
# Generation of transgenic lines using piggyBac transposons in the clonal raider ant

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Ants exhibit a fascinating range of developmental and behavioral traits, including the generation of distinct morphological castes from a single genome, complex chemical communication, and the flexible behavioral division of labor that underlies colony life. In recent years, new technologies such as whole genome sequencing, RNASeq, and CRISPR/Cas9 have advanced our ability to study these interesting phenomena on a molecular level. However, a complete genetic toolkit also requires the ability to create transgenic organisms by inserting custom-designed DNA sequences into the genome. To address this, we established an efficient piggyBac transposon-based gene integration protocol for the clonal raider ant (*Ooceraea biroi*) and used this protocol to generate the first transgenic ant lines. This protocol allows us to rapidly generate transgenic lines in clonal raider ants, which can then be propagated directly via asexual reproduction. We generated multiple independent lines that broadly express the fluorescent protein DsRed under the control of the baculovirus promoter *ie1*. Fluorescence from this transgene is visible in live animals from the larval stage through adulthood under epifluorescence. These marker lines allow parentage and reproductive output to be established visually in an otherwise clonal background, and can be used to address questions about reproductive division of labor in the clonal raider ant. Moreover, this protocol can now be applied for generating additional transgenic tools in ants, such as fluorescent reporters under tissue-specific promoters or genetically-encoded biosensors and modulators. Implementing these tools will open new opportunities for investigating the molecular mechanisms of physiology and behavior in clonal raider ants.