

Genetic regulation of compost and plant degradation mechanisms in *Agaricus bisporus*

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1. Introduction

Agaricus bisporus (common button mushroom) is an economically significant mushroom with an annual global value in excess of \$4.7 billion (Eastwood et al, 2015). When commercially grown, *A. bisporus* mushrooms are mostly picked from the first and second flush. This is due to the third flush resulting in reduced yields (Royse and Sanchez, 2008), which are also often more prone to disease. This occurs despite significant nutrients and nitrogen being available in the compost for *A. bisporus* to utilise. To further understand why this is occurring, microarray analysis was carried out on compost samples throughout a full commercial growth cycle, with the aim of identifying genes that may be responsible for this reduction in yield.



Fig. 1: First flush of *A. bisporus*.

2. Project Overview

- Four replicates of A15 compost samples were collected every 48hrs from before 1st flush up until the end of the 3rd flush, from across an entire Commercial Mushroom Farm shelf.
 - RNA was isolated and hybridized to Microarrays.
- Data analysis was carried out to generate gene expression profiles, by comparing each samples expression against Day 11 (Pinning).

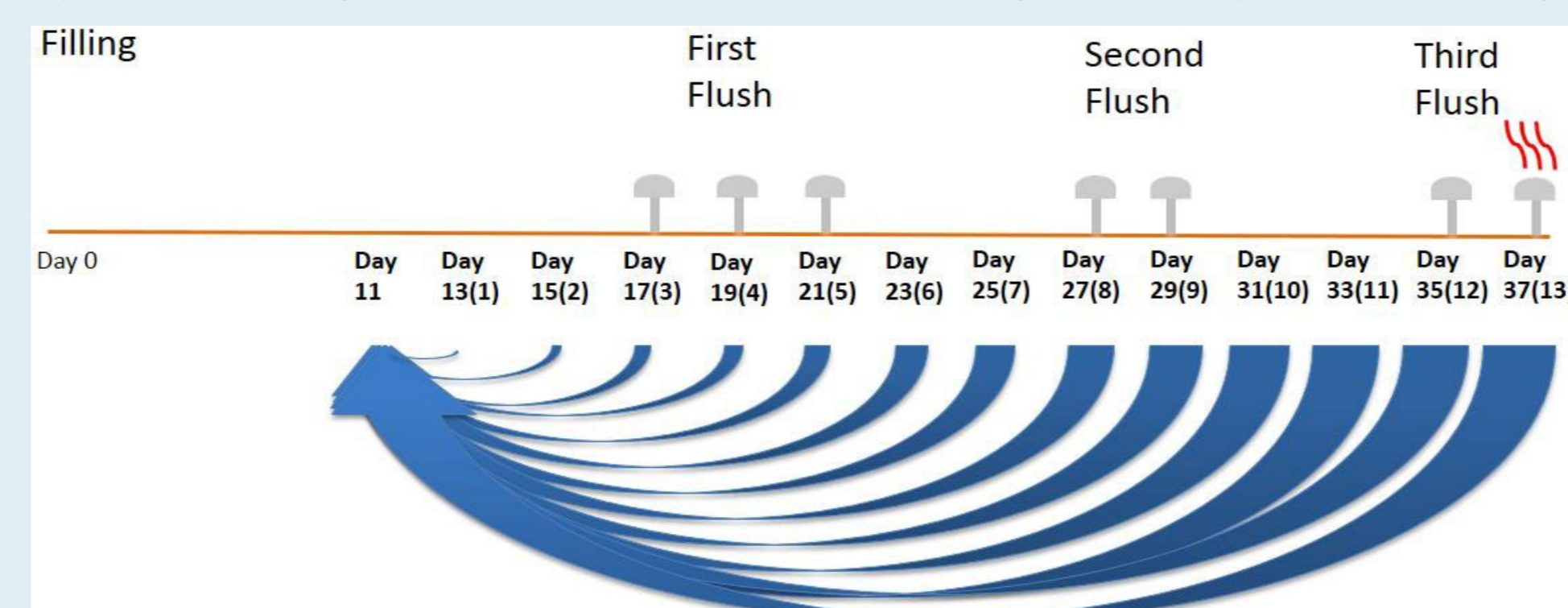


Fig 2: A schematic representing the gene expression comparisons of all time points against Day 11, the first sample point.

