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Expanding the *C. elegans* toolbox into a toolshed

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Since Sydney Brenner's proposal to exploit *Caenorhabditis elegans* to unravel the genetic basis of metazoan development and the nervous system [1], technological developments have turned this model organism into one of the premier resources for studying multiple facets of cell biology. Several excellent compendia outline some of these methodological advancements [2–8]. In addition, the WormMethods section of WormBook (http://www.wormbook.org/toc_wormmethods.html) provides a rich compilation of *C. elegans* methodology. The goal of this edition of Methods is to highlight recent innovations that have expanded the repertoire of techniques available to *C. elegans* investigators, and to update methods not covered extensively by these other references.

The study of gene function by transgenesis is one of the most powerful technologies available to *C. elegans* investigators. Recently, the application of genome editing has enhanced significantly the scope and precision of these methods. The first 8 articles of this edition of Methods provide added insight into these technologies. Waaijers and Boxem, discuss practical considerations in the use of the CRISPR/Cas9 system to engineer the genome. They describe non-homologous end-joining or homologous recombination methods in combination with modified DNA templates [9]. As an alternative approach, Yi et al. review the use of transcription activator-like effector nucleases (TALENs) for genome editing, especially in somatic tissues [10]. Another, powerful strategy for genome manipulation employs the *Mos1* transposon to introduce single copy transgene insertions, gene deletions or gene conversions. These techniques were recently reviewed in Methods [11] and in other journals, and will not be described here [12–15]. Kage-Nakadai et al. provide a simplified method for single or low copy transgene integration from extrachromosomal arrays by treatment with trimethylpsoralen and less DNA damaging, long-wave ultraviolet illumination [16]. Antibiotic selection for transgene maintenance or

integration, a mainstay of mammalian transgenic systems, has finally made its way into *C. elegans*. Two groups report on the use of single or dual drug selection on solid or liquid media and after microinjection or microparticle bombardment [17,18]. Of major interest in *C. elegans* transgenesis is better spatial and temporal control of transgene expression. Hubbard describes intersectional expression strategies using the FLP or Cre recombinases to direct cell specific re-arrangement of transgenes flanked by FRT and *loxP* recombination sites, respectively [19]. Okazaki et al. detail an optogenetic technique that utilizes transgenes harboring light-driven proton pumps to selectively silence subsets of cells [20]. Adaptation of a microscope to deliver a laser pulse at the appropriate wavelength could be used to activate optogenetic transgenes or ones driven by heat-shock promoters in order to direct expression in single cells [21].

Forward genetic screens in *C. elegans* continue to provide important unbiased insights into biological pathways. While the screens can be tedious and labor-intensive, the biggest bottleneck has been mapping and identifying the mutants. Whole-genome DNA sequencing has helped streamline and accelerate this process. Hu outlines two different crossing strategies for maximizing the chances of identifying the correct mutation without SNP mapping [22].

C. elegans was one of the first organisms used to study the genetic basis of aging, especially in response to different types of stressors. One article examines methods for performing genotoxic stress assays using different mutagens [23], while two manuscripts enumerate techniques used to induce and assay proteotoxicity: heat shock [24] and transgenes encoding aggregation-prone proteins [25]. The effect of these and others stressors can have dramatic effects on the lifespan or healthspan of animals. Two papers describe the multiple methods for determining these parameters including assays amenable to automation [26,27].

Translational medicine attempts to solve clinical problems by bridging the gap between basic bench research and the bedside. Traditionally, translational medicine has relied on mammalian models of human disease. The ability to study normal and human disease-related physiological processes in *C. elegans* has transformed this organism into a powerful translational tool [28]. For example, elevations of blood pCO₂ occur in a number of respiratory illnesses and this increase can have profound effects on cellular metabolism. Noam et al. outline several techniques to study the effects of hypercapnea in *C. elegans* and show that many of these responses are conserved in higher eukaryotes [29]. Obesity is a major public health concern. Wahlby et al. expand upon their earlier studies and describe Oil Red O staining and automated imaging methods to study fat metabolism in the nematode [30]. *C. elegans* has been used a model to study muscle physiology and movement extensively. In contrast, *C. elegans* can also be used to study quiescence, and Nagy et al. outline mounting and imaging technologies that permit quantitative analysis of this important behavioral phenotype [31]. Derangements of intracellular pH, redox status and levels of ATP, NADPH and calcium are important indicators of cellular injury and stress. Wang et al. show how these types of parameters can be studied in *C. elegans* in real-time by automated imaging using fluorescent ratiometric reporters [32]. Similarly, endocytosis plays a crucial role in diverse cellular processes including nutrient intake and cell signaling. Wang

and Audhya describe transgenic approaches using fluorescently-tagged proteins and different imaging techniques to follow several steps in endomembrane trafficking [33].

High-throughput and high-content drug screening, once the domain of protein biochemists and cell biologists, are now being adapted for use in *C. elegans* for modeling different aspects of human disease or mammalian physiology [34]. Benson et al. details assay development for a semi-automated high-content drug screen [35]. While these phenotype-based screens are extremely powerful in identifying hits for lead development, they do not yield the actual drug targets. Miedel et al. provide a combined biochemical purification and mass spectroscopy approach to this problem by constructing a triple affinity tag that can be adapted for detection of drug-target or protein–protein interactions [36]. Live animal imaging also has become an important part of the drug discovery process in *C. elegans*, especially during the validation phase. Luke et al. describe a simple non-microfluidic means of immobilizing animals for relatively long periods of time that allow for widefield or confocal imaging following drug treatments [37].

Collectively, the authors of these manuscripts have shed more light on the vast array of experimental techniques now available to conduct studies in *C. elegans*. Combined with forward and reverse genetic approaches, *C. elegans* will continue to be one of the premier tools for understanding basic cellular biology under physiological and pathological conditions.

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