MyTaq™ Extract-PCR Kit | Fast Mouse Genotyping

Rodents serve as useful animal models in biochemical research, with short gestation periods and large litters providing a means of observing genetically rare phenotypes. The large numbers of rats or mice used in experiments however raise a problem – very small neonates need to be screened quickly for particular genotypes. Template DNA for genotyping is most commonly prepared from ear or tail biopsy samples. MyTaq™ Extract-PCR Kit provides a rapid, low cost DNA extraction method without the need for columns or phenol. This is coupled to MyTaq HS Mix for fast and highly-specific PCR amplification in a convenient single-tube reaction which minimizes contamination while maximizing yield.

MyTaq Extract-PCR can be used in routine genotyping experiments, for example the sex determination of neonatal mice. This is often a time-consuming process, even in adults, involving the measurement of the anogenital distance[i], but a PCR method first described by Clapcote and Roder[ii] can be used to screen large colonies much more rapidly. The assay uses two primers to amplify sequences from both the X-chromosome-specific gene *Jarid1c* and the Y-chromosome-specific gene *Jarid1d*. This is possible because exons 9 and 10 of *Jarid1c* have a high degree of sequence similarity with exons 9 and 10 of *Jarid1d*, and discrimination between the two genes can be made on the basis of size alone. In *Jarid1c*, the intron between the two exons is 114 bp long but in *Jarid1d* this distance is only 85 bp, a difference between the genes of 29 bp. Consequently a PCR directed to this region to genomic DNA from male mice would amplify two DNA fragments simultaneously (331 bp from *Jarid1c* and 302 bp from *Jarid1d*), but would yield only a single fragment of 331 bp in female mice[ii].

In order to test this we extracted DNA using MyTaq Extract-PCR from tail snips of a small colony of mice, then performed a PCR reaction using MyTaq HS and the *Jarid* primers. We compared the results obtained from PCR to the genital morphology of the mice.

MATERIALS AND METHODS

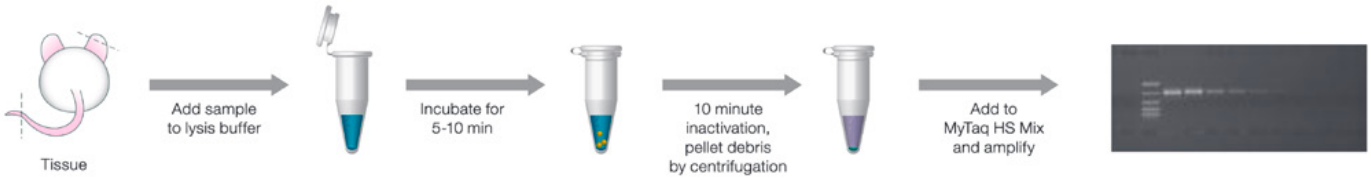

Fig 1. Overview of the workflow, tissues can be ready for PCR in 15min.

A 2 mm snip of tail tissue was removed from each mouse and placed into 20 µL Buffer A, 10 µL Buffer B and 70 µL water. The reactions were incubated at 75°C for 5 minutes, followed deactivation at 95°C for 10 minutes to give the DNA Extract. Each 25 µL of PCR reaction consists of 1x MyTaq HS mix, 1 µL DNA extract, water and 0.4 µM of the *Jarid* primer set[ii]:

Forward: **5'-CTGAAGCTTTTGGCTTTGAG-3'**

Reverse: **5'-CCGCTGCCAAATTCTTTGG-3'**

Reactions were incubated at 95°C for 3 minutes, followed by 35 cycles of 95°C for 15 sec., 60°C for 15 sec. and 72°C for 20 sec. Following thermal cycling, the whole reaction from each PCR product was mixed with 6 µL 5x loading dye buffer and 15 µL of each mix loaded onto a 2% (W/V) agarose gel. Negative controls are not shown, but controls for PCR inhibition and a positive control of genomic DNA from a known female mouse were included. The PCR inhibition controls consisted of the same proportion of MyTaq Extract-PCR Kit reagents, *Jarid* set primer, MyTaq HS Mix and water but were also spiked with 100 ng of female mouse DNA.

RESULTS AND DISCUSSION

After PCR and agarose gel electrophoresis (Fig.2), a single band was seen in five of the nine reactions performed, and a doublet in the remaining lanes. The PCR product sizes corresponded exactly to a visual determination of the sex of the individual mice. We did not observe any inhibition of PCR due to the lysate.

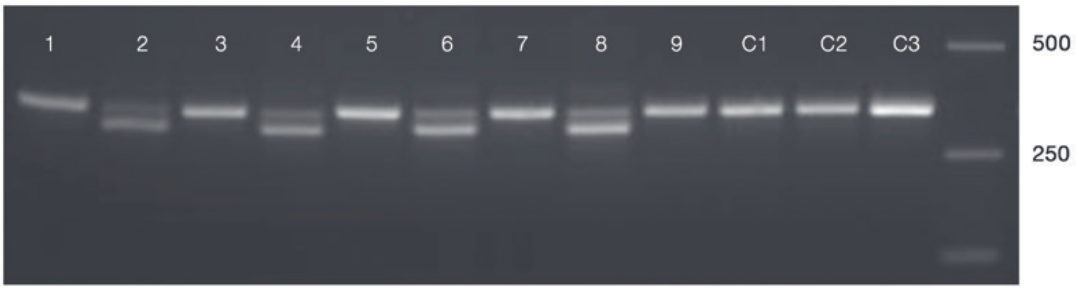

Fig 2. sex determination in neonate mice
PCR products obtained by simultaneous amplification of X- and Y- chromosome-specific genes. Lanes 1, 3, 5, 7 and 9 female adult mice *(Mus musculus)*, lanes 2, 4, 6, and 8 male adult mice. Lanes C1, C2 and C3 female adult controls. Lanes C1 and C2 inhibition PCR controls and lane C3 PCR positive control. M is EasyLadder I.

SUMMARY

The MyTaq Extract-PCR Kit provides a rapid, reliable and robust means of isolating DNA from mouse tail snips, with results in less than two hours. The MyTaq Extract-PCR buffer minimized the inhibitory effects normally seen when using mouse tail lysates.

REFERENCES

i Greenham L. W., & Greenham V. Sexing mouse pups. *Lab Anim.* (NY), **11(3)**: 181-438 (1977)
ii Clapcote, J & Roder J. C. Simplex PCR assay for sex determination in mice. *BioTechniques*, **38 (5)**: 702-706 (2005)