Optimizing DNA extraction from blood using *prep*GEM™ and *forensic*GEM™ Blood kits

ZyGEM NZ Ltd, Waikato Innovation Park, Ruakura Road, Hamilton, New Zealand.

In general, DNA does not need extensive purification for the polymerase chain reaction (PCR). The key to simplifying nucleic acid preparation is to develop procedures that minimize labour and cost, but are sufficiently rigorous to remove inhibitory substances.

Blood is notoriously difficult to work with because it contains numerous inhibitors. These include haem, IgG, lactoferrin and other blood components (1). As these substances are highly inhibitory to *Taq* DNA polymerase, most nucleic acid extraction methods include complex purification steps to remove them.

Difficulties are compounded with mammalian blood where the bulk of cells - the red blood cells - are not nucleated, and so yields of DNA are relatively low. Yields are lowered further by the solid material in the extract sequestering high molecular weight DNA.

ZyGEM Blood extraction kits rely on a thermophilic proteinase and proprietary buffers that reduce inhibition to the point where a simple, clarified lysate can be used directly in a PCR, qPCR and forensic profiling. The simplicity of the method makes it easily-automated, less prone to error and low cost. Furthermore, being a closed-tube reaction, forensic samples are protected from extraneous contamination, and with clinical samples, laboratory staff are protected from pathogens.

A key to success with the ZyGEM kit is matching the extraction times for the volume of blood being used. Some PCR inhibitors are not intrinsic to blood itself, but are the products of over-digestion with proteinase. The intention is to digest for long enough to release nucleic acids, but not so long that a soup of short, inhibitory peptides is generated.

Two blood DNA extraction kits are produced by ZyGEM; *forensic*GEM Blood and *prep*GEM Blood. Both kits use the same chemistry, but *forensic*GEM has been validated and QCed for forensic use. All of the information in this document is applicable to both kits.

We provide methods that will give the best results from the ZyGEM blood kits for different blood volumes.

Reference

1. Al-Soud WA, Rådström P. Purification and characterization of PCR-inhibitory components in blood cells. J. Clin. Microbiol. 2001; 39(2): 485–493.

One Step Lysis

Fresh, Heparin, Ctirate Blood (1 - 25 µl)

1. Depending on the blood volume you wish to use, make up the extraction cocktail as recommended in the Blue box.

Blood	*RED Buffer*	*prepGEM*	*Water*	*Minutes at 75°C*	*ng/µl Yield**
1	10	1	88	2	0.3
2.5	10	1	86.5	2	1.1
5	10	1	84	2	2.2
7.5	10	1	81.5	5	3.0
10	10	2	78	5	3.6
15	10	2	73	10	4.5
20	10	2	68	15	7.0
25	10	2	63	15	8.0

* Blood from a healthy individual

2. Incubate the smples at 75°C for the time shown in the table above.
3. Incubate at 95°C for 5 min.
4. Centrifuge at 20,000 r.c.f. for 5 min.
5. Pipette the supernatant to a new tube. This contains the DNA.

The DNA is now ready to use. We recommend that no more that 1 µl of undiluted extract should be used in a 25 µl PCR

EDTA Blood (1 - 10 µl)

1. Depending on the blood volume you wish to use, make up the extraction cocktail as recommended in the Blue box.

Blood	*RED Buffer*	*prepGEM*	*CaCl2*	*Water*	*Minutes at 75°C*	*ng/µl Yield**
1	10	1	2.5	85.5	5	0.3
2.5	10	1	2.5	84	10	1.0
5	10	1	5	79	15	2.0
7.5	10	1	5	76.5	15	3.0
10	10	2	5	73	15	3.6

* Blood from a healthy individual

2. Incubate the smples at 75°C for the time shown in the table above.
3. Incubate at 95°C for 5 min.
4. Centrifuge at 20,000 r.c.f. for 5 min.
5. Pipette the supernatant to a new tube. This contains the DNA.

The DNA is now ready to use. We recommend that no more that 1 µl of undilute extract should be used in a 25 µl PCR

FOR MORE INFORMATION CONTACT: support@zygem.com

Typical Results

qPCR Amplification

Extracts were amplified using primers for the glyceraldehyde phosphate dehydrogenase (GAPDH) gene; (Forward: TTCACCACCATGGAGAAGGCTGGG and Reverse: GTCCACCACCCTGTTGCTGTAGCC).

5 µl of a 1:5 dilution of the extract was added to a 25 µl PCR using Platinum® SYBR® Green pPCR SuperMix-UDG with Rox (Invitrogen).

Figure 1 shows the qPCR profiles of DNA extracted from 2.5 µl of blood extracted using the One Step Lysis method. Note how over-digestion affects the slope and end-point of the profile.

