until well coated. Using buttered spatula or wax paper evenly press mixture into 13 x 9 x 2-inch pan coated with cooking spray. Cool. Cut into 2-inch squares. Enjoy.

ACKNOWLEDGEMENT: No elves were harmed during this research project.

Competing Interests: The authors declare competing financial interests: DNA2.0 performs protein engineering, GeneGPS optimization and gene synthesis to a global customer base. Better, smarter and faster than anybody. Better looking too.

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Wikipeidia (2013) *Althaea officinalis*, or Marsh Mallow, is a species indigenous to Africa, which is used as a medicinal plant and ornamental plant. A confection made from the root since ancient Egyptian time evolved into today's marshmallow treat.

Happy Holidays
and
Merry New 2014