植物石蜡切片 BCIP/NBT 显色原位杂交（CISH）实验报告

1.实验器材及试剂

1.1 实验器材

名称	厂家	型号
脱水机	DIAPATH	Donatello
载玻片	Servicebio	
盖玻片	江苏世泰实验器材有限公司	10212432C
病理切片机	上海莱卡仪器有限公司	RM2016
无酶离心管	Servicebio	EP-150-M
摇床(钟摆式)	Servicebio	TSY-B
涡旋混匀器	Servicebio	MV-100
移液枪	Dragon	KE0003087/KA0056573
免疫组化笔	Servicebio	G6100
冰箱	青岛海尔股份有限公司	BCD-192TGN
显微镜	CIC	XSP-C204
正置光学显微镜	日本尼康	NIKON ECLIPSE CI
成像系统	日本尼康	NIKON DS-U3
恒温箱	LABOTERY	GSP-70
高压灭菌锅	松下健康医疗	MLS-3751L-PC
包埋机	武汉俊杰电子有限公司	JB-P5
冻台	武汉俊杰电子有限公司	JB-L5
组织摊片机	浙江省金华市科迪仪器设备有限公司	KD-P

1.2 主要实验试剂

试剂	厂家	货号	稀释比
DEPC	Amresco	E174	
FAA 固定液（DEPC 水）	Servicebio	G1112	
石蜡	Sakura		
无水乙醇	国药集团化学试剂有限公司	100092683	
环保型脱蜡透明液	Servicebio	G1128	
PBS	Servicebio	G0002	
20×SSC 洗脱液	Servicebio	G3016-4	
BSA	Servicebio	G5001	

蛋白酶 K 储存液	Servicebio	G1234
甘油明胶	Servicebio	G1402
杂交缓冲液	Servicebio	G3016-3
anti-DIG-AP	jackson	200-052-156
BCIP/NBT 显色剂	博士德	AR1023

2.植物石蜡切片 BCIP/NBT 显色原位杂交实验步骤

2.1 组织固定：组织取出洗净后立即放入固定液（DEPC 水配制）中固定 12h 以上。真空泵抽气。

2.2.脱水：组织固定完成后经梯度酒精脱水后浸蜡，包埋。脱水过程真空泵抽气。

2.3.切片：石蜡经切片机切片，摊片机捞片，62°烤箱烤片 2h。

2.4.石蜡切片脱蜡至水：依次将切片放入脱蜡透明液I15min-脱蜡透明液II15min-无水乙醇I5min-无水乙醇II5min,自然晾干，DEPC 水浸泡。

2.5.消化：基因笔画圈，根据不同组织不同指标特性，滴加蛋白酶 K(20ug/ml) 37°消化 min 纯水冲洗后 PBS 洗 3 次×5min。

2.6.预杂交：滴加预杂交液，37°C 孵育 1h。

2.7. 杂交：倾去预杂交液，滴加含探针_____杂交液，浓度____，恒温箱_____度杂交过夜。

2.8.杂交后洗涤：洗去杂交液，2×SSC，37°C 洗 10min，1×SSC，37°C 洗 2×5min，0.5×SSC 室温洗 10min。若非特异杂交体较多，可以增加甲酰胺洗涤。

2.9.滴加封闭液：滴加封闭血清 正常兔血清 。室温 30min。

2.10.滴加鼠抗地高辛标记碱性磷酸酶（anti-DIG-AP）：倾去封闭液，滴加 anti-DIG-AP。37°C 孵育 40min，后 TBS 洗 4 次×5min。

2.11.BCIP/NBT 显色：滴加 BCIP/NBT 显色液，显微镜观察阳性。后纯水冲洗。

2.12.封片：甘油明胶封片。

2.13.显微镜检，图像采集分析。

3.植物石蜡切片 BCIP/NBT 显色原位杂交实验结果判读

BCIP/NBT 显出的阳性表达为蓝紫色。mRNA 原位杂交显示结果理论为胞浆阳性，少数核阳性属正常。micRNA 与 lncRNA 不同指标表达定位不同。根据表达量显色有深浅。

注：上述涉及到的所有试剂，仪器等在 RNA 原位杂交实验时都需使用 DEPC 处理后的 Rnase free 环境。

附表 1 探针信息

NBT/BCIP-ISH Assay Protocol (Plant Paraffin Setion)