- 5 candidate genes were selected based on their expression profile for further characterisation;
 - Cellulose, Hemicellulose/pectin, Oxidoreductase, Laccase and an "Unknown" gene, that is unique to *A. bisporus*.
- The promoters of these genes have been linked to eGFP through Gibson Assembly. A promoter analysis will now be carried out by expressing these plasmids in the model basidiomycete *Coprinopsis cinerea* and culturing the transformants on a variety of substrates and monitoring eGFP fluorescence.
- Down regulation studies will also be carried out.

3. Plasmid Design

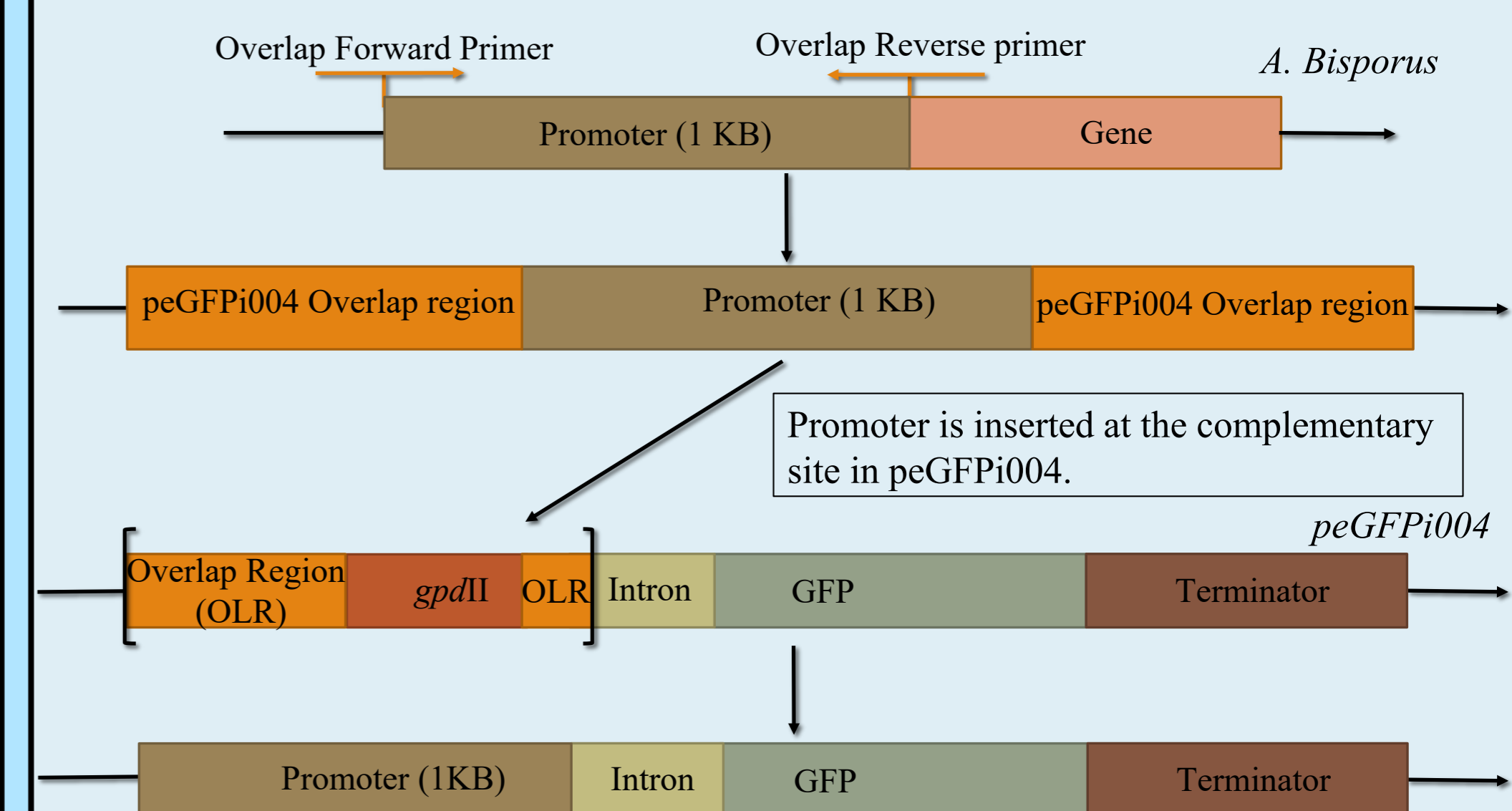


Fig 3: Schematic plan for the insertion of the each genes promoter into the plasmid peGFPi004.

