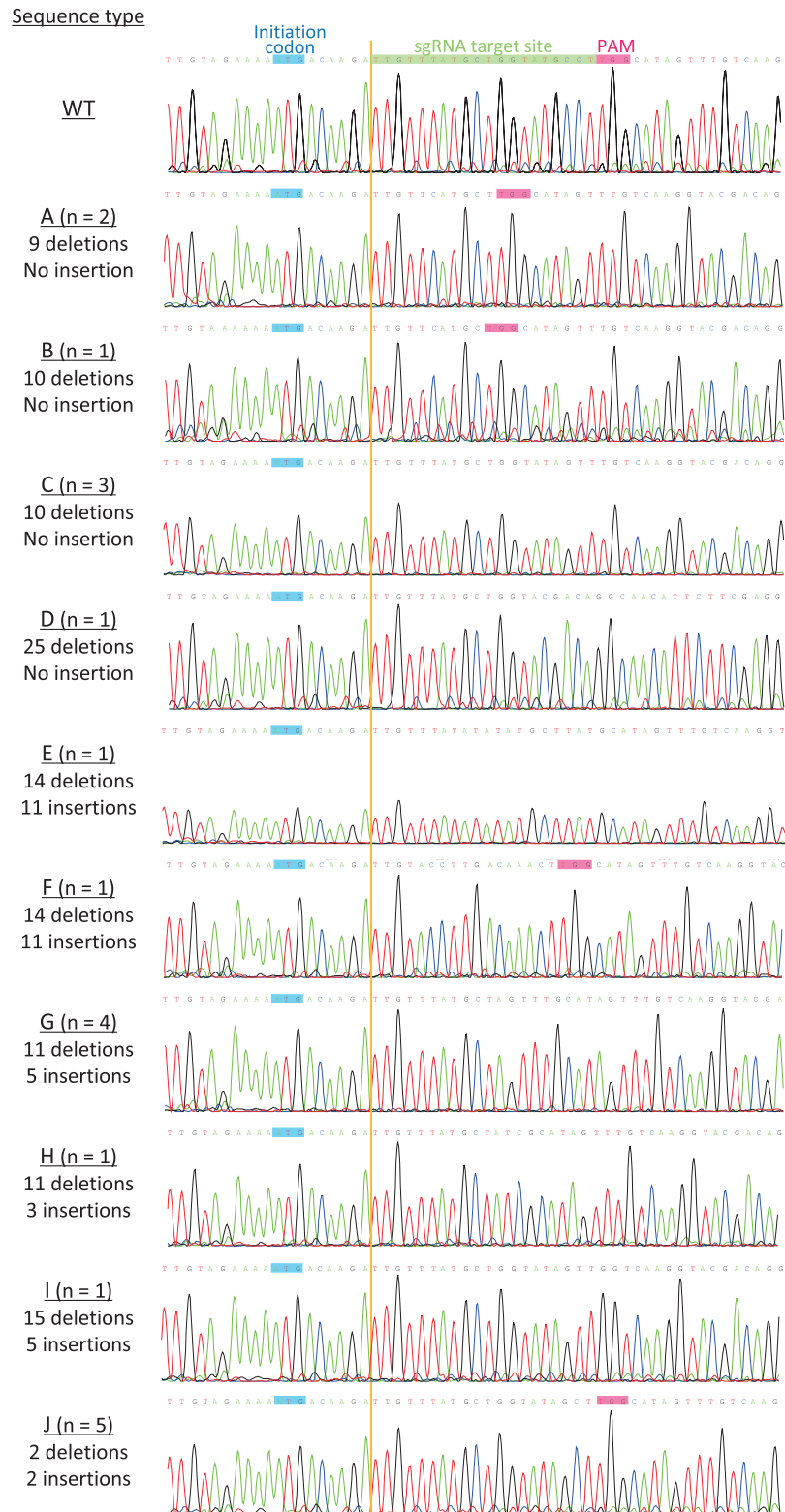


Supplementary Fig. S1. Overview of experimental procedures for creating genome-edited drones. The overview of experimental procedures to create genome-edited drones is shown along the time (day) axis. Experimental procedures for injection, queen rearing, and drone production are indicated in white boxes with yellow, red, and blue frames, respectively. The gray boxes indicate the day of the manipulations shown in the boxes to which the gray boxes are attached.



