news

Singling-out point mutations

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A highly sensitive molecular technique for detecting and quantifying single-base mutations has been developed by researchers at Johns Hopkins University School of Medicine (http://www.hopkinsmedicine.org). The LigAmp technique is still being refined but it has the potential to become an important tool for diagnosing and managing diseases involving point mutations.

There are already several methods for detecting single-base mutations but, according to study leader James Eshelman, 'the strengths of LigAmp are its sensitivity (~1 mutant molecule in 10,000 wild-type molecules), its quantitative nature, and its ability to detect multiple mutations simultaneously'.

Ligate and amplify

The LigAmp approach (so called because it involves ligation – joining – and amplification steps) essentially scales up single-base differences into something more detectable [1]. It involves two oligonucleotides that match the target gene: one is specific at its 3′ end for the mutant base; the other matches the gene starting just 3′ to the mutant base. Both oligonucleotides bind to both mutant and wild-type versions of the gene, but only the mutant allele binds the upstream oligonucleotide tightly enough for the two oligonucleotides to ligate. The resulting strand can then be amplified and detected using quantitative PCR. Detection depends on a probe sequence built into one of the oligonucleotides, which is amplified to detectable levels only in if ligation has occurred. Ligation and amplification have already been combined in a similar way in the multiplex ligatable probe amplification (MLPA) assay but, says Eshlemen, LigAmp is different as it focuses on point mutations rather than large deletions or duplications.

Eshelman's team tested their method on two important mutations: one in *KRAS2*, a gene implicated in most pancreatic cancers, and the K103N mutation of HIV-1, which confers drug resistance. They were able to detect and quantify both mutations in samples containing mutant and wild-type DNA in a ratio of 1:10,000. They have also shown that the technique is effective for pancreatic juice and plasma taken from patients, highlighting its diagnostic potential. And there is scope for multiplexing: by building different probe sequences into different versions of the upstream oligonucleotides, Eshleman and colleagues detected and quantified two different mutated genes at once, at up to 1:1000 dilution.

> **'LigAmp is different as it focuses on point mutations rather than large deletions or duplications'**

Practicalities

As with any new technique, things are not yet perfect. Mike Makrigiorgos of the Dana Farber–Brigham and Women's Cancer Center (http://www.brighamandwomens.org/bwhcancer/) expressed concerns that LigAmp could prove costly. This was echoed by Vanessa Hayes of the Garvan Institute of Medical Research (http://www.garvan.org.au/). And, although she sees LigAmp as having potential, she thinks it is 'limited by the fact that it cannot detect unknown mutations'.

Furthermore, as Mike Makrigiorgos points out, LigAmp might not work as well for other known single-base mutations as it does for K103N and the *KRAS2* mutation. 'The usefulness

of this particular method will depend on the ability of ligase to distinguish correctly between 'match' and 'mismatch', which is significantly dependent on sequence context'. Eshleman agreed that detection of some mutations could prove more difficult but, considering his team's success so far, he expects that the sensitivity of LigAmp will be 'as high for the vast majority of mutations'. Even with K103N and the *KRAS2* mutation, LigAmp is not foolproof – some nonspecific amplification and false-positive results were reported.

But both Eshleman and Hayes stress that this is normal for such assays, and Eshleman feels that the accuracy of LigAmp results is already 'sufficient for most applications'. He and his team are currently working on refining their technique, for example by testing a panel of different ligases, but he believes that LigAmp is already 'relatively close' to being used as a diagnostic tool.

Reference

1 Shi, C. *et al.* (2004) LigAmp for sensitive detection of single-nucleotide differences. *Nat. Methods* 1 DOI:10.1038/NMETH713 (Epub. ahead of print; http://www.nature.com/nmeth)

New hope for mechanism-based treatment of Parkinson's disease

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US researchers have discovered that rifampicin, an antibiotic used to treat tuberculosis and leprosy, inhibits the formation of α-synuclein fibrils and disaggregates fibrils that have already formed. Because the aggregation of α-synuclein in dopaminergic neurons is a critical step in the pathogenesis of Parkinson's disease (PD), rifampicin or a related compound could provide a new approach to the treatment of PD [1].

α-synuclein and Parkinson's disease

PD is a common neurodegenerative disorder caused by the progressive loss of dopaminergic neurons in the substantia nigra. Current treatments are symptomatic – patients are usually given levodopa to improve motor symptoms. A better approach, notes Anthony Fink, Professor of Chemistry and Biochemistry at the University of California, Santa Cruz, 'would be to get at the root of the disease and prevent any further progression.' And if a

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