# Creation of an opsin library for neurophysiology research

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## Introduction

In neurophysiology, optogenetic techniques can be used to impart light sensitivity to cells.

This project will support the large, cross-disciplinary "Controlling Abnormal Network Dynamics using Optogenetics" (CANDO) project that aims to create a cortical implant with the capacity to regulate neural cell activity[1].

Opsin genes derived from algae can be integrated into human cells by gene therapy. These genes cause cells to express light sensitive ion channels into the cell membrane in addition to ion channels already present that allow the excitation or inhibition of neurons dependent by light stimulation on the properties of the channel[2].

## Aims

- Identify sequence, origins and characteristics of 6 opsin genes; Chronos, H134R, oChIEF, eArchT 3.0, SFO, CatCh
- To design cloning plans to obtain the genes from an appropriate source and clone them into a suitable lentivector plasmid from which to create virus.
- Sub-clone opsins into lentivector for testing.
- Create a library of these constructs for use by CANDO

## Planning the library

The DNA sequences for each opsin were determined from NCBI and addgene online databases.

Source plasmids were identified from addgene. PCR primers were designed in order to amplify the opsin from the plasmid for restriction cloning.

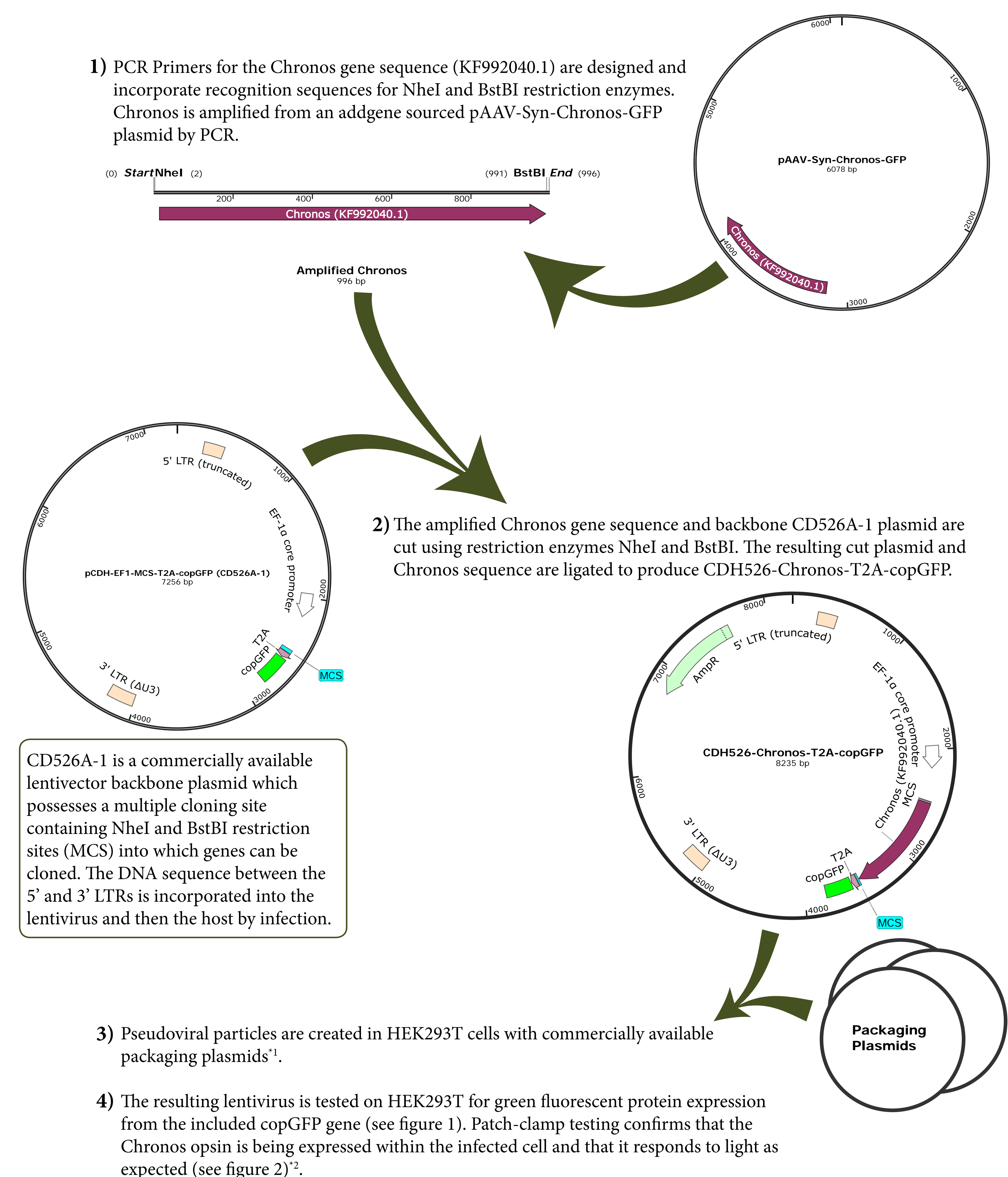
Opsin characteristics were documented such their peak response wavelength, transport kinetics, light sensitivity and whether they act to excite or inhibit neurons was documented along with material source and sequence (summarised in Table 1).

