APPLICATION NOTE

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Robust Amplification of Uracil-Containing and Bisulfite-Converted DNA using DeCodiFi™ High-Fidelity DNA Polymerase

Goal

To demonstrate the robust uracil tolerance and amplification capabilities of DeCodiFi™ High-Fidelity Polymerase for challenging sequencing workflows, specifically evaluating its performance with uracil-containing primers and templates, uracil incorporation, and highly fragmented bisulfite-converted DNA from mouse models.

Application Benefits

- **Amplification with uracil-containing primers:** Amplifies long DNA fragments (20.6 kb) using uracil-containing primers, performing with comparable efficiency to standard primers without uracil.

- **Performance with uracil-containing templates and uracil incorporation:** Amplification of degraded or bisulfite-treated DNA without stalling at uracil bases.

- **Amplification of bisulfite-converted DNA:** Consistently amplifies challenging bisulfite- treated targets (429 bp) across samples, including wild-type neuronal DNA and CRISPR-generated knockout mouse model DNA.

Felipe Galleguillos and Fernanda Vera performed the bisulfite conversion case study in the laboratory of Fernando Bustos at Universidad Andrés Bello, Chile.
Please email sales@kurabiotech.com for more information.

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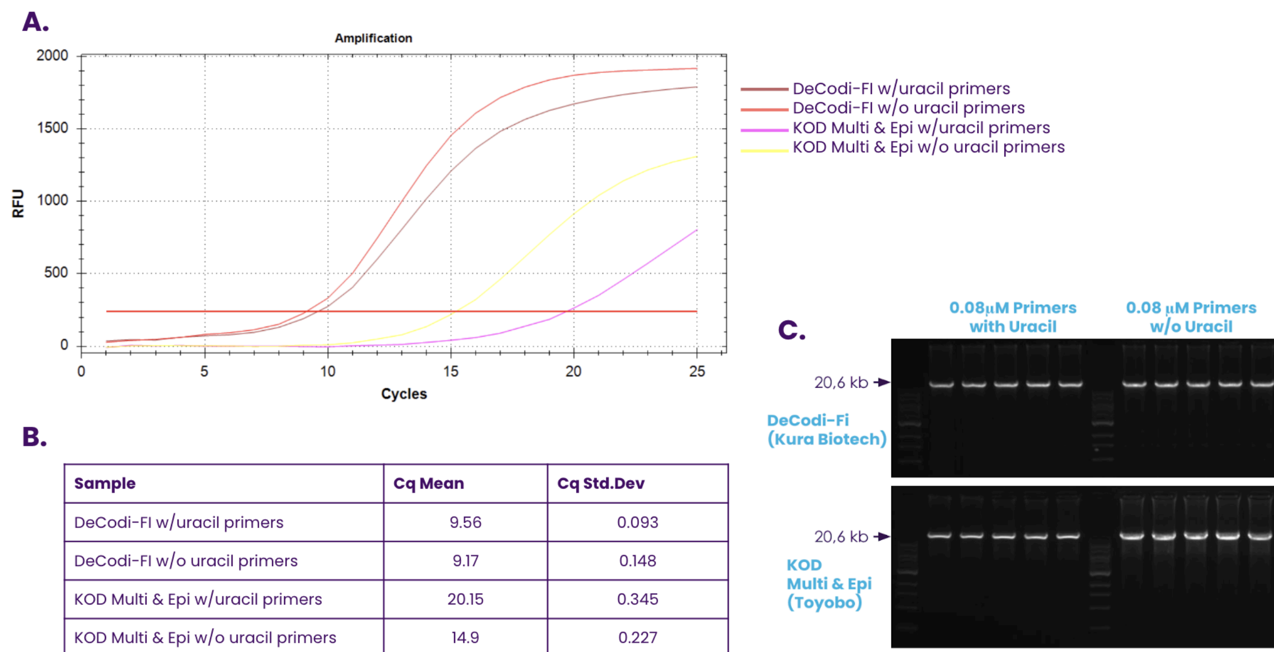
APPLICATION NOTE

Amplification with DeCodiFi™ using uracil-containing primers, uracil-containing templates, and incorporation of dUTPs

20 kb DNA amplification with DeCodiFi™ using uracil-containing primers:

Standard high-fidelity polymerases often stall at uracil residues, whereas DeCodiFi™ efficiently amplifies with uracil-containing primers. Comparable amplification is observed with or without uracil; for uracil-containing primers, a final concentration of 0.08 µM is recommended instead of the standard 0.2 µM. In comparison, KOD Multi & Epi(Toyobo) showed reduced efficiency in the presence of uracil, with a delayed mean Cq when using uracil-containing primers (Figure 1, Table B). This performance could be advantageous for applications such as Kinnex PCR, where efficient amplification with uracil-containing primers is required.



