

MicroGEM 快速入门指南

使用

PDQeX

forensicGEM Sperm

的DNA萃取



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所有产品均受限制使用许可。请参阅我们网站上的产品文档。

QSG_008_190531_PDQeX forensicGEM Sperm

关于本指南

本快速入门指南提供了从液体精液或污渍萃取DNA的方法。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。在52°C时，嗜温顶体降解酶发挥作用。然后75°C步骤激活嗜热蛋白酶、裂解细胞、杀死核酸酶和剥离核小体的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。

液体试剂的制备和存储

- Acrosolv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	XSC0050	0.55 ml
100	XSC0100	1.1 ml
500	XSC0500	5.5 ml
1000	XSC1000	11.0 ml

试剂储存： forensicGEM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，forensicGEM和Acrosolv应储存在-20°C以防止意外污染。ORANGE+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。

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- PDQeX *forensic*GEM Sperm 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 Sperm 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 Sperm can be quantified using a qPCR or by using fluorescent dyes like Pico Green, iQuant, Qubit assays or the like. With mixed samples, a Y-specific qPCR is recommended. 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.
- 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.

Stains and Swabs

The processing of the sample will vary dependent on sample type. For liquid samples, try to keep the volume of the liquid below 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 *forensicGEM*

10 µl *Acrosolv*

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 'Sperm' program for the extraction:

52°C 5 mins

75°C 3 mins

95°C 2 mins

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