



# PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NORTHPOLE

## Metabolic engineering of *Picea abies* for receptor mediated induction of fluorescence and olfactory signaling

Santa Claes<sup>1\*</sup>, Feliz Navidad<sup>1</sup>, Tomte Ness E.<sup>2</sup>, Ten N. Baum<sup>2</sup>,  
Hanukkah Harry<sup>1,2</sup>, Bada Din Sridhar<sup>2</sup>, and Jeremy Elf<sup>1</sup>

<sup>1</sup>University of NorthPole, Dept Bioengineering, Arctic Lane 1, Santa's Secret Village, NorthPole

<sup>2</sup>University of NorthPole, Department of Cool, Cold Tundra Avenue 365, Santa's Secret Village, NorthPole

\*Corresponding author: [santa@DNA20.com](mailto:santa@DNA20.com)

Received 28 June 2008, received in revised form 25 October 2008, accepted 24 December 2008

---

### Abstract

Reoccurring seasonal harvesting of *Picea abies* (Norwegian spruce) and the subsequent attachment of light fixtures to the tree followed by composting after only 3 weeks of minimal use is not only a waste but also leads to diminishing spruce forests, release of carbon dioxide, global warming, increased energy consumption and a political burden to the ongoing diplomatic efforts of Santa Claus. We here present a synthetic biology solution to address this key concerns for winter celebrations – A song induced Christmas tree with endogenous light and odor emission. Advances in synthetic biology, tissue engineering and metabolic engineering have now provided new insights and techniques for controlling whole organism signaling and bio-compound production *in situ*, and recent efforts in synthetic biology have demonstrated that complex regulatory and metabolic networks can be designed and engineered in microorganisms and simple eukaryotes. Leading researchers at the NorthPole Bioscience Engineering Council here disclose how sonic-induced signals received through tissue engineered tympanal organs are incorporated into regulating the metabolic distribution of fluorescent and olfactory signals in *Picea abies*. Upon induction, the cellular nodality of the tree branches emit light at predefined wavelength due to engineered luciferases mTwinkle (yellow), mWonderbread (white) and mSnoBalls (pink). The synthetic induction system also triggers the production and emission of seasonal olfactory signals including trans-cinnamaldehyde (cinnamon), 6-gingerol (ginger), and methyl salicylate (mint). The synthetic pathway is hereby denoted *xmas1*.

© 2008 NorthPole Inc. All rights reserved

*keywords:* synthetic biology, *Picea abies*, chipotle, snow, sled, tissue engineering, Cindy Lauper

---

## Introduction

In the current political climate where Santa and the elves are being challenged by increasingly outspoken non-believers who propose that there is no Santa (Myers 2005) it is instrumental for the North Pole region to emphasize how Christmas can be properly integrated within the social fabric of the industrialized and developing nations through new technology development and social responsibility (Johnson and Johnson 2003). The North Pole Economic Development Council has accordingly made a concerted effort to roll out financial and regulatory support for expansion into the areas of synthetic biology, systems biology, tissue engineering, metabolic engineering and cleantech with a focus on near-term commercial applications. The North Pole has accordingly been declared a HUBZone to facilitate the necessary financial investments (Geithner, Obama *et al.* 2008).

There are approximately 500,000 acres of Christmas trees (usually *Abies alba*, *Abies procera*, *Abies plastica* and related species) planted in the US alone (Silver and Fir 2006). With a tree growth cycle of 10 years, the resources required to fulfill the seasonal need of pine scent is significant (Deere, Massey-Ferguson *et al.* 2008). Global costs associated with the rearing, upkeep and recycling of pine trees used for the short holiday season also include the annual release of 2,430 metric ton of CO<sub>2</sub> and an estimated 28% increased energy consumption to light up the trees during holiday season (Alka and Seltzer 2001).