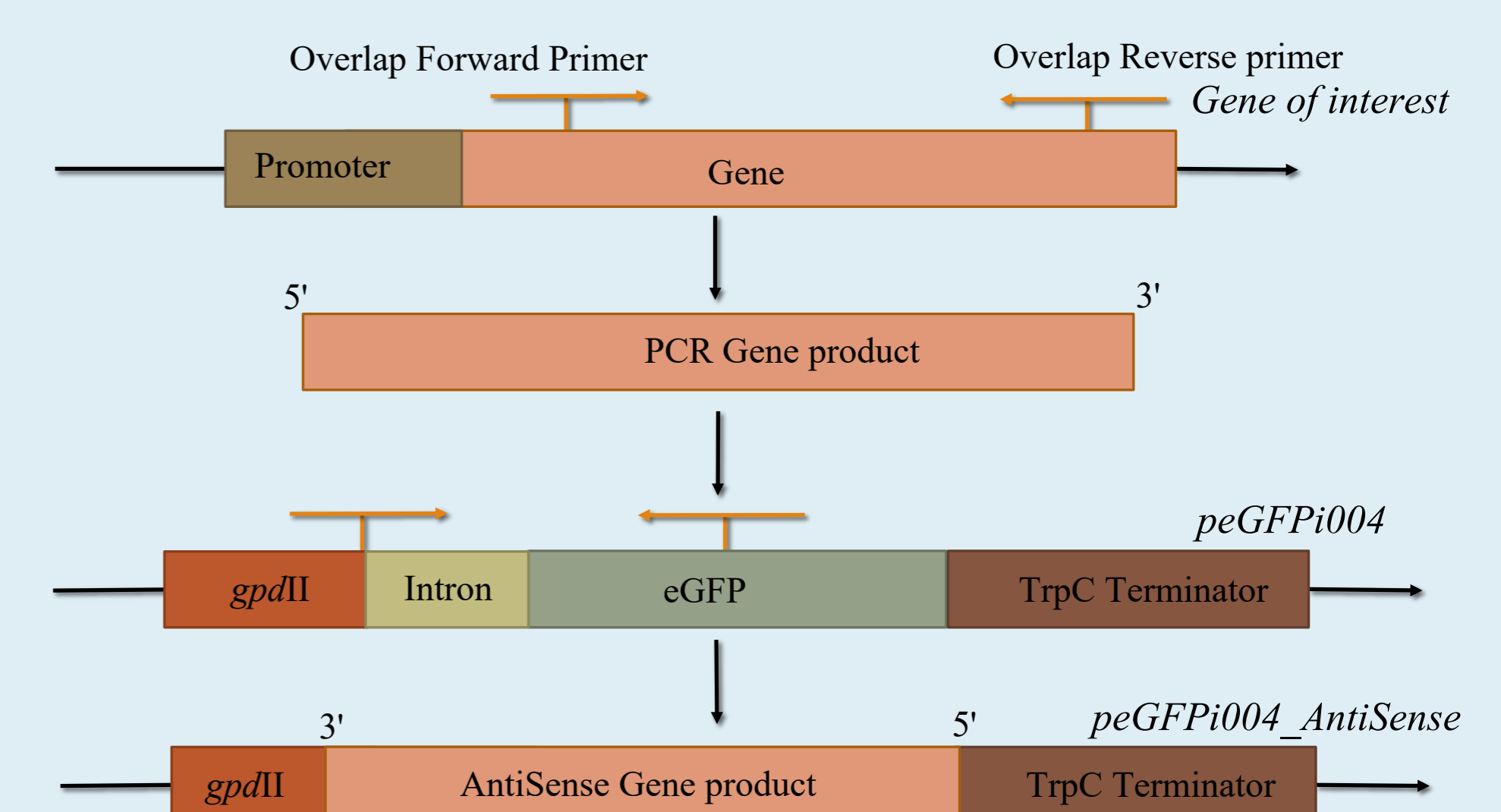


Fig 4: Schematic plan for the insertion of the anti-sense gene fragment into the plasmid peGFPi004.

4. Results

- Microarray results were analysed using R statistics after normalisation.
 - The data was filtered to select for genes that were significant ($P < 0.01$) in 25% of the arrays and clustered using k-clustering and soft clustering from the Bioconductor package mFuzz.
- Several genes of interest were identified, some of which are believed to be critical in yield control as well as a gene unique to *A. bisporus*.
- Cluster analysis demonstrated that several gene expression profiles followed the commercial cropping cycle (Fig. 5 and 6).

