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DNA Extraction Using **livestockGEM™ Ear Punch** (Ear Tag Collection Tubes)

ZyGEM Quick-Start Guide

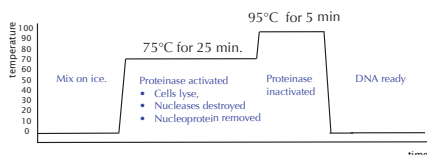
livestockGEM™ Ear Punch

The following method is recommended for extracting DNA from animal ear punches processed within the collection tube.

The key difference to the other ZyGEM methods is that this procedure assumes that the collection tube does not fit into a thermal cycler and so must be incubated in an air oven. The slower heat exchange means that longer incubations are needed.

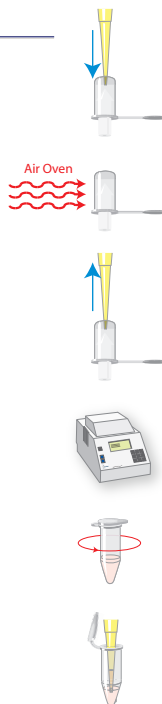
- All manipulations should be performed in a clean-room or a PCR hood.
- Use only certified DNA-free tubes and reagents.
- Wash any equipment that will come into contact with the sample in 0.05% hypochlorite bleach. Rinse thoroughly with DNA-free water.

Procedure Outline



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Extraction Method

1. Add through the septum of the collection tube:

61.5 µl DNA-free water.
7 µl of 10 x Buffer **GOLD**
1.5 µl *livestockGEM™*

2. In an oven, incubate the tubes at 75°C for 25 minutes.
3. Transfer the liquid to a PCR compatible tube and Incubate at 95°C for 5 minutes.

A thermal cycler can be used for this step.

For long term storage, sediment the debris by centrifuging and full speed for 2 minutes and decant the sample to a new tube.

The sample is now ready for quantification and analysis.

Typically DNA should be at approximately 5 ng / µl

In general, no more than 5 µl of a 1:10 dilution of the extract should be used in a PCR.

Technical tips for sample management

- *livestockGEM™ Ear Punch* is a preparative method for DNA extraction from animals. The *livestockGEM™* 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 for quantitative, multiplex and end-point PCR.
- DNA extracted using *livestockGEM™* is largely single-stranded because of the 95°C heat step.
- For accurate yield assessment, a qPCR is recommended. The DNA produced by *livestockGEM™* is approximately 90% single-stranded. If standard fluorescent cheating dyes are to be used for quantification, then this factor should be taken into consideration.
- 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.
- 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 *livestockGEM™* 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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