We here present a synthetically engineered *Picea abies* (Norwegian spruce). Sonic sensors were transplanted from the tympanal organ of the African locust (*Schistocerca gregaria*) and tuned to the wavelength of children emitting shrills. The signal cascades via cinnamaldehyde-induced apoptosis in *Picea* PLC/PRF/5 cells through activation of the proapoptotic Bcl-2 family

proteins and MAPK pathway and activate a set of luciferases emitting light at three different wavelengths. The luciferases are clustered at the node of each tree branch. The same tympanal organ signal transduction pathway also activates the release of trans-cinnamaldehyde (smell of cinnamon), 6-gingerol (smell of ginger) and methyl salicylate (mint-like smell).

## Materials and methods

Synthetic genes were designed by and purchased from DNA2.0 (Menlo Park, CA). Growth media and standard molecular biology buffers were purchased from Teknova (Morgan Hill, CA). Restriction enzymes were purchased from New England Biolabs (Ipswich, MA). All materials and chemicals not otherwise noted were purchased from The Elves Workshop Supplies Inc. (Santa's Secret Village, NorthPole) and were of analytical grade or equivalent.

### *Heterologous expression host*

*Picea abies* strain Marve (F<sup>-</sup> *mcrA* Δ (*mcrBC*-*hsdRMS*-*mrr*) *recA1* *endA1* *lon* *gyrA96* *thi* *supE44* *relA1* λ<sup>-</sup> Δ(*lac-proAB*) (Fleksnes and Wesenlund 1972) was used as host for the engineering of the synthetically introduced *xmas1* pathway. The Norwegian spruce was chosen for having a metabolic network that could easily be deceived into accepting non-natural biological building blocks. Truth be told, the Norwegian spruce is clearly the stupidest of spruces (Keillor 2005). This unique Norwegian characteristic is very helpful when re-engineering the underlying framework without disclosing any information to the host system (see also (Opsahl 1974)).

### *Molecular biological procedures*

Standard procedures were used for cloning, manipulation and analysis of DNA, PCR, electroporation, and transformation

(Sambrook, Fritsch *et al.* 1989). All oligonucleotide synthesis and DNA sequencing was performed by DNA2.0 using instruments and consumables from Applied Biosystems (Foster City, CA).

### Sequence analysis

DNA sequence tracefiles were analyzed, automatically assembled into full length contiguous sequences and finished using the Staden package (Staden, Judge *et al.* 2001). Gene DNA sequence design and codon optimization was carried out using the Gene Designer software (Villalobos, Ness *et al.* 2006). Homology searches were performed with the Blast algorithm (Altschul, Madden *et al.* 1997) implemented on a local server.

### Induction Assay

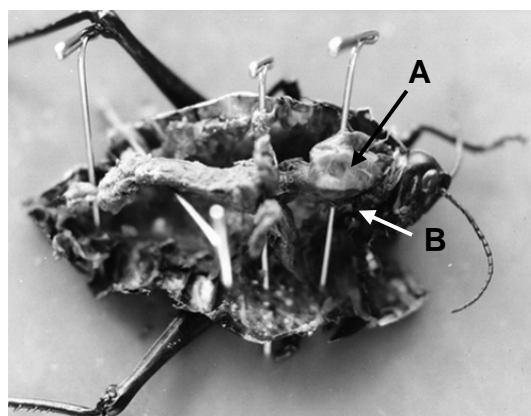
Sonic induction of the synthetic system was carried out using 109 children ranging from 4 to 8 years of age (*Cantatrix falsetto*) singing various Christmas carols (and 'This land is your land'). Extended noise level was sustained by dispersing Sour Patch Kids (Cadbury Adams, Whippany, NJ) and Tootsie Rolls (Tootsie Roll Industries, Chicago, IL). The children were furnished by the Fletcher-Maynard Elementary School (Cambridge, MA). Anecdotal evidence suggested these children to be able to emit extraordinarily high-pitched sounds (H.H. – data not shown). The researcher utilized noise-canceling headphones (Panasonic RP HC500) and halothane vapor induced anesthesia during the induction assay. No child was harmed during the experiment.

