普通 PCR 操作步骤

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
PCR 仪	北京东胜创新生物科技有限公司	东胜龙 ETC811
超净工作台	苏净安泰	SW-CJ-1FD
电泳仪	Servicebio	FW-600
凝胶成像系统	上海天能科技有限公司	Tanon-1600R
标准试剂型纯水仪	青岛富勒姆科技有限公司	FBZ2001-up-p

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
琼脂糖	Servicebio	G5056
2×Fast Pfus PCR Master Mix	Servicebio	G3305
无水乙醇	国药集团化学试剂有限公司	10009218
MarkerI DNA ladder	Servicebio	WGM1100
离心管	Servicebio	
TIP 头	Servicebio	
引物	Servicebio	

2 实验步骤

2.1 基因组 DNA 提取

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 15 秒，室温放置 5 分钟；

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；

若为血液，取 200 μl 新鲜血液；

2.1.2 加入 30 μl 蛋白酶 K 溶液，混匀。

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

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

2.1.5 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 30 秒，倒掉废液，将吸附柱 CB3 放回收集管中。

2.1.6 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 30 秒，倒掉废液，将吸附柱 CB3 放回收集管中。

2.1.7 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 30 秒，倒掉废液，将吸附柱 CB3 放回收集管中。

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

2.1.9 将吸附柱 CB3 放回收集管中，12000 g 离心 2 分钟，倒掉废液，室温放置 10 分钟，以彻底晾干吸附材料中残余的漂洗液。

2.1.10 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 50 µl TE 洗脱缓冲液，室温放置 5 分钟，12000 g 离心 2 分钟，将溶液收集到离心管中。

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

2.2 PCR 扩增

2.2.1 PCR 体系：

2 x Fast Pfus PCR Master Mix	25µl
Forward Primer (10 µM)	1µl
Reverse Primer (10 µM)	1µl
模板	Variable
ddH2O	Add to 50µL

2.2.2 PCR 扩增程序设定

预变性	98℃，2min	
变性	98℃，20s	←┐
退火	55℃，20s	│30×循环
延伸	72℃，10s	┘
末段延伸	72℃，5min	
降温	16℃，2min	

2.3 琼脂糖凝胶电泳

2.3.1 制备 3%琼脂糖凝胶:称取 1.5 g 琼脂糖置于锥形瓶中,加入 50ml 1×TAE,瓶口倒扣小烧杯.微波炉加热煮沸 3 次至琼脂糖全部融化,摇匀,即成 3.0%琼脂糖凝胶液.

2.3.2 胶板制备:取电泳槽内的有机玻璃内槽(制胶槽)洗干净,晾干,放入制胶玻璃板.取透明胶带将玻璃板与内槽两端边缘封好,形成模子.将内槽置于水平位置,并在固定位置放好梳子.将冷却到 65℃左右的琼脂糖凝胶液混匀小心地倒入内槽玻璃板上,使胶液缓慢展开,直到整个玻璃板表面形成均匀胶层.室温下静置直至凝胶完全凝固,垂直轻拔梳子,取下胶带,将凝胶及内槽放入电泳槽中.添加 1×TAE 电泳缓冲液至没过胶板为止.

2.3.3 加样:在点样板或 parafilm 上混合 DNA 样品和上样缓冲液,上样缓冲液的最终稀释倍数应不小于 1X.用 10 ul 微量移液器分别将样品加入胶板的样品小槽内,每加完一个样品,应更换一个加样头,以防污染,加样时勿碰坏样品孔周围的凝胶面.(注意:加样前要先记下加样的顺序).

2.3.4 电泳:加样后的凝胶板立即通电进行电泳,电压 60-100V,样品由负极(黑色)向正极(红色)方向移动.电压升高,琼脂糖凝胶的有效分离范围降低.当溴酚蓝移动到距离胶板下沿约 1cm 处时,停止电泳.

