TOOLBOX

Technique probes gene expression in intact tissue

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Risk site: Only some cells in breast cancer tissue express high levels of HER2 (red), which can cause the disease, compared with a control protein (blue).

A new method allows researchers to highlight mutations in individual cells without first isolating the cells from the surrounding tissue, according to a study in the August *Nature Methods*[1].

Organs, including the brain, are made up of diverse cell types, each expressing its own set of proteins. To examine the patterns of expression, researchers need to first isolate each cell type. This step prevents researchers from seeing how expression in these individual cell types fits into the organ as a whole, however.

The new method allows researchers to sequence messenger RNA (mRNA), the genetic message that produces proteins, in individual cells within a slice of tissue. They can use this method to look for patterns of gene expression, or to identify mutations in single DNA base pairs.

Researchers first prepare tissue slices so that the cells are permeable. They then add the components needed to derive DNA from an mRNA of interest. Next they add a probe that circularizes when it binds to a target sequence on the newly created DNA. The same enzyme that duplicates DNA during cell division can copy this circle of DNA multiple times, amplifying it within the cell.

The researchers then add a library of DNA probes that stick to a region of interest, such as the site of a potential mutation, or a specific region on a protein. The library contains four kinds of probes, each attached to a different fluorescent molecule and varying by one DNA nucleotide. For example, if a site in the region of interest contains an adenine, a red probe will stick to it, but if it contains a thymine instead, a green probe will stick.

To demonstrate the method's potential, the researchers used it to identify cells that express higher-than-normal levels of HER2 — which can lead to breast cancer — in a slice of cancerous breast tissue. Within the slice, 27 cells, all of them inside the tumor, express excess HER2, compared with 298 cells that express a control protein.

The method can also be used on brain tissue — for example, to look at autism-specific alterations in certain brain regions. It can also be used to identify **mosaic mutations**, which arise after fertilization and so affect only a subset of cells in the body.

References:

1: Ke R. *et al. Nat. Methods* **10**, 857-860 (2013) **PubMed**