Feb 15, 2020

Quantitative analysis method of mRNA expression using dual RNA *in situ* hybridization by comparison with housekeeping genes

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.badcia2w

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ABSTRACT

In retrospective formalin-fixed paraffin-embedded (FFPE) samples, RNA quality of FFPE tissues showed variation following the storage period and fixation process. So we suggest quantitative analysis by dual detection of target mRNA and housekeeping genes in one section and calculated the target gene/housekeeping gene ratio by two open-source image analysis programs.
This assay potentially allows for reliable quantification of mRNA expression levels in retrospective FFPE samples.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0229031

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Seung B, Cho S, Kim S, Lim H, Sur J (2020) Quantitative analysis of *HER2* mRNA expression by RNA *in situ* hybridization in canine mammary gland tumors: Comparison with immunohistochemistry analysis. PLoS ONE 15(2): e0229031. doi: 10.1371/journal.pone.0229031

GUIDELINES

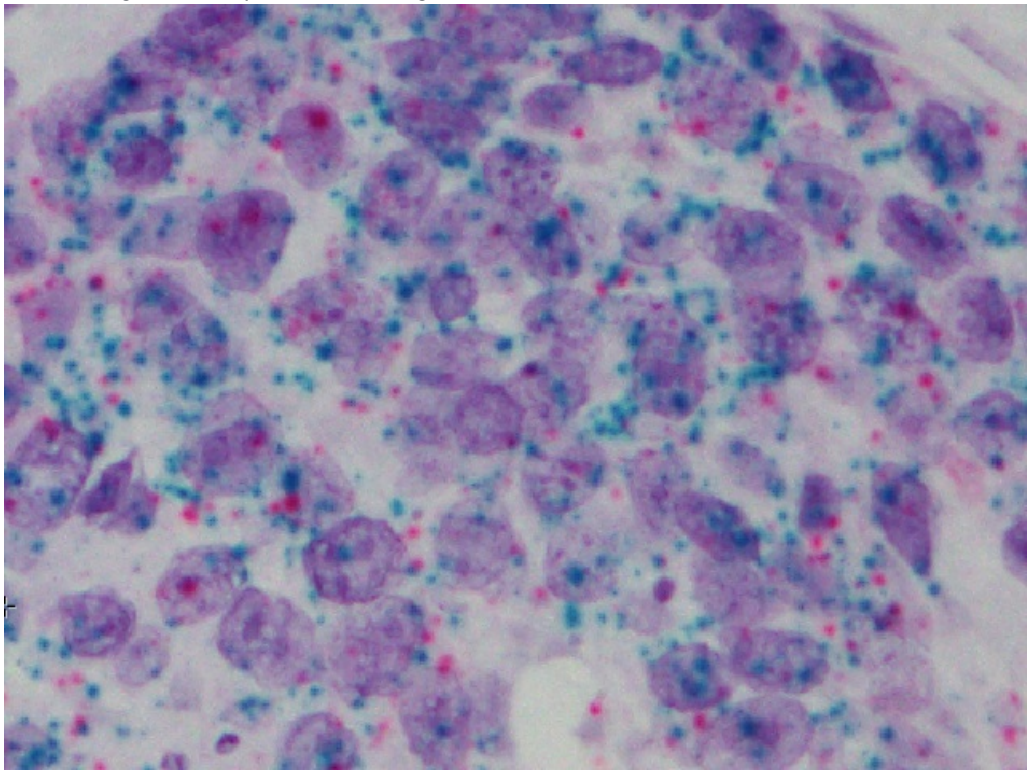
RNA-ISH method was performed manually on FFPE tissues using the RNAscope® duplex assay (Advanced Cell Diagnostics, Hayward, CA, USA) according to the manufacture's instructions.

