The Best Polymerase for PCR Genotyping

TITANIUM™ *Taq* outperforms the competition!

- **Robust**
- **Unrivaled sensitivity**
- **Minimal optimization required**
- **Room temperature set-up**

TITANIUM *Taq* DNA Polymerase is a highly sensitive, robust enzyme that's ideal for amplifying targets from any DNA template, including bacterial and plasmid DNA, cDNA, and complex genomic DNA. This versatile enzyme is perfect for all challenging PCR applications.

TITANIUM *Taq* contains an N-terminal deletion mutant of *Taq* polymerase that lacks 5'-exonuclease activity (1). This novel enzyme also contains carefully engineered amino acid substitutions that increase its solubility. For maximum performance, the enzyme has been blended with **TaqStart® Antibody,** a hot start antibody that provides increased specificity and yield (2), making TITANIUM *Taq* a highly sensitive and robust PCR polymerase for many applications.

TITANIUM Challenge

We recently challenged researchers at Columbia University to compare TITANIUM *Taq* to any other general-use PCR polymerase on the market. Two researchers, Dr. Li Song and Dr. Charles LeDuc, used TITANIUM *Taq* to genotype transgenic mice by PCR. Both scientists found TITANIUM to be a vast improvement over the other enzymes they tried.

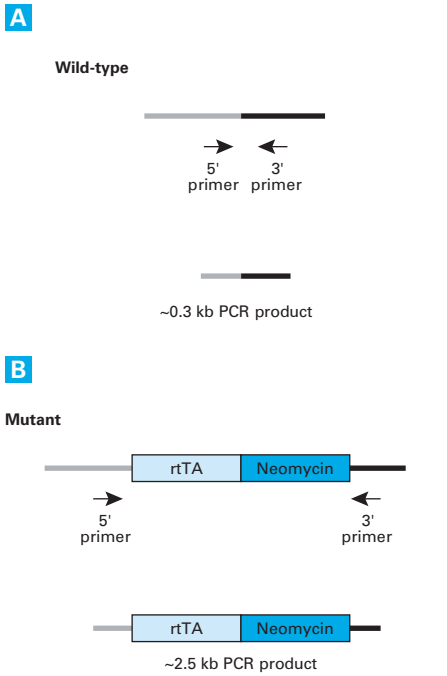

Figure 1. Genotyping strategy. Wild-type and mutant alleles were identified by PCR using gene-specific primers that flanked the insertion site of the rtTA knock-in gene. According to this strategy, the wild-type allele should generate a 0.3 kb PCR product (**Panel A**), while mutant alleles containing the rtTA-neo cassette should generate a 2.5 kb PCR product (**Panel B**). Note: The figure is not to scale.

Dr. Song performed a PCR genotyping assay to distinguish wild-type mice from heterozygous and homozygous rtTA (Tet-On®) knock-in mice. As shown in Figure 1, the primers used in the assay flanked the insertion site of the rtTA-neomycin cassette used to create the mutation. Accordingly, wild-type alleles (i.e., those lacking the rtTA-neo cassette) were expected to generate a 0.3 kb PCR product (Figure 1, Panel A), while mutant alleles (i.e., those containing the rtTA-neo cassette) were expected to generate a 2.5 kb PCR product (Figure 1, Panel B).

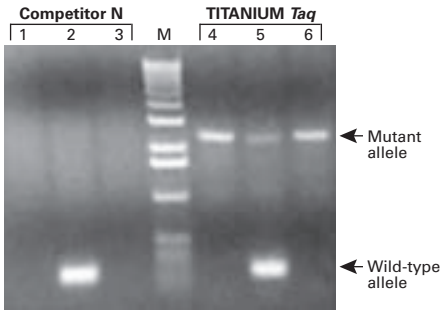

Figure 2. PCR Genotyping—TITANIUM *Taq* vs. a competitor polymerase. Genomic DNA from three different mice was analyzed by PCR in an attempt to distinguish between wild-type and mutant alleles. Using the genotyping strategy described in Figure 1, PCR was carried out with gene-specific primers and either TITANIUM *Taq* DNA Polymerase or a competitor enzyme (according to manufacturer specifications). TITANIUM *Taq* successfully amplified the mutant allele present in all three samples (Lanes 4–6). Competitor N, on the other hand, failed to amplify the mutant allele in any of the samples (Lanes 1–3). Lane M: DNA size marker.

In his assay, Dr. Song used these primers and either TITANIUM *Taq* or a competitor enzyme to amplify genomic DNA from three different mice, in order to see how well the two enzymes amplified the wild-type and mutant alleles (Figure 2). TITANIUM *Taq* successfully amplified both alleles, allowing the homozygous rtTA knock-in mice (Figure 2, Lanes 4 and 6) to be easily distinguished from the heterozygous rtTA knock-in mouse (Lane 5). The competitor enzyme, on the other hand, failed to amplify the mutant allele in any of the DNA samples. In fact, Dr. Song commented that he had never been able to see both bands until he used TITANIUM *Taq*.

The Best Polymerase for PCR Genotyping...continued

TITANIUM outperforms the competition

In Dr. LeDuc's assay, PCR was used to detect homozygous E-cadherin floxed alleles in transgenic mice. Such mutant alleles contain two *loxP* sites flanking exons 6–10 of the mouse E-cadherin gene (*Cdh-1;* 3). Floxed alleles were identified through the use of gene-specific primers that flanked the *loxP* site in intron 10 (see Figure 3); these mutant alleles were expected to generate a 980 bp amplicon, whereas wild-type alleles would generate a 900 bp amplicon. To amplify the region of interest, Dr. LeDuc used these primers and either TITANIUM *Taq* or one of two competitor polymerases (Figure 4). It is clear from Dr. LeDuc's data that TITANIUM *Taq* was the only enzyme able to amplify the desired region (Figure 4, Lanes 1 and 4). Both competitor enzymes failed completely (Figure 4, Lanes 2, 3, 5, and 6).

Dr. Song's and Dr. LeDuc's data clearly show what we've always known: TITANIUM *Taq* DNA Polymerase provides robust and reliable results for even the most challenging assays. This saves you time and money, and allows you to focus on what is most important—your research, not experimental design. Try TITANIUM *Taq,* and see how it can benefit your research.

References

1. Barnes, W. M. (1992) *Gene* **112**(1):29–35.
2. Kellogg, D. E. *et al.* (1994) *Biotechniques* **16**(6):1134–1137.
3. Boussadia, O. *et al.* (2002) *Mech Dev.* **115**(1–2):53–62.

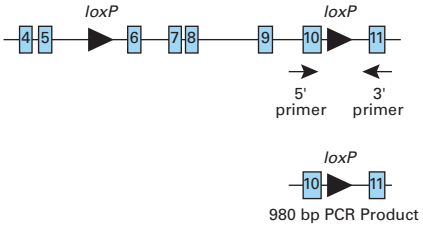

Figure 3. Strategy for the detection of E-cadherin floxed mice. Mutant alleles were detected by PCR using gene-specific primers flanking the *loxP* site in intron 10 of the E-cadherin gene (above). Amplification of the indicated region of the floxed allele should generate a 980 bp PCR product. In the schematic representation above (adapted from Reference 3), the *loxP* sites are represented by black arrowheads, and the exons by boxes. Note: The figure is not to scale.

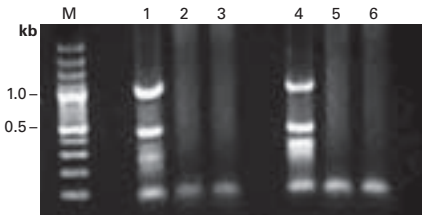

Figure 4. Detection of E-cadherin floxed mice—TITANIUM *Taq* versus two competitor enzymes. Genomic DNA from two E-cadherin floxed mice was analyzed by PCR using the primers described in Figure 3, and either TITANIUM *Taq* DNA Polymerase (Lanes 1 and 4), Competitor I polymerase (Lanes 2 and 5), or Competitor P polymerase (Lanes 3 and 6), according to each manufacturer's specifications. TITANIUM *Taq* successfully amplified the 980 bp target region (Lanes 1 and 4), whereas both competitor enzymes failed completely. Lane M: 100 bp DNA size marker.

Product	Size	Cat. No.
TITANIUM *Taq* DNA Polymerase		
	100 rxns*	639208
	500 rxns*	639209
	1000 rxns*	639242
TITANIUM *Taq* PCR Kit		
	30 rxns*	639211
	100 rxns*	639210

* Based on a reaction volume of 50 µl.

Components

- TITANIUM™ *Taq* DNA Polymerase
- 5X Reaction Buffer

Related Products

- Advantage® 2 Polymerase Mix and Kits (Cat. Nos. 639201, 639202, 639206 & 639207)

Notice to Purchaser

Please see the TITANIUM™ PCR Products, Hot Start Antibody, and PCR licensing statements on page 40.