1. Apparatus and reagents

1.1 Apparatus

Name	Producer	Model
Dehydrator	WHJJ	JJ-12J
Paraffin embedding machine	WHJJ	JB-P5
Pathologic microtome	Leica	RM2016
Frozen flat	WHJJ	JB-L5
Water Bath-Slide	Kedee	KD-P
RNase-freeGlass microscope slides	Servicebio	
Micro-centrifuge	Servicebio	D1008E
Rocker	Servicebio	TSY-B
Vortex	Servicebio	MV-100
Pipettor	Dragon	KE0003087/KA0056573
Microscopy	CIC	XSP-C204
Liquid Blocker PAP Pen	Servicebio	G6100
Refrigerator	HAIER	BCD-192TGN
incubator	LABOTERY	GSP-70
autoclave	PANASONIC	MLS-3751L-PC

1.2 Major reagents

reagent	manufacturer	article number
Ethanol	SCRC	100092683
BioDewax and Clear solution	Servicebio	G1128
PBS	Servicebio	G0002
Proteinase K	Servicebio	G1234
4% of paraformaldehyde（DEPC water）	Servicebio	G1113
DEPC	Amresco	E174
20×SSC solution	Servicebio	G3016-4
BSA	Servicebio	G5001
Nuclear fast red dye	Servicebio	G1035
Glycerin-gelatin	Servicebio	G1402
Hybridization buffer	Servicebio	G3016-3
anti-DIG-AP	Jackson	200-052-156

2. The steps of the experiment

2.1. **Organization fixed**: Take out the organization, wash clean, then Immediately put in the fixed fluid (DEPC) to fix above 12h.

2.2. **Dehydration**: The tissue is dehydrated by gradient alcohol, paraffin, embedding and Vacuum pumping in dehydration process.

2.3. **Section**: The paraffin is sliced through the slicer, the piece of the slice machine and the 62 - degree oven roast for 2 hours.

2.4. **Dewaxing and dehydration:** Soak sections in 2 changes of Dewaxing Transparent Liquid, 15 minutes each. Dehydrate in 2 changes of pure ethanol for 5 minutes each. Then, followed respectively by dehydrating in gradient ethanol of 85%and 75% ethanol 5 minutes each. Wash in DEPC dilution.

2.5. **Digestion**: Mark the objective tissue with liquid blocker pen, according to the characteristics of tissues, add proteinase K(20ug/ml) working solution to cover objectives and incubate at 37℃ for___min. Washing with pure water, then wash three times with PBS (pH 7.4) in a r0ocker device, 5 min each.

2.6. **Pre-hybridization**: Add Pre-hybridization solution to each section and incubate for 1 h at 37℃.

2.7. **Hybridization**: Discard the pre-hybridization solution, add the probe hybridization solution ,concentration_____, and incubate the section in a humidity chamber and hybridize overnight at___℃.

2.8. **Washing**: Remove the hybridization solution. Wash sections with 2×SSC for 5 min at 37℃, Wash sections in 1×SSC two times for 5 min each at 37℃, and wash in 0.5×SSC for 10 min at room temperature. Formamide washing can be added if there are more non-specific hybrids.

2.9. **Blocking**: add blocking solution (Rabbit serum) to the section and incubate at room temperature for 30 min.

2.10. Add the Alkaline Phosphatase-conjugated IgG Fraction Monoclonal Mouse Anti-Digoxin Antibody: (anti-DIG-AP): Remove the blocking solution and add anti-DIG-HRP. Incubate at 37 ℃ for 40 min, then Wash sections in TBS four times for 5 min each.

2.11. **NBT/BCIP developing**: dry sections slightly, add fresh prepared NBT/BCIP chromogenic reagent to marked tissue. Manage reaction time by observing in microscopy until positive expression appears shows blue-purple. Then stop developing reaction by wash in running tap water.

2.12. **Mounting:** Natural air dry and mount with Glycerol Jelly mounting medium.

2.13. Image the results using a brightfield fluorescence microscope. Store slides at RT.

3. Interpretation of the results

The positive expression of BCIP/NBT was blue-purple. The results of mRNA in situ hybridization showed that cytoplasm was positive and a few nuclear positive were normal. The expression of micRNA and lncRNA was different. According to the expression, the color is very light.

Attached table 1 probe information.