MATERIALS

NAME	CATALOG #	VENDOR
RNAscope® duplex assay		

RNA-ISH original image

1 RNA-ISH images were acquired at ×400 magnification.



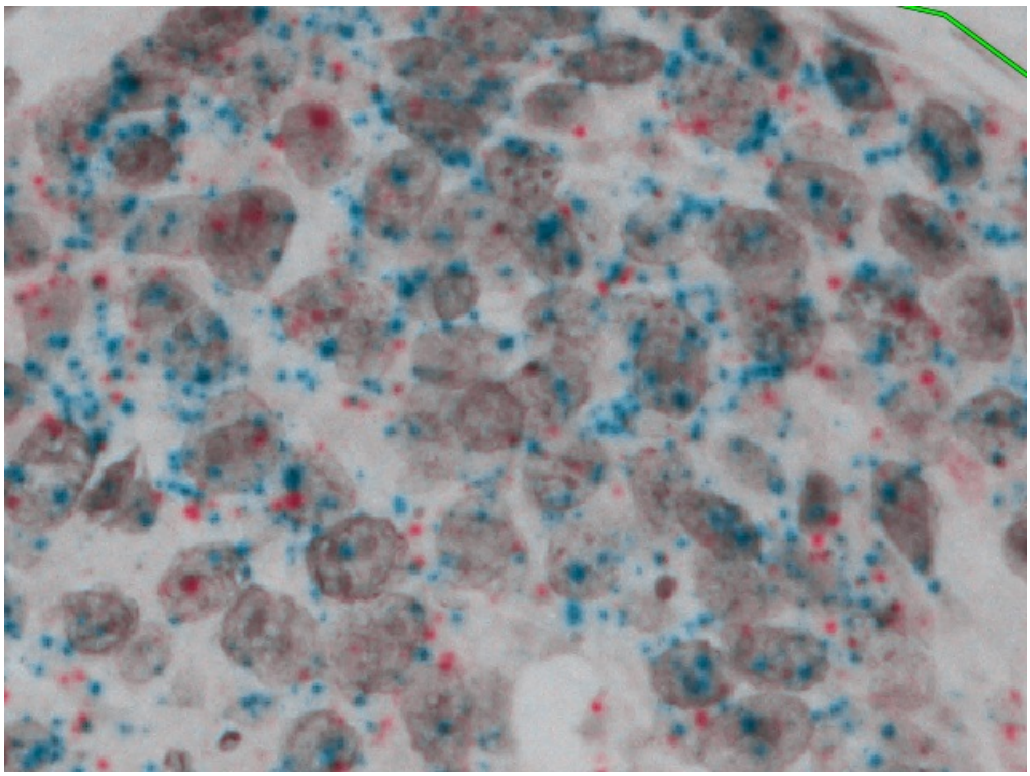
Example of zoomed image of RNA-ISH duplex image (×400 magnification)

Counting the blue dots

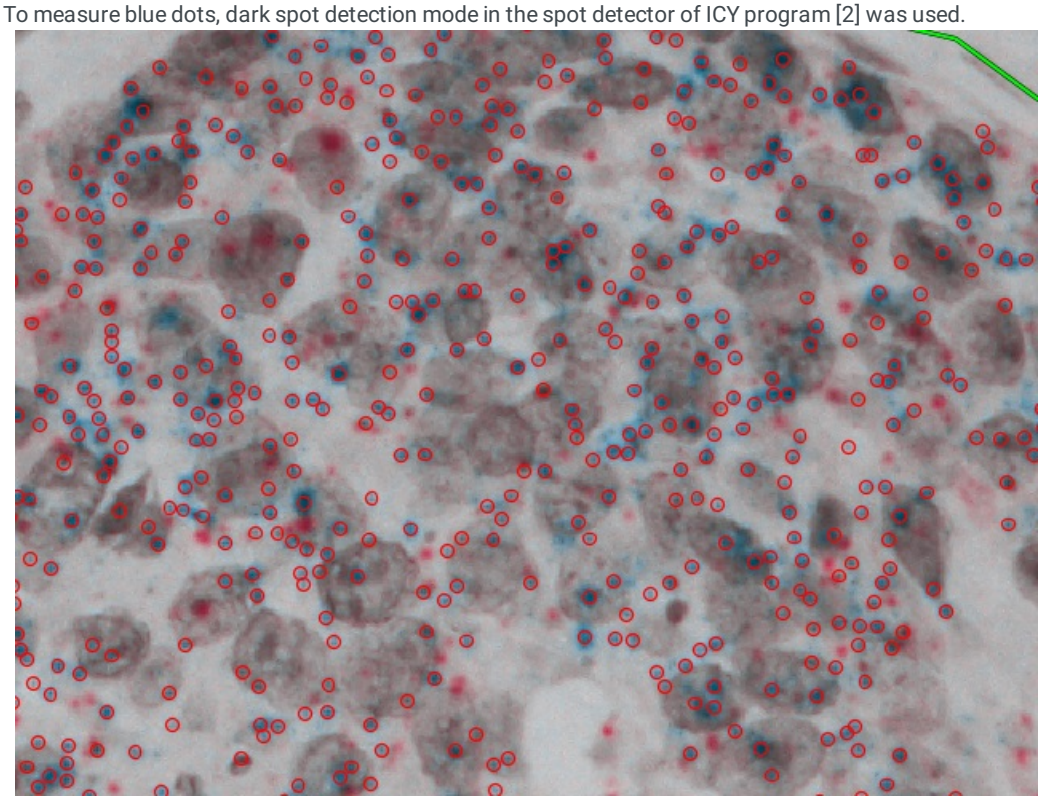
2 The original images were converted using Fiji program [1].

