DNA extraction: Proteinase K versus *prep*GEM

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Introduction

Life in the laboratories is becoming increasingly hectic on a daily basis. During the current pandemic, lab personnel have been overwhelmed with requests for faster and more reliable results. Turn-around-time is getting shorter and deadlines are becoming more and more challenging.

In this application note, we leverage the unique characteristics of eXymes's *prep*GEM™ Universal chemistry (#PUN1000) for rapid temperature-driven extraction (TDE) of viral DNA from infected cells. Furthermore, we compare eXymes's workflow with the "traditional" proteinase K (PK) digestion, a protocol widely used for its low cost and reliability.

PK has been extensively used for low-cost extraction of DNA. However, its low price comes with several limitations, such as the requirment of (i) large quantity of starting material, (ii) long incubation time, and (iii) the use of ionic detergents. These are necessary for denaturing proteins and need to be then removed before downstream analysis can occur. All of this results in significant increases in the time required to obtain the necessary starting material (e.g., expansion of single cell clones). This method also results in the production of chemical waste and has a high rate of plastic consumption.

In contrast, by using the *prep*GEM enzyme, a thermophilic proteinase which has optimal activity at 75°C, thus denaturing proteins in absence of ionic detergents, we show a novel workflow to significantly reduce time, plastic consumption, and costs for extraction of viral DNA from infected cells. The folllowing table reports the main differences between

	*prep*GEM	proteinase K
Inactive	<45°C and >95°C	>65°C
Working temperature	75°C	20-65°C
Require ionic detergents	NO	YES
Lysozyme	Compatible	Incompatible

Table 1: Main differences between *prep*GEM and proteinase K.

Methods and Results

Viruses were grown using adherent cells as hosts, seeded at different densities, specifically 30,000, 3,000, and 1,500 cells/well.

After 2 days in culture:

1) cells were trypsinized, washed with PBS, and extractions were performed on the cell pellet, *or*
2) the cell medium was removed and the extraction mixes were added directly to the plate on the cell monolayer.

Viral DNA was then extracted from infected cells using the following protocols.

Traditional (PK) protocol
Cells were incubated in 50 µl of in house-made Lysis Buffer containing 0.2 mg/ml of PK for 30 minutes at 55°C and 5 minutes at 95°C .

eXymes protocol
Cells were incubated in 50 µl of extraction mix for 5 minutes at 75°C and 2 minutes at 95°C.

Extraction Mix	Volume
Nuclease-free Water	44 µl
10X **BLUE** Buffer	5 µl
*prep*GEM	1 µl

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In all cases, 4 µl of template was loaded for amplification in PCR with specific primer for the viral DNA (final volume 25 µl) and with the 2x HotStarTaq Master Mix (Qiagen, #203446).

Reagents	Amount/sample
2xHotStarTaq Master Mix	12.5 µl
2 µM Forward Primer	2.5 µl
2 µM Reverse Primer	2.5 µl
Nuclease-free Water	3.5 µl
Template	4 µl

The reaction was run in a thermocycler (Bio-Rad, T100) with the following program:

Heat activation DNA polymer-	95°C	15 min	
Denaturation	94°C	30 sec	
Annealing	60°C	1 min	35x
Extension	72°C	1 min	
Final extension	72°C	10 min	
Ending	4°C	hold	

Amplified products were run on a 2% agarose gel with Gelred (Biotium, #41003) and the image was acquired on a Chemidoc MP imaging system (Bio-Rad, Figure 1).

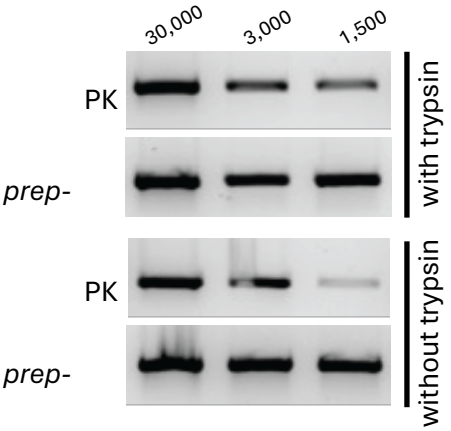

Figure 1:Amplified products from the different conditions. PK stands for proteinase K, which represents the "traditional" proto-

All approaches used to obtain input material for the PCR were successful and resulted in the specific amplification of viral DNA. Both the lysis of trypsinized cells (top) and the lysis of adherent cells directly in the plate (bottom) produced a sufficient quantity of DNA in order to detect the viral DNA using PCR. However, the *prep*GEM Universal kit resulted in stronger bands, especially when lower cell numbers were used as input. Furthermore, the *prep*GEM proteinase worked more efficiently in a shorter time (Figure 2).