## Results and discussion

### Sonic reception system

Signal input to the synthetic *Picea abies* system was achieved through orthogonal transplantation of insect tympanal ostium tissue and the immediately adjacent tracheal tubing to the tapetal layer

in the innermost of four sporophytic layers encapsulating the meristem of the plant. Tympanal organs are the hearing organ in insects, consisting of a membrane stretched across a frame backed by an air sac. Sounds vibrate the membrane, and the vibrations are sensed by the chordotonal organ. We reasoned that the tympanal and chordotonal organs from the infamous African locust *Schistocerca gregaria* should be a conducive inducer of stimuli considering the breadth, history and scope of the swarming locust (Mohammed 1787; Joel 1924). It is clear that the communication signal system of the locust must be well developed so that it can both coordinate the swarming process and sing 'When You Wish upon a Star', although not necessarily simultaneously (Cricket and Disney 1940).



**Fig.1.** Dissected *Schistocerca gregaria*. The tympanal organ (A) and the underlying chordotonal organ (B) that transmit the signal is clearly visible in the anterior of the insect. Glenfiddich anesthesia (Coca and Cola 2001) was utilized during the dissection process to constrain the movement of the tissue donor

The tympanal and chordotonal tissue from 96 geographically separated locust individuals were HLA typed with predominant type I pneumocytes on frozen sections. Locust donors with alveolar epithelium expressed HEA-125, class I MHC. antigens HLA-DR (total of four individuals) were chosen as donors of tympanal and chordotonal tissue. The assumption was that this would mitigate any cerebral functions in the host. The dissected

donor (Fig 1) did not object. Organs were subsequently transplanted into the meristem of *Picea abies* using previously described procedures (Einstein, Zweistein *et al.* 1974). The tree was treated aggressively with immunosuppressive agents including Cyclosporin, Tacrolimus (FK506), and Rapamycin to minimize the rejection of the tympanal and chordotonal tissue transplants.

#### *Emitting seasonal light*

The luciferase gene used in this study was originally derived from a small population of reindeer (*Rangifer tarandus*) having red noses (Pret & Zel 1995; Stroganoff and Lindström 2004). The light emission spectra of the wild type gene was engineered using the ProteinGPS technology of DNA2.0 to display light in the yellow (mTwinkie), pink (mSnoBalls) and white (mWonderbread) spectra as previously described (Hostess, Tour *et al.* 2003; Liao, Warmuth *et al.* 2007).

The three monomeric luciferase analogs were codon optimized for maximal protein expression level in *Picea abies* chloroplasts using the Welch algorithm (Gustafsson, Govindarajan *et al.* 2004). The synthetic constructs were preceded by a chordotonal inducible CMV promoter and a Shine-Dalgarno (SD) sequence (GGAGG) located 4 to 12 nucleotides upstream of the initiator AUG. The monomeric luciferase genes were immediately followed by another SD and genes encoding the leaf Npr1 signaling pathway (Gross and Simmons 2005). This construction ensures that the dicistronic operon is to be expressed only at the node of each branch as it emits the light. The vector also encoded kasugamycin resistance for selection purposes.

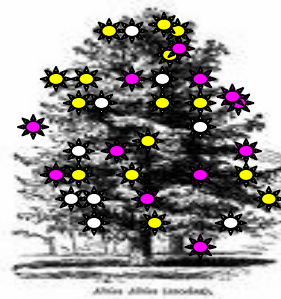
Plastid transformation offers several unique advantages compared with nuclear genome transformation, such as high level of transgene expression within plastids, expressing multiple transgenes as operons,

lack of position effect due to site-specific transgene integration, and reducing risks of gene flow via pollen due to maternal inheritance of the plastid genome.

Chloroplast containing *Picea abies* tissue was transformed with the vectors expressing the monomeric luciferase constructs using standard agrobacterium transfection methodology.

