MicroGEM

Mount Erebus, Antarctica - the source of *prep*GEM® (seen here with Castle Rock)

Rapid, reliable DNA prep for mouse genotyping using *prep*GEM®

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Small animal genotyping

Biomedical research frequently uses mice and rats as animal models. In many cases, a precise genotype of the animal is necessary. Often this is a time-consuming process, and when large numbers of animals are required to generate supported data, reagent and labour costs can become prohibitive. Clearly, an efficient, low-cost DNA extraction is needed that is capable of producing DNA template suitable for a broad range of analytical techniques.

This application note describes the benefits of using a DNA extraction kit from ZyGEM NZ that allows hundreds of samples to be processed in an hour whilst still producing DNA of a suitable quality for analysis.

*prep*GEM® 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 thermal cycler. The *prep*GEM kit uses less plasticware (tips and tubes) and generates less chemical waste than any other commercial extraction system.

*prep*GEM® features:

- Thermostable enzyme & optimized buffer
- Simple two-step temperature profile (75°C & 95°C)
- DNA ready for PCR, qPCR, SNP testing in 20 min
- No overnight incubations or centrifugation required
- Compatible with PCR or detection reagents

Introduction

The sex of a mouse can be determined by a PCR method developed by Clapcote and Roder [1]. This method relies on a difference 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 standard PCR and gel electrophoresis.

Material and methods

To demonstrate the performance of the *prep*GEM kit for the type of heterozygosity 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 *prep*GEM Tissue (1 µl *prep*GEM, 10 µl Buffer Gold, 89 µl H_2O, 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:

FORWARD	5'-CTGAAGCTTTTGGCTTTGAG
REVERSE	5'-CCGCTGCCAAATTCTTTGG

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 *prep*GEM Tissue kit against similar commercially available kits, the same diagnostic assay was used on DNA templates generated using two other quick DNA-extraction kits referred to 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 $MgCl_2$, 0.2 mM primers, 0.1 mM dNTPs, 0.24 mg/ml BSA, 2x SYBR Green, 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 visualised using agarose gel electrophoresis and the yield and levels of inhibition determined by qPCR. Validation of *prep*GEM 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.

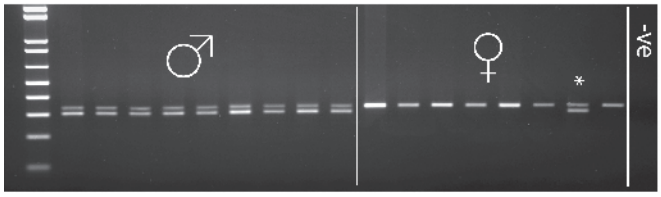

Figure 1. Three month old culled mice tail tips DNA-extracted with *prep*GEM® Tissue and analysed on a 1.5% agarose gel (10 µl of PCR product was run without clean-up). Amplification of the Jarid1 locus from sexed male and reportedly female mice. * Incorrect determination by AGD in lane 17. Lane 1: 1kb+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

Extraction comparisons

To compare the performance of *prep*GEM Tissue against other methods, six mouse tail tips were extracted using *prep*GEM and two commercially available kits for rapid DNA extraction. Again, the *Jarid1* locus was amplified with either Platinum® *Taq* (Life Technologies) or the optimised PCR reagents supplied with the extraction kit.

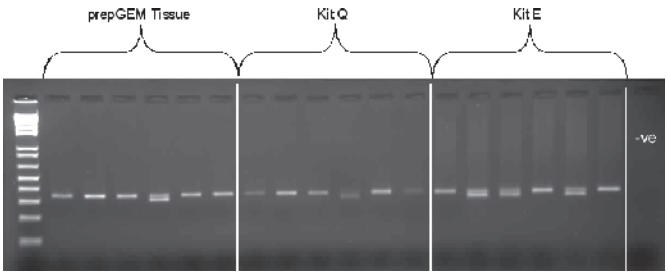

Figure 2. Three month old mice tail tips DNA-extracted and the *Jarid1* locus amplified. *prep*GEM® Tissue lanes 2-7, amplified with Platinum *Taq*; lanes 8-14 kit Q, amplified with manufacturers' optimised PCR reagents; lanes 15-20 with kit E, amplified with Platinum *Taq*, and analysed on a 1.5% agarose gel (10 µl of PCR product was run without column clean up. Lane 1: 1kb+ ladder (Invitrogen)

PCR products were visualised by agarose gel electrophoresis (Figure 2). The *prep*GEM 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 manufacturer's 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:

- *prep*GEM Tissue: 100.0 ng / mg of tissue from 2 mm tail tips
- Kit E samples: 6.43 ng / mg of tissue from 5 mm tail tips
- Kit Q samples: 1.83 ng / mg of tissue from 5 mm tail tips

In addition to differences in the yields, it is notable from the plots that Kit E produced DNA which was inhibitory to the PCR. This problem is demonstrated by the lower end-point fluorescence and slope of the plots when the Quanta and 'homebrew' reagents are used, and failure with the Invitrogen qPCR reagents.

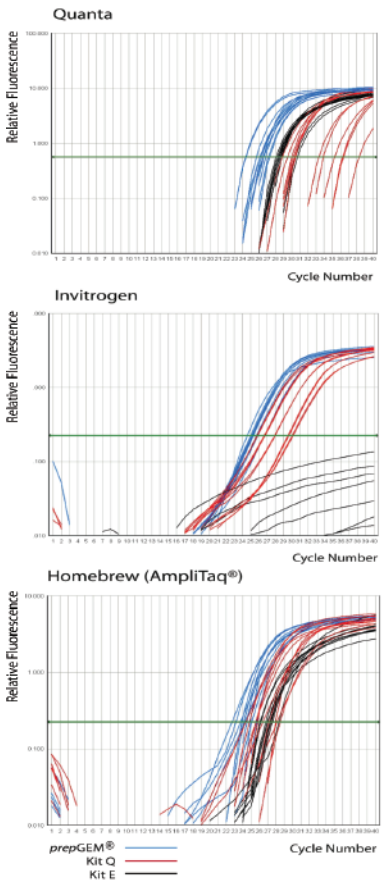

Figure 3.

qPCR from the three different extraction methods.

*prep*GEM® (Blue)

Kit Q (Red)

Kit E (Black).

The assay was performed using three different qPCR mastermixes: Quanta PerfeCTa™ SYBR® Green FastMix™ (Top).

Invitrogen Platinum® SYBR® Green pPCR SuperMix-UDG with Rox (Middle).

'Homebrew' reagent mix with AmpliTaq® DNA polymerase with Buffer II from Applied Biosystems (Bottom)

Conclusions

The *prep*GEM Tissue kit offers researchers a tool for rapid, robust and reliable DNA extraction which generates DNA templates that outperform other rapid extraction 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 *prep*GEM Tissue which offers researchers the flexibility to choose their own diagnostic reagents according to need.

The likely reason for the improved performance of the *prep*GEM Tissue kit 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

[1] Clapcote S. J. and Roder J. C. (2005) Simplex PCR assay for sex determination in mice. Biotechniques 38: 702-70.

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