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LAMP in situ complete

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MATERIALS TEXT

FAA Solution

For 100mL add the following:

Ethanol (95%)	50mL
Glacial Acetic Acid	5mL
Formalin (37% Formaldehyde)	10mL
diH2O	35mL

10xTE

For 1L add the following:

1M Tris pH 7.5	100mL	Final Concentration: 100mM
0.5M EDTA pH 8.0	20mL	Final Concentration: 10mM

10xMAB

For 1L add the following:

Maleic Acid	116g	Final Concentration: 1M
Sodium Choloride	87.7g	Final Concentration: 1.5M
Sodium Hydroxide	40g	
H2O	800mL	

Adjust pH to 7.5, add H2O to 1L and autoclave

10xTBS

For 1L add the following:

Sodium Choloride	87.7g	Final Concentration: 1.5M
Tris	60.6g	Final Concentration: 0.5M
DEPC H2O	800mL	

Adjust pH to 7.5, autoclave. *DEPC treated water must be used when making this solution*

10xBlocking buffer

For 50mL add the following:

10xMAB	40mL
Triton-x-100	150uL
Non fat dry milk	5g

Mix throughly with stir bar and plate, adjust to 50mL with 10xMAB

NBT/BCIP Solution

NBT/BCIP tablets from Roche, 1 tablet dissovled in **10 ml** DEPC H2O, aliquoted into **1 ml** volumes, stored in the dark at **4 °C**

Tissue Fixiation

1 Harvest tissue and submerge in an excess of FAA solution, pull a vacuum for ~2 min and agitate samples gently. Hold under vacuum for 2-3 min before slowly releasing vacuum. Once the vacuum is released the samples should sink, if tissue samples float to the surface, agitate gently and repeat vacuum step if necessary.

2 Incubate samples at 🌡 **4 °C** for 10 to 14 hours 14h

Dehydration and Embedding 10m

3 2 washes in 50% EtOH, 5 min per wash

4 2 washes in 50% EtOH, 30 min per wash 1h

5 Incubate 10 min in 75% EtOH at 🌡 **60 °C** 10m

5.1 ⏸

Can be held in 75% EtOH overnight at 🌡 **4 °C**

6 Incubate 10 min 85% EtOH at 🌡 **60 °C** 10m

7 Incubate 10 min 95% EtOH at 🌡 **60 °C** 10m

8 2 washes in 100% EtOH, 10 min per wash at 🌡 **60 °C** 20m

9 Incubate 10 min 3:1 EtOH (100%) : Histoclear (or xylenes) at 🌡 **60 °C** 10m

10 Incubate 10 min 1:1 EtOH (100%) : Histoclear (or xylenes) at 🌡 **60 °C** 10m

11 Incubate 10 min 1:3 EtOH (100%) : Histoclear (or xylenes) at 🌡 **60 °C** 10m

12 Incubate 5 min Histoclear (or xylenes) at 🌡 **60 °C** 5m

12.1 Place tissue molds and tweezers/tools in oven (or preffered heating element)

13 Incubate 10 min 1:1 Histoclear (or xylenes) : Paraffin at 60 °C 10m

14 5 washes in paraffin at 60 °C 30 min per wash 2h 30m

14.1 ⏸

Can be held overnight in paraffin

15 Transfer tissue into pre-warmed tissue molds, fill molds with paraffin and align samples into desired orientation. Wait for bubbles to disperse, accelerate dispersal with gentle agitation if desired. Remove molds from oven and allow to cool

Sectioning

16 Tissue sectionsing is a skill and a well angeled and extremely sharp blade is critical. An excellent guide to the technique can be found within "Plant Mircotechnique and Microscopy" by Steven Ruzin 1999

16.1 Use the initial sections to adjust the microtome such that sections ribbon together. When approaching the perceived area of interest reduce the number of sections per slide. Float section ribbon on 45 °C diH2O, capture on slide leaving a wide margin around sample.

17 Bake tissue sections overnight at ~ 45 °C

Dewaxing 10m

18 Incubate slides in Histoclear (or xylenes) for 10 min at 55 °C

19 3 washes of Histoclear (or xylenes) at 55 °C for 5 min per wash 15m

20 3 washes of 100% EtOH at 55 °C for 2 min per wash 6m

21 Incubate 2 min in 95% EtOH 2m

22 Incubate 2 min in 70% EtOH 2m

23 Incubate 2 min in 50% EtOH 2m

24 Incubate 2 min in DEPC H2O 2m

25 Incubate 2 min in 1xTBS 2m

25.1

Can be held overnight in 1xTBS

Tissue Prep

26 Prepare Proteinase K buffer (1xTE, 0.5% Triton-x-100, 20ug/uL Proteinase K), vortex before adding Proteinase K.

27 Remove slide, drain excess buffer, and outline tissue with hydrophobic pen/wax pencil

28 Add **250 µl** Proteinase K buffer to each slide and place in humidity chamber at **37 °C** for 20 min 20m

29 Wash 3 times with 1xTBS for 5 min per wash 15m

29.1

Can be held overnight at **4 °C**

30 Detecting DNA? Proceed to LAMP Reaction
Detecting RNA? Remove genomic background first

Remove Genomic Background

31 Prewarm humidity chamber to **37 °C**

32 Prepare a **40 µl** DNase I reaction per slide according to manufacturers (Thermo Scientific) instructions

33 Apply DNase I reaction mix drop-wise onto tissue sections, pitch and roll slide to ensure the reaction covers all tissue samples. Place slide into humidity chamber and incubate at **37 °C** for 1 hour 1h

33.1

To prevent the reaction mix from pooling and encourage more uniform coverage of tissue samples, every 5-7 min gently pitch and roll the humidity chamber

34 Wash 3 times in 1xTBS, 5 min per wash, proceed to LAMP reaction 15m

34.1

Can be held overnight at **4 °C**

LAMP reaction

35 Prewarm humidity chamber to 65 °C

36 Prepare a 30 µl LAMP reaction per slide as outlined in the manufacturers (New England Biosciences) instructions.

36.1 For colorimeteric detection add 0.15 µl of digoxigenin-11-dUTP, 25nmol (25uL) from Roche, this replaces a portion of the H2O in the reaction mix

36.2 For fluorescent detection use fluorescently labeled loop primer mixture in the standard LAMP reaction

37 Apply desired LAMP reaction (colorimeteric or fluorescent) mix drop-wise to the tissue samples, pitch and roll the slide to encourage uniform distribution. Place in humidity chamber and incubate for an empirically determined amount of time, typically between 30 min to 1 hour 45m

37.1 ⚠

To avoid pooling and encourage even distribution of the reaction mix, every 5-7 min gently pitch and roll the humidity chamber for the duration of incubation

37.2 For fluorescent detection procced to step 43

38 2 washes in 1xMAB, 5 min per wash, proceed to detection 10m

38.1 ⏸

Can be held overnight at 4 °C

Colorimeteric Detection 1h

39 Incubate slides in 1xBlocking buffer with gentle rocking at room temperature for 1 hour

40 Flood slide with 250 µl of a 1:5000 anti-DIG alkaline phosphatase conjugated antibody in 1xBlocking buffer, place in humidity chamber and incubate at room temperature for 1 hour 1h

41 Wash 5 times in 1xMAB, 5 min per wash 25m

42 Flood slides with 250 µl NBT/BCIP, incubate at room temperature until reaction develops to satisfactory levels, typically 20-30 min 30m

43 Stop reaction by flooding slides with diH2O, mount a slide cover with your prefered method