细胞涂片 SABER-荧光原位杂交（TSA）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	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	
无水乙醇	国药集团化学试剂有限公司	100092683	
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	Jackson	G3016-3	
anti-DIG-HRP	WANWU	200-032-156	
FITC-TSA			

2.细胞涂片地高辛荧光原位杂交实验步骤

2.1.细胞涂片固定：细胞涂片置于 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 6min。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 has-circ-0071023 浓度 500nM），42 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

2.6.二标孵育：滴加含二标探针杂交液，稀释比 1：400.42°孵育 3h。后 2×SSC，37°C 洗 10min，1×SSC，37°C 洗 2×5min，0.5×SSC 37°洗 10min。

2.7.滴加封闭液：滴加封闭血清 正常兔血清 。室温 30min。

2.8.滴加鼠抗地高辛标记过氧化物酶（anti-DIG-HRP）：倾去封闭液，滴加 anti-DIG-HRP。37°C 孵育 50min，后 PBS 洗 3 次×5min。

2.9.滴加 FITC-TSA：滴加 FITC-TSA 试剂，避光室温反应 5min。后 PBS 洗 3 次×5min.

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.细胞爬片地高辛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（FITC）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

Has-circ-0071023 probe:

5'-GCCCACACCCTGTCCTTCTCATGCAATCC

（ttt CATCATCAT ACATCATCAT）$_{30}$ -3'

二标探针：

5'-DIG-tt ATGATGATGT ATGATGATGT -3'

Cell climbing –DIG -TSA -ISH protocol

1. Apparatus and reagents

1.1 Apparatus

Name	Producer	Model
Coverslip	Citotest	10212432C
Enzyme-free centrifuge tube	WANWU	EP-150-M
Shaker	WANWU	TSY-B
Vortex	WANWU	MX-F
Pipettor	Dragon	KE0003087/KA0056573
Liquid blocker 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
Ethanol	SCRC	100092683
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		G1401

hybridization buffer	WANWU	G3016-3
anti-DIG-HRP	Jackson	G3016-3
FITC-TSA	WANWU	200-032-156

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

2.7. 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.

2.8 **TSA developing**: Dry sections slightly, add fresh prepared TSA 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.

2.9. **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

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.