RNA-FISH Technique: Probe Cocktails vs Single Probes [P4/11]

Ricardo Vieira [1], António Pereira [1,2], António Candeias [1,2], Ana Teresa Caldeira [1,2], Marina González-Pérez [1]

[1] HERCULES Laboratory, Évora University, Portugal
[2] Chemistry Department, School of Sciences and Technology, Évora University, Portugal

RNA-Fluorescence *In Situ* Hybridization (RNA-FISH) has become a widely used powerful tool in environmental microbiology. It enables to analyze and visualize the microorganisms of interest within microbial communities in their natural environments by fluorescent labelling of specific RNA sequences. Poor accessibility or low content of the RNA target region can cause false positives/negatives due to low fluorescence of the cells. To reduce the chances of this occurring, probe cocktails – i.e. mixture of several probes that hybridize to different regions of the target RNA- has been proposed as an alternative to single probes use for increasing the Fluorescence Intensities (FI) [1]. However, is this really a good solution? To answer this question a comparative study of the RNA-FISH results obtained using two probe cocktails (EUK-Mix-6-FAM-(2)=EUK516+EUK1379 and EUK-Mix-6-FAM-(3)"=EUK-Mix-6-FAM(2)+EUK1195) and the corresponding single probes separately, in various concentrations (from 0.75 to 8.37 ng/µL), was accomplished in this work. *Rhodotorula* sp. was used as biological model. The microscopic and fluorimetric analysis, for all the probes and probe cocktails tested, evidenced that the totality of the cells present at the end of each assay exhibits fluorescence and that the FI detected increases with the probe/probe cocktail concentration. The key finding of this work was that the use of probe cocktails is not always a good solution for maximizing the FI as at high concentrations the single probe EUK516-6-FAM yielded higher FI than the probe cocktails.

Reference:

1. Müller E., Drewello U., Drewello R., Weißmann R. and Wuertz S. (2001). J Cult Herit., 2(1), 31–42.

Acknowledgments:
This work was funded by National Funds through FCT through MICROTECH-ART project (PTDC/BBB-IMG/0046/2014) and postdoctoral grant SFRH/BPD/100754/2014 as well as through HIT3CH project (ALT20-03-0246-FEDER-000004), co-funded by FEDER through ALENTEJO 2020.

Keywords: Fluorescence in situ hybridization, probe cocktails, RNA-FISH, Rhodotorula sp.