组织芯片制作实验报告

一、实验器材及试剂

1、实验器材

名称	厂家	型号
脱水机	DIAPATH	Donatello
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
病理切片机	上海徕卡仪器有限公司	RM2016
组织摊片机	浙江省金华市科迪仪器设备有限公司	KD-P
烤箱	天津市莱玻瑞仪器设备有限公司	GFL-230
防脱载玻片	Wanwu	G6012-1-50
正置光学显微镜	日本尼康	NIKON ECLIPSE E100
成像系统	日本尼康	NIKON DS-U3
组织芯片融合仪	Wanwu	JX-10
组织芯片取样手柄	Wanwu	SH-10,15,20,30,50
受体底座蜡块	Wanwu	RP-10,15,20,30,50
包埋底模	Wanwu	B-1

2、主要实验试剂

试剂	厂家	货号
二甲苯	国药集团化学试剂有限公司	10023418
无水乙醇	国药集团化学试剂有限公司	100092683
HE 染液套装	Wanwu	G1005
盐酸	国药集团化学试剂有限公司	10011008
氨水	国药集团化学试剂有限公司	10002118
中性树胶	国药集团化学试剂有限公司	10004160

二、石蜡切片制作

1、取材：新鲜组织用固定液固定24h以上。将组织从固定液取出在通风橱内用手术刀将目的部位组织修平整，将修切好的组织和对应的标签放于脱水盒内。

2、脱水浸蜡：将脱水盒放进脱水机内依次梯度酒精进行脱水。75%酒精4h-85%酒精2h-90%酒精2h-95%酒精1h-无水乙醇I 30min-无水乙醇II 30min-醇苯5-10min-二甲苯I 5-10min-二甲苯II 5-10min-65℃融化石蜡I 1h-65℃融化石蜡II 1h-65℃融化石蜡III 1h。

3、包埋：将浸好蜡的组织于包埋机内进行包埋。先将融化的蜡放入包埋框，待蜡凝固之前将组织从脱水盒内取出按照包埋面的要求放入包埋框并贴上对应的标签。于-20℃冻台冷却，蜡凝固后将蜡块从包埋框中取出并修整蜡块。

4、切片：将修整好的蜡块，放入-20℃冻台冷却，再将冷却的蜡块置于石蜡切片机切片，厚4μm。切片漂浮于摊片机40℃ 温水上将组织展平，载玻片将组织捞起，60℃ 烘箱内烤片。水烤干蜡烤化后取出常温保存备用。

三．HE染色与定位

1、石蜡切片脱蜡至水：依次将切片放入二甲苯I20min-二甲苯II20min-无水乙醇I5min-无水乙醇II5min-75%酒精5min，自来水洗。

2、苏木素染色：切片入苏木素染3-5min，自来水洗，分化液分化，自来水洗，返蓝液返蓝，流水冲洗。

3、伊红染色：切片依次入85%、95%的酒精脱水各5min，入伊红染液中染色5min。

4、脱水封片：将切片依次入无水乙醇I5min -无水乙醇II5min -无水乙醇III5min-二甲苯I5min -二甲苯II5min透明，中性树胶封片。

5、显微镜镜检，确定芯片取点部位。

四、组织芯片制作

1、根据组织点的制备要求选取相应规格的组织芯片取样器和受体蜡块；

2、根据HE定位用取样器对目的部位的组织点进行取样，得到供体的组织柱。按照芯片矩阵要求，将所取的供体组织柱点按既定的顺序依次注入到相应受体蜡块孔内；

3、利用组织芯片融合仪对供体组织柱点和受体蜡块进行多次反复融合，使二者完全融合制作成组织芯片蜡块。

五、组织芯片切片

1、将修整好的组织芯片蜡块置于石蜡切片机上切片，片厚4μm。 切片漂浮于摊片机40℃温水上将组织展平，用载玻片将组织捞起，并放进60℃烘箱内烤片。待水烤干蜡烤化后取出常温保存备用。