| Opsin | Peak Response Wavelength (nm) | Properties |
|---|---|---|
| Chronos | 500 | Excitatory, ultrafast kinetics |
| H134R | 450 | Excitatory |
| oChIEF | 450 | Excitatory, superior expression and membrane localisation |
| eArchT 3.0 | 566 | Inhibitory |
| SFO | 470/590 | Excitatory, step function - activated at 470nm, deactivated at 590nm |
| CatCh | 474 | Excitatory, enhanced light sensitivity |

*Table 1. Opsins characterised in the library. Peak response gives the wavelength which elicits the strongest response from the channel. Key properties of each opsin are included.*

## Building the library

For each opsin, a plan was devised to obtain the gene and sub-clone it into a 3rd generation lentivector plasmid which would then be used to produce pseudoviral particles. We illustrate this using the Chronos opsin.




1) PCR Primers for the Chronos gene sequence (KF992040.1) are designed and incorporate recognition sequences for NheI and BstBI restriction enzymes. Chronos is amplified from an addgene sourced pAAV-Syn-Chronos-GFP plasmid by PCR.

2) The amplified Chronos gene sequence and backbone CD526A-1 plasmid are cut using restriction enzymes NheI and BstBI. The resulting cut plasmid and Chronos sequence are ligated to produce CDH526-Chronos-T2A-copGFP.

CD526A-1 is a commercially available lentivector backbone plasmid which possesses a multiple cloning site containing NheI and BstBI restriction sites (MCS) into which genes can be cloned. The DNA sequence between the 5' and 3' LTRs is incorporated into the lentivirus and then the host by infection.

3) Pseudoviral particles are created in HEK293T cells with commercially available packaging plasmids*1.

4) The resulting lentivirus is tested on HEK293T for green fluorescent protein expression from the included copGFP gene (see figure 1). Patch-clamp testing confirms that the Chronos opsin is being expressed within the infected cell and that it responds to light as expected (see figure 2)*2.

## Chronos construct testing results

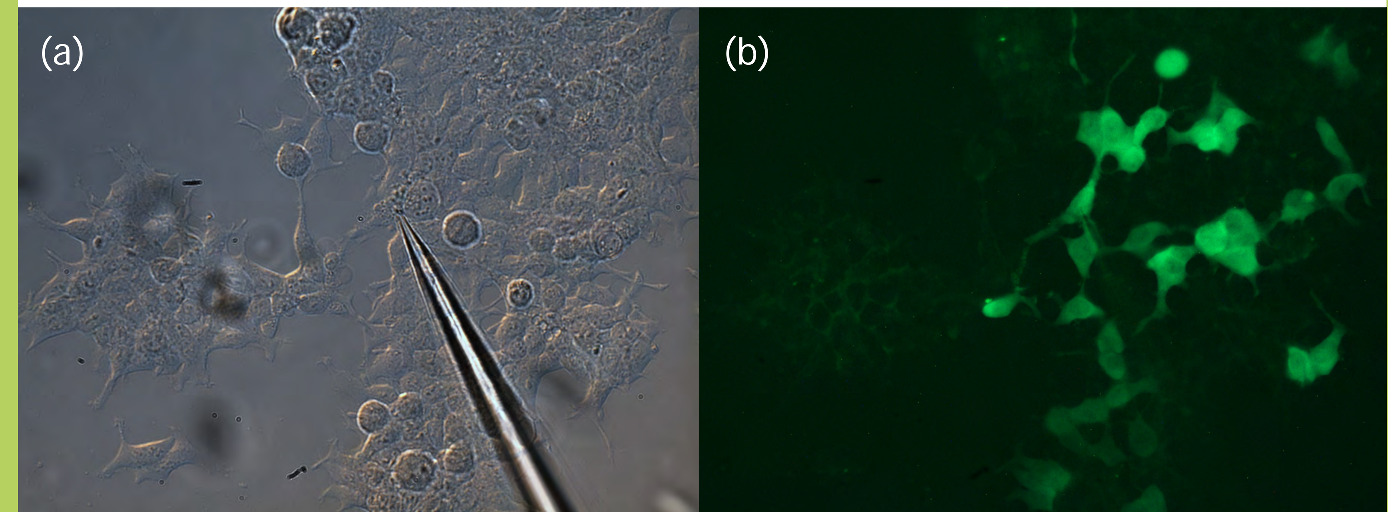



*Figure 1. Lentivirus created*1 *using the CDH-EF1-Chronos-T2A-copGFP vector plasmid was used to infect HEK293T cells and gave a strong **green fluorescent signal***2*. Image (b) shows the observed fluorescence from the cells in (a). This demonstrates expression of the plasmid construct within the cell line.*

