*ZyGEM Quick-Start Guide*

# DNA Extraction Using *prep*GEM™ Insect

See information at www.zygem.com
or email support@zygem.com

## *Technical tips for sample management*

- *prep*GEM™ Insect is a preparative method for DNA extraction - it is not a purification protocol. The *prep*GEM™ method lyses cells and removes nucleoproteins from the DNA. Extracted DNA can be used for many types of genotyping including SNP and STR analysis as well as quantitative, multiplex and end-point PCR.
- DNA extracted using *prep*GEM™ is largely single-stranded because of the 95°C heat step.
- For accurate yield assessment, a qPCR is recommended. The DNA produced by *prep*GEM™ is approximately 90% single-stranded. If standard fluorescent chelating dyes are to be used for quantification, then this factor should be taken into consider-ation.
- As with any preparative method for nucleic acid extraction, for best results prepare and manage samples at 4°C, or on ice, before and after extraction.
- Depending on the type of insects present in a sample, and their state of desiccation, grinding and/or re-hydration may be required to achieve optimal yield.
- The presence of EDTA in the sample can reduce enzyme performance in the extraction. This problem can be overcome by adding $CaCl_2$ to a final concentration of 200 μM.
- When storing sample after extraction, aspirate the supernatant from the tissue and store separately.
- 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 *prep*GEM™ reagents are stable at 4°C but after tubes have been opened and for longer term storage, reagents should be stored at -20°C.

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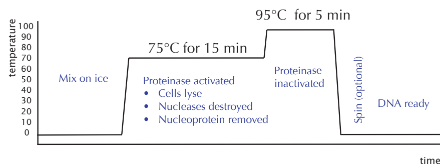
## *prep*GEM™ Insect

The following method is recommended for extracting DNA from insects or insect parts using *prep*GEM™ Insect.

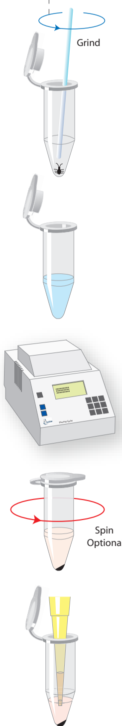
- All manipulations should be performed in a clean-room or a PCR hood.
- Use only certified DNA-free tubes and reagents.
- Wash forceps and scalpels and dissection surfaces in 0.05% Hypochlorite bleach. Rinse thoroughly with DNA-free water.

### *Procedure Outline*




### *Sample Preparation*

- Insects, or parts of insects should be placed in a thin-walled PCR tube for extraction. Approximately 1-2 $mm^3$ of total tissue should be used.
- For older tissue, the sample should be crushed.




### *Extraction Method*

1. Add to the material:
   35 μl of PCR grade water,
   4 μl of 10x Buffer **BLACK**
   1 μl *prep*GEM™

For small insects, these volumes can be reduced further. Solutions can be made as a mastermix and stored on ice.

2. Incubate at:
   75°C for 15 minutes
   95°C for 5 minutes

A thermal cycler can be used for this step

3. Transfer supernatant to a new tube

The DNA is in this solution. **Do not discard**.

Centrifugation is undesirable for automation and should not normally be needed. However with some matierial, two minutes at 13,000 x *g* may assist in clarifying the extract.

The sample is now ready for PCR.

Depending on the age and quality of tissue, 0.1 - 5 μl of extract is recommended for a 25 μl PCR

To order *prep*GEM™ Insect visit www.zygem.com
or email: info@zygem.com or contact your local distributor

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