Frequence of crispr mutations in a photoreceptor gene in salmon salar

Using plasmid vectors in bacteria to amplify and sequence a gene to determine mutation frequency

Øyvind kjærgård Masdal
Christine Horne
Ståle Ellingsen

BACKGROUND

Nonvisual photoreception is the mechanism where photoreceptors, cells able to detect light, but not involved in visual perception triggers various biological responses.

Photoreceptor cells have been found in the pineal gland of salmon, where light are able to reach them by passing through the pineal window in the skull(Nordtug,1990).

This non-visual photoreception is suggested to be involved in the circadian cycle of Salmon, by influencing production of the melatonin hormone.(Falcon,199)

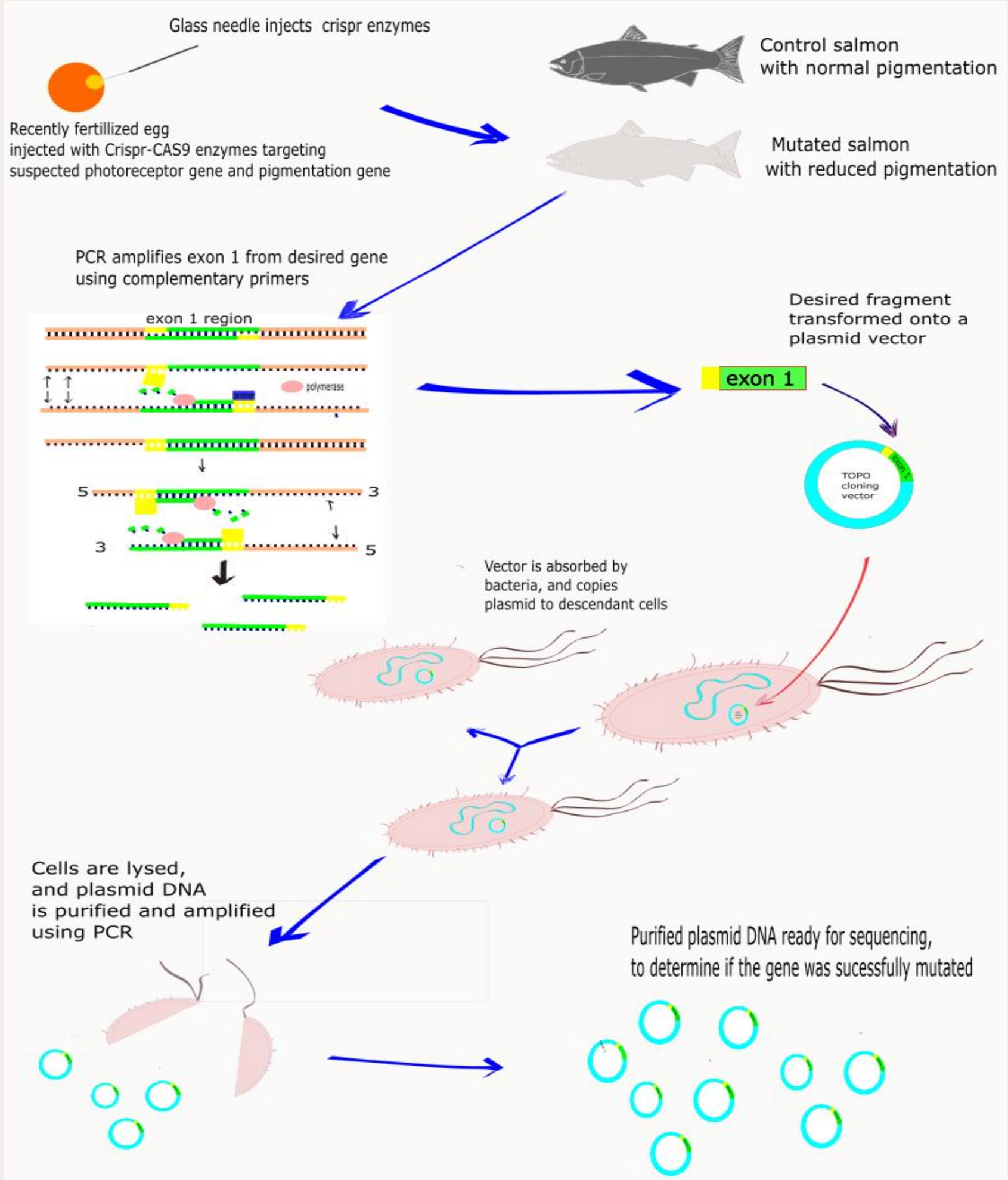
The technique Crispr-cas9 utilizes guide-mRNAs to bind to specific sequences, which guides a Cas9 enzyme to induce a double strand break. The resulting strand repair often results in a mutation and/or inactivation of the targeted sequence. This technique was therefore used in attempt to mutate a targeted photoreceptor gene along with a pigmentation gene acting as a control, to render them inactive.

PROJECT

We genotyped Crispr-Cas9 modified salmon individuals, to determine the frequency of successful mutation of the selected exorhodopsin gene.

Methods

Figure 1: Using complementary primers, we amplified the first known exon in the photoreceptor gene using PCR. The exon fragment was transformed onto a plasmid vector. Compatible bacterial cells absorbed the vector, and bacterial growth increased the available quantity of plasmid for sequencing.



SUMMARY

By examining if the targeted sequence had been mutated in the modified individuals across 40 samples, we determined the frequency of successful Crispr-Cas9 mutation of the targeted gene to be 65%.

```
  1 CCATGAACGGCACAGAGGGCCCAAACTTCTACGTGCCCATGTCTAACAAG     50
    ||||||||||||||||||||||||||||||.|||||||||||||
  6 CCATGAACGGCACAGAGGGCCCAAACTTCTATGTGCCCATGTCTA-----     50

 51 ACAGGGGTGGTGAGGAGCCCCTTTGAGCACCCTCAGTACTACCTAGCTGC    100
    ||||||||||||||||||||||||||||||||||||||||||||||||||
 51 ACAGGGGTGGTGAGGAGCCCCTTTGAGCACCCTCAGTACTACCTAGCTGC    100
```

Figure 2: Alignment between the exon strand(lower) found in a sequenced plasmid display a deletion in the 50th base pair region compared to the WT Exon sequence (upper).

Results

A total of 40 samples sequenced

23 had exon successfully embedded into the plasmid.
15 out of the 23 embedded exons had mutations around targeted base pairs. yieldig a mutation frequency of 65.2%

References:
Nordtug, T., & Berg, O. K. (1990). Optical properties of the pineal window of Atlantic salmon (Salmo salar L.). *Fish Physiology and Biochemistry*, *8*(6), 541–546. https://doi.org/10.1007/BF00003412

Falcón, J. (1999). Cellular circadian clocks in the pineal. In *Progress in Neurobiology* (Vol. 58, Issue 2, pp. 121–162). Elsevier Ltd. https://doi.org/10.1016/S0301-0082(98)00078-1
Illustrasjon: Øyvind Masdal
¨mal Powerpoint: av Helge Grønahug

UNIVERSITY OF BERGEN