# 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 |
|---|---|---|
| 组织/细胞/血液基因组 DNA 提取试剂盒 | Servicebio | G3633 |
| 2×Fast Pfus PCR Master Mix | Servicebio | G3305 |
| 高纯度低电渗琼脂糖（Agarose） | Servicebio | G5056 |
| GN100bp DNA Ladder II | Servicebio | G3366 |
| 1.5mL 离心管（无菌无酶） | Servicebio | EP-150-J |
| SerRed 核酸染料 | Servicebio | G3606 |
| Recombinant Proteinase K | Servicebio | G1234 |
| 红细胞裂解液 | Servicebio | G2015 |
| 50*TAE | Servicebio | G3001 |
| 6× DNA Loading Buffer | Servicebio | G3011 |
| Water Nuclease-Free | Servicebio | G4700 |
| 无水乙醇 | 国药集团化学试剂有限公司 | 10009218 |
| 异丙醇 | 国药集团化学试剂有限公司 | 80109218 |

## 2 实验步骤

### 2.1 基因组 DNA 提取

2.1. 1 处理材料

**a)** 取 50-100 μL 无核红细胞全血（含抗凝剂），置于 1.5 mL 离心管中，补加溶液 **Buffer GA** 至 **200 μL**，使用涡旋振荡器涡旋混匀，接着进行第 3 步（**注意：如果处理更大体积血液，在样品中加入 3 倍体积红细胞裂解液（客户自备，目录号：G2015）颠倒混匀，室温放置 5 min，期间再颠倒混匀几次，1，000 rpm 离心 5 min，使用移液器轻轻吸弃上清，留下白细胞沉淀，加入 200 μL 溶液 Buffer GA，使用涡旋振荡器涡旋混匀，接着进行第 3 步**）；

**b)** 取 5-20 μL 有核红细胞全血（含抗凝剂），置于 1.5 mL 离心管中，补加溶液 Buffer GA 至 200 μL，使用涡旋振荡器涡旋混匀，接着进行第 3 步；

**c)** 取 $10^6$-$10^7$ 细胞悬液，置于 1.5 mL 离心管中，1，000 rpm 离心 5 min，使用移液器轻轻吸弃上清；

**d)** 取 2-30 mg 的动物组织（切勿使用超过最大起始量的组织），置于 1.5 mL 离心管中，用剪刀尽可能地剪成碎块，对于一些质地坚硬的组织也可以进行液氮研磨；

2.1.2. 向离心管中加入 **200 μL 溶液 Buffer GA**，使用涡旋振荡器重悬样品；

2.1.3. 向离心管中加入 **20 μL 溶液 Proteinase K Stock Solution**，56℃孵育 30-60 min（每 10 min 涡旋混匀），较难裂解的材料可以适当延长裂解时间（**注意：**如果有未彻底裂解的样品，会导致提取量和纯度偏低）；

2.1.4. 向离心管中加入 **200 μL 溶液 Buffer GB**，充分上下颠倒混匀 6-8 次，70℃放置 10 min，溶液应变清亮（**注意：**加入缓冲液 GB 时可能会产生白色沉淀，一般 70℃放置时会消失，不会影响后续实验。如溶液未变清亮，说明细胞裂解不彻底，可能导致提取 DNA 量少和提取出的 DNA 不纯）；

2.1.5. 向离心管中加入 **200 μL 无水乙醇**，涡旋振荡混匀 15 sec，若是溶液中有沉淀，则 10，000 g 离心 5 min，收集的上清液转移至新的离心管中，注意尽量不要吸取沉淀；若是没有沉淀，则直接进行下一步；

2.1.6. 如果需要去除 RNA，可选**步骤 a）或 b）**

**a)** 向离心管中加入 **50μL RNase A（客户自备，目录号：G3405）**，吹打均匀后常温放置 5 min；

**b)** 第 12 步结束后的洗脱产物，向离心管中加入 **1μL RNase A（客户自备，目录号：G3405）**，吹打均匀后常温或 37℃放置 5-30 min 即可；

2.1.7. 将吸附柱（Bind DNA Mini Columns）置入收集管（Collection Tubes）中，向吸附柱中加入上一步所得产物，10，000 g 离心 30 sec，倒掉废液，将吸附柱放回收集管中；

2.1.8. 向吸附柱中加入 **500 μL 溶液 Buffer PD**，10，000 g 离心 1 min，倒掉收集管中的废液，将吸附柱放入收集管中；

2.1.9. 向吸附柱中加入 **600 μL 溶液 Buffer PW**（**使用前请先检查是否已加入无水乙醇**），10，000 g 离心 1 min，倒掉收集管中的废液，将吸附柱放入收集管中（**注意：**如果回收的 DNA 是用于盐敏感的实验，例如酶切或 PCR 等，建议 Buffer PW 加入后静置 2-5 min 再离心）；

2.1.10. 重复操作步骤 9；

2.1.11. 将吸附柱放入收集管中，10，000 g 离心 2 min，尽量除去残留的液体；将吸附柱置于室温放置 5 min 或者 37℃培养箱放置 3 min，彻底晾干（**注意：**漂洗液中乙醇的残留会影响后续的酶切、PCR 等实验）；

2.1.12. 将吸附柱放入一个干净离心管中，向吸附中的膜中间位置**悬空滴加 50-100μL 溶液 Elution Buffer 或 dd$H_2O$**（60-65℃预热 Elution Buffer 或 dd$H_2O$ 后再使用效果更佳），室温放置 2 min，10，000 g 离心 2 min，收集 DNA 溶液（**注意：**洗脱液的体积不应少于 50 μL，体积过少会影响回收的效率。为了提高 DNA 的回收量，可将离心得到的溶液重新加回离心吸附柱中，室温放置 2 min，10，000 g 离心 2 min，将 DNA 溶液收集到离心管中。洗脱液的 pH 值对于洗脱效率有较大影响，其 pH 值在 7.0-8.5 范围内，pH 值低于 7.0 会降低洗脱效率；DNA 产物应保存在-20℃，以防 DNA 降解）。

