# 细胞爬片 SweAMI-荧光原位杂交（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 |
|---|---|---|
| 盖玻片 | 江苏世泰实验器材有限公司 | 10212432C |
| 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 |
| 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 |

1.2.主要实验试剂

| 试剂 | 厂家 | 货号 | 稀释比 |
|---|---|---|---|
| DEPC | Amresco | E174 | |
| 4%多聚甲醛（DEPC 水） | Servicebio | G1113 | |
| PBS | Servicebio | G0002 | |
| 20×SSC 洗脱液 | Servicebio | G3016-4 | |
| BSA | Servicebio | G5001 | |
| 蛋白酶 K | Servicebio | G1234 | |
| DAPI | Servicebio | G1012 | |
| 抗荧光淬灭封片剂 | Servicebio | G1401 | |
| 杂交缓冲液 | Servicebio | G3016-3 | |

## 2.细胞爬片 SweAMI 荧光原位杂交实验步骤

**2.1.细胞爬片固定：**细胞爬片置于 4%多聚甲醛（DEPC）固定 20min，于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。

**2.2.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）消化②min。纯水冲洗后 PBS 洗 3 次×5min。.

**2.3.预杂交：**滴加预杂交液 37°恒温箱 1h。

**2.4.杂交：**倾去预杂交液，滴加杂交液（含探针__③浓度 _④__），_____⑤_度杂交过夜。

**2.5.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 37°洗 10min。若非特异杂交体较多，可以增加甲酰胺洗涤

**2.6 杂交 2：**轻轻甩干切片，滴加已预热的探针混合物 2 杂交液（60 μL），水平置于湿盒内 40℃杂交 45 min。此过程中在湿盒底部加入 50ml 2× SSC，防止干片。

**2.7 杂交后洗涤：**倾去杂交液，将切片依次用 40° C 预热的 2× SSC、1× SSC、0.5× SSC、0.1× SSC 于 40° C 漂洗 5 min。注：若非特异性杂交体较多，可以在此步增加洗涤时间、次数及调整杂交液中甲酰胺浓度。

**2.8 信号杂交：**轻轻甩干切片，滴加已预热的信号探针杂交液（60 μL），水平置于湿盒内 40℃杂交 45 min。此过程中在湿盒底部加入 50 ml 2× SSC，防止干片。

**2.9 杂交后洗涤：**倾去杂交液，将切片依次用 40° C 预热的 2× SSC、1× SSC、0.5× SSC、0.1× SSC 于 40℃漂洗 5 min。注：若非特异性杂交体较多，可以在此步增加洗涤时间、次数及调整杂交液中甲酰胺浓度。

**2.10.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。

**2.11.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.细胞爬片 SweAMI 荧光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。Cy3 标记的红光。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**

# Cell Climbing –SweAMI-FISH Protocol

## 1. Apparatus and reagents

1.1 Apparatus

| Name | Producer | Model |
|---|---|---|
| Coverslip | Citotest | 10212432C |
| Enzyme-free centrifuge tube | 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 | Nikon | NIKON ECLIPSE CI |
| Imaging system | Nikon | NIKON DS-U3 |
| Incubator | LABOTERY | GSP-70 |
| Autoclave | PANASONIC | MLS-3751L-PC |

1.2 Major reagents

| reagent | manufacturer | article number |
|---|---|---|
| DEPC | Amresco | E174 |
| 4% of paraformaldehyde（DEPC water） | Servicebio | G1113 |
| Ethanol | SCRC | 100092683 |
| PBS | Servicebio | G0002 |
| 20×SSC solution | Servicebio | G3016-4 |
| BSA | Servicebio | G5001 |
| Proteinase K | Servicebio | G1234 |
| DAPI | Servicebio | G1012 |
| Anti-fluorescence quenching sealing tablets | Servicebio | G1401 |
| hybridization buffer | Servicebio | G3016-3 |
| anti-DIG-HRP | Jackson | G3016-3 |
| FITC-Tyramide | Servicebio | G1222 |

**2. The steps of the experiment**

2.1. **Cell climbing fixation**：Cell climbing was fixed in 4% paraformaldehyde (DEPC) for 20 min, washed 3 times with PBS (pH 7.4) on a decolorizing shaker for 5 min each time.

2.2. **Digestion**: Mark the objective tissue with liquid blocker pen, according to the characteristics of different tissues and different indicators, Add proteinase K (20 ug/ml) to cover tissues and incubate at 37℃ for_____minutes. Washed with sterilized water, then washed three times with PBS，5 min each time

2.3. **Prehybridization:** Add hybridization buffer onto specimen and incubate at 37℃ for 1h.

2.4. **Hybridization**: Remove the pre-hybridization solution, add the probe hybridization solution with concentration of ___, and incubate the section in a humidity chamber and hybridize overnight at___℃.

2.5. **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.

**2.6 Imaging oligo (DIG)**: Add the hybridization solution containing Imaging Oligo (DIG), Dilution ratio 1:400. 42 ° FOR 3 hours. 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.

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

2.8. Add the mouse anti-digoxigenin-labeled peroxidase (anti-DIG-HRP): remove the blocking solution and add anti-DIG-HRP. Incubate at 37 ℃ for 40 min, then Wash sections in PBS four times for 5 min each.

2.9 Tyramide **developing**: Dry sections slightly, add fresh prepared Tyramide chromogenic reagent to marked tissue. Reaction in dark for 5 min at room temperature. Then wash sections in TBST three times for 10 min each and use PBS wash for 5 min.

2.10. **Stain cell nuclei (counter stain)**: Incubate with DAPI for 8min in the dark, and then mounting.

2.11 **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**

The nuclear stained by DAPI were blue under ultraviolet excitation, and the positive expression was a kind of fluorescence labeled by corresponding luciferin. FAM (488) appears green, cy3

appears red. The results of mRNA in situ hybridization were cytoplasmic positive and a few nuclear positive were normal. MicRNA and lncRNA were expressed differently. According to the expression, Different fluorescence brightness is strong or weak.

Note: All reagents, instrument need RNase free.

**Attached table 1 probe information.**