Xpert directXtract PCR Kit

Mouse Genotyping Application Note

Introduction

The Xpert directXtract PCR Kit combines a simple but efficient DNA extraction method with direct PCR amplification in a convenient and easy-to-use manner. This kit eliminates the need of time-consuming and costly DNA purification methods. It can be used with a wide variety of samples, including whole blood, mouse tails, FTA-cards, and FFPE tissue, and is thus ideal for genotyping and screening. In this application note, the easiness, fastness and efficiency of the Xpert directXtract PCR kit for genotyping of Atrx knockout/knock-in mice using 2mm^2 ear punches are demonstrated.

Ear punches and primers were kindly provided by Dr. João Vinagre, IPATIMUP, Porto

METHODS

DNA Extraction - 15 minutes

Using DNase-free microtubes, ear punches (2mm^2) from individual mice were covered with 100µl of lysis buffer consisting of Xpert directXtract buffers A and B and incubated at 75ºC for 5 minutes (lysis). Upon heat-inactivation at 90ºC for 10 minutes, samples were centrifuged at 14,000-16,000g for 1 minute and the cleared supernatant was transferred to a new DNase-free microtube.

PCR - 60 minutes

One microliter of 10x-diluted cleared supernatant was used as template in a 25µl PCR reaction using 12.5µl Xpert Fast Hotstart Mastermix (2x) with dye [included in this kit], together with 1µl of each primer (10pmol). Xpert Fast Hotstart DNA polymerase, with its extension rate of 4-8 kb/min, allows for fast PCR. After the initial hot-start at 95ºC for 3 minutes, targets were amplified by 40 cycles of 95ºC for 15 seconds (denaturation), 60ºC for 12 seconds (annealing) and 72ºC for 15 seconds (extension), followed by a final extension at 72ºC for 3 minutes and cooling down to +4ºC.

Electrophoresis - 15 minutes

Immediately following PCR, ten microliters of PCR reaction mixture were directly loaded onto a 2% agarose gel, prepared with GRS Agarose LE, without the need of adding loading buffer. Electrophoresis was carried out using SGTB DNA Electrophoresis buffer, which allows for extreme high voltages without melting the gel and thus extreme fast electrophoresis with excellent separation and high resolution. Total run time was 15 minutes at 250V (minigel: ~17V/cm).

RESULTS

Optimization

Although the combination of Xpert directXtract buffers and Xpert Fast Hotstart DNA polymerase allows for efficient DNA amplification even in the presence of relative high concentrations of PCR inhibitors, it is recommended to use a 10-fold dilution of the cleared supernatant as sample, as this not only dilutes the target DNA but inhibitors as well. Amount and nature of PCR inhibitors depend on the type of sample and in the case of mouse ear punches, it was found not to have a great effect, as amplification in the case of undiluted sample was similar to 2x, 5x, 10x, 20x diluted samples as deduced by visual examination of gel bands.

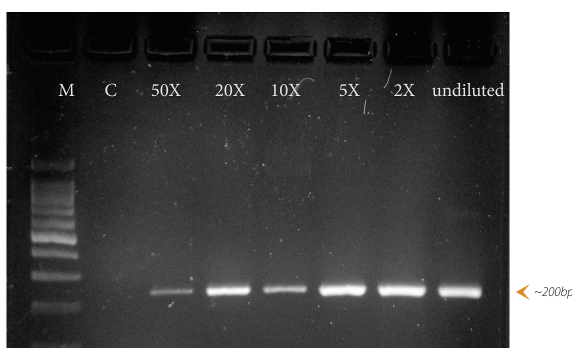

Fig 1. *Amplification using primer set A of a ~200bp and/or ~1200bp target from a serial dilution of cleared supernatant obtained by DNA extraction from an ear punch from mouse nº22 (normal ~200bp). All dilutions, including 50x, amplified as expected, whereas negative control did not. Strong bands, no non-specific bands and size as expected (Marker in lane 1 is GRS Ladder 100bp).*

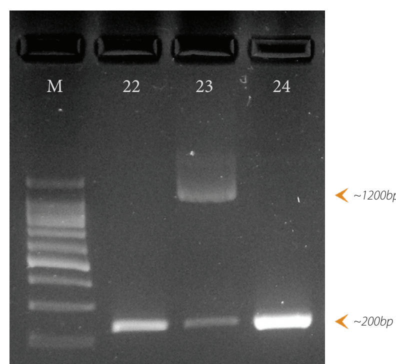

Fig 2. *Amplification using primer set A. All bands have expected sizes as mice nº22 and nº24 are normal whereas mouse nº23 is heterozygote KI. (Marker in lane 1 is GRS Ladder 100bp).*

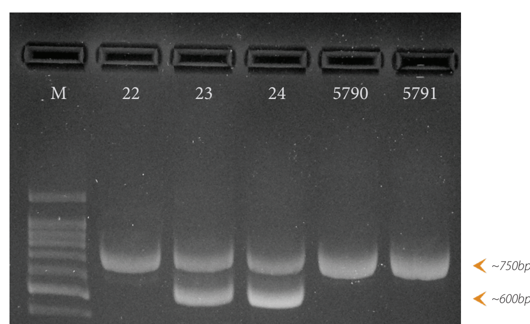

Fig 3. *Amplification using primer set B. All bands have expected sizes as mice nº22, 5790 and 5791 are normal and mice nº23 and 24 are heterozygote KO. (Marker in lane 1 is GRS Ladder 100bp).*

Genotyping of Atrx KO/KI mice

DNA was extracted from ear punches from 5 different mice (nº 22, 23, 24, 5790 and 5791) and 10-fold dilutions of cleared supernatant were used in 3 different and separated PCR reactions. In the first PCR, primer set A was used with expected amplicon size of ~200bp and/or ~1200bp. In the second PCR, primer set B was used with expected amplicon size of ~600bp and/or ~750bp, whereas in the final PCR, primer set C was used with expected amplicon size of ~300bp and/or ~400bp.

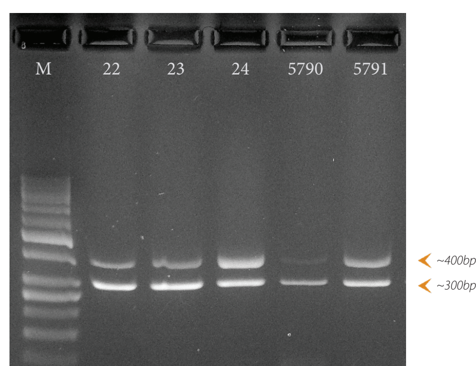

Fig 4. *Amplification using primer set C. All bands have expected sizes as mice nº5790 is normal and all others are heterozygote KI. (Marker in lane 1 is GRS Ladder 50bp).*

CONCLUSION

DNA extraction is carried out in a single tube, without the need of multiple washing steps, therefore minimizing the risk of contamination. DNA is ready for PCR in just 15 minutes! Xpert Fast Hotstart DNA polymerase, with its extension rate of 4-8 kb/min, not only allows for fast PCR, but in combination with optimized buffer ensures high yield and sensitivity. Upon completion of PCR, the reaction is ready for direct loading onto an agarose gel without the need of adding loading buffer, making this an ideal product for mouse genotyping, which can be completed in as little as one and a half hours.