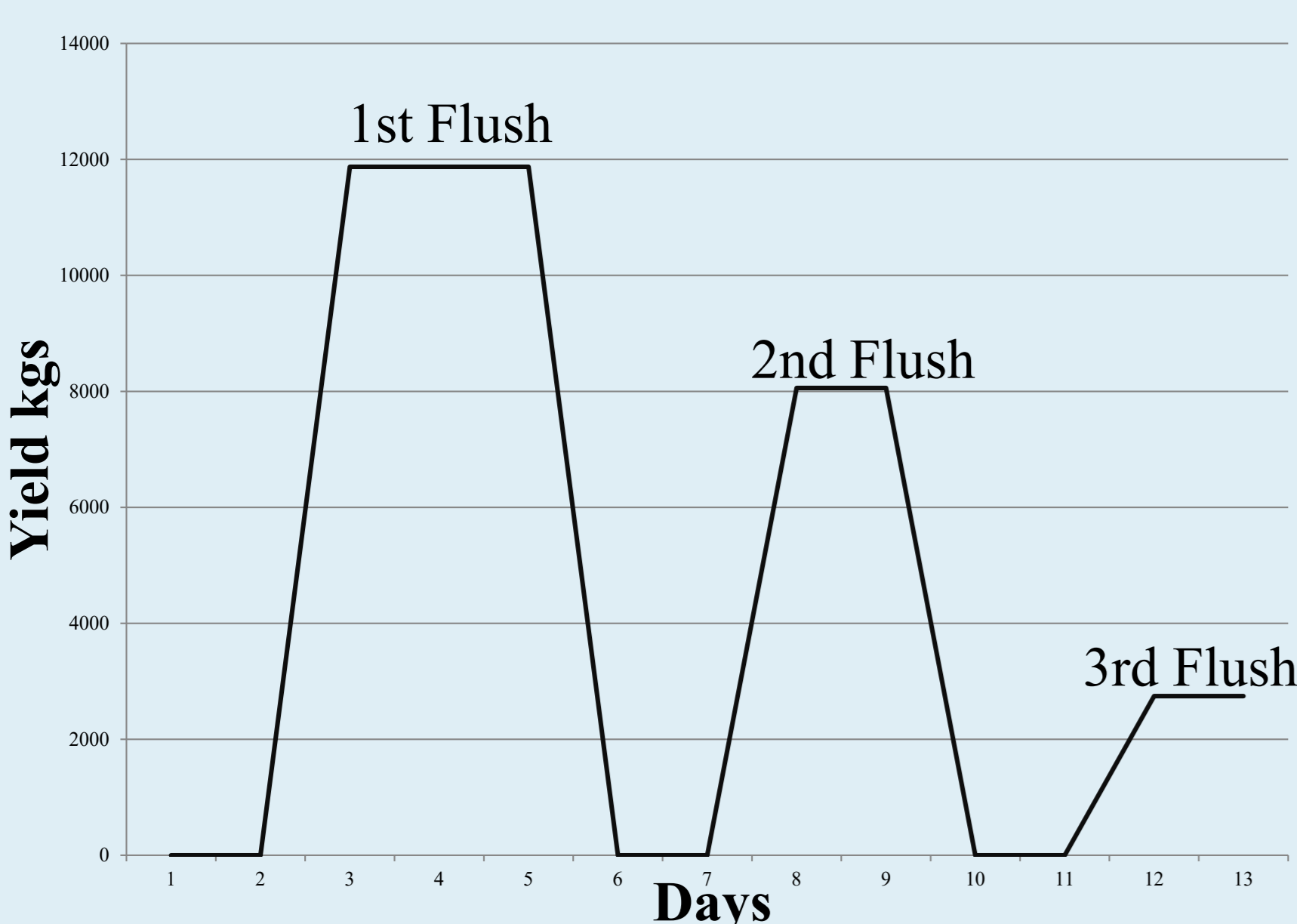


Fig. 5: Total yield for the first, second, and third flushes of *A. bisporus* during a commercial growth cycle.

