

Evaluation Challenges in the Validation of B7-H3 as Oral Cancer Prognosticator

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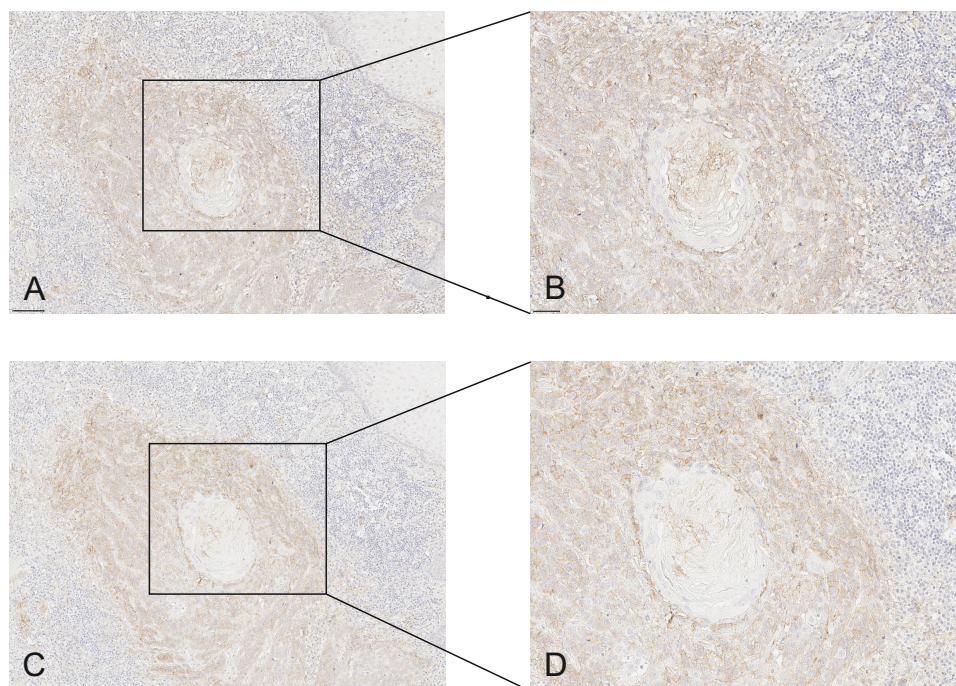
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SUPPORTING INFORMATION



Online resource 1. Two antibodies used in this yielded a similar staining pattern. A-B: goat anti-human B7-H3, C-D: rabbit anti-human B7-H3. Scale bar A and C, 100 μm ; B and D, 50 μm .

A.

 Watershed cell detection

Setup parameters

Choose detection image: Hematoxylin

Requested pixel size: 0.5 μm

Nucleus parameters

Background radius: 0 μm

Median filter radius: 1 μm

Sigma: 1 μm

Minimum area: 10 μm^2

Maximum area: 400 μm^2

Threshold: 0.1

Max background intensity: 2

☒ Split by shape

☒ Exclude DAB (membrane staining)

Cell parameters

Cell expansion: 8 μm

☐ Limit cell expansion by nucleus size

☒ Include cell nucleus

B.

 Create detection classifier

Classifier

Classifier type: Random Trees (default) Edit Auto-update Build & Apply

► Advanced options

Intensity

Intensity feature: Membrane: DAB OD mean

Threshold 1+: 0.05

Threshold 2+: 0.13

Threshold 3+: 0.23

☐ Use single threshold

Details

Classifier: Random Trees

Classes: [Stroma, Tumor]

Normalization: None

Online resource 2. Scoring parameters for A. cell and membrane detection (threshold 0.1), and for B. membranous DAB classifier (threshold mild staining 0.05, moderate staining 0.13, and strong staining 0.23).

Online Resource 3.groovy

```
setImageType('BRIGHTFIELD_H_DAB');
setColorDeconvolutionStains('{ "Name" : "20180228_analysis", "Stain 1" :
"Hematoxylin", "Values 1" : "0.59942 0.56599 0.56599 ", "Stain 2" : "DAB",
"Values 2" : "0.27679 0.48359 0.83038 ", "Background" : " 242 241 241 " }');
selectAnnotations();
runPlugin('qupath.imagej.detect.nuclei.WatershedCellMembraneDetection',
'{"detectionImageBrightfield": "Hematoxylin", "requestedPixelSizeMicrons": 0.5,
"backgroundRadiusMicrons": 0.0, "medianRadiusMicrons": 1.0, "sigmaMicrons":
1.0, "minAreaMicrons": 10.0, "maxAreaMicrons": 400.0, "threshold": 0.1,
"maxBackground": 2.0, "watershedPostProcess": true, "excludeDAB": true,
"cellExpansionMicrons": 8.0, "limitExpansionByNucleusSize": false,
"includeNuclei": true, "smoothBoundaries": false, "makeMeasurements": true}');
runClassifier('C:\\Users\\meris\\Desktop\\b7-h3\\Oulu cases - Priscilla\\with -
Safecopy\\classifiers\\classifier13319_2.qpclassifier');
```

Online resource 3. Script for Qupath to analyse FFPE slides.

Online Resource 4.groovy

```
setImageType('BRIGHTFIELD_H_DAB');
setColorDeconvolutionStains('{ "Name" : "20180228_analysis", "Stain 1" :
"Hematoxylin", "Values 1" : "0.59942 0.56599 0.56599 ", "Stain 2" : "DAB",
"Values 2" : "0.27679 0.48359 0.83038 ", "Background" : " 242 241 241 " }');
selectTMACores();
runPlugin('qupath.imagej.detect.nuclei.WatershedCellMembraneDetection',
'{"detectionImageBrightfield": "Hematoxylin", "requestedPixelSizeMicrons": 0.5,
"backgroundRadiusMicrons": 0.0, "medianRadiusMicrons": 0.0, "sigmaMicrons":
1.0, "minAreaMicrons": 10.0, "maxAreaMicrons": 1000.0, "threshold": 0.1,
"maxBackground": 2.0, "watershedPostProcess": true, "excludeDAB": true,
"cellExpansionMicrons": 8.0, "limitExpansionByNucleusSize": false,
"includeNuclei": true, "smoothBoundaries": false, "makeMeasurements": true}');
selectTMACores();
runClassifier('C:\\Users\\meris\\Desktop\\b7-h3\\Oulu cases - Priscilla with -
Safecopy\\classifiers\\classifier13319_2.qpclassifier');
```

Online resource 4. Script for Qupath to analyse TMA slides.
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