



DNA extraction from honey bee intestinal bacteria and pollen

With kind permission of Keith Browne, Irish Research Fellow.
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APPLICATION

The mite *Varroa destructor* is largely responsible for the catastrophic reduction in viable colonies of the Dark Northern European honey bee *Apis mellifera mellifera*. Furthermore, introgression from imported southern sub-species is thought to have altered the genetic integrity of this bee to the point where it is now considered extinct across most of Europe, but Ireland remains a stronghold (Hassett et al., 2018, Henriques et al., 2018). The Dark Northern honey bee is adapted to colder, wetter weather and a shorter foraging season compared to the southern sub-species. These adaptations allow the production of honey in northern Europe and efficient pollination of crops and wild flora. Research focuses on the adaptive evolution of this honeybee including how some bees display resistance/tolerance to parasitism. Observed colony traits and local environmental conditions, combined with genomics, genotyping and morphometric analysis of the bees have been used to determine those strains & traits offering greatest tolerance to *Varroa* whilst retaining local adaptations. This information will be employed to guide a breeding program for *Varroa*-resistant native Irish honey bees.

SAMPLE SETUP

Using a mix of various grinding material sizes, the MiniG[®] was used to disassociate the walls of plant pollens brought into the hives of honey bees. This was one of the initial stages to enable the extraction of the pollen DNA. This DNA was then bioinformatically analyzed to determine the diversity and abundance of pollens used by the bees over a year.



The MiniG[®] was also used to the same effect on the guts of honey bees in order to extract DNA from the bacterial content. As per the pollen, a bioinformatics analysis was conducted to investigate regional and temporal variation in this microbiome.

2mL disruption tubes loaded with 100um silica which was chosen for pollen samples due to the high proportion of sub-10um pollen. Up to 24 disruption tubes were inserted into a foam holder (#1680) and clamped into the MiniG[®]. The samples were then homogenized for 10 minutes at 1500 rpm.

:: APPLICATION NOTE SP029

DNA extraction from plant and animal tissue.

:: APPARATUS

1600 MiniG[®]

:: APPLICATION

DNA extraction



RESULTS AND DISCUSSION

Bead beating pollen presented a challenge due to a sample mass larger than typically recommended for 2 mL tubes and this required optimization. However, because the 1600 MiniG[®] can be easily loaded and adjusted, this only took a short time experimenting with different bead/speed/time combinations to establish the optimum protocol.

The homogenized samples were checked using light microscopy which showed a very satisfactory level of pollen disruption ready for DNA extraction.

CONCLUSIONS

Data was successfully generated from bees sampled from across the island of Ireland. Mitochondrial DNA data identified different variants with a large proportion of bees having sequences identical to three European variants but all other variants being novel. Further analyses indicated that the Irish population is genetically diverse and that almost 100% of sampled bees were determined to be pure *A. m. mellifera*.

The 1600 MiniG[®] fitted into already established protocols since, as a relatively small benchtop machine, it could be strategically placed to optimize workflow and avoid excessive movement between workstations. The ease of sample loading combined with straightforward speed/time adjustment also assisted this optimization. The MiniG[®] has been shown to be reliable, uncomplicated, and helps avoid making mistakes in repetitive work such as DNA extractions.

REFERENCES

- Hassett, J., Browne, K. A., McCormack, G. P., Moore, E., Soland, G., Geary, M. and Native Irish Honey Bee, S. (2018) 'A significant pure population of the dark European honey bee (*Apis mellifera mellifera*) remains in Ireland', *Journal of Apicultural Research*, 57(3), 337-350.
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For more information on the 1600 MiniG[®] Please visit www.spexsampleprep.com/minig.



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