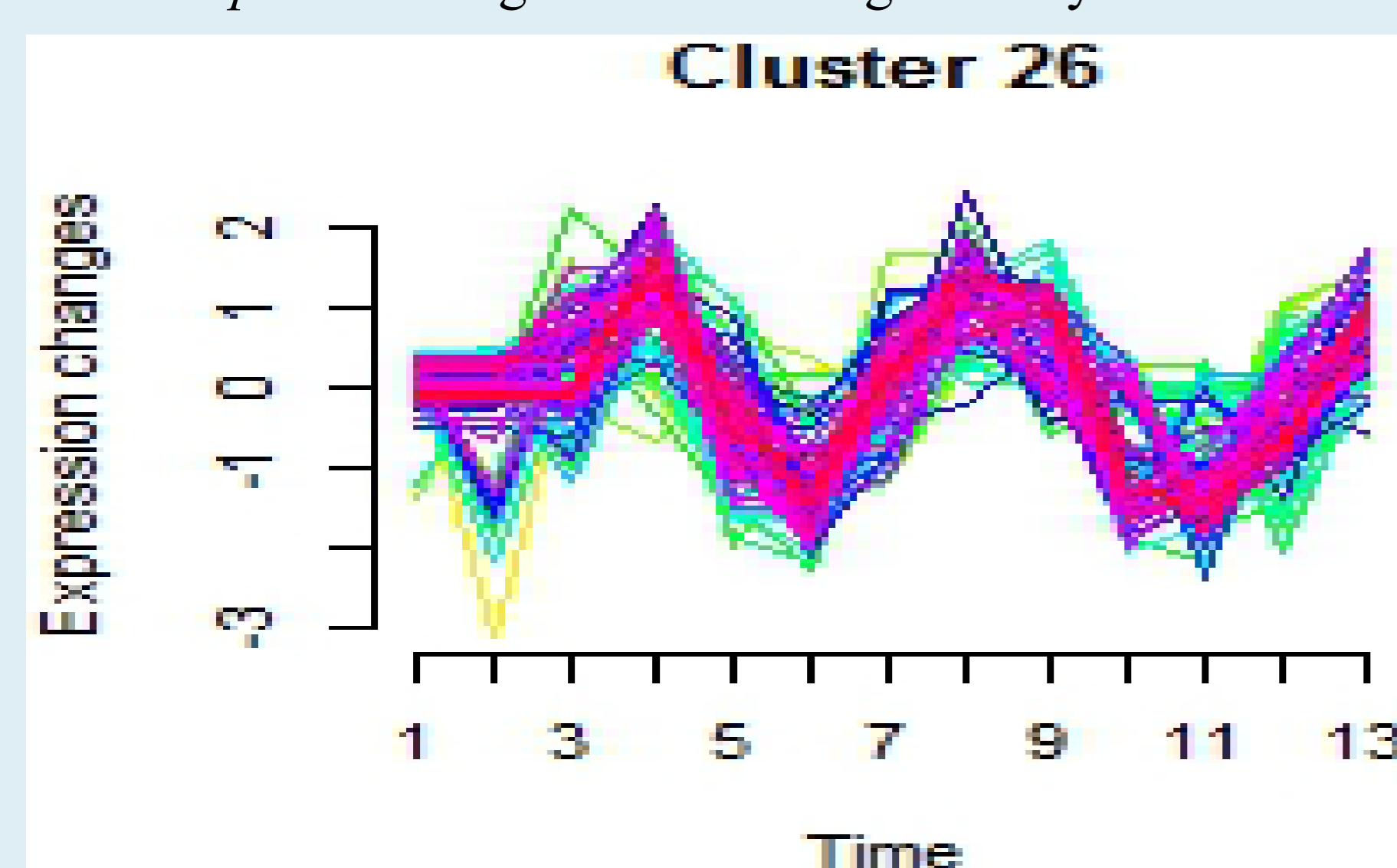


Fig. 6: Gene expression changes across a commercial crop of *A. bisporus*.

- 4 out of 5 of the candidate genes have had their promoters isolated.
- 3 promoters have already been inserted into peGFPi004 and are in the process of been transformed into *C. cinerea*.
- PCR optimisation for the isolation of antisense fragments is underway.
- A large scale bioinformatics analysis of all the promoters present in *A. bisporus* is also being conducted.

