冰冻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
冰冻切片机	Thermo	Cryotome E
载玻片	Wanwu	
盖玻片	江苏世泰实验器材有限公司	10212432C
无酶离心管	Wanwu	EP-150-M
摇床(钟摆式)	Wanwu	TSY-B
涡旋混匀器	Wanwu	MX-F
移液枪	Dragon	KE0003087/KA0056573
Gene tech pen	Gene tech	GT1001
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）	Wanwu	G1113	
OCT 包埋剂	Wanwu	G6059-110ML	
无水乙醇	国药集团化学试剂有限公司	100092683	
二甲苯	国药集团化学试剂有限公司	10023418	
蔗糖	Wanwu	G5031	
PBS 缓冲液（DEPC）	Wanwu	G0020	
20×SSC 洗脱液	Wanwu	G3016-4	
BSA	Wanwu	G5001	
蛋白酶 K	Wanwu	G1205	
DAPI	Wanwu	G1012	
抗荧光淬灭封片剂	Wanwu	G1401	
杂交缓冲液	Wanwu	G3016-3	

2.冰冻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 4%多聚甲醛（DEPC 水配制）固定 12h 以上。

2.2.脱水：固定后组织放入 15%蔗糖溶液中 8h，后换 30%蔗糖溶液中过夜。

2.3.冰冻切片固定：冰冻切片室温晾干，置于 4%多聚甲醛（DEPC）固定 10min，于 PBS（PH7.4）中在脱色摇床上晃动洗涤 3 次，每次 5min。

2.4.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___。纯水冲洗后 PBS 洗 3 次×5min。

2.5.预杂交：滴加预杂交液，37°C 孵育 1h。

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

2.7.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。

2.8.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。

2.9.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.冰冻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标记的荧光。FAM(488)为绿光，cy3 为红光。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

Frozen Tissue–fluorescence probe-FISH protocol

1. Apparatus and reagents

1.1 Apparatus

Name	Producer	Model
Frozen slicer	Thermo	Cryotome E
Glass slide	Wanwu	
Coverslip	Citotest	10212432C
Enzyme-free centrifuge tubes	Wanwu	EP-150-M
Shaker	Wanwu	TSY-B
Vortex	Wanwu	MX-F
Pipettor	Dragon	KE0003087/KA0056573
Gene tech pen	Gene tech	GT1001
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）	Wanwu	G1113
OCT embedding agent	Wanwu	G6059-110ML
Ethanol	SCRC	100092683
Xylene	SCRC	10023418
Sucrose	Wanwu	G5031
PBS solution (DEPC)	Wanwu	G0020
20×SSC solution	Wanwu	G3016-4
BSA	Wanwu	G5001
Proteinase K	Wanwu	G1205
DAPI	Wanwu	G1012
Anti-fluorescence quenching sealing tablets	Wanwu	G1401

2. The steps of the experiment

2.1. **Tissue fixation:** tissue was washed and fixed with 4% paraformaldehyde for 12 h or more.

2.2. **Tissue dehydration:** Transfer tissue to 15% sucrose solution for 8 h and were subsequently to a 30% sucrose solution overnight.

2.3. **Frozen section fixation:** The frozen sections were dried at room temperature, fixed in 4% paraformaldehyde (DEPC) for 10 min, and shaken on the shaker in PBS (pH 7.4) three times for 5 min each.

2.4. **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 and then washed three times with PBS for 5 min each time.

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

2.6. **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___℃.

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

2.8. **Stain cell nuclei (counter stain)**: Incubate with DAPI for 8min in the dark, and then mounting with anti-fluorescence quenching sealing tablets.

2.9. **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.