基因组 DNA 提取实验报告

1 实验器材及试剂

1.1 实验器材

名称	厂家	型号
台式高速冷冻型微量离心机	DragonLab	D3024R
超净工作台	苏净安泰	SW-CJ-1FD
标准试剂型纯水仪	青岛富勒姆科技有限公司	FBZ2001-up-p
超微量分光光度计	Thermo	NanoDrop2000

1.2 主要实验试剂及耗材

试剂	厂家	货号
血液/细胞/组织基因组 DNA 提取试剂盒	Tiangen Biotech(Beijing) CO.,LTD	DP304
异丙醇	国药集团化学试剂有限公司	80109218
离心管	Wanwu	
TIP 头	Wanwu	

2 基因组 DNA 提取步骤

2.1 样本前处理

2.1.1 将组织处理为细胞悬液，10000 g 离心 1min，倒尽上清，加 300μl 缓冲液 GA，振荡 15s，室温放置 5min。

2.1.2 若为悬浮细胞，收集细胞沉淀，若为贴壁细胞，弃掉培养基，用 PBS 洗一遍，胰酶消化，PBS 洗一遍，收集细胞沉淀，加入 300 μl GA，振荡 15s，室温放置 5min。

2.1.3 若为血液样本，取 300 μl 新鲜血液。

2.2 加入 20 μl 蛋白酶 K 溶液，混匀，56℃处理 1-3h。

2.3 加入 300 μl 缓冲液 GB，充分颠倒混匀，70℃放置 10min，简短离心以去除管盖内壁的水珠。

2.4 加入 300 μl 无水乙醇，充分振荡混匀 15s，简短离心以去除管盖内壁的水珠。

2.5 将上一步所得溶液和絮状沉淀都加入一个吸附柱 CB3 中（吸附柱放入收集管中），12000 g 离心 30s，倒掉废液，将吸附柱 CB3 放回收集管中。

2.6 向吸附柱 CB3 中加入 500 μl 缓冲液 GD（已加入无水乙醇），12000 g 离心 30s，倒掉废液，将吸附柱 CB3 放回收集管中。

2.7 向吸附柱 CB3 中加入 700 μl 漂洗液 PW（已加入无水乙醇），12000 g 离心 30s，倒掉废液，将吸附柱 CB3 放回收集管中。

2.8 向吸附柱 CB3 中加入 500 μl 漂洗液 PW，12000 g 离心 30s，倒掉废液。

2.9 将吸附柱 CB3 放回收集管中，12000 g 离心 2min，倒掉废液，室温放置 10min，以彻底

晾干吸附材料中残余的漂洗液。

2.10 将吸附柱 CB3 转入一个干净的离心管中，向吸附膜的中间部位悬空滴加 100 µl TE 洗脱缓冲液，室温放置 5min，12000 g 离心 2min，将溶液收集到离心管中。

2.11 将离心得到的溶液再加入吸附柱 CB3 中，室温放置 2min，12000 g 离心 2min，将溶液收集到离心管中。

Genomic DNA extraction Lab Report

1 Laboratory Equipment and Reagents

1.1 Laboratory equipment

Equipment	Manufacturers	Model
Centrifuge	DragonLab	D3024R
Clean bench	Suzhou Antai Air Tech Co., Ltd	SW-CJ-1FD
Standard reagent type pure water meter	Qingdao Fulum Technology Co., Ltd	FBZ2001-up-p
Ultramicro spectrophotometer	Thermo	NanoDrop2000

1.2 Reagents

Reagents	Manufacturers	Order
TIANamp Genomic DNA Kit	Tiangen Biotech(Beijing) CO.,LTD	DP304
Isopropyl alcohol	Sinopharm Group Chemical Reagent Co., Ltd.	80109218
Centrifuge tube	Wanwu	
TIP	Wanwu	

2 Genomic DNA Extraction Experimental steps

2.1 Samples preparation

2.1.1 For blood, please use 200 µl fresh, frozen or anticoagulantadding blood. If less than 200 µl, please make up with buffer GA to 200 µl.

2.1.2 If the sample is blood from poultry, birds, amphibians, of which red blood cells have nucleolus, the amount should be reduced to 5-20 µl and adjust the volume to 200 µl with buffer GA.

2.1.3 The adherent cells should be treated to cell suspension first, then centrifuge the cells for 1 min at 10,000 rpm (~11,200 × g), then discard the flow-through and re-suspend cell pellet in 200 µl buffer GA.

2.1.4 Animal tissue (spleen＜10mg) should be treated to cell suspension first, then centrifuge the cells for 1 min at 10,000 rpm (~11,200 × g), then discard the flow-through and re-suspend cell pellet in 200 µl buffer GA.

2.2 Add 20 µl Proteinase K, mix thoroughly by vortex. If the sample is tissue: incubate at 56°C until the tissue is completely lysed.

2.3 Add 200 µl Buffer GB to the sample, mix thoroughly by vortex, and incubate at 70°C for 10 min to yield a homogeneous solution. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

2.4 Add 200 µl ethanol (96-100%) to the sample, and mix thoroughly by vortex for 15 s. A white

precipitate may form on addition of ethanol. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

2.5 Pipet the mixture from step 4 into the Spin Column CB3 (in a 2 ml collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard flow-through and place the spin column into the collection tube.

2.6 Add 500 µl Buffer GD (Ensure ethanol (96-100%) has been added) to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s, then discard the flow-through and place the spin column into the collection tube.

2.7 Add 600 µl Buffer PW (Ensure ethanol (96-100%) has been added) to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through and place the spin column into the collection tube.

2.8 Repeat Step 7.

2.9 Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely. Note: The residual ethanol of buffer PW may have some affection in downstream application. 10. Place the Spin Column CB3 in a new clean 1.5 ml microcentrifuge tube, and pipet 50-200 µl Buffer TE directly to the center of the membrane. Incubate at room temperature (15-25°C) for 2-5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g).