BIOSEARCH TECHNOLOGIES
*Advancing Nucleic Acid Technology℠*

# Stellaris™ fluorescence *in situ* hybridization (FISH) probes: a powerful tool for mRNA detection

The Stellaris™ FISH technology is a new mRNA detection method that enables simultaneous detection, localization and quantification of individual RNA molecules at the cellular level. This technique is ideal for applications that analyze transcription site activity, protein-RNA interactions and mRNA translocation events, and holds promise for future diagnostic applications.*



Sensitive enough to detect individual molecules of mRNA, Stellaris FISH probe sets consist of multiple singly labeled oligonucleotides (generally 30–48) designed to hybridize along targeted RNA transcripts. Each discrete transcript is observed as a diffraction-limited spot by conventional fluorescence microscopy. The relatively large number of probes used to detect a single mRNA molecule makes this technology less prone to false negatives, since a sufficient fraction of probes will still bind the target mRNA and produce signal despite partial mRNA degradation or obstruction by RNA-binding proteins. The potential for false positives is low because the signal is only detectable when tens of probes are bound. Off-target probe binding generates only weak and diffuse fluorescence that is well below the threshold for specifically targeted mRNA detection. This *in situ* technology provides direct detection of individual molecules of mRNA without amplification. Furthermore, Stellaris FISH probes can be labeled with any of a number of different dyes, allowing for simultaneous detection of several mRNA targets. The entire procedure can be carried out in a single day, including cell fixation, probe hybridization and imaging with a wide-field fluorescence microscope.

## Positive controls and active sites of RNA transcription

To supplement Stellaris assays designed for custom targets, positive control probe sets targeting well-expressed mRNAs such as GAPDH (glyceraldehyde 3-phosphate dehydrogenase) were designed and verified. Two distinct Stellaris probe sets targeting the coding sequence of either human (**Fig. 1a**) or mouse (**Fig. 1b**) GAPDH provided robust signals and revealed mRNAs predominantly localized to the cytoplasmic compartment of the cells, with active sites of transcription appearing in the nucleus as larger and significantly brighter spots due to the accumulation of nascent mRNAs.

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In order to further analyze and verify the nuclear spots as active sites of transcription, mRNA transcripts of the *HER2* (human epidermal growth factor receptor 2; also known as NEU and ERBB2) gene in SK-BR-3 breast cancer cells were interrogated. In 25% to 30% of all human breast cancers, *HER2* is found to be amplified and/or its protein product overexpressed[1], and it has emerged as a major biomarker for invasive breast cancer. SK-BR-3 cells contain approximately 11 copies of the *HER2* gene[2]. SK-BR-3 cells probed with Stellaris probes to HER2 mRNAs revealed hundreds of cytoplasmic mRNA transcripts and, strikingly, multiple bright spots of active transcription within the nuclei, often totaling 10 or 11 (**Fig. 1c**). Furthermore, with the addition of the histone deacetylase inhibitor TSA (trichostatin A), *HER2* transcription ceased, as evidenced by the disappearance of these bright nuclear spots (**Fig. 1d**). These observations are consistent with reports that TSA inhibits synthesis of and selectively destabilizes *HER2* transcripts[3].

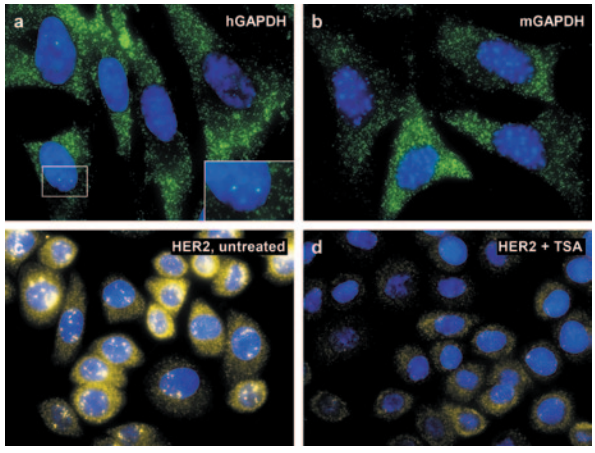


**Figure 1 |** Detection of mRNAs in human and mouse fibroblasts. (**a,b**) Maximum-intensity images showing fluorescent spots corresponding to GAPDH mRNA in normal human lung fibroblasts (HEL 299) (**a**) and in a mouse embryonic fibroblast cell line (NIH/3T3) (**b**); the inset shows the sites of active RNA transcription. (**c,d**) SK-BR-3 human breast cancer cells probed for HER2 mRNAs were either untreated (**c**) or treated for 6 h with 1 μM TSA (**d**).

# APPLICATION NOTES

## Multiplexing with different probe sets

Two or more distinctly labeled Stellaris FISH probe sets can be combined and used to analyze different mRNA transcripts simultaneously. The potential to multiplex is limited only by the microscope filter set and the equipment's ability to optically separate various fluorochrome signals. As an example, in SK-BR-3 cells, HER2 mRNAs (red) were again visualized using Stellaris FISH probes (**Fig. 2a**), and the same cells were simultaneously probed for *ACTB* transcripts coding for β-actin (green) (**Fig. 2b**). Probing with the *HER2* FISH probes produced the anticipated pattern of mRNA localization, including the intense nuclear spots indicative of active transcription. In the cytoplasm, ACTB and HER2 mRNAs exhibited similar patterns of high expression; however, the number of nuclear spots of active transcription differed substantially (*ACTB* is not amplified in SK-BR-3 cells). In addition, the fluorescent spots of cytoplasmic mRNA for EGFR2 and β-actin overlapped minimally, emphasizing the specificity of each Stellaris FISH probe set (**Fig. 2**).

