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Probing the connectivity of neural circuits at single-neuron resolution using high-throughput DNA sequencing

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There is growing excitement in determining the complete connectivity diagram of the brain—the "connectome". So far, the complete connectome has been established for only one organism, *C. elegans*, with 302 neurons connected by about 7000 synapses—and even this was a heroic task, requiring over 50 person-years of labor. Like all current approaches, this reconstruction was based on microscopy. Unfortunately, microscopy is poorly suited to the study of neural connectivity because brains are macroscopic structures, whereas synapses are microscopic. Nevertheless, there are several large-scale projects underway to scale up high-throughput microscopic approaches to the connectome.

Here we present a completely novel method for determining the brain's wiring diagram based on high-throughput DNA sequencing technology, which has not previously been applied in the context of neural connectivity. The appeal of using sequencing is that it is getting faster and cheaper exponentially: it will soon be routine to sequence an entire human genome (~3B nucleotides) within one day for $1000.

Our approach has three main components. *First*, we express a unique sequence of nucleotides—a DNA "barcode"—in individual neurons. A barcode consisting of a random string of even 30 nucleotides can uniquely label $4^{30} = 10^{18}$ neurons, far more than the number of neurons in a mouse ($<10^9$ neurons) brain. *Second*, we use a specially engineered transsynaptic virus to transport “host” barcodes from one neuron to synaptically coupled partners; after transsynaptic spread, each neuron contains copies of "invader" barcodes from other synaptically coupled neurons, as well its own "host" barcode. *Third*, we join pairs of host and invader barcodes into single pieces of DNA suitable for high-throughput sequencing.

Modern sequencing technology could in principle yield the connectivity diagram of the entire mouse brain. Similar approaches can be applied to *Drosophila* and *C. elegans*.