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Iterative ATAC-seq to home in on specific neuron subsets.

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Small neuronal subpopulations, and even individual neurons, can have profound effects on many different behaviors. Thus, numerous studies focus on identifying which specific subpopulations underlie various behavioral outputs and on understanding how these neurons function. Previous approaches in *Drosophila* were based on large collections of (split-)Gal4 lines, which are often generated in a random shotgun manner, followed by extensive staining and screening. Here, we set out to generate Gal4 lines to isolate and manipulate the ~300 dopaminergic neurons of the fly brain. To do so, we hypothesize that surveying the open chromatin landscape by ATAC-seq (assay for transposon-accessible chromatin) will yield genomic enhancer elements that drive expression in subsets of neurons assayed. For proof of principle, we compared open chromatin in neurons to whole body. We cloned peaks more open in neurons, or in whole body and generated transgenic animals. As hypothesized, DNA segments derived from open neuronal chromatin drive expression in various neuronal sub-sets, while 'body segments' show little neuronal expression. We then picked a neuronally-expressed transgenic line and determine open chromatin in those neurons to isolate enhancer DNA elements to further subdivide this neuronal pattern. To apply this approach to dopamine neurons, we have determined open chromatin in these neurons by bulk, and by single-cell ATAC-seq. This iterative ATAC-seq approach can be applied to any cell type and yields enhancer elements open in the sequenced input neurons, thus leading to an informed approach in tool generation for the isolation, characterization and manipulation of specific neuronal sub-types.