User Guide

Surveyor® Mutation Detection Kit for Standard Gel Electrophoresis

This guide applies to catalog numbers: **706025, 706020, 706021**

www.IDTDNA.com/Surveyor

Revision: 1

For Research Use Only

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Introduction

IDT Surveyor Mutation Detection Kits use a mismatch-specific DNA endonuclease to scan for known and unknown mutations and polymorphisms in heteroduplex DNA. Surveyor Nuclease, the key component of the kit, is an endonuclease that cleaves DNA with high specificity at sites of base-substitution mismatch and other distortions[1]. These DNA endonucleases cut both strands of a DNA heteroduplex on the 3'-side of the mismatch site. Insertion/deletion mismatches and all base-substitution mismatches are recognized, but the efficiency of cleavage varies with the sequence of the mismatch[1].

Surveyor Nuclease has been shown to be a robust and reproducible tool for mutation detection[1-3]. It has been used to detect accurately a variety of mutations and polymorphisms in the human, mammalian, bacterial and plant genomes (see Appendix D)

The Surveyor Mutation Detection Kit for Standard Gel Electrophoresis has been designed to cleave unlabeled DNA fragments at mismatched sites for subsequent analysis by agarose gel electrophoresis or polyacrylamide gel electrophoresis (PAGE). DNA 200 to 4000 bp long can be analyzed using agarose gel electrophoresis while smaller fragments (<1000 bp) can be analyzed using polyacrylamide gel electrophoresis (PAGE).

The Surveyor Mutation Detection Kit was developed for use with Transgenomic Optimase or Maximase DNA polymerases and reaction conditions have been optimized for the reaction buffers included with those enzymes. However, the Surveyor Mutation Detection Kit will perform well with many other commercially available polymerase buffers, with some minor adjustments. Digestion reaction conditions for DNA amplified by PCR using most commercially available DNA polymerases are provided in Appendix A; these reaction conditions increase the amounts of mismatch cleavage products generated by Surveyor Nuclease from DNA containing genetic variations and reduce non-specific enzymatic activity.

To use this kit successfully, we strongly recommend that you:

- **Read this manual thoroughly and carefully follow the instructions and guidelines provided.**
- **Verify the compatibility of your PCR reaction buffers and conditions with Surveyor Mutation Detection kits by referring to Appendix A (page).**
- **Perform the control experiments outlined in the section *Control Experiments—Using Control C and Control G Plasmid DNA.***

If you have further questions or need assistance, please contact IDT Customer Care at:

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Kit Components and storage

The Surveyor Mutation Detection Kit for Standard Gel Electrophoresis is available in three sizes:

- 25-Reaction Kit (Catalog No. 706025)
- 100-Reaction Kit (Catalog No. 706020)
- 1000-Reaction Kit (Catalog No. 706021)

Component	25-Reaction Kit (706025) Amount Provided	100-Reaction Kit (706020) Amount Provided	1000-Reaction Kit (706021) Amount Provided
Surveyor Nuclease S	30 µL	4 x 30 µL	1 x 1.0 mL
Surveyor Enhancer S	30 µL	4 x 30 µL	1 x 1.0 mL
0.15 M $MgCl_2$ Solution	250 µL	3 x 250 µL	3 x 1.5 mL
Stop Solution	250 µL	3 x 250 µL	3 x 1.5 mL
Control C	20 µL	20 µL	20 µL
Control G	20 µL	20 µL	20 µL

Store the kit at –20°C. When stored correctly, the kit will perform up to 6 month after purchase.

Detecting Mutations with the IDT Surveyor Mutation Detection Kit

An Overview

Mutation detection and confirmation with Surveyor Nuclease involves four steps:

Step 1—Prepare PCR amplicons from mutant (test) and wild-type (reference) DNA.

Step 2—Mix equal amounts of test and reference DNA; hybridize them by heating and cooling the mixture to form hetero- and homoduplexes.

Step 3—Treat the annealed heteroduplex/homoduplex mixture with Surveyor Nuclease. The reference DNA alone, treated similarly, serves as a negative control.

Step 4—Analyze the DNA fragments by agarose gel electrophoresis or polyacrylamide gel electrophoresis. The formation of new cleavage products, due to the presence of one or more mismatches, is indicated by the presence of additional bands. The relative size of these cleavage products indicates the location of the mismatch or mismatches.

