# Nuclear Envelope Associated Tether (NEAT) system

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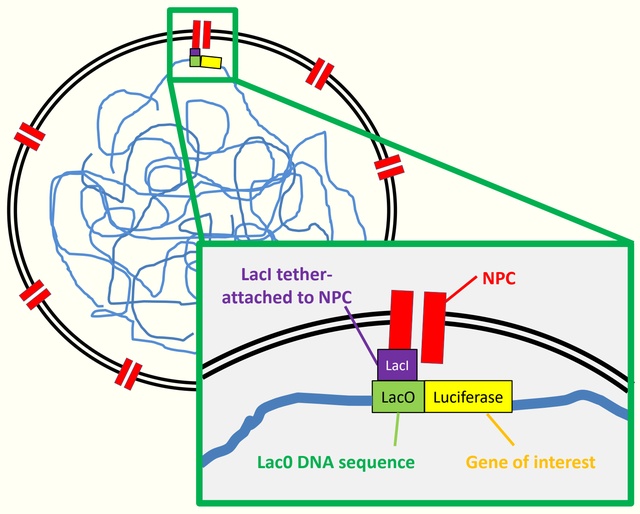
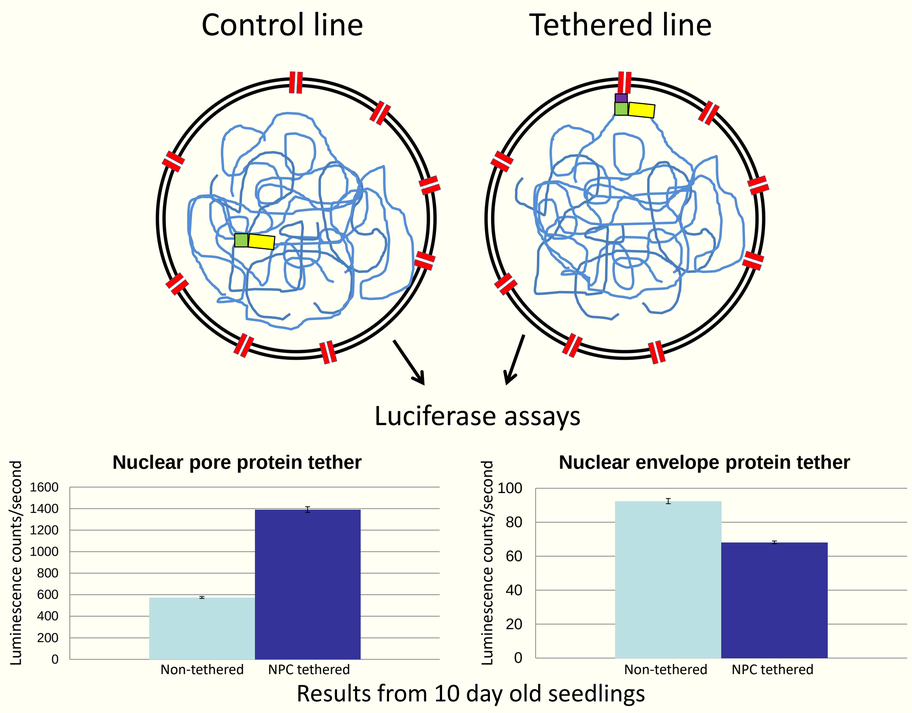
- Creating transgenic plants relies on the random insertion of genes into DNA. Insert position within the genome can play a huge role in the expression level of the transgene.
- In other model systems, studies have shown that the physical position of the chromatin within the nucleus may alter gene expression.
  - ➢ Chromatin positioning at the nuclear pore (NPC) is associated with enhanced expression of genes.
- We have used a LacO-LacI system to tether a luciferase reporter gene to the NPC and are checking expression levels by luciferase assays.




The Lac0-LacI system has been used to tether the luciferase reporter gene to the nuclear pore complex.

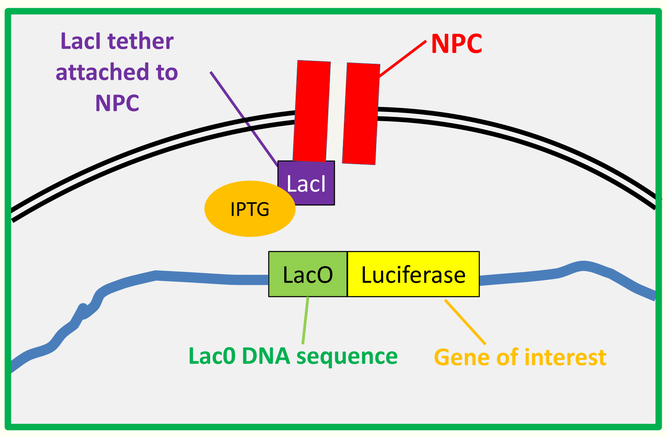
A number of NPC and NE proteins were tagged with LacI.




Results from 10 day old seedlings

The nature of the nuclear pore tether altered whether levels of luciferase were increased or decreased.

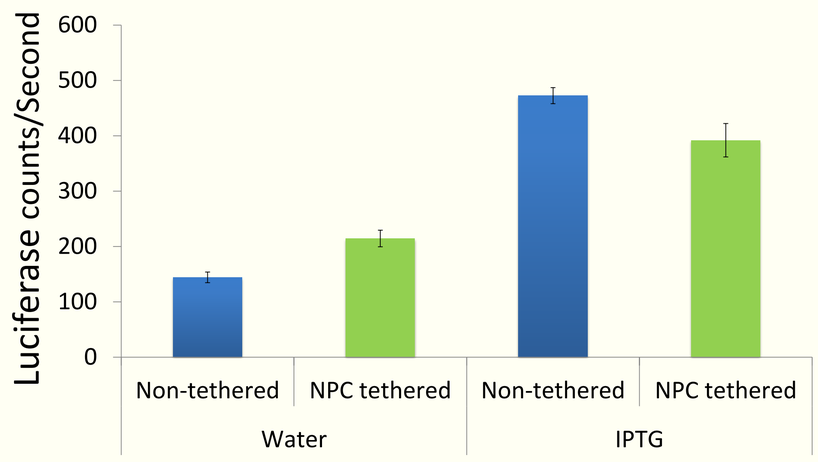
One nuclear tether showed an increase in luciferase activity in 10 day old seedlings when compared to control lines.



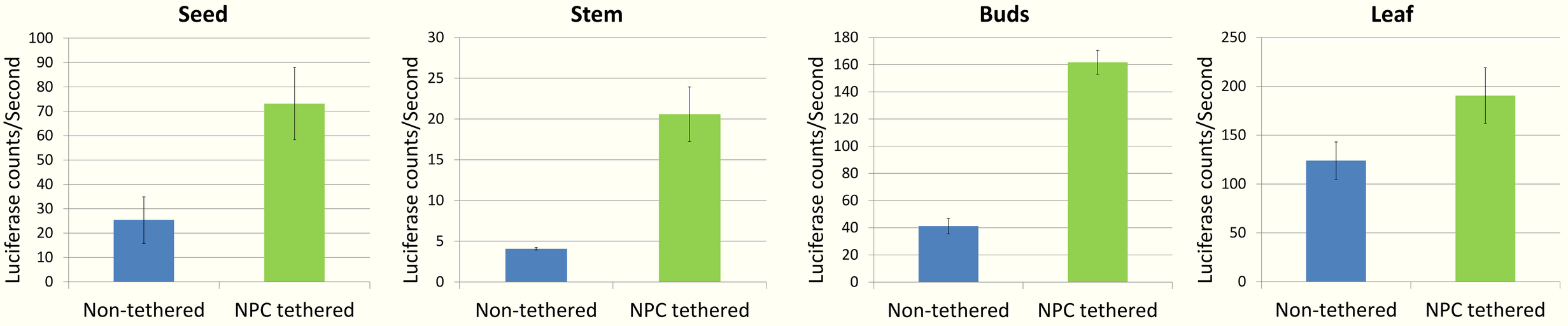

IPTG disrupts the Lac0-LacI interaction

## Disrupting the tether with IPTG

Treating seedlings with IPTG abolishes the increase in luciferase activity seen in the tethered lines. This is presumably due to disruption of the LacO-LacI tether and a return of the luciferase gene to its 'normal' nuclear position.

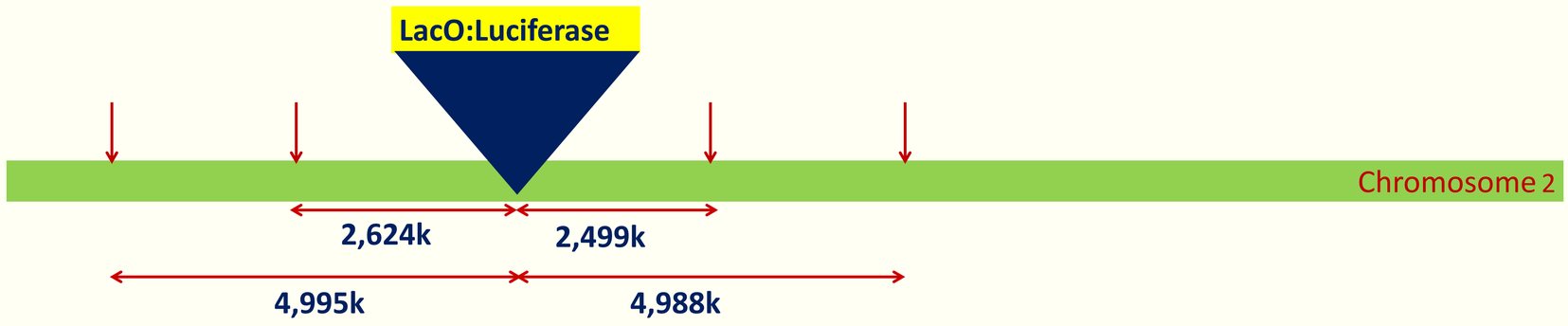



## Luciferase activity in mature plants




## Analysis of genes flanking Luciferase site by Q-PCR

What proportion of the chromatin surrounding the insert site is affected by the NPC tethering?




## Other future perspectives

- Confirming results of luciferase assays with Q-PCR and western blots
- Testing a range of different reporter systems, including GFP
- The Lac0-LacI tether was used as a proof on concept approach. However this is a large and complex system which is not viable for use in crop plants. For these reasons we are working towards identifying natural DNA tethers for the NPC protein using a chromatin precipitation approach.

**References:**
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**Dieppois and Stutz,** 2010. Connecting the transcription site to the nuclear pore: a multi-tether process that regulates gene expression. *Journal of Cell Science:* **123**, 1989-1999
**Rosin *et al.,*** 2008. Genome-wide transposon tagging reveals location-dependent effects on transcription and chromatin organization in Arabidopsis. *The Plant Journal*: **55**, 514–525