TECHNOLOGY

No-bake recipe for DNA

Modern genetics relies so heavily on PCR that it is hard to imagine laboratory life without it. However, although PCR was indisputably one of the biggest breakthroughs in genetics — transforming fields from forensic science to disease diagnosis — the thermal cycling at the heart of this technique makes it hungry for energy, time and special equipment. By using a bacterial helicase instead of heat to denature the template, Huimin Kong and colleagues have now overcome the limitations of PCR by devising a means to amplify DNA at a single temperature. This opens the door to the development of quick, hand-held diagnostic devices that could move DNA analysis from the bench into the field.

The new amplification technique, called helicase-dependent amplification (HDA), mimics a process that occurs in nature. A helicase — in this case the *Escherichia coli* UvrD helicase — is first used to separate the dsDNA template molecules. The separated strands are then coated with ssDNA binding proteins to keep them apart, allowing primers to anneal and be extended by a polymerase. The reaction occurs at 37°C, from start to finish, and can achieve over a million-fold amplification of genomic DNA. It is also sensitive enough to pick up DNA from intact cells and to detect pathogen DNA in blood.

HDA is not the first isothermal reaction to be proposed, but the alternatives involve more complex reactions and rely on an initial denaturing step that requires heat. Essential tweaks are now being carried out to improve the efficiency of HDA. By experimenting with the reaction components — nature has hundreds of helicases to choose from — and their concentrations, Kong and colleagues hope to bring reaction times down to 15 minutes. Machine-free DNA amplification 'while-you-wait' might be just around the corner.

Tanita Casci

References and links

ORIGINAL RESEARCH PAPER Vincent, M., Xu, Y. & Kong, H. Helicase-dependent isothermal DNA amplification. *EMBO Reports* 9 July 2004 (doi:10.1038/sj.embor.7400200)

WEB SITE

New England Biolabs: http://www.neb.com

GENOME EVOLUTION

Mutations: more common than you thought

Mutation rate, whether per locus or genome-wide, features as a variable in many calculations that underlie evolutionary genetic hypotheses. But a true experimental estimate of mutation rate is difficult to obtain. To this end, several groups have used 'mutation-accumulation' lines in both *Drosophila melangaster* and *Caenorhabditis elegans*: in an attempt to minimize selection pressure, genetically identical flies or worms are maintained for many generations in a benign environment. Such assays are long and tedious: for one collection, scientists even separated each worm in each generation from everyone else to avoid any selection pressure induced by the stressful dating scene, and instead, self-fertilize.

Previous studies have used fitness-based assays to assess the number of mutations that developed in each line, yielding an indirect estimate of mutation rate. For a more direct estimate, Denver and colleagues have now revisited a collection of these special worm lines and sequenced more than 4 Mb of loci scattered around the genome. In a recent paper in *Nature*, they report the total haploid genomic mutation rate to be approximately 2.1 mutations per genome per generation — an estimate that is an order of magnitude higher than previous best guesses, and 2 orders higher than the indirect estimate from the same collection (although the previous estimates referred to deleterious mutation rate, whereas these authors estimate total mutation rate). Not only that, but the more frequently observed mutations were insertions, in contrast to reports based on pseudogenes that indicated that most naturally occurring mutations in worms are deletions.

So, with one study, Denver and colleagues prompt the entire field to rethink the process of mutation over time and our measurement of it. It does not take long for this to generate controversy: in a thought-provoking News and Views piece, Rosenberg and Hastings speculate on the mechanisms at work in the Denver study. Either previous estimates were wrong because they only detected mutations that produce phenotypes, as Denver *et al.* would suggest, or the new study uses methods that push the worms to develop more mutations even in the absence of deleterious selection, maybe even through triggering stress-response pathways. Future studies might have to bring the worms out of their posh retirement to settle the question.

Chris Gunter, Senior Editor, Nature

References and links

ORIGINAL RESEARCH PAPER Denver, D. R. *et al.* High mutation rate and predominance of insertions in the *Caenorhabditis elegans* nuclear genome. *Nature* **430**, 679–682 (2004)

FURTHER READING Rosenberg, S. M. & Hastings, P. J. Worming into genetic instability. *Nature* **430**, 625–626 (2004)

WEB SITE

NemATOL: http://nematol.unh.edu/index.php