**Small animal genotyping**

Biomedical research frequently uses mice and rats as animal models. In many cases, a precise genotype of the animals is necessary but often this step is time-consuming. With the number of animals required to generate supported data, reagent and labour costs can become prohibitive and so efficient, low-cost DNA extraction is needed. However, such a method should still be capable of providing template suitable for a broad range of analytical techniques.

Here we describe the benefits of using a DNA extraction kit from ZyGEM Corporation that allows tens or hundreds of samples to be processed in an hour while still producing DNA of a suitable quality for analysis.

The prepGEM kits are designed to be automated on any liquid handling workstation that can carry PCR or peltier plates, or semi-automated using a simple workstation and a thermal cycler. The prepGEM kit uses less plasticware (tips and tubes) and generates less chemical waste than any other commercial extraction system.

**Introduction**

The sex of a mouse can be determined by a PCR method developed by Clapcote and Roder (2005). This method relies on a differential in the intron sizes of two homologous X and Y linked genes, Jarid1c and Jarid1d. Both loci can be amplified using a single primer pair to generate amplicons of 331 and 302 bp. Sex is ascertained either by qPCR and melt curve analysis, or by PCR and gel electrophoresis.

**Material and methods**

To demonstrate the performance of the prepGEM kit for the type of hetero- and homozygosity assays commonly required in transgenic mouse facilities, 9 male and 7 female 3 month old mice were gender-tested after scheduled culling. DNA was extracted using the standard procedure for prepGEM Tissue (1 µl prepGEM, 10 µl Buffer Gold, 89 µl H2O, incubate 75°C for 15 min and 95°C for 5 min). From the supernatant, 5 µl was added to a PCR mix and amplified using the primers described by Clapcote and Roder (2005):

**FORWARD** 5' - CTGAAGCTTTTGGCTTTGAG

**REVERSE** 5' - CGGCTGCCAATTTTGGG

These primers generate amplicons from the Jarid1c (Y-chromosome) and Jarid1d (X-chromosome) loci of 331 and 302 bp in males and a single band of 331 bp in females.

To evaluate the performance of the prepGEM® Tissue kit against similar commercially available kits, the same diagnostic assay was used on DNA templates generated using two other quick DNA-extraction kits cited here as Q and E. The procedures for these kits were followed according to manufacturers’ specifications, and where necessary, amplification was performed using the manufacturer-provided amplification kit.

**qPCR Reagents**

In order to demonstrate the ability of the extracted DNA to perform with PCR reagents from different vendors, three different qPCR methods were used:

- PerfeCTa® SYBR® Green FastMix® (Quanta Bioscience).
- Platinum® SYBR® Green qPCR SuperMix-UDG with Rox (Invitrogen).
- Home-brew mixture with AmpliTaq® DNA polymerase with Buffer II (Applied Biosystems) as follows: 1X Buffer II, 4 mM MgCl2, 0.2 mM primers, 0.1 mM dNTPs, 0.24 mg/ml BSA, 1:200 SYBR, 0.04 mM ROX, 0.02 U AmpliTaq.

Cycling conditions were as follows: 95°C, 2 min; (95°C, 30 sec; 60°C 30 sec; 72°C 30 sec) x 40; Dissociation curve.

**Results and discussion**

Amplification products were visualized using agarose gel electrophoresis and the yield and levels of inhibition determined by real-time qPCR. Validation of prepGEM Tissue in conjunction with gender determining PCR can be seen in Figure 1. A doublet was seen for all male animals and single bands for the females. The one exception had been incorrectly sexed by the anogenital distance (AGD) method.

**Figure 1.** Three month old culled mice tail tips extracted with prepGEM® Tissue and analysed on a 1.5% agarose gel (10 µl of PCR product was run without clean-up). Amplification of the Jarid1 locus from sexed male and reportedly female mice. * Incorrect determination by AGD in lane 17. Lane 1: 1kb+ ladder. Lane 19: Negative control.
Extraction comparisons

To determine comparative performance of the prepGEM Tissue kit against commercially available kits with procedures similar to prepGEM Tissue, six mouse tail tip samples were extracted using prepGEM and two other rapid DNA extraction kits. Again, the Jarid1 locus was amplified with either Platinum® Taq (Invitrogen, Carlsbad) or the optimised PCR reagents supplied with the extraction kit.

PCR products were visualised by agarose gel electrophoresis (Figure 2). The prepGEM Tissue kit yielded readily-amplified DNA with no migration distortion or smearing; kit Q showed a reduced yield of PCR product, as determined by band intensity. Kit E showed better yield of PCR using the manufacturers’ optimised PCR reagents but the high-molecular weight smearing above the PCR amplicon could compromise quantification.

Quantification of DNA yield using different qPCR reagents

In order to determine the absolute yield of each extraction kit, qPCR was performed using three different qPCR methods (see Materials and Methods). Standards were generated using a Qiagen DNeasy Kit and quantified with a 260/280 nm absorbance assay. The results of the qPCR reactions, performed on an Eppendorf epGradient S can be seen in Figure 3. Yield from each DNA prep method was calculated. The average yields were:

- prepGEM Tissue: 98.0 ng / mg of tissue from 2 mm tail tips.
- Kit E samples: 0.34 ng / mg of tissue from 5 mm tail tips.
- Kit Q samples: 2.87 ng / mg of tissue from 5 mm tail tips.

In addition to the differences in the yields, it is notable from the plots that Kit E produced DNA which was inhibitory to the PCR.

This is demonstrated by the lower end-point fluorescence and slope of the plots when the Quanta and Homebrew reagents are used, and the failure with the Invitrogen qPCR reagents.

Conclusions

The prepGEM Tissue kit offers researchers the tools for a rapid, robust and reliable DNA extraction that generates DNA templates that outperform other rapid commercial kits in both standard and quantitative PCR assays.

Kit Q routinely gave lower DNA yields and although Kit E performed adequately using the vendor’s own reagents, it underperformed when alternative reagents were used. This result suggests that the kit would have less utility than prepGEM Tissue which offers researchers the flexibility to choose their own diagnostic reagents according to need.

The likely reason for the improved performance of the prepGEM Tissue kits is that the high temperature proteinase releases DNA from the sample without requiring chemical denaturants such as alkali, chaotropic salts or detergents. These substances can carry over into downstream reactions and cause reduced performance.

Reference