2.3.5 电泳完毕后,取出凝胶,用含有 0.5 ug/ml 的溴化乙锭 1×TAE 溶液染色约 20 min,再用清水漂洗 10 min.

2.3.6 观察照相:在紫外灯下观察,DNA 存在则显示出红色荧光条带,采用凝胶成像系统拍照保存。

PCR amplification and Agarose Gel Electrophoresis

1 Laboratory equipment and reagents

1.1 Laboratory equipment

Equipment	Manufacturers	Model
Centrifuge	DragonLab	D3024R
PCR instrument	Beijing Dongsheng Innovation Biotechnology Co., Ltd.	ETC811
Constant temperature oscillation shaker	Changzhou Aohua Instrument Co ., Ltd	SHZ-82A
Clean bench	Suzhou Antai Air Tech Co., Ltd	SW-CJ-1FD
Electrophoresis	Servicebio	FW-600
Gel imaging system	Tanon Science & Technology Co., Ltd.	Tanon-1600R
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
2×Fast Pfus PCR Master Mix	Servicebio	G3305
Agarose	Servicebio	G5056
50*TAE	Servicebio	G3001
MarkerI DNA ladder	Servicebio	WGM1100
Anhydrous ethanol	Sinopharm Group Chemical Reagent Co., Ltd.	10009218
Centrifuge tube	Servicebio	
TIP	Servicebio	
Primer	Servicebio	

2 Experimental steps

2.1 Genomic DNA Extraction Experimental steps

2.1.1 Samples preparation

2.1.1.1 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.

2.1.1.2 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.

2.1.1.3 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.

2.1.1.4 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.

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

2.1.3 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.

2.1.4 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.

2.1.5 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.

2.1.6 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.

2.1.7 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.

2.1.8 Repeat Step 7.

2.1.9 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

2.1.10 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).

2.2 PCR amplification

2.2.1 0.2ml PCR tube, 20ul pcr amplification system.

2 x Fast Pfus PCR Master Mix	25µl
Forward Primer (10 µM)	1µl
Reverse Primer (10 µM)	1µl
Template	Variable
ddH2O	Add to 50 µL

2.2.2 PCR amplification

Pre-denaturation	98℃，2min	
Denatured	98℃，20s	30×cycle
Annealing	55℃，20s	
Extended	72℃，10s	
End extension	72℃，5min	

3 Agarose gel electrophoresis：

3.1 Melt 3g agarose in 100ml of 1X TAE in the microwave until all agarose is dissolved and there are no stringy pieces. Cool the liquid under cold running water for 10-15 seconds or allow to cool by letting it sit at RT until it is not too hot to hold. Add a very small amount of ethidium bromide (1ul per 20ml is sufficient). You can do this simply by dipping the pipet tip into the stock solution and then swirling this into the liquid agar. Ethidium bromide is used to visualize the DNA when viewed under UV light.

3.2 Pour the gel into the gel mold held in place by the clamp, with the desired comb in place. Immediately rinse the flask in RO water and place on drying rack. Allow the gel to dry for about 15 to 20 minutes.

3.3 Remove comb and transfer gel in gel mold to gel box with TBE buffer, making sure that the gel is completely submerged (do not fill past max fill line).

3.4 Load the gel.Each sample needs loading buffer, which should be used at a ratio of 1:6. Loading buffer is used as a marker for migration rates and also to give the sample density so that it sinks to the bottom of the well.

3.5 After all samples and ladder(s) have been loaded, place the lid on the gel box, make sure it is plugged into the powersource, and turn the powersource on. Run it at about 92 volts for 30-45 minutes, or until loading buffer bands are where you want them.

3.6 Check the gel when it is done using the Gel imaging system. If it's not spread out enough, run longer. Remember to turn off the power when you disconnect the lid from the box! Also, when not in use, please make sure the lid is on to minimize evaporation of buffer and to keep dust out. When you have taken a picture or saved the image, throw the gel away in the trash.