



## 植物石蜡切片荧光探针原位杂交（FISH）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            |
| 正置荧光显微镜 | 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    | 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     |     |

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|          |            |         |
|----------|------------|---------|
| 蛋白酶 K    | Servicebio | G1234   |
| 抗荧光淬灭封片剂 | Servicebio | G1401   |
| 杂交缓冲液    | Servicebio | G3016-3 |
| DAPI     | Servicebio |         |

## 2.植物石蜡切片荧光探针原位杂交实验步骤

**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II5min-脱蜡透明液II5min-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 6ng/ul，恒温箱 37 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.DAPI 复染核：**切片滴加 DAPI 染液，避光孵育 8min，冲洗后滴加抗荧光淬灭封片剂封片。

**2.10.镜检拍照：**切片于尼康正置荧光显微镜下观察并采集图像。（紫外激发波长 330-380nm，发射波长 420nm,发蓝光；FAM(488)绿光激发波长 465-495nm，发射波长 515-555 nm，发绿光；CY3 红光激发波长 510-560，发射波长 590nm，发红光。

## 3.植物石蜡切片荧光探针原位杂交实验结果判读

DAPI 染出来的细胞核在紫外的激发下为蓝色，阳性表达为相应荧光素（488）标记的绿光。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。根据表达量不同荧光亮度有强弱。

**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



## Fluorescence Probe-FISH Assay Protocol (Plant Paraffin Section)

### 1. Apparatus and reagents

#### 1.1 Apparatus

| Name                               | Producer   | Model               |
|------------------------------------|------------|---------------------|
| Dehydrator                         | DIAPATH    | Donatello           |
| RNase-free Glass microscope slides | Servicebio |                     |
| Coverslip                          | Citotest   | 10212432C           |
| Pathologic microtome               | Leica      | RM2016              |
| Enzyme-free centrifuge tubes       | Servicebio | EP-150-M            |
| Shaker                             | Servicebio | TSY-B               |
| Vortex                             | Servicebio | MV-100              |
| Pipettor                           | Dragon     | KE0003087/KA0056573 |
| Liquid Blocker PAP Pen             | Servicebio | G6100               |
| Refrigerator                       | HAIER      | BCD-192TGN          |
| Microscopy                         | CIC        | XSP-C204            |
| Fluorescent Microscopy             | NIKON      | Nikon Eclipse ci    |
| Imaging system                     | NIKON      | Nikon DS-U3         |
| Incubator                          | LABOTERY   | GSP-70              |
| autoclave                          | PANASONIC  | MLS-3751L-PC        |
| Paraffin embedding machine         | WHJJ       | JB-P5               |
| Frozen flat                        | WHJJ       | JB-L5               |
| Tissue spreader                    | Kedee      | KD-P                |

#### 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        |

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|                      |            |         |
|----------------------|------------|---------|
| BSA                  | Servicebio | G5001   |
| Nuclear fast red dye | Servicebio | G1035   |
| hybridization buffer | Servicebio | G3016-3 |

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## 2. Procedure

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(20 ug/ml) working solution to cover objectives and incubate at 37°C 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°C.

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\_\_\_°C.

2.8. **Washing:** Remove the hybridization solution. Wash sections with 2×SSC for 5 min at 37°C, Wash sections in 1×SSC two times for 5 min each at 37°C, 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 **Stain cell nuclei (counter stain):** incubate with DAPI for 8min in the dark, and then mounting.

2.10 **Microscopic examination and photography:** to take photos with positive fluorescence microscope. DAPI glows blue by UV excitation wavelength 330-380 nm and emission wavelength 420 nm; FAM glows green by excitation wavelength 465-495 nm and emission wavelength 515-555 nm; CY3 glows red by excitation wavelength 510-560 nm and emission wavelength 590 nm.

## 3. Interpretation of the results



武汉赛维尔生物科技有限公司

Wuhan Servicebio Technology Co., Ltd.

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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.**

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