MicroGEM 快速入门指南

使用

PDQeX

*forensic*GEM Universal

的DNA萃取

MicroGEM

走向极端
毫不费力的DNA萃取

更多详细信息，请访问
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QSG_007_190531_PDQeX forensicGEM Universal

关于本指南

本快速入门指南提供了从一系列法医样本萃取DNA的方法。由于犯罪现场样本差异很大，我们可以帮助开发任何特定方法。请通过 info@microgembio.com联系我们。

程序概述： MicroGEM萃取产品为使用嗜热和嗜温酶的独特混合物。若方法里包含了52°C步骤，则允许嗜温酶Histosolv减弱并降解细胞物质。然后，75°C步骤激活嗜热蛋白酶裂解细胞、杀死核酸酶并剥离核小体的DNA。最后，95°C步骤使PDQeX内管收缩，阀门打开并将萃取物推进滤嘴，从而除去蛋白酶和抑制剂。

液试剂的制备和存储

- *Histosolv* 是有助于消化的试剂混合物。它以冻干粉末形式提供。它应该重新悬浮于无DNA水中，如下所示：

套件尺寸 (Rxn)	编码	加入的水的体积
50	XFU0050	0.55 ml
100	XFU0100	1.1 ml
500	XFU0500	5.5 ml
1000	XFU1000	11.0 ml

试剂储存： *forensic*GEM试剂在室温下是稳定的，但收货后应储存在4°C。且试剂打开后，*forensic*GEM酶和*Histosolv*应储存在-20°C以防止意外污染。缓冲液可以储存在4°C以方便使用。

一般说明

- 所有操作需在洁净室或PCR工作台中进行。
- 应始终穿戴洁净服、手套和发网。
- 仅使用经过认证的无核酸试管和试剂。
- 使用0.5%漂白剂清洗会与样本接触的设备。用蒸馏水彻底冲洗。

注意事项

- 若控制屏幕指示温度高于35°C，请勿加载PDQeX仪器。
- 确保收集抽屉和加热模块清洁且无DNA。
- 确保收集抽屉尽可能插入且笔直。
- 若计划运行少于24个反应，请确保放置在收集抽屉的PCR管与加热模块中使用的通道相对应。

MicroGEM Reagent QC

MicroGEM reagents are manufactured and quality tested following ISO 18385:2016. Our reagents are made from certified DNA-free chemicals and solutions, and all buffers and enzymes are treated with DNAse and UV before shipment.

Important Technical Tips

- PDQeX *forensic*GEM Universal is a method that lyses cells and removes nucleoproteins from the DNA.
- There is no concentration step in the procedure and so the concentration of the extract is dependent on: 1) The quality of the sample; 2) In the case of swabs, the type of swab and the volume of water used to wash the swab; 3) The extraction volume (which in some cases can be scaled).
- DNA extracted using the PDQeX *forensic*GEM Universal kit is suitable for High-Throughput Sequencing (HTS) and many types of genotyping including SNP and STR analysis as well as quantitative, multiplex and end-point PCR.
- DNA extracted using PDQeX *forensic*GEM Universal can be quantified using a qPCR or by using fluorescent dyes like Pico Green, iQuant, Qubit assays or the like. Nanodrop is incompatible with PDQeX reagents.
- As with any preparative method for nucleic acid extraction, best results are obtained when samples are handled at 4°C, or on ice, before and after extraction.
- The haem colouration carries through to the DNA leaving the sample slightly pink. This does not cause inhibition of PCR, qPCR or human profiling.
- For long term storage of the extracted DNA, add TE buffer to 1x (10 mM Tris, pH 7.5, 1 mM EDTA) and store at -20°C.

Buccal - Liquid, Swabs and Stains

The processing of the sample will vary dependent on sample type. For liquid samples, volumes should be between 5-20 µl. With cotton swabs, add 1/4 of the swab directly to the PDQeX cartridge. Stained fabric can be swabbed or small portions added directly to the tube.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **BLUE** Buffer
2 µl	*forensic*GEM
	DNA-free water - Add to a final volume of 100 µl
	(Note - this will vary depending on whether you add any liquid with your sample).