#### *Emitting seasonal odor*

The formation of cinnamaldehyde and gingerol are related to the phenylpropanoid metabolic pathways, *i.e.* the transformation of phenylalanine to cinnamic acid and its hydroxylated derivatives coumaric acid, caffeic acid, gingerol and ferulic acid. We used a phenylalanine oxidase (CinA) and a phenylalanine deaminase (CinB) identified in the wasp genome (*Nasonia vitripennis*) to oxidize the carboxylic group to an aldehyde and to remove the amine from phenylalanine. The GenBank sequence identifier NM\_001129280 and XM\_001601513 denotes the original open reading frames. The amino acid sequences were codon optimized for maximal protein expression level in *Picea abies* chloroplasts using the Welch algorithm (Gustafsson, Govindarajan *et al.* 2004).

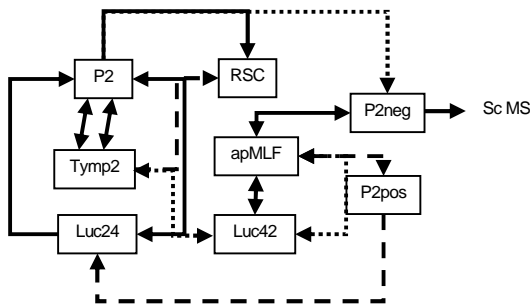


**Fig.2.** *Picea abies* strain Marve encoding the expressing mTwinkie, mSnoBalls and mWonderbread monomeric luciferase genes. The tree was exposed to 30 minutes of children singing Christmas carols. Maximal induction could be seen after 1hr.

The enzymes CinA and CinB were engineered for increased  $K_{cat}/K_M$  and

substrate specificity so that ~30% of all non-bound phenylalanine in the cell would be converted to trans-cinnamaldehyde and released through the stoma in conjunction with transpiration.

The gingerol synthesis was driven by GinT, an enzyme originating from the ginger plant (*Zingiber officinal*) where the coumarate is reduced and hydroxylated to form Gingerol (the ginger smelling compound). GinT and its enzymatic properties have previously been described (Thomson, Preston *et al.* 2007). Similarly to the CinA and CinB enzymes, the GinT enzyme was engineered and expressed at sufficient levels through codon optimization.



**Fig.3.** Tentative model describing the signal transduction pathway for the *xmas1* control system. Solid lines signifies activation, dotted lines signifies inhibition, dashed lines signifies interrogatory functionality. The model is further described in the text and elsewhere (Wait and See 2008).

### Conclusion

Synthetic biologists engineer complex artificial biological systems to investigate natural biological phenomena and for a variety of applications. We here outline an immediate application of synthetic biology that integrates many levels of control and signal transduction with the goal of reducing the environmental impact of Christmas.

We specifically show methods for successfully designing and constructing

engineered cells with novel functions in a framework of an abstract hierarchy of biological devices, modules, cells, and multicellular systems. To achieve predictability and reliability, strategies for engineering *Picea abies* must include the notion of cellular context in the functional definition of devices and modules, use rational redesign and directed evolution for system optimization, and focus on accomplishing tasks using cell populations rather than individual cells.

Christmas trees with endogenous emission of light and smell that is induced by the sound of singing children projects the future for ongoing efforts at Santa's laboratories©. Future efforts include the incorporation of sound emission from the tree. This goal has so far been proven elusive as our intent to clone the famous Ho Ho Ho from Santa himself has failed despite several serious attempts. We now have lowered our initial goal and instead are proposing the cloning and heterologous expression of the sound derived from chattering elves. We are currently exploring different methods for grinding up the elves to isolate the relevant factors and expect to be able to report this exciting extension of our synthetic biology Christmas tree in due course.

### Acknowledgements

The pretympalal follicles were surgically removed from the pre-pubertal female locust by Drs Olesiuk, tenBosch and Wang. We sincerely appreciate their carving skills. Assistance with managing the principal of the Fletcher-Maynard Elementary School came from Dr. Villalobos. Drs Spofforth and Pimentel proposed the product launch and validated the emission signals. Financial support for the work came from the DNA2.0 Research Council grant #002.