[1] Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012;9(7):676-82.

3 Original images were converted using "Image→Color→Dichromacy→Tritanope" by Fiji program
Save as Tiff file.



4 Select regions of interest (ROI) in Tritanope filtered images.
To measure blue dots, dark spot detection mode in the spot detector of ICY program [2] was used.



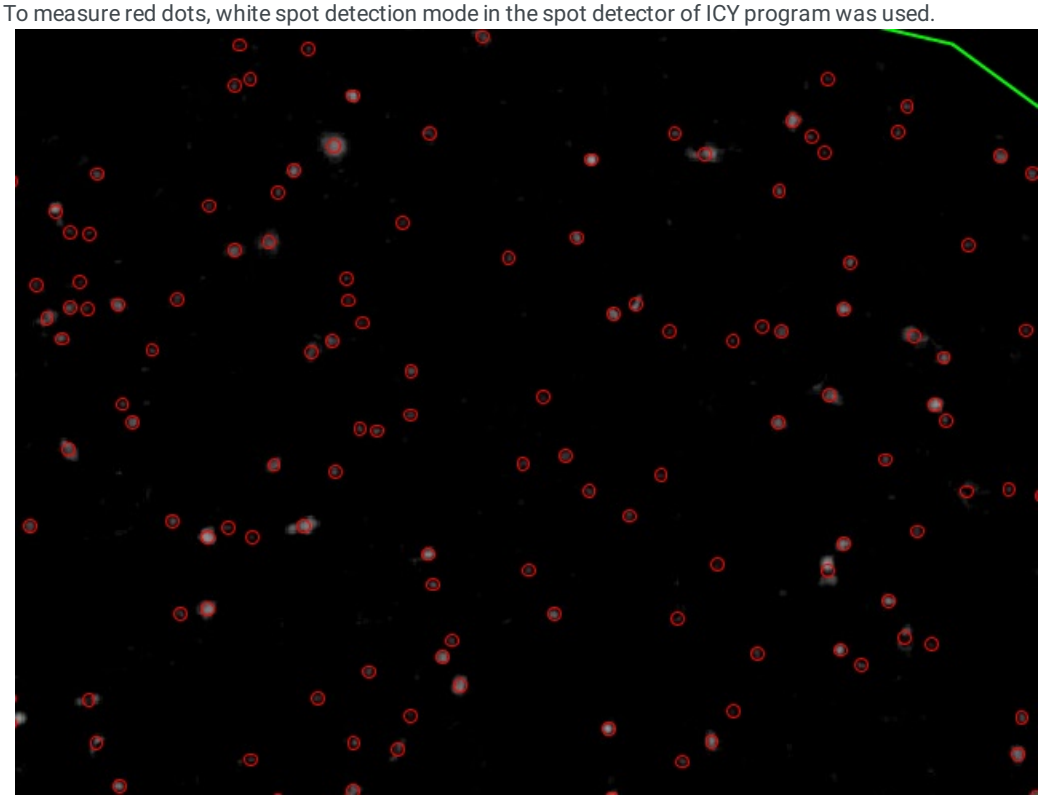
Size of spots to detect (scale 2).
Find adequate filter conditions for your research.

[2] De Chaumont F, Dallongeville S, Chenouard N, Hervé N, Pop S, Provoost T, et al. Icy: an open bioimage informatics platform for extended reproducible research. Nat Methods. 2012;9(7):690-6.

Counting the red dots

5 Tritanope-filtered images were converted to a CIELAB (RGB to CIELAB) and 'a' channel images were acquired as jpg files using Fiji program.

6 Select regions of interest (ROI) in 'a' channel images.
To measure red dots, white spot detection mode in the spot detector of ICY program was used.



Size of spots to detect (scale 3).
Find adequate filter conditions for your research.