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Oetection of seeded pathology using tyramide amplification

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ABSTRACT

This protocol details the detection of seed pathology using tyramide amplification.

ATTACHMENTS

956-2484.docx

MATERIALS

TBST:

A	В
Tris-HCl pH 7.4	20 mM
NaCl	150 mM
Triton X 100	0.05%

OPEN ACCESS



DOI:

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Protocol status: Working We use this protocol and it's working

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PROTOCOL integer ID: 94352

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NINDS

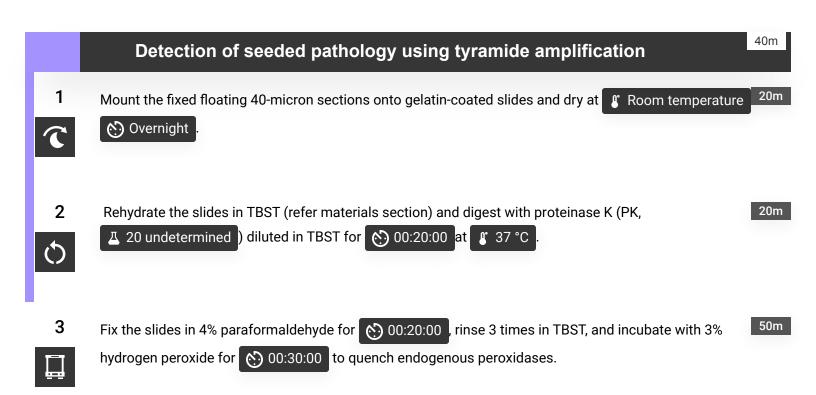
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Place the slides in blocking buffer (TBST, 3% bovine serum albumin, 2% goat serum) for 69 01:00:00 and 2h 4 § 4 °C in blocking buffer containing anti-PSER129 antibody EP1536Y then incubate it Overnight at (Abcam) diluted 1:50,000. 5 Next day, wash the slides 3 times in TBST and incubate with biotinylated anti-rabbit antibody (Vector Labs) 1h diluted 1:400 in blocking buffer for (5) 01:00:00 6 Wash the slides 3 times in TBST and incubate with avidin-biotin complex (ABC) reagent (Vector labs) diluted the in blocking buffer for (5) 01:00:00 7 Wash the slides twice with borate buffer [M] 0.1 Mass Percent | Sodium tetraborate | QH 8.5 | and incuba | 30m in borate buffer containing 0.003% hydrogen peroxide and [M] 5 micromolar (µM) biotinyl tyramide (Sigma-Aldrich) for (5) 00:30:00 8 1h Wash the slides 3 times in TBST and incubate with ABC reagent for 01:00:00



ABC reagent can increase detection sensitivity.

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Wash the slides in TBST and develop using nickel-enhanced 3,3'-Diaminobenzidine DAB as previously described (refer references section).



- 11 Counterstain the slides with methylgreen (Sigma), dehydrate with graded alcohols, clear with xylenes, and cover the slides using cover slipps with cytoseal 60 (Fisher Scientific).
- 12 Perform the Brightfield microscopy using Nikon A1 laser scanning microscope.



Perform the density analysis including binary masks and region of interest (ROI) analyses using Elements software (Nikon).