Sample	Cq Mean	Cq Std.Dev
DeCodi-FI w/uracil primers	9.56	0.093
DeCodi-FI w/o uracil primers	9.17	0.148
KOD Multi & Epi w/uracil primers	20.15	0.345
KOD Multi & Epi w/o uracil primers	14.9	0.227

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Figure 1: Amplification of a 20.6 kb lambda target using primers with and without uracil. A 20.6 kb λ DNA fragment was amplified from 2 ng of input DNA using DeCodiFi™ (Kura Biotech) and KOD Multi & Epi (Toyobo). Reactions were performed in five replicates using 0.08 µM primers with or without uracil. PCR conditions included 25 cycles, a 60°C annealing temperature, and a 10-minute extension. Gel electrophoresis was performed by loading 2 µL of PCR product per well alongside a 1 kb ladder.

(A) qPCR amplification curves comparing DeCodiFi™ amplification with and without uracil-containing primers, and KOD Multi & Epi with and without uracil-containing primers.
(B) qPCR results showing the Cq mean and standard deviation for both enzymes and primer conditions.
(C) Agarose gel electrophoresis confirming amplification of the expected 20 kb product for both enzymes with both primer types.

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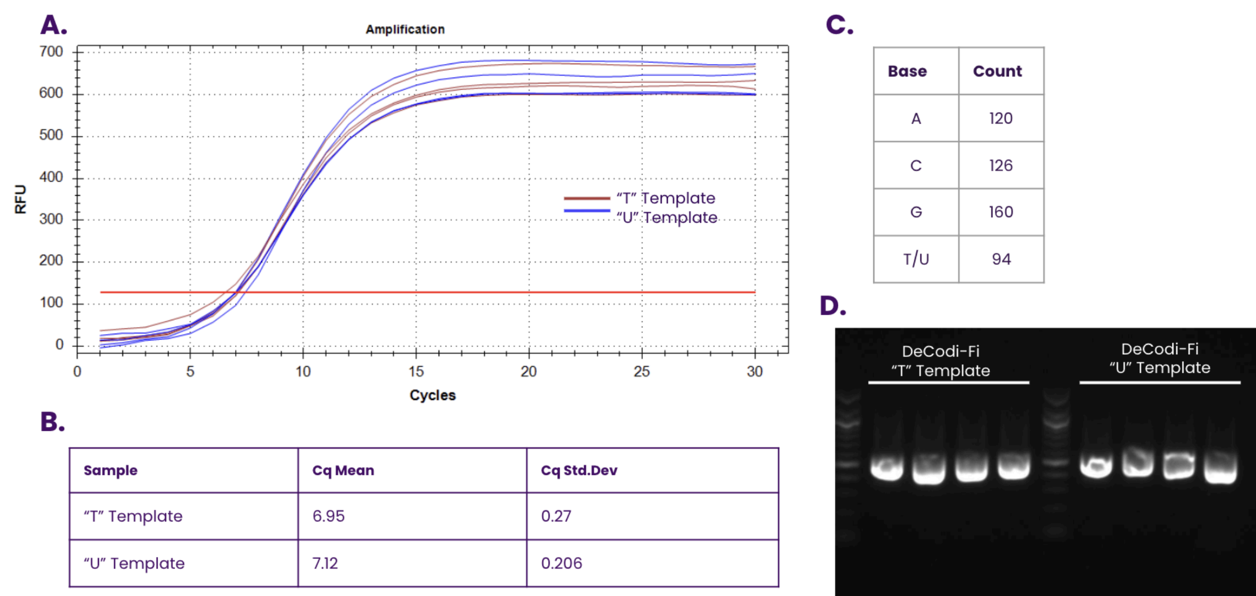
Amplification of uracil-containing templates with DeCodiFi™:

DeCodiFi™ was evaluated for its ability to amplify uracil-containing templates. PCR was performed using a 500 bp dsDNA template with either standard bases (ACG**T**) or uracil-substituted bases (ACG**U**), both containing 94 T/U positions. Reactions (25 µL, n=4) used standard primers and dNTPs and were run for 30 cycles.