Experimental report on tissue chip fabrication

1. Experimental equipment and reagents

1.1 Experimental equipment

name	manufactor	model
Dehydrator	DIAPATH	Donatello
Embedding machine	Wuhan Junjie Electronics Co., Ltd	JB-P5
Frozen platform	Wuhan Junjie Electronics Co., Ltd	JB-L5
Pathology slicer	Shanghai Leica Instrument Co., Ltd	RM2016
KD-P Water Bath	Kedee	KD-P
oven	Tianjin Laibo Rui Instrument Equipment Co., Ltd	GFL-230
Slides	Wanwu	G6012-1-50
Orthostatic microscope	NIKON, JAPAN	NIKON ECLIPSE E100
Image system	NIKON, JAPAN	NIKON DS-U3
Tissue chip fusion instrument	Wanwu	JX-10
Tissue chip sampling handle	Wanwu	SH-10,15,20,30,50
Recipient base wa	Wanwu	RP-10,15,20,30,50

1.2 Main experimental reagents

Reagent name	Manufactor	Article number
xylene	National Pharmaceutical Group Co., Ltd	10023418
Anhydrous ethanol	National Pharmaceutical Group Co., Ltd	100092683
He dye suit	Wanwu	G1005
hydrochloric acid	National Pharmaceutical Group Co., Ltd	10011008
ammonia	National Pharmaceutical Group Co., Ltd	10002118
neutral balsam	National Pharmaceutical Group Co., Ltd	10004160

2.Preparation of paraffin section

2.1Tissue fixation: Dissect tissue as fast as possible, then immerse in fixative immediately. Trim tissue sample appropriately in chemical hood after fixation (at least 24 hours).

2.2 Dehydration and paraffin infiltration:

75% alcohol--4 h

85% alcohol--2 h

90% alcohol--2 h

95% alcohol--1 h

100% alcohol I--0.5 h

100% alcohol II--0.5 h

Alcohol and Xylene mix--5 to 10 min

Xylene I--5 to 10 min

Xylene II--5 to 10 min

Paraffin (65° C) I--1 h

Paraffin (65° C) II--1 h

Paraffin (65° C) III--1 h

2.3Embedding: Process tissue sample in melted paraffin in cassettes. Adjust sample to desired orientation according to client's request and label it. Freeze at -20° C until paraffin solidify completely. Take out paraffin block and trim accordingly.

2.4 Section: Cool at-20 ° freezing table, cut 4μm sections using microtome, pick them up with a paint brush and place them on the surface of 40° C deionized water bath. Float sections onto the histological slides. Dry slides in oven (60° C). Label slides and store in room temperature.

3.H&E stain and location

3.1Deparaffinize and hydrate to water:

Xylene I--20 min

Xylene II--20 min

100% alcohol I--5 min

100% alcohol II--5 min

75% alcohol--5 min

Rinse in water

3.2 Stain in haematoxylin solution: Immerse slides in haematoxylin solution for 3 to 5 minutes; rinse them in water. Then differentiate slides with acid alcohol, rinse again. Blue up sections with ammonia solution, wash in slowly running tap water.

3.3 Stain in eosin:

85% alcohol--5 min

95% alcohol--5 min

Eosin solution--5 min

3.4 Dehydrate and mount:

100% alcohol I--5 min

100% alcohol II--5 min

100% alcohol III--5 min

Xylene I--5 min

Xylene II--5 min

Mount with resin

3.5Locate target area using microscope.

4.Prepare tissue micro array slide

4.1Choose recipient paraffin block with appropriate core size (based on requirement) and corresponding puncher.

4.2Take our target area with puncher from original tissue block (donor block) based on H&E result. Inject to core of recipient block according to required sequence.

4.3.Use a tissue chip fusion instrument to repeatedly fuse the donor tissue column and the acceptor wax block to make the two completely fused to form a tissue chip wax block.

5. Tissue chip slicing

5.1 slice the modified tissue chip wax block on the paraffin slicer, the slice thickness is 4μm. Slice float on the spreader 40°C warm water to spread the tissue flat, with a slide to remove the tissue, and put into 60°C oven baking sheet. After baking, remove and store at room temperature.