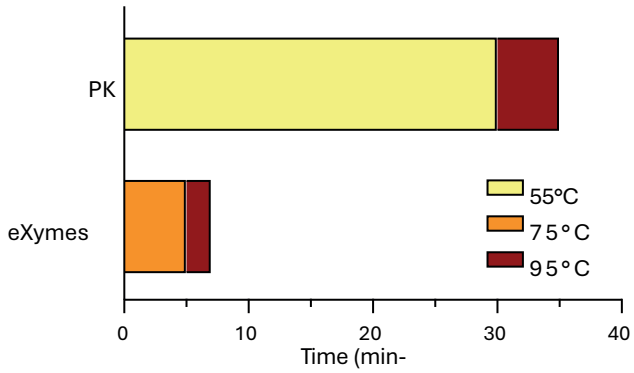

Figure 2: Comparison of the duration of the "traditional" (PK, 35 minutes) and the eXymes (7 minutes) protocols. eXymes performs faster and better.

To further test the *prep*GEM proteinase performance and speed up the viral DNA detection, we also evaluated an approach to simultaneously perform both the extraction and the PCR amplification in a single tube.
This approach was tested on (i) one pellet of 1,500 cells and (ii) 4 µl of a cell solution in PBS brought to a concentration of 2,500 cells/µl (total number of cells = 10,000).

Specifically, 1.5 µl of extraction mix and the PCR master mix were added in the same tube, as detailed below:

Reagents	Amount/sample
2xHotStarTaq Master Mix	12.5 µl
2 µM Forward Primer	2.5 µl
2 µM Reverse Primer	2.5 µl
Nuclease-free Water	3.15 µl
10X BLUE Buffer	0.35 µl
*prep*GEM	0.167 µl

One 5 minutes at 75°C step was included before the PCR program of interest, and run in a thermocycler (Bio-Rad, T100).

*prep*GEM activation	75°C	5 min	
Heat activation DNA polymer-	95°C	15 min	
Denaturation	94°C	30 sec	
Annealing	60°C	1 min	35x
Extension	72°C	1 min	
Final extension	72°C	10 min	
Ending	4°C	hold	

Amplified products were run on a 2% agarose gel with Gelred (Biotium, #41003) and the image was acquired on a Chemidoc MP imaging system (Bio-Rad, Figure 3). Interestingly, this approach also produced a clear signal for low cell numbers, while simultaneously greatly reducing the number of pipetting steps, hands-on-time, and plastic consumption.

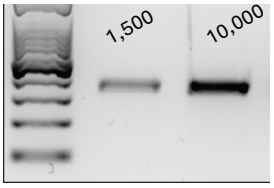

Figure 3: image from agarose gel of the single-tube extraction-PCR approach. Extraction and PCR amplification were performed in the same tube.

Conclusion

Having a rapid and reliable methodology for DNA extraction is a game-changer in most laboratories.

Here, we show that eXymes's *prep*GEM Universal kit (#PUN1000) compared to the traditional proteinase K led to outstanding results in a fraction of the time, even when starting from low abundant material.

This is of key importance, in all those case for which the amount of starting material is a limiting factor, such as primary cells or slow dividing cells (for example, after transfection or selection). For example, in CRISPR genotyping, this approach will allow to significantly decrease the time from single clone expansion to DNA extraction and Sanger sequencing for clone selection from 3-6 weeks to 24-48h, drastically speeding up the workflow for generation of CRISPR cell lines (Figure 4).

In addition, we provide an innovative approach for single-tube extraction and amplification, which will further decrease the hands-on-time and the plastic consumption in several different applications.

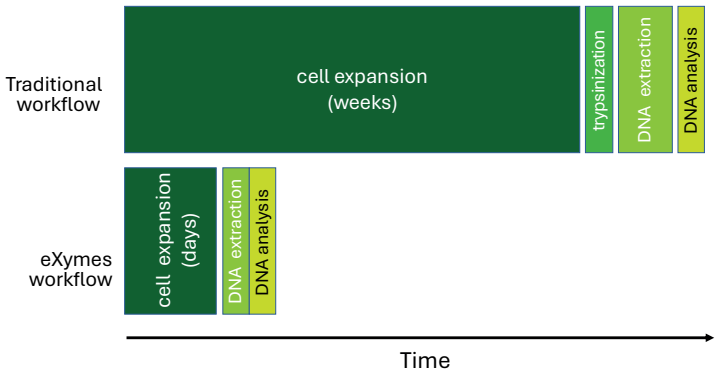

Figure 4: Overview of the differences between a traditional workflow and the workflow with eXymes's *prep*GEM.

In conclusion, using *prep*GEM Universal kit (#PUN1000) allows:

- Lower hands-on-time per reaction.
- Lower plastic consumption (both tubes and filtered tips).
- Lower time of analysis due to the higher performance of the *prep*GEM proteinase approach.
- Lower input material.
- Lower costs for plastic consumables associated with the extraction.
- No chemical waste and its associated costs.
- Increased turn-around-time and high throughput capac-