

A Diagnostic Microarray for Honeybee Viruses

Introduction

The honeybee, *Apis mellifera*, plays a vital role in agriculture by assisting with the pollination of many different crops. The pollination activity of bees in the UK alone has been estimated at £200million. Numerous pathogens and parasites threaten bee health. Most honeybee viruses are single stranded RNA viruses and are very similar in size and shape, making them difficult to distinguish from each other using physical characteristics.

Materials and methods

Sequences for eight bee viruses were available from GenBank: Deformed wing virus (DWV), Kashmir bee virus (KBV), Acute paralysis virus (APV), Varroa destructor virus (VDV), Sacbrood virus (SBV), Chronic paralysis virus (CPV), Apis iridescent virus (AIV) and Black queen cell virus (BQCV). Initial design of 50-mer oligonucleotides to bee virus sequence was carried out using OligoArray 2.0 (Rouillard *et al.*, 2003), followed by manual selection of promising sequences (figure 1). Between 6 and 10 oligonucleotides were selected for inclusion on the microarray. Four oligonucleotides were designed to the 18S ribosomal gene of *Apis mellifera* as internal controls. Dye-labelled cDNA was created using the CyScribe post-labelling kit (Amersham Biosciences) and all samples hybridised overnight at 42°C.

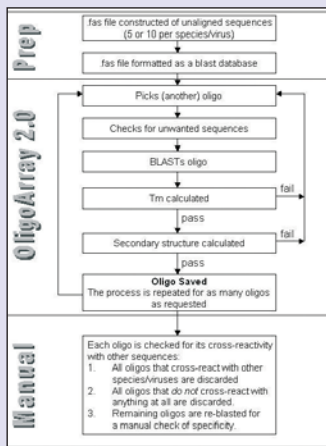


Figure 1: Flowchart showing the process for selecting oligonucleotides for inclusion on the array

Results

Initial testing of this low-density microarray has been very successful with 60/61 of the oligonucleotide capture probes tested hybridising with the correct target DNA. Only 1 oligonucleotide has so far shown cross-hybridisation with an incorrect virus (circled in figure 4). Testing of bees from infected hives has shown that extracting total RNA from only one bee is insufficient for a positive identification. The distribution of some bee viruses, for example deformed wing, can be varied throughout a comb and as such five bees or more are required for testing.

Reference

Rouillard, J.M., Zuker, M., and Gulari, E. (2003) OligoArray 2.0: Design of oligonucleotide probes for DNA microarrays using a thermodynamic approach. *Nucleic Acids Research* 31(12):3057-3062.

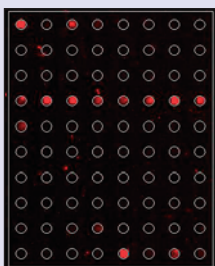


Figure 2: Deformed wing virus

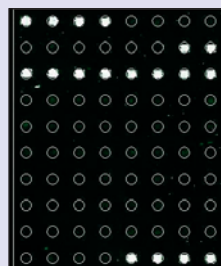


Figure 3: Black queen cell virus

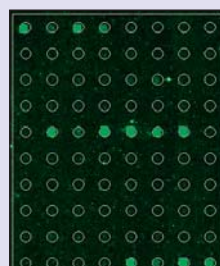


Figure 4: Kashmir bee virus

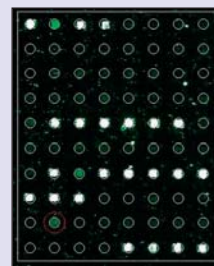


Figure 5: Mixed infection with Kashmir bee virus and Sacbrood virus



Authors

Rachel Glover
Neil Boonham

Address

Central Science Laboratory,
Sand Hutton,
York,
YO41 1LZ.
UK.

Acknowledgement

We gratefully acknowledge Defra (Department for the Environment, Food and Rural Affairs).



CSL is an Executive Agency of Defra