**2.2 PCR 扩增**

2.2.1 PCR 体系：

| | |
|---|---|
| 2 x Fast Pfus PCR Master Mix | 25 μL |
| Forward Primer (10 μM) | 1 μL |
| Reverse Primer (10 μM) | 1 μL |
| 模板 | 1.5 μL |
| Water Nuclease-Free | Add to 50 μL |

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 配制 3%琼脂糖凝胶液：称取 1.5 g 琼脂糖置于锥形瓶中，加入 50 mL 1×TAE，微波炉加热煮沸至全部融化，摇匀。

2.3.2 制备点样凝胶块：将制胶槽洗干净，晾干，放入制胶底座，在固定位置插好梳子。将制胶底座置于水平位置，将冷却到 65℃左右的琼脂糖凝胶液倒入胶槽中，胶液缓慢展开，直到整个胶槽内铺成均匀胶层，室温下静置直至完全凝固，垂直轻拔梳子，将凝胶及胶槽放入电泳槽中，添加 1×TAE 电泳缓冲液至没过凝胶表面为止。

2.3.3 加样：在点样板中按照 6：1 的比例混匀 DNA 样品和 6×Loading Buffer。用 10 ul 移液器分别将样品按顺序加入对应的孔中。每加完一个样品，更换一个吸头，以防污染，加样时勿碰坏样品孔周围的凝胶面。

2.3.4 电泳：设定电压 60-100V，时间 30-45min。（注：样品应由负极(黑色)向正极(红色)方向移动）当溴酚蓝移动到距离胶板下沿约 1cm 处时，停止电泳。

2.3.5 观察照相：在紫外灯下观察，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 |
|---|---|---|
| Servicebio Genomic DNA Kit | Servicebio | G3633 |
| 2×Fast Pfus PCR Master Mix | Servicebio | G3305 |
| 2×Fast Pfus PCR Master Mix | Servicebio | G3305 |
| Agarose | Servicebio | G5056 |
| GN100bp DNA Ladder II | Servicebio | G3366 |
| Centrifuge tube(1.5 mL) | Servicebio | EP-150-J |
| TIP | Servicebio | |
| Recombinant Proteinase K | Servicebio | G1234 |
| Red Blood Cell Lysis Buffer | Servicebio | G2015 |
| 50*TAE | Servicebio | G3001 |
| 6× DNA Loading Buffer | Servicebio | G3011 |
| SerRed | Servicebio | G3606 |
| Water Nuclease-Free | Servicebio | G4700 |
| Ethanol absolute | Sinopharm Group Chemical Reagent Co., Ltd. | 10009218 |

**2. Experimental Steps**

**2.1 Genomic DNA Extraction Experimental steps**

2.1 Samples preparation

2.1.1 For blood, please use 50-100 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 5 min at 1,000 rpm, then discard the flow-through and re-suspend cell pellet in 200 μL **buffer GA**.

2.1.4 Animal tissue (2-30mg) should be treated to cell suspension first, then centrifuge the cells for 1 min at 12,000 rpm, then discard the flow-through and re-suspend cell pellet in 200 μL **buffer GA**.

2.2 Add 20 μL **Proteinase K Stock Solution**, mix thoroughly by vortex. If the sample is tissue: incubate at 56°C for 30-60 min until the tissue is completely lysed(mix thoroughly by vortex per 10 min).

2.3 Add 200 μL **Buffer GB** to the sample, mix thoroughly for 6-8 times, 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 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 Bind DNA Mini Columns (in a 2 mL collection tube) and centrifuge at 10000g for 30 s. Discard flow-through and place the spin column into the collection tube.

2.6 Add **500 μL Buffer PD** to Bind DNA Mini Columns, and centrifuge at 10000g for 1 min , then discard the flow-through and place the spin column into the collection tube.

2.7 Add **600 μL Buffer PW** (Ensure ethanol has been added) to Bind DNA Mini Columns, and centrifuge at 10000g for 1 min. Discard the flow-through and place the spin column into the collection tube.

2.8 Repeat Step 7.

2.9 Centrifuge at 10000g for 2 min to dry the membrane completely. Note: The residual ethanol of buffer PW may have some effection in downstream application.

2.10. Place the Bind DNA Mini Column in a new clean 1.5 mL microcentrifuge tube, and pipet 50-100 µL **Elution Buffer or dd$H_2O$** 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 10000g.

**2.2 PCR amplification**

2.2.1 0.2mLPCR tube， 20ul pcr amplification system.

| | |
|---|---|
| 2×Fast Pfus PCR Master Mix | 25 µL |
| Forward Primer (10 µM) | 1 µL |
| Reverse Primer (10 µM) | 1 µL |
| Template | 2 µL |
| Water Nuclease-Free | Add to 50 µL |

2.2.2 PCR amplification

| | | |
|---|---|---|
| Pre-denaturation | 98℃，2min | |
| Denaturation | 98℃，20s | 30×cycle |
| Anealing | 55℃，20s | |
| Extension | 72℃，10s | |
| End extension | 72℃，5min | |

3. **Agarose gel electrophoresis：**

3.1 Melt 3g agarose in 100 mL 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 SerRed (1ul per 20mLis sufficient). You can do this simply by dipping the pipet tip into the stock solution and then swirling this into the liquid agar. SerRed 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. 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 TAE 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 power， and turn the power 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.