Rapid Dispatches Highlights

Red/ET Meets Poly Q

At the genetic level, Huntington's disease (HD) is characterized by an expansion of glutamine residues (poly Q) at the N-terminus of the huntingtin gene. The length of the poly Q tract is associated with disease phenotype: longer poly Q regions result in earlier disease onset. There exists a great interest in studying HD using animal models. However, generating knock-in mice with expanded poly Q tracts within the huntingtin gene can be a cloning challenge owing to its repetitive nature. However, in a new article at *Rapid Dispatches*, Stephanie Hager and colleagues from Gene Bridges in Heidelberg, Germany, and Mount Sinai Medical Center in New York, NY, detail a novel Red/ET strategy that facilitates the cloning of difficult fragments, such the long poly Q repeats associated with HD. Hager et al.'s approach relies on the creation of chimeric primers harboring a PCR binding site along with a bifunctional homology arm. These fragments can be used as primers to amplify antibiotic resistance cassettes which can then be inserted on either side of the huntingtin cDNA. From here, recombination is used to engineer BAC transgenes with varying lengths of the poly Q tract. The authors describe the generation of four such BAC transgenes, and go on to generate four new transgenic mouse models of HD. The work described in this article will not only go far in enhancing our understanding of Huntington's disease through these new animal models, but the recombination approach developed to generate these transgenic animals should also have applications in other areas of biological research as well. See **"Red/ET recombination with chimaeric oligonucleotides allows rapid generation of BAC transgenes harbouring full-length or truncated huntingtin cDNA."**

Category: Molecular Biology

Tunable RNAi

Knocking-down gene function has proven an important molecular biology technique for exploring biological processes. One very popular approach to gene knockdown is through the use of short interfering RNAs (siRNAs). These experiments can even be followed by phenotype rescue to confirm the knockdown. Here, the target protein is transiently re-expressed to rescue to siRNA phenotype. A challenge with this approach is controlling the level of transient protein expression—too much can lead

Quest for Virulence

Understanding bacterial pathogenesis is important to deciphering the many ways in which bacteria establish an infection in a host. Traditionally, bacterial mutants harboring targeted gene disruptions are generated and then tested in animal models to assess effects on virulence in comparison to wild-type bacteria. Although this approach is effective in identifying genes and proteins crucial in pathogenesis, it is also time-consuming and requires large numbers of animals. In a Report appearing in *Rapid Dispatches*,

Didier Cabanes and colleagues from Universidade do Porto in Porto, Portugal, describe a new strategy for screening targeted bacterial mutants which is fast and requires significantly fewer animals than traditional approaches. Taking advantage of the speed and power of PCR amplification, Cabanes et al. employ a PCR-based screen wherein careful design of amplification primers enables bacterial discrimination from a pool of mixed bacterial mutants in infected mouse organs. First, combinations of bacterial mutants are pooled together, and then, using mutant specific PCR primers, the input pool is amplified and analyzed. This mutant pool is then orally inoculated into mice and, after 72 hours, the liver and spleen are recovered and bacterial cells plated. Total colonies are then resuspended in sterile PBS and PCR is performed to assess the bacterial mutant output pool. Surprisingly, the intensity of the PCR bands correlated very well with the quantity of the corresponding bacterial strain in each pool, making the approach relatively quantitative. In the end, this new method provides a rapid and efficient new way to assess multiple bacterial virulence factors simultaneously. The authors also suggest that the approach can be adapted to different bacterial systems, providing a uniform methodology for studying host-pathogen infections. See **"PCR-based screening of targeted mutants for the fast and simultaneous identification of bacterial virulence factors."**

Category: Molecular Biology/Microbiology/PCR Methods

Strategy for PCR-based screening of targeted mutants.

to inhibition of the pathway of interest while too little will not provide rescue. In a Benchmark published at *Rapid Dispatches*, Wesley Sundquist and colleagues at the University of Utah detail their construction of a series of seven mammalian expression vectors designed specifically for the fine control of protein expression levels. Sundquist et al. first generated a series of nested deletions within the CMV promoter transcription factor binding site and then combined these within an siRNA resistant expression construct. The authors tested their new vectors by rescuing the HIV-1 budding phenotype in cells lacking endogenous CHMP2 protein (ESCRT-III protein family). These seven new expression vectors should find much utility with researchers looking to "tune" the expression of their

protein of interest. See **"Attenuated Protein Expression Vectors for Use in siRNA Rescue Experiments"**.

Category: Cell Biology/Molecular Biology/Virology

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