## Reference

- Alka, J. and M. Seltzer (2001). "Mapping of various things for various reasons." Hum. Genet. **32**(33): 33-44.
- Altschul, S. F., T. L. Madden, et al. (1997). "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs." Nucleic Acids Res **25**(17): 3389-402.
- Coca, C. and P. Cola (2001). "Gardening is the art that uses flowers and plants as paint, and the soil and sky as canvas." J. of Electron Microscopy **23**(3): 66-78.
- Cricket, J. and W. Disney (1940). "When you wish upon a star." Pinocchio **1**(23): 1-2.
- Deere, J., N. Massey-Ferguson, et al. (2008). "Hanging Automobile Air Freshener, Mountain Pine. Great long-lasting scent with elastic hang cord." Tractor Supply Inc. 2008 Catalog SKU 1050391.
- Einstein, A., B. Zweistein, et al. (1974). "Spatial integration in the temporal cortex." Res. Proc. Neurophysiol. Fanatic Soc. **54**: 33-8.
- Fleksnes, M. and R. Wesenlund (1972). "Fleksnes fataliteter." NRK - Hele Saken **145**(11): 1-25.
- Geithner, T., B. Obama, et al. (2008). "Doppietta del sorprendente Brighi e a una punizione perfetta di Totti." J Line Item Veto **08**(62354): 6565.
- Gross, T. and G. Simmons (2005). "Fresh air kiss." J. Environmental Entertainment **54**(6): 56-9.
- Gustafsson, C., S. Govindarajan, et al. (2004). "Codon bias and heterologous protein expression." Trends Biotechnol. **22**(7): 346-53.
- Hostess, Z., O. Tour, et al. (2003). "Genetically targeted luciferase-assisted light activation." Nat Biotechnol. **21**(12): 1505-8.
- Joel (1924). What the gnawing locust has left, the swarming locust has eaten. Bible, The Holy Spirit. **1**.
- Johnson, R. M. and R. R. Johnson (2003). "Benchmarks for the next decade. Measuring the competitiveness of Christmas." North Pole Economic Development Report **3**(1): 37-6428.
- Keillor, G. (2005). "The Sons of Knute, Norwegian fraternal organization." Lake Wobegon Herald Star **16**(4): 27.
- Liao, J., M. K. Warmuth, et al. (2007). "Engineering proteinase K using machine learning and synthetic genes." BMC Biotechnol. **7**: 16.
- Mohammed (1787). Locusts moving in swarms. They will emerge from their graves with downcast eyes, like swarming locusts.
- Myers, P. Z. (2005). "Flying Spaghetti Monster - An alternative model for explaining the appearance of toys during winter holiday season." Journal of Social Anthropology **3434**(45): 456-75.
- Opsahl, A. (1974). "Volvo Sonett har sju forgassere." NRK review **5454**(4): 4.
- Pret, A. and B. Zel (1995). "Red luciferase reporter from Rangifer tarandus: a sensitive tool for detection and assay." Mol Microbiol. **15**(6): 1055-67.
- Sambrook, J., E. F. Fritsch, et al. (1989). Molecular cloning. A laboratory manual. Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory Press.
- Silver, B. and C. Fir (2006). "Division of Christmas Trees. Program for the Detainment of Elves." United States Department of Agriculture. 2006 Census **06**: 1-400.
- Staden, R., D. P. Judge, et al. (2001). "Sequence assembly and finishing methods." Methods Biochem Anal. **43**: 303-22.
- Stroganoff, S. and K. Lindström (2004). "Laser treatment of rosacea in reindeer: a genetic study." Arch Dermatol. **140**(11): 1345-9.
- Thomson, M. A., P. W. Preston, et al. (2007). "Lime dermatitis from gin and tonic with a twist of lime." Contact Dermatitis **56**(2): 114-5.
- Villalobos, A., J. E. Ness, et al. (2006). "Gene Designer: A synthetic biology tool for constructing artificial DNA segments." BMC Bioinformatics **7**: 285.
- Wait, H. and T. See (2008). "Banana split in four dimensions." Clin. Bull. **6**(78): 95-106.