

# Multiscale Genome Engineering: Genome-Wide Screens and Targeted Approaches

Neville E. Sanjana

**Abstract** New advances in genome engineering technologies, such as efficient programmable CRISPR nucleases, have enabled new advances in forward and reverse genetic studies. Here, I discuss recent work from our group combining top-down approaches like genome-wide loss-of-function screens and bottom-up approaches like disease variant modeling in human stem cells and stem cell-derived cortical neurons.

## Introduction

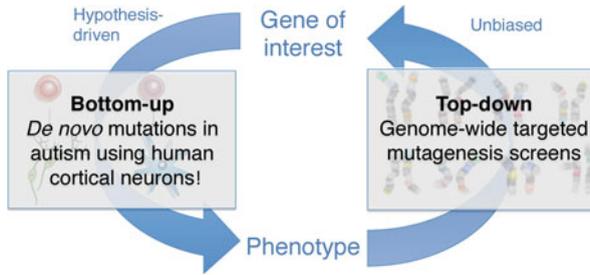
Patient-sequencing studies have yielded large lists of disease-associated gene variants but it has been difficult to establish a causal role based solely on genetics. New methods are needed for rapidly understanding the effects of these variants and ascertaining whether the variants directly influence disease-related phenotypes. At the IPSEN meeting “Genome Editing in Neurosciences,” I presented two approaches (top-down and bottom-up) for harnessing new genome engineering techniques to decipher the roles of genetic variants in human health and disease.

Top-down approaches utilize large-scale pooled libraries of genome-engineering reagents to start with a large, minimally biased hypothesis space and identify relevant variants via a single phenotypic selection (Fig. 1, right). In contrast, bottom-up approaches start with a handful of genetic variants nominated by strong genetic data, e.g., genome-wide association studies, case-control, family linkage studies, etc., and they examine a wide range of phenotypes (Fig. 1, left).

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N.E. Sanjana (✉)  
New York Genome Center, New York, NY, USA

Department of Biology, New York University, New York, NY, USA  
e-mail: [neville@sanjanalab.org](mailto:neville@sanjanalab.org)



**Fig. 1** Schematic diagram of the dynamic interplay between top-down and bottom-up genetic approaches. Top-down approaches (*right*) identify new gene candidates that can shape/reduce the space of hypotheses for more detailed bottom-up (*left*) cellular models and phenotyping. Top-down approaches are unbiased or minimally biased to cast a wide net of possible genetic hypotheses and use phenotypic selection to identify putative disease-associated gene variants. Bottom-up approaches focus on a smaller set of variants but usually provide a more detailed phenotypic analysis of different molecular/cellular/circuit aspects of each genetic variant. Candidate variants for the bottom-up approach can be derived from either genetic evidence (e.g., patient sequencing studies) or from top-down approaches like genome-wide CRISPR screens

## Top-Down Approaches Using Genome-Wide CRISPR Screens

The microbial CRISPR-Cas9 nuclease from *S. pyogenes* can be guided to specific DNA sequences using a 20 bp guide sequence. Given the short length of the guide sequence, we have been able to use oligonucleotide array synthesis techniques to create libraries of thousands of guide sequences in a pooled format. By designing pooled libraries to target all genes in a specific genome, we have created a new tool for functional genomic screens (Sanjana 2016). Genome-scale CRISPR knock-out (GeCKO) screens use the consistent phenotypic enrichment of *multiple* CRISPR reagents targeting the same gene to lend evidence to the gene's role in a particular disease. Using a GeCKO library targeting ~18,000 genes with 64,751 guide sequences, we have found loss-of-function mutations that confer resistance to the BRAF inhibitor vemurafenib in human melanoma cells (Shalem et al. 2014). Using a second-generation GeCKO library in a mouse model, we performed an *in vivo* screen to identify driver mutations that trigger metastasis to the lung (Sanjana et al. 2014; Chen et al. 2015). Recently, we have expanded the scope of CRISPR pooled screens to also include noncoding regions of the genome (Sanjana et al. 2016; Wright and Sanjana 2016).

## Bottom-Up Approaches Using Exome Sequencing in Autism

Whole exome and whole genome sequencing have ushered in a revolution in identifying rare, disease-associated variants. Several whole exome sequencing studies have pinpointed rare *de novo* variants associated with autism spectrum disorder by examining exomes from autistic individuals and comparing them to parental

exomes (Iossifov et al. 2012; Neale et al. 2012; O’Roak et al. 2012; Sanders et al. 2012). The next logical step is to create relevant cellular models to better understand the mechanisms through which these variants work and to serve as a platform for drug screens and therapeutic testing. There are two major roadblocks for building these kinds of cellular models. The first one concerns gene editing and, over the past 3 years, has become largely historical: Until recently, genome engineering in human stem cells and neurons has been challenging but transfection of CRISPR plasmids or ribonucleoproteins provides an easy, efficient technique for engineering human cells (Peters et al. 2008; Swiech et al. 2015). The second major hurdle has been neural differentiation. Common protocols to differentiate neurons from human stem cells, such as dual SMAD inhibition or embryoid body differentiation, require months to create mature neurons (Zhang et al. 2001; Chambers et al. 2009). Recently, we and others have demonstrated that viral overexpression of Neurogenin 1 or Neurogenin 2 can rapidly drive stem cells into a homogeneous culture of mature cortical neurons (Zhang et al. 2013; Busskamp et al. 2014). These neurons display robust electrophysiological activity within just 2–3 weeks after the start of differentiation, making them ideally suited for synaptic assays, calcium imaging and neurophysiology. We are now moving forward with phenotypic analyses of de novo mutations in autism using the combined CRISPR-Neurogenin platform for rapid mutagenesis and human neuron profiling.

Taken together, these new technologies in genome engineering—enabled in large part by CRISPR nucleases and related transformative methods—have improved our ability to perform forward and reverse genetic assays in relevant model systems. A major challenge for neuroscience is finding clear phenotypes that accurately reflect complex diseases, such as schizophrenia or autism. Despite these challenges, a combination of top-down and bottom-up approaches will pave the way for a clearer understanding of the human brain in healthy and disease states.

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