

KAPA Express Extract & KAPA SYBR FAST

Mouse Genotyping

To demonstrate the use of KAPA Express Extract for fast and reliable real-time PCR template preparation, DNA was extracted from commonly used mouse tissues and used as template in four mouse genotyping qPCR assays with KAPA SYBR FAST. KAPA Express Extract reactions were performed as outlined in **Tables 1** and **2**. DNA extracts were used as template in qPCR with KAPA SYBR FAST 2X Universal MasterMix, performed as outlined in **Tables 3** and **4** using the Eppendorf realplex qPCR instrument. Amplicons used in this study ranged in size and GC content from 231 – 360 bp and 55 – 64% GC.

Results (**Figure 1**) obtained with DNA extracted from mouse tail and ear with KAPA Express Extract was compared to those obtained with 1 ng of purified mouse genomic DNA. All four amplicons were successfully amplified with KAPA SYBR FAST. Best results (earliest Ct scores) were obtained with DNA extracted from mouse tails with KAPA Express Extract. Results with DNA extracted from mouse ears with KAPA Express Extract were equal or better than those obtained with 1 ng of mouse genomic DNA.

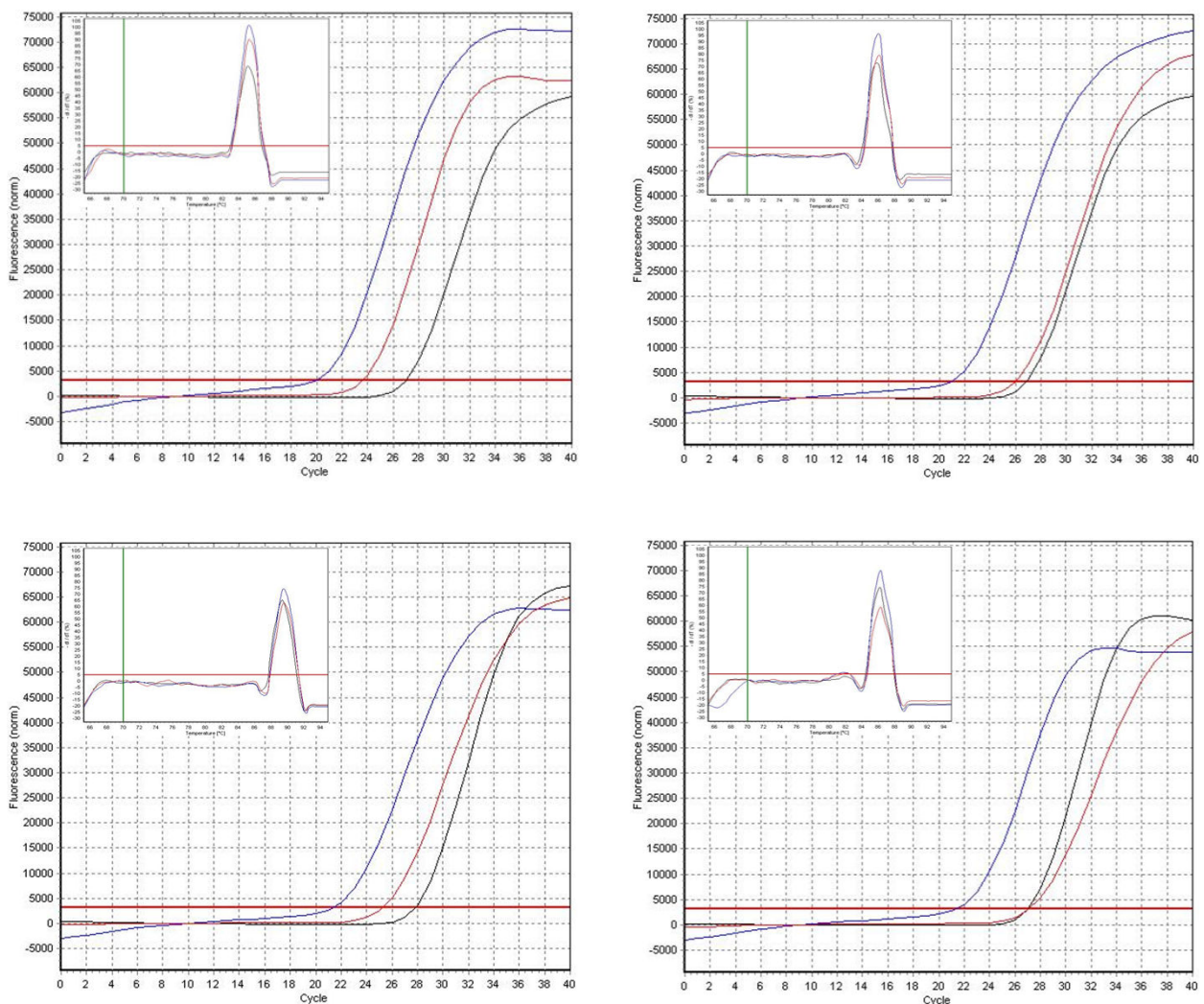


Figure 1: Amplification plots and melt curves of amplicon 1 (top left, 231 bp and 55% GC), amplicon 2 (top right, 360 bp and 55% GC), amplicon 3, (bottom left, 297 bp and 58% GC) and amplicon 4 (bottom right, 312 bp and 64% GC). Blue = 1 µl DNA extracted from mouse tail with KAPA Express Extract; Red = 1 µl DNA extracted from mouse ear with KAPA Express Extract; Black = 1 ng of purified mouse genomic DNA.

Table 1: KAPA Express Extract protocol