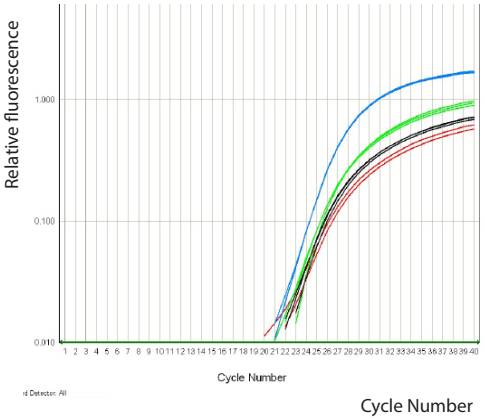

Figure 1 GAPDH qPCR profiles from 2.5 µl blood digested with 1 µl of *prep*GEM for different times at 75°C. BLUE = 2 min, GREEN = 5 min, BLACK = 10 min, RED = 15 min.

Promega PowerPlex® 18D Profiling

3 µl of a DNA extract from 10 µl of blood was amplified in a 12.5 µl reaction using the PowerPlex 18D System (Promega). Profiles were visualised using an ABI 3130 Genetic Analyser and analysis undertaken using the GeneScan® Analysis, and GeneMapper® software (Applied Biosystems).

The clean profile demonstrates that the direct lysis method can be used for quality, high-multiplex forensic DNA profiling (Figure 2).

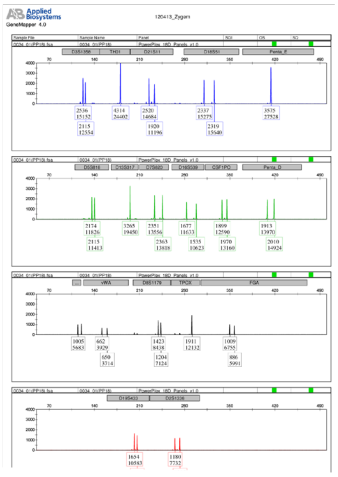

Figure 2 Promega PowerPlex 18 profiles obtained from 10 µl of blood extracted with *prep*GEM using the recommended incubation time.

Two Step Lysis - RBC Pre-lysis

All blood types (10 - 50 µl)

For larger volumes of blood, it is advisable to remove the red blood cells prior to the proteolytic digestion.

1. Pipette between 10 and 50 µl of blood into a 1.6 ml tube.
2. Add 10 x volume of water the tube and the tube and vortex.
3. Hold the tubes at room temperature for 2 mins.
4. Centrifuge at 10,000 r.c.f. for 5 mins.
5. Carefully remove the supernatant and discard.

It is a good idea to give the tubes another quick spin to force the last drop of liquid to the bottom of the tube where it can be removed with a pipette.

6. Add the following extraction reagents to the tube:
   - 10 µl of 10x RED Buffer
   - 1 µl *prep*GEM for 10 - 25 µl of Blood used
   - 2 µl *prep*GEM for 26 - 50 µl of Blood used
   - DNA-free water to 100 µl
7. Incubate at 75°C 5 minutes.
8. Incubate at 95°C for 5 min.
9. Centrifuge at 20,000 r.c.f. for 5 min.
10. Pipette the supernatant to a new tube. This contains the DNA.

Expected Yields (healthy individual)

Blood (µl)	Yield (ng / µl)
10	4
20	8
30	15
40	19
50	24

Technical tips

- *prep*GEM and *forensic*GEM Blood are preparative kits for DNA extraction. The methods lyse cells and remove nucleoproteins from the DNA. Extracted DNA can be used for many types of genotyping including SNP and STR analysis as well as for qPCR, multiplex and end-point PCR.
- DNA extracted using the kits are largely single-stranded because of the 95°C heat step.
- For accurate yield assessment, a qPCR is recommended. Fluorescent chelating dyes and OD 260/280 are NOT reliable ways to quantify ZyGEM DNA extracted from blood..
- 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.
- The reagents are stable at 4°C but after tubes have been opened and for longer term storage, reagents should be stored at -20°C.
- When storing the sample after extraction, aspirate the supernatant from the precipitated residue. Be careful not to disturb the pellet.
- Choose your PCR polymerase carefully. Some Taq derivatives perform better with blood-extracted DNA

Centrifugation Tips

- The ZyGEM buffer is a proprietary formulation that precipitates PCR inhibitors. The solid material should not be disturbed when removing the supernatant.
- Typically, 5 minutes at 13,000 r.c.f is sufficient to give a well-packed pellet. Longer spins should be used for lower r.c.f. centrifugations. For example, a typical 96-well plate, swing out rotor rated at 3,000 r.c.f. should be spun for 10 minutes. Centrifugation should be performed immediately after extraction.