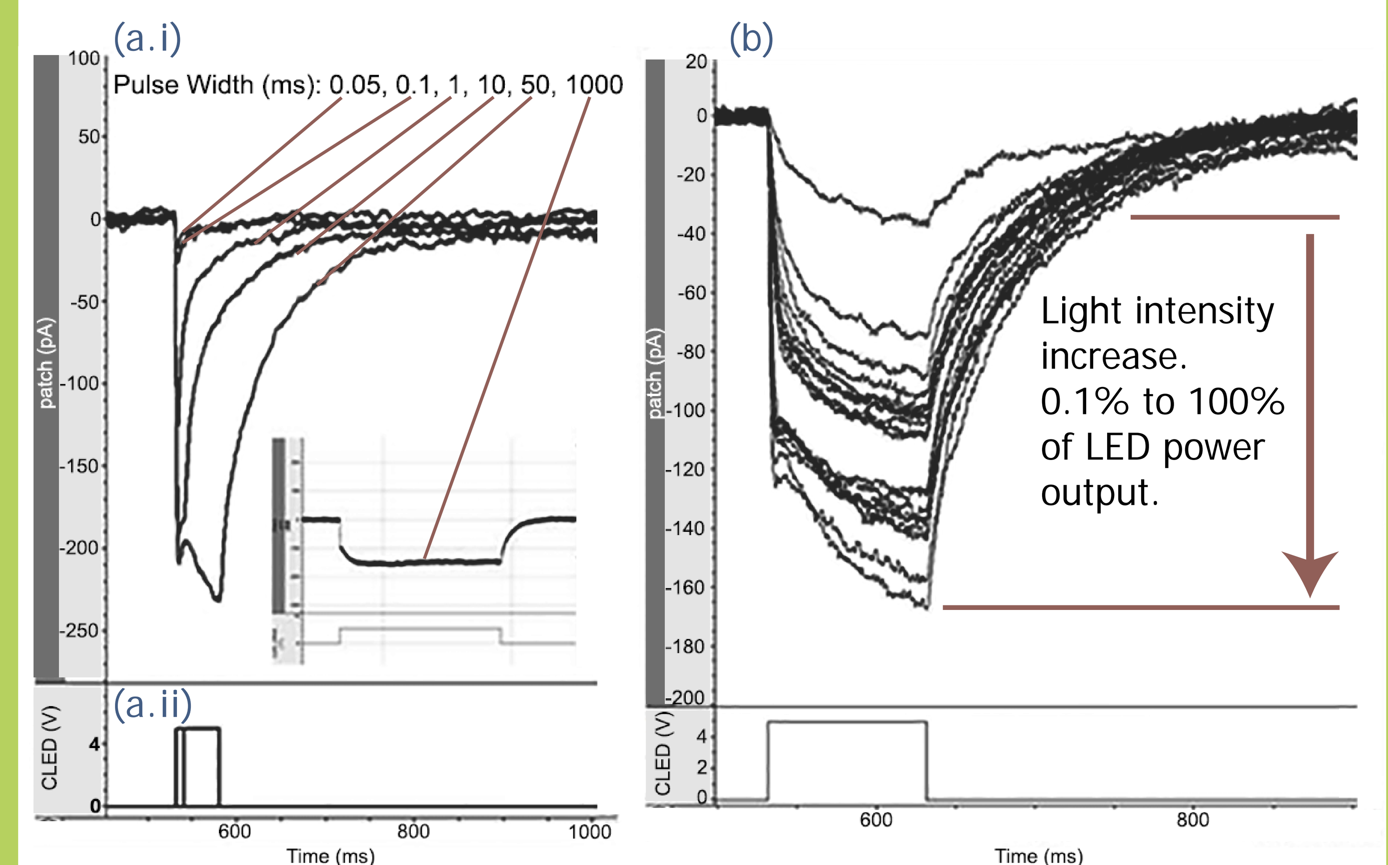



*Figure 2. Patch-clamp testing measured the voltage change caused by the Chronos channelrhodopsin opening in response to light at 470nm. (a.i) shows the change in spike and recovery against to the duration of light pulse from the LED (a.ii). (b) shows the change in response when the intensity of light is altered.*

## Discussion

We have sub-cloned a range of opsins into lentivector plasmids. We have also demonstrated the functionality of each construct as illustrated here with Chronos.

In addition, we have started work on a new backbone plasmid. This will facilitate further research into tissue specific promoters and allow us to make use of non-commercial packaging plasmids.

The successful creation of the library and development of the new lentivector plasmid provides a template on which further research can be done.

## Acknowledgements

This project was funded by Newcastle University Research Scholarship. *1Thanks to Dr Elizabeth Stoll for creating the lentivirus used in testing.
*2Thanks to Dr Rolando Berlinguer-Palmini for conducting the electrophysiology experiments and for allowing the use of the results.

## References

[1] Newcastle University (2014) 'About CANDO', About CANDO. Available at: http://www.cando.ac.uk/aboutcando/ (Accessed: 02/07/2015).
[2] Mattis, J. et al. (2012) 'Principles for applying optogenetic tools derived from direct comparative analysis of microbial opsins', Nat Methods, 9(2), pp. 159-72.