Step	Description
Reaction setup	<ol style="list-style-type: none"> 1. Transfer samples directly into individual 0.2 ml PCR tubes 2. Prepare a bulk lysis solution by combining KAPA Express Extract Buffer, Enzyme and PCR grade water, as outlined in Table 2. 3. Add 100 µl bulk lysis solution to each sample
Lysis	<ol style="list-style-type: none"> 1. Place tubes in a thermocycler 2. Incubate at 75 ° for 10 min. During this step, cells are lysed, nucleases and proteins degraded and DNA released
Heat-inactivation	Incubate tubes at 95 °C for 5 min to inactivate the thermostable KAPA Express Extract Enzyme
Sample recovery	<ol style="list-style-type: none"> 1. Centrifuge tubes for 1 min to pellet debris. 2. Recover DNA-containing supernatant.

<ご注意>

“血液サンプル”をKapaExpressExtractキットで使用された場合、溶解液をリアルタイムPCRに使用されることはお勧めしておりません。
理由:血液由来の“ヘム”が、SYBRGreenの蛍光シグナルを消失させるためです。

Table 2: KAPA Express Extract reaction setup

Reaction component	Final concentration	Per 100 µl reaction	Bulk solution (for 100 rxns)
PCR grade water	-	88.0 µl	8.8 ml
10X KAPA Express Extract Buffer	1X	10.0 µl	1.0 ml
KAPA Express Extract Enzyme (1 U/µl)	20 mU/µl	2.0 µl	0.2 ml

Table 3: KAPA SYBR FAST reaction setup

Reaction component	Final concentration	Per 20 µl reaction
PCR grade water	-	8.2 µl
KAPA SYBR FAST 2X qPCR MasterMix	1X	10.0 µl
Forward primer (10 µM)	200 nM	0.4 µl
Reverse primer (10 µM)	200 nM	0.4 µl
KAPA Express Extract DNA extract	-	1.0 µl

Table 4: KAPA SYBR FAST cycling protocol

Step	Temperature	Duration	Cycles
Enzyme activation	95 °C	3 min	Hold
Denature	95 °C	5 sec	40
Anneal/extend	60 °C	30 sec	40
Dissociation	According to instrument guidelines		