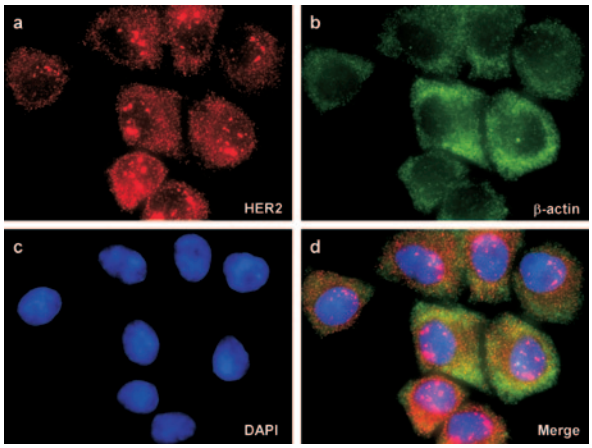


**Figure 2 |** Simultaneous imaging of two different mRNAs in mammalian cells. (**a**) Fluorescent spots correspond to mRNAs of HER2 (red) in SK-BR-3 cells. (**b**) Fluorescent spots correspond to mRNAs of β-actin in the same cells. (**c**) Nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole). (**d**) A merge of the three different channels.

## Sensitivity, quantification and probing of tissue sections

The Stellaris FISH method has been demonstrated to be effective at visualizing well-expressed mRNA transcripts in most tissues and cells. It also provides sufficient sensitivity to detect rare transcripts. When visualized with Stellaris FISH probes and quantified by automated spot counting, HER2 mRNA was shown to be highly overexpressed in SK-BR-3 cells, averaging 1,118 total transcripts (**Fig. 3a**). This number far exceeded those observed in two other breast cancer cell lines: MCF-7 (**Fig. 3b**) and MDA-MB-231 (**Fig. 3c**). MCF-7 cells averaged 67 transcripts, and MDA-MB-231 cells averaged 20 transcripts. These data are consistent with western blot measurements of protein levels in these three different breast cancer cell lines[4]. In contrast to the semiquantitative nature of western blotting, the Stellaris FISH technique provides consistent and discrete numbers of total mRNA.

Along with immunohistochemistry (IHC) and genomic DNA FISH, Stellaris FISH can thus serve as a valuable companion method to assess the status of *HER2* in suspected tumors. By using Stellaris FISH we have successfully confirmed overexpression of HER2 mRNA in formalin-fixed paraffin embedded (FFPE) human breast cancer tissue (**Fig. 3d**). Use of Stellaris FISH probes to detect HER2 mRNA in routine clinical specimens may eventually help clinicians decide on the appropriate and most effective regimen of patient treatment.

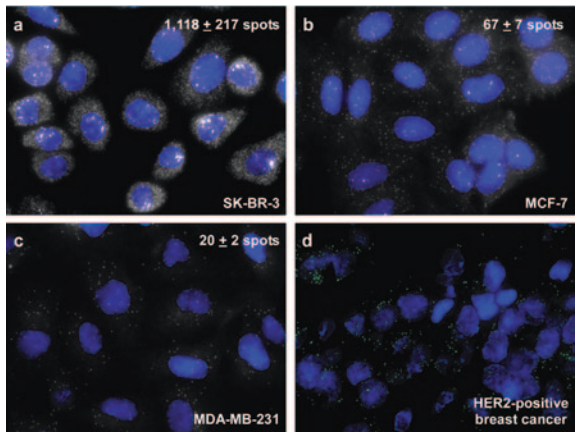


**Figure 3 |** Quantification of mRNAs in various human breast cancer cell lines. (**a–c**) mRNAs of HER2 in SK-BR-3 (**a**), MCF-7 (**b**) and MDA-MB-231 cells (**c**). (**d**) HER2 mRNA was also detected in human breast cancer tissue.

## Conclusion

The power and simplicity of the Stellaris FISH method makes it well suited to the detection of mRNA molecules *in situ*; no unusual chemicals, procedures or equipment are required. More importantly, this technology allows for the measurement of absolute numbers of mRNA molecules, thus providing strong quantitative data, in contrast to traditional western blotting and northern blotting techniques, which provide relative levels of protein and RNA, respectively. Not only does the Stellaris method of RNA detection perform successfully in mammalian cells, but it also works well in human FFPE tissue preparations as well as in other model organisms, for example, *Saccharomyces cerevisiae, Caenorhabditis elegans* and *Drosophila melanogaster.*

1. Murphy, C.G. & Modi, S. HER2 breast cancer therapies: a review. *Biologics* **3**, 289–301 (2009).
2. Millson, A. *et al.* Comparison of two quantitative polymerase chain reaction methods for detecting HER2/neu amplification. *J. Mol. Diagn.* **5**, 184–190 (2003).
3. Scott, G.K. *et al.* Transcriptional repression of ErbB2 by histone deacetylase inhibitors detected by a genomically integrated ErbB2 promoter-reporting cell screen. *Mol. Cancer Ther.* **1**, 385–392 (2002).
4. Tuna, M., Chavez-Reyes, A. & Tari, A.M. HER2/neu increases the expression of Wilms' Tumor 1 (WT1) protein to stimulate S-phase proliferation and inhibit apoptosis in breast cancer cells. *Oncogene* **24**, 1648–1652 (2005).