Successful amplification was observed for both templates by gel electrophoresis. Cq values were comparable between conditions (**T**: 6.95 ± 0.27(SD); **U**: 7.12 ± 0.21(SD)), confirming efficient amplification of uracil-containing DNA.



Sample	Cq Mean	Cq Std.Dev
"T" Template	6.95	0.27
"U" Template	7.12	0.206

Base	Count
A	120
C	126
G	160
T/U	94

Figure 2. Amplification of standard versus uracil-containing templates using DeCodiFi™.
A 500 bp target was amplified from templates containing either standard bases (ACGT) or uracil-substituted bases (ACG**U**), using 0.5 ng of input DNA. Reactions were performed in triplicate using standard primers and dNTPs (0.3 mM), and amplification was stopped after 30 cycles.

(A) qPCR amplification curves comparing T- and U-containing templates.
(B) qPCR quantitative results showing the Cq mean and standard deviation for both conditions.
(C) Base composition of the templates.
(D) Agarose gel electrophoresis confirming amplification of the expected 500 bp product for both templates.

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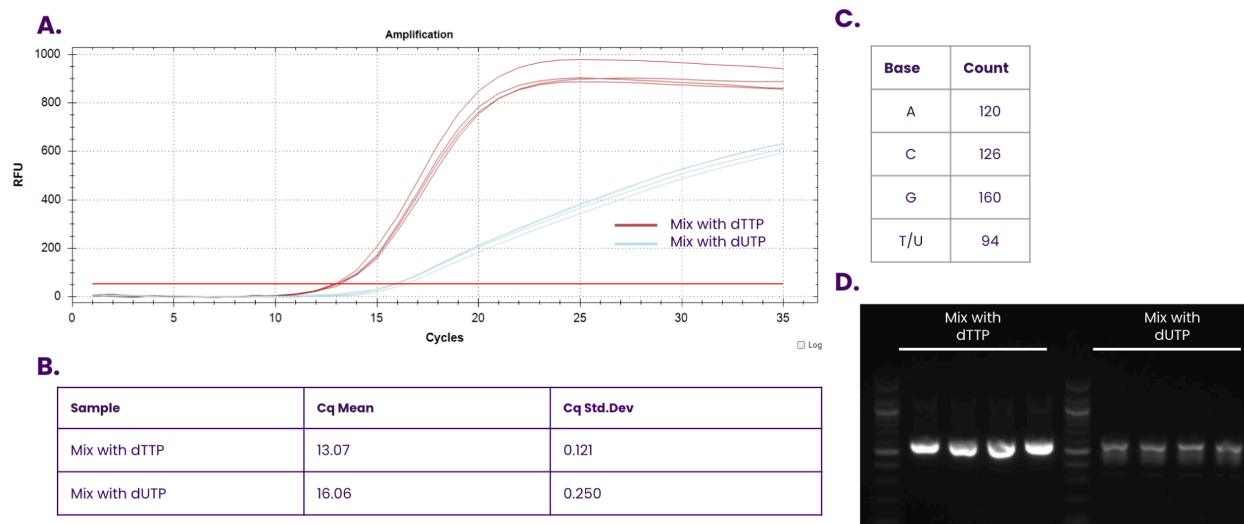
DeCodiFi™ dUTP incorporation efficiency:

DeCodiFi™ dUTP incorporation efficiency was evaluated by amplifying a 500 bp fragment from 2 ng of Lambda gDNA in 25 µL reactions. Amplifications were performed in quadruplicate for 35 cycles using standard primers and either a conventional dNTP mix (A, C, G, **T**) or a dUTP-containing mix (A, C, G, **U**) without dTTP.

Gel electrophoresis confirmed successful amplification under both conditions. Cq analysis showed a mean of 13.07 (±0.12) for standard dNTPs and 16.06 (±0.25) when dUTP replaced dTTP, indicating functional incorporation with an expected decrease in amplification performance.



Sample	Cq Mean	Cq Std.Dev
Mix with dTTP	13.07	0.121
Mix with dUTP	16.06	0.250

Base	Count
A	120
C	126
G	160
T/U	94

Figure 3. Amplification using standard dNTPs(dTTP) versus dUTP-substituted dNTPs(dUTP) with DeCodiFi™.

A 500 bp target was amplified from 2 ng of Lambda gDNA using standard primers and either standard dNTPs (A, C, G, **T**) or dUTP-substituted dNTPs (A, C, G, **U**) at a final concentration of 0.3 mM. Reactions were performed in tetraplicate, and amplification was carried out for 35 cycles.

(A) qPCR amplification curves comparing standard dNTPs(dTTP) and dUTP-substituted dNTPs(dUTP).
(B) qPCR quantitative results showing the Cq mean and standard deviation for both conditions.
(C) Base composition of the 500 bp amplicon.
(D) Agarose gel electrophoresis confirming amplification of the expected 500 bp product under both conditions.

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Case Study: Amplification of Bisulfite-Converted Mouse DNA

Bisulfite treatment is widely used to study DNA methylation and epigenetic changes. However, this process introduces uracil residues and fragments the DNA, making these samples more challenging to amplify. Collaborating researchers used DeCodiFi™ to amplify bisulfite-converted DNA to assess epigenetic changes in CRISPR-generated autism mouse models. The target was the TSS1 promoter region of the *Kcc2b* isoform (429 bp) from bisulfite-treated samples, including neuronal DNA at 3 and 15 days in vitro (3 DIV and 15 DIV) and two autism knockout models (VPS50 KO and MeCP2 KO).

KAPA 2G(non-proofreading), previously used for bisulfite sequencing, was included for comparison. Overall, DeCodiFi™ showed good yield, reduced smearing, and required a lower primer amount (0.2 µL per reaction), which may also help reduce nonspecific amplification and primer-dimer formation—an advantage when generating clean PCR products for downstream applications such as cloning.

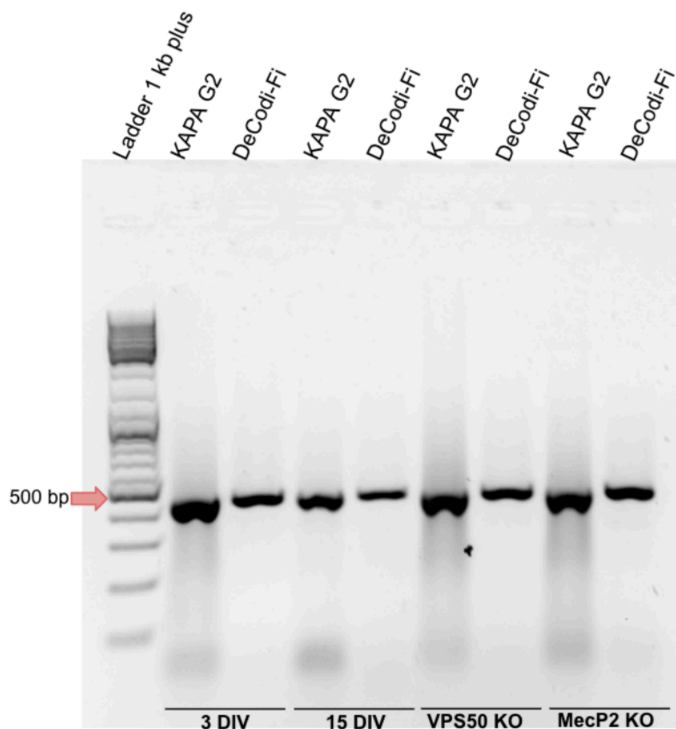

Figure 4: Comparison of conventional PCR amplification using DeCodiFi™ and KAPA G2. A ~429 bp fragment corresponding to the TSS1 region of *Kcc2b* isoform was amplified from bisulfite-treated DNA. Samples included neuronal DNA at 3 and 15 days in vitro (3 DIV and 15 DIV) and two knockout models (VPS50 KO and MeCP2 KO). PCR conditions followed the recommended protocols for each enzyme. Including an annealing temperature of 62 °C and a recommended 0.4 µM final concentration primers, and 58 °C and 0.2 µM, for KAPAG2 and DeCodiFi™, respectively. Products were analyzed by agarose gel electrophoresis alongside a 1 kb Plus DNA ladder.

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Conclusion

DeCodiFi™ High-Fidelity Polymerase demonstrates consistent amplification capabilities for applications involving uracil. It efficiently amplifies uracil-containing templates and supports uracil incorporation, successfully generating 20.6 kb fragments with uracil-containing primers while maintaining higher efficiency and lower Cq values than KOD Multi & Epi. It also robustly amplifies bisulfite-converted DNA from mammalian models (wild-type and knockout), providing the performance required for downstream sequencing-based applications.

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