

BEEGENETICS

Sampling protocol: males

Material provided

- Numbered sample tubes (containing a buffer solution)
- Sampling clamp: 1 different clamp per colony
- Sampling Spreadsheet (digital)

Method

For each colony, a sample is taken from a single hind leg of **15 male nymphs** (drones).

Male nymphs are used because adult males may come from other colonies.

You can use a magnifying glass, magnifying glasses and a headlamp or other powerful light source to facilitate sampling.

1. Remove a frame containing operculated male brood from the colony, and eliminate all bees **by brushing or gently shaking them.**



The male brood cells have bulging opercula, unlike worker cells which are flat.

Place yourself on a flat surface, away from the hive, and position the frame so that you can easily access the cells.



1. Using the handle of the clamp, carefully **remove the operculum** of a cell and inspect the male nymph. Perform the samples only on nymphs **with colored eyes** (pink, purple or darker).

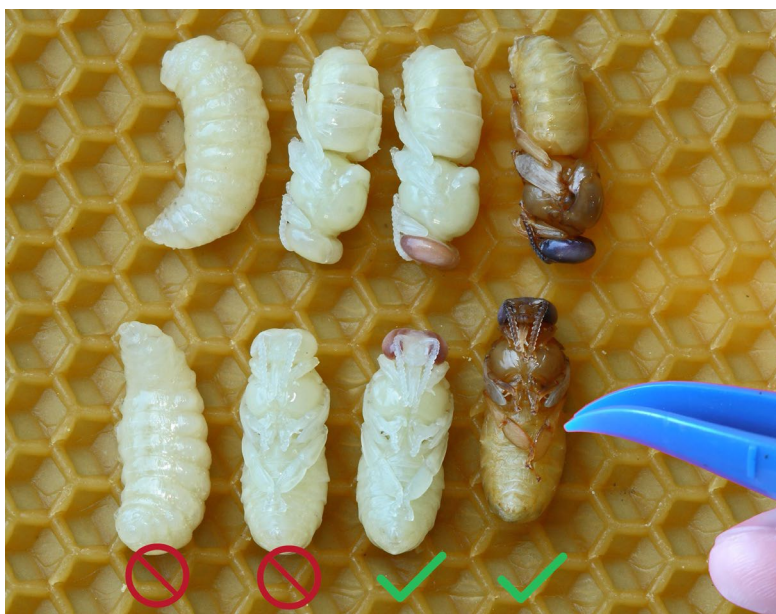


*Cell unharmed with a male
at the right stage of development (purple-eyed nymph)*

1. Carefully insert the open clamp into the cell to gently grasp the nymph by the sides of the thorax and slowly pull the male out of the cell.

If the nymph does not emerge cleanly from the alveolus, move on to another male.

2. Using the tongs, gently remove one of the back legs of the nymph. You can use another clean tool, such as a pin or toothpick, to stabilize the male.



Males at the correct stage of development on the right



3. Place the tab in the tube. You can use another **clean** tool to detach the tab from the clamp.
4. Do not put other part of the bee in the tube. There is no problem if the leg has broken into several parts.
5. Repeat steps 3 to 7 until you get **15 legs** (1 per nymph). Close the tube carefully.
6. Tap the bottom of the closed tube on a hard surface to thoroughly immerse the legs in the buffer solution.
7. Replace the frame in the hive. The bees will repair open cells and remove damaged nymphs.
8. Fill out the sample information sheet for this colony.
9. Let the tube rest in the refrigerator for 6 to 8 hours, so that the legs are well saturated by the buffer solution, before sending.

Do not freeze!

Important : if you take several colonies, **use a different clamp for each hive to avoid cross-contamination.**

Note : *The return of the clamps is optional. Nevertheless, if they are returned, they can be cleaned and reused, thus limiting the use of single-use plastic.*

