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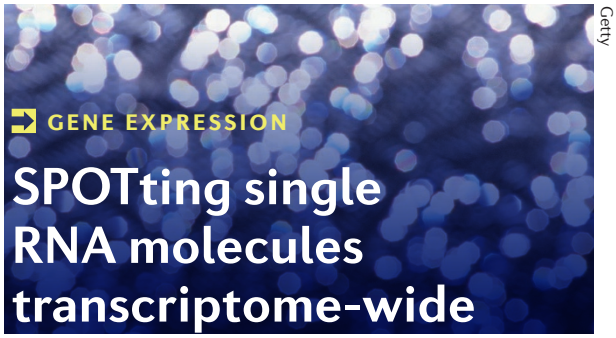

Getty

> an accurate, cost-effective alternative to sequencing techniques

The standard method for profiling the transcriptome of a biological sample is by RNA sequencing (RNA-seq), whereas fluorescence *in situ* hybridization (FISH) has traditionally been used to image and quantify individual RNA molecules in cells or tissues on a coverslip. A new technique developed by Eng *et al.*, called RNA sequential probing of targets (SPOTs), now enables transcriptome-wide mRNA quantification using FISH technology.

The new method is based on sequential FISH, which can image multiple transcripts simultaneously *in situ* thanks to sequential barcoding. By contrast, RNA SPOTs is an *in vitro* method that starts by capturing mRNA extracted from a biological sample (here mouse fibroblasts and embryonic stem cells) on poly(dT)-coated coverslips. These are hybridized to pools of 28–32 different primary probes per mRNA (a total of more than 300,000 probes to cover all 10,212 mouse mRNAs), which are then detected in five sequential rounds of barcoding and imaging.

Key to the method is the probe design. The centre of each 149-nucleotide (nt) long primary probe — a 25-nt sequence that is complementary to the target mRNA — is flanked by four 20-nt overhang sites (two on each side) each of which has one of 12 possible sequences that in combination form the unique barcode for each target mRNA. In each round of barcoding, overhang sites are detected using readout oligos coupled with one of three fluorescent dyes (Cy3b, Alexa594 or Alexa647) in four iterations of hybridization, imaging and extinction (cleavage of the dye), which results in a 12 'pseudocolour' scheme (3*4) after which all readout probes are completely removed. Five sequential rounds of barcoding are performed using 60 different readout oligos (that is, 12 per barcoding round): four rounds to ensure coverage of the transcriptome plus an additional round to correct for potential errors. The pseudocolour images from five barcoding rounds are ultimately decoded to reveal the identity of each individual mRNA. Because each spot corresponds to one molecule, relative quantification of mRNAs is possible.

Data obtained using RNA SPOTs showed high concordance with RNA-seq data transcriptome-wide, as well as with single-molecule FISH results for individual mRNAs. An obvious advantage of RNA SPOTs is the potential to target only a certain set of genes of interest in favour of increasing the number of cells or samples analysed, thus making it an accurate, cost-effective alternative to sequencing techniques.

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Nature Communications

ORIGINAL ARTICLE Eng, C. -H. L. *et al.* Profiling the transcriptome with RNA SPOTs. *Nat. Methods* http://dx.doi.org/10.1038/nmeth.4500 (2017)