Supplementary Fig. S2. Results of genotyping analysis of wild-type and genome-edited drones. The results of sequence analysis around the sgRNA target site of wild-type and genome-edited drones are shown as waveform data. A representative result is shown if many drones exhibited the same genotype. Letters in blue, green, and magenta boxes indicate the initiation codon, sgRNA target site, and PAM, respectively. Yellow solid line indicates the 5' end of the sgRNA target site of wild-type drone (top panel), and sequence data of other drones (lower panels) are aligned according to the solid line. Note that, although automatic sequencing data indicate that the sixth nucleotide from the 5' end for sequence type B in this figure is "A", it is actually judged as "G" based on the real waveform data, and thus is shown as "G" for sequence type B in Fig. 3.

Supplementary Table S1. Overview of mosaic queen (No. 2) lifetime. The overview of events and manipulations conducted during the lifetime of a mosaic queen (No. 2) is shown. The mosaic queen (No. 2) hatched on 8 October 2015 from an egg laid on 5 October, began laying unfertilized (drone) eggs around the beginning of December 2015, after transient anesthesia with CO₂, and was alive until 29 December, 2015, until it was discarded from the hive. All offspring of the mosaic queen (No. 2) were sampled from the colony into which the queen had been introduced on 7 January, 2016. The colony in which the queen (No. 2) was maintained was exchanged for a newly established queenless colony with enough workers on 20 November, because the queen (No. 2) had not laid any eggs in the previous colony that had been set in the flight room for over a month without a sufficient supply of workers, and therefore activities of this colony were too low. A brood (larvae and/or pupae) comb was added to colony in the flight room to maintain activities of the colony on 15 and 25 December, 2015 and 5 January, 2016.

| | | | |
|--------|---|--------|--|
| 2015 | | ↓ | |
| Oct 5 | •Egg oviposition •Injection of sgRNA and Cas9 mRNA into embryo | Dec 7 | Drone larvae were observed in No. 2 queenright colony. |
| ↓ | | Dec 8 | Sampling of drone larvae from No. 2 queenright colony |
| Oct 8 | Larval hatching | ↓ | |
| ↓ | | Dec 15 | A brood (larvae & pupae) comb was added to No. 2 queenright colony. |
| Oct 12 | Larva was introduced into queenless colony set in the flight room (FR). | ↓ | |
| ↓ | | Dec 25 | A brood (larvae & pupae) comb was added to No. 2 queenright colony. |
| Oct 20 | Queen cell was retrieved from colony set in FR. | ↓ | |
| ↓ | | Dec 27 | An adult drone was discarded outside of colony. |
| Oct 23 | Queen emergence | Dec 28 | An adult drone was discarded outside of colony. •Three adult drones were discarded outside of colony. |
| ↓ | | Dec 29 | •Queen was discarded outside of colony. •Queen was dissected and stored at -80°C. |
| Oct 28 | Queen was anesthetised with CO ₂ . | Dec 30 | Three adult drones were discarded outside of colony. |
| Oct 29 | Queen was anesthetised with CO ₂ . | Dec 31 | Two adult drones were discarded outside of colony. |
| ↓ | | ↓ | |
| Nov 6 | •Queen was confined in a small cage with some workers. •The small cage was put inside of queenless colony set in FR. | 2016 | |
| ↓ | | Jan 3 | Four adult drones were discarded outside of colony. |
| Nov 9 | Queen was released from the small cage. (No eggs & larvae were observed.) | ↓ | |
| ↓ | | Jan 5 | A pupal comb was added to the colony (queenless). |
| Nov 20 | •Queen was confined in a small cage with some workers. •The small cage was put inside of another queenless colony set in FR. | ↓ | |
| ↓ | | Jan 7 | •Four adult drones were discarded outside of colony. •Sampling of all drones larvae and pupae from the colony |
| Nov 27 | Queen was introduced into colony after transient CO ₂ treatment to all workers. | | |