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
   - 24 well plate
   - 8 strip tubes
   - Individual tubes
4. Put the drawer in place.
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

MAKE SURE THE PDQEX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL- OTHERWISE YOU WILL LOSE YOUR SAMPLE

7. Select the 'Buccal' program for the extraction:

75°C	5 mins
95°C	2 mins

- **Times may be adjusted by internal laboratory optimisation.**
- **Changes to the default temperatures are not recommended.**

Solid Tissue

Cut the tissue into cubes of approximately 1- 2 mm^3. With hair follicles, use 1-3 hairs. Cut off the shaft 4 mm above the follicle.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **ORANGE+** Buffer
2 µl	*forensic*GEM
10 µl	*Histosolv*
78 µl	DNA-free water

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
    - 24 well plate
    - 8 strip tubes
    - Individual tubes
4. Put the drawer in place.
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

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7. Select the 'Tissue' program for the extraction:

52°C	5 mins
75°C	10 mins
95°C	2 mins

- **Times may be adjusted by internal laboratory optimisation.**
- **Changes to the default temperatures are not recommended.**

Saliva on Storage Cards

Depending on the storage card, it is typical that the preservatives in the card are inhibitory to *Taq* DNA polymerase and so a pre-wash is recommended prior to DNA extraction. For storage cards without preservatives (e.g. MicroGEM Storage cards, Whatman 903), the pre-wash step is likely not necessary, and may potentially cause a loss of sample. Test a pre-wash step before running precious samples.

Pre-Wash (Optional)

1. Remove one 3 mm disc from the card-stored sample and place into a thin-walled PCR tube.
2. Wash the disk in 100 µl of DNA-free water by incubating at room temperature for 15 min.
3. Aspirate the water from the disc and discard the water.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **BLUE** Buffer
2 µl	*forensic*GEM
88 µl	DNA-free water

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
    - 24 well plate
    - 8 strip tubes
    - Individual tubes
4. Put the drawer in place
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

MAKE SURE THE PDQEX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL- OTHERWISE YOU WILL LOSE YOUR SAMPLE

7. Select the 'Buccal' program for the extraction:

75°C	5 mins
95°C	2 mins

- **Times may be adjusted by internal laboratory optimisation.**
- **Changes to the default temperatures are not recommended.**

Blood - Liquid, Swabs and Stains

The processing of the sample will vary dependent on sample type. For liquid samples (e.g. fresh, EDTA, heparin, citrate), volumes should be between 1-10 µl. With cotton swabs, add 1/4 of the swab directly to the PDQeX cartridge. Stained fabric can be swabbed or small portions added directly to the tube.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **ORANGE+** Buffer
2 µl	*forensic*GEM
10 µl	*Enhancer*
	DNA-free water - Add to a final volume of 100 µl (Note - this will vary depending on whether you add any liquid with your sample).

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
   - 24 well plate
   - 8 strip tubes
   - Individual tubes
4. Put the drawer in place.
5. Insert the PDQeX cartridges into the heating block.
6. Cover the cartridges with the hinged flap and close the sliding door.

MAKE SURE THE PDQEX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL- OTHERWISE YOU WILL LOSE YOUR SAMPLE

7. Select the 'Blood' program for the extraction:

75°C	10 mins
95°C	2 mins
105°C	2 mins

- **Times may be adjusted by internal laboratory optimisation.**
- **Changes to the default temperatures are not recommended.**

Blood on Storage Cards

Depending on the storage card, it is typical that the preservatives in the card are inhibitory to *Taq* DNA polymerase and so a pre-wash is recommended prior to DNA extraction. For storage cards without preservatives (e.g. MicroGEM Storage cards, Whatman 903), the pre-wash step is likely not necessary, and may potentially cause a loss of sample. Test a pre-wash step before running precious samples.

Pre-Wash (Optional)

1. Remove one 3 mm disc from the card-stored sample and place into a thin-walled PCR tube.
2. Wash the disk in 100 µl of DNA-free water by incubating at room temperature for 15 min.
3. Aspirate the water from the disc and discard the water.

Preparing the extraction mixture

For each extraction, make up:

10 µl	10x **ORANGE+** Buffer
2 µl	*forensic*GEM
10 µl	*Enhancer*
78 µl	DNA-free water

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl).
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7. Select the 'Blood' program for the extraction:

75°C	10 mins
95°C	2 mins
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