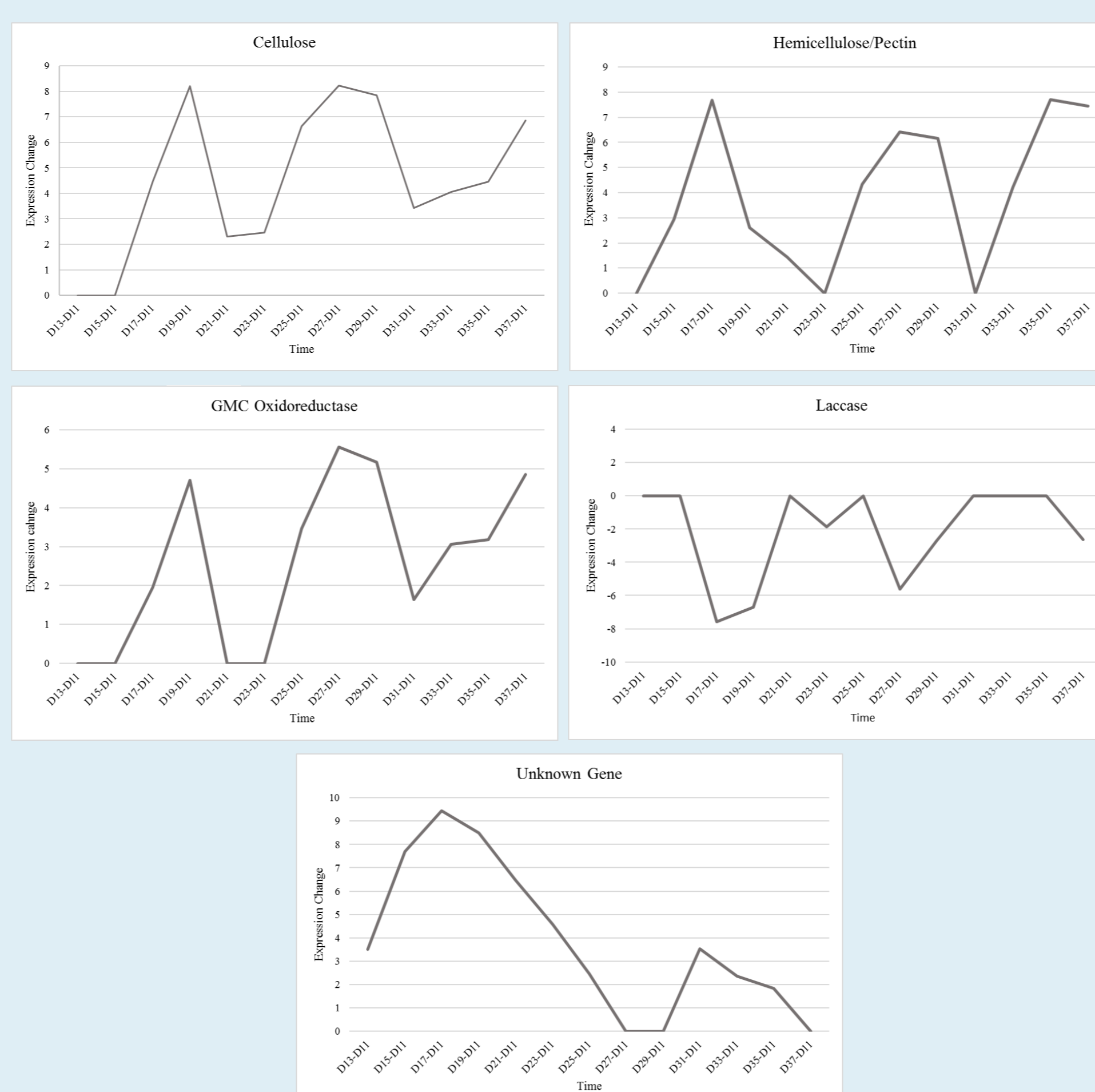


Fig 7: Gene expression profiles of the 5 selected genes for promoter analysis.

5. Future work

- Promoter analysis of identified genes using the available *A. bisporus* and *C. cinerea* transformation systems.
 - Identify specific regions within promoters which are stimulated by certain substrates and identify compounds which may be inhibitory to these.
 - Down regulation of genes potentially involved in compost utilisation to determine gene function.
- Complete the large scale bioinformatic analysis of *A. bisporus* promoters.

Bibliography

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Royse, D. and Sanchez, J. (2008). Supplementation of 2nd break mushroom compost with isoleucine, leucine, valine, phenylalanine, Fermenten® and SoyPlus®. *World Journal of Microbiology and Biotechnology*, 24(10), pp.2011-2017.

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