Factors Affecting the Quality of Results

The following factors influence the quality of results when using Surveyor Nuclease.

- **The quality of the genomic DNA to be amplified**

High quality DNA (from fresh or frozen cells or tissue) should be used. The DNA should have a concentration of >5 ng/µL as determined by absorbance at 260 nm, have an absorbance ratio at 260/280 nm of >1.7 and be >90% DNA (i.e. free of most tRNA and rRNA contamination as judged by appearance on an agarose gel). Store DNA samples at –20°C.

If the DNA template is extracted from paraffin-embedded tissue, several additional precautions can be taken. The extracted DNA can be treated with uracil DNA glycosylase to prevent

amplification of DNA fragments containing deaminated C residues[4]. Often a high percentage of the A_{260} adsorbing material extracted from paraffin-embedded tissue does not amplify well during PCR. Using a larger amount of starting DNA, e.g. ~50 ng versus 10 ng, may help to produce a reasonable amplification product.

- **The quality and quantity of the PCR amplified DNA**

PCR should produce a sufficiently high yield (>25 ng/µL) of a **single** amplified species of the correct size. Verify each amplified DNA product produces a single band of the expected size by gel electrophoresis or other methods. We strongly recommend the use of a proofreading DNA polymerase (e.g., Optimase Polymerase; Transgenomic) to reduce the amount of base misincorporation during PCR (which leads to the generation of 'false' mutations and spurious Surveyor Nuclease cleavage fragments). Similarly, non-specific PCR fragments can be interpreted as mutations and can mask Surveyor Nuclease mismatch cleavage products. If possible, a reference DNA should be digested with Surveyor Nuclease and run to exclude spurious background by visual comparison. Primer-dimers should be strenuously avoided as their presence dramatically inhibits Surveyor Nuclease cleavage at mismatch sites. The amount of DNA used as substrate for Surveyor Nuclease digestion should be at least 200 ng and as much as 400 ng. All of the digested DNA should be analyzed during gel electrophoresis.

- **Intensity of transilluminator fluorescence signal for the intercalating dye used to stain the DNA sample**

We recommend the use of ethidium bromide (EtBr) to stain DNA, but alternative fluorescent dyes such as SYBR® Gold, can also be used with the appropriate detection system. For EtBr-stained DNA, a transilluminator platform emitting light at the appropriate wavelength (i.e., 250–300 nm) must be used.

- **The sensitivity of the photography system used to capture the image of the gel**

The sensitivity of commercial digital photography systems vary. Differences in signal intensities captured by two different photography systems are shown in Figure 1. Additional suggestions for photographing gels are given in *Appendix B: Agarose Gel Electrophoresis* and *Appendix C: Polyacrylamide Gel Electrophoresis*.

- **Suppression of DNA end nicking**

In addition to its mismatch-specific activity, Surveyor Nuclease also has 5' exonuclease activity that attacks the ends of double-stranded DNA increasing background signal during extended incubations. This activity can be suppressed by the addition of extra $MgCl_2$ prior to Surveyor Nuclease digestion. See the table in Appendix A to verify reaction conditions for many commercial PCR enzymes.

- **The composition of the PCR buffer**

Commercially available PCR buffers vary dramatically in content and the contents are often not defined by suppliers. A few buffers are **NOT** compatible with Surveyor Nuclease due to pH or due to the presence of additives, surfactants or other proprietary ingredients. **Surveyor Nuclease reaction conditions have been defined for a large number of different PCR buffers that improve signal intensity significantly (1.5 to 4 fold, depending upon the buffer) while maintaining low background. These reaction conditions are included in the protocols**

described in this User Guide (see *Step 3—Treatment with Surveyor Nuclease* and *Appendix A: Surveyor Nuclease Reaction Conditions*).

- **Signal to Noise ratio**

The signal to noise ratio is generally high enough to detect mutations present at a low percentage of the total DNA template. It is possible to detect 1%–20% mutant DNA mixed with Wild-Type depending upon the particular DNA amplicon, its size, the number and type(s) of mutation(s) and the analysis platform. Figure 1 shows the digestion products generated with homoduplex and heteroduplex Control DNA (included in this kit) and from genomic DNA containing normal and mutant APC genes (not provided in this kit) analyzed by agarose gel electrophoresis. The mutation-specific cleavage products are clearly seen as two new bands of the expected sizes when compared to the DNA size marker.

Examples of Results

Examples of results obtained using the Surveyor Mutation Detection Kit for Standard Gel Electrophoresis for agarose gel electrophoresis or PAGE are shown in Figures 1 through 3 below.

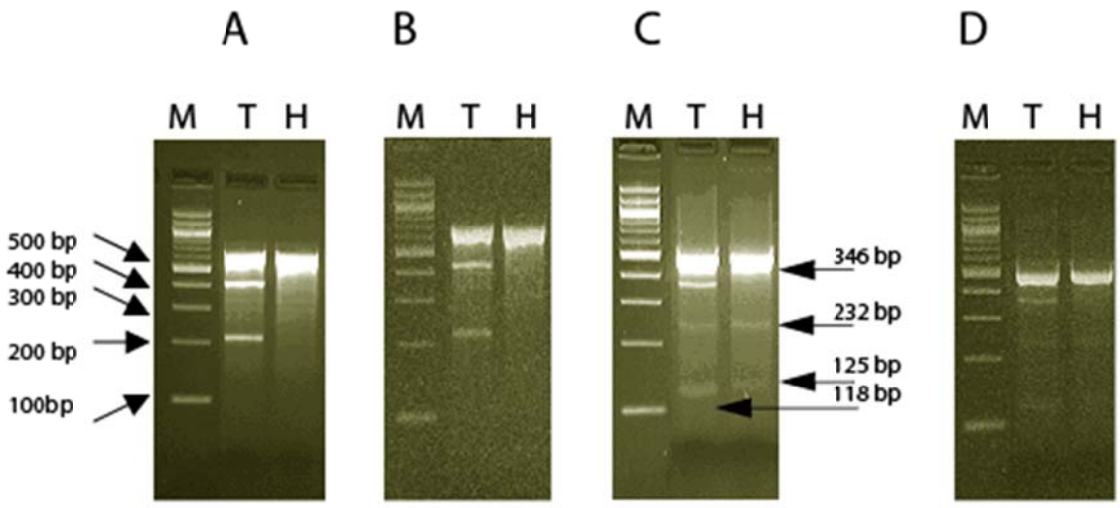


Figure 1. Surveyor Nuclease digestion products of amplicons derived from two different genes.

Panel A and B: A 632 bp amplicon was PCR amplified from 2 µL of Control G and Control C plasmid DNA (contained in this kit) with Optimase Polymerase. Control G/C heteroduplex/ homoduplex was formed by hybridizing equal amounts of Control G and Control C homoduplex PCR product and contains homoduplexes and C/C and G/G mismatched hetero-duplexes. Control G PCR product was self-hybridized to serve as a homoduplex control. Control G/C heteroduplex/homoduplex DNA (**lane T**) and Control G homoduplex DNA (**lane H**) (400 ng) were digested in PCR buffer with 1 µL of Surveyor Nuclease S. Incubation was for 20 min at 42°C. Surveyor Nuclease cleavage at the mismatches produces products of 217 bp and 416 bp. Digestion products were analyzed by gel electrophoresis on a 2.5% agarose gel in 1X TBE buffer. Marker DNA (100 bp DNA Ladder; New England BioLabs) is shown in **lane M**.

Panel C and D: A 464 bp amplicon from the human APC gene was PCR amplified from 100 ng of human cell line SW-480 DNA (mutant) and normal human genomic DNA (Promega) (wild-type) with Optimase Polymerase. The APC gene amplicons were hybridized in equal amounts and the normal amplified DNA was self-hybridized to form a control. APC gene cross-hybridized mutant/wild-type DNA (**lane T**) and self-annealed wild-type DNA (**lane H**) (400 ng) were digested with 2 µL of Surveyor Nuclease S. Incubation was for 20 min at 42°C. Surveyor Nuclease cleaves at a C>T point mutation to generate cleavage products of 118 bp and 346 bp. A SNP present in the APC gene in both mutant and wild-type DNA is cut by Surveyor Nuclease to produce two cleavage products of ~232 bp. Both mutant and wild-type DNA also contain a PCR artifact DNA migrating at 125 bp that was present before Surveyor Nuclease treatment. Digestion products were analyzed by gel electrophoresis on a 2.5% agarose gel in 1X TBE buffer. Marker DNA (**lane M)** as above. In all four panels DNA was stained with EtBr and was transilluminated at 254 nm for capture of the gel image by digital photography. In **Panel A** and **Panel C** the image was captured with an AlphaImager™ 2200 Imaging System (Alpha Innotech Corp.). In **Panel B and D** the image was captured with an EDAS 290 System (Kodak).

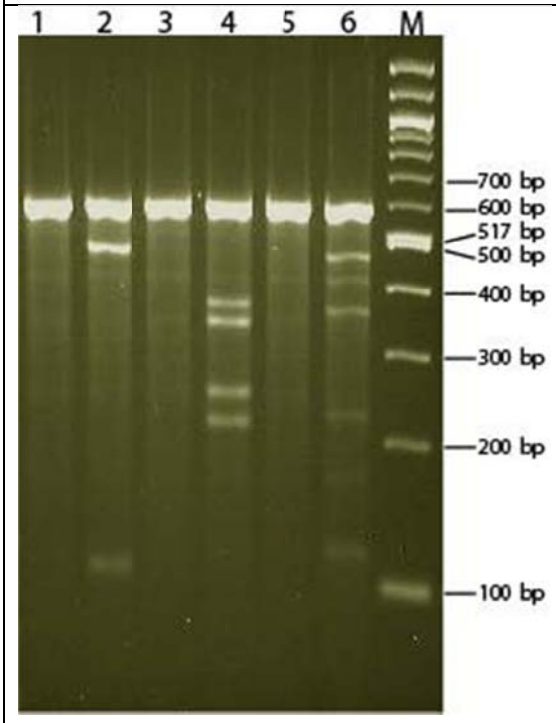
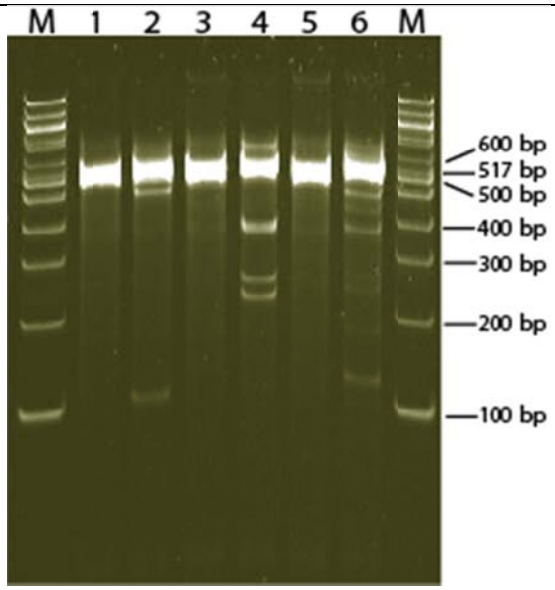
A. Agarose Gel Electrophoresis Using a 2% Agarose Gel	B. Polyacrylamide Gel Electrophoresis Using a 10% Polyacrylamide Gel
	

Figure 2. Surveyor Nuclease digestion products of amplicons derived from three mutants and the wild type sequence of the *E. coli lacZa* gene. The 584 bp amplicons were PCR amplified with Optimase® Polymerase (Transgenomic). Mutant amplicons were annealed either alone or in combination with the wild-type amplicon. Approximately 200 ng of the amplicon DNA were digested by adding 0.5 µL Surveyor Nuclease S directly to the PCR product mix, and incubating this digestion reaction for 20 min at 42°C. Digestion products were analyzed by standard gel electrophoresis using a 2% Agarose gel run in 1X TBE (**Panel A**), or a 10% polyacrylamide gel run in 1x TBE (**Panel B**). Mutant #8 (lane 1, homoduplex; lane 2, hetero/homoduplex) was shown to have one mutation (a single-base deletion); the cleavage products were 109 + 474 bp long. Mutant #10 (lane 3, homoduplex; lane 4, hetero/ homoduplex) was shown to have two mutations (T>C and C>T; the cleavage products were 220 + 364 bp and 248 + 336 bp long). Mutant #13 (lane 5, homoduplex; lane 6 hetero/homoduplex) was shown to have three mutations (single-base deletion, T>C, and C>T; the cleavage products were 120 + 464 bp, 170 + 414 bp, and 226 + 358 bp long). Lane M shows a 100 bp DNA Ladder (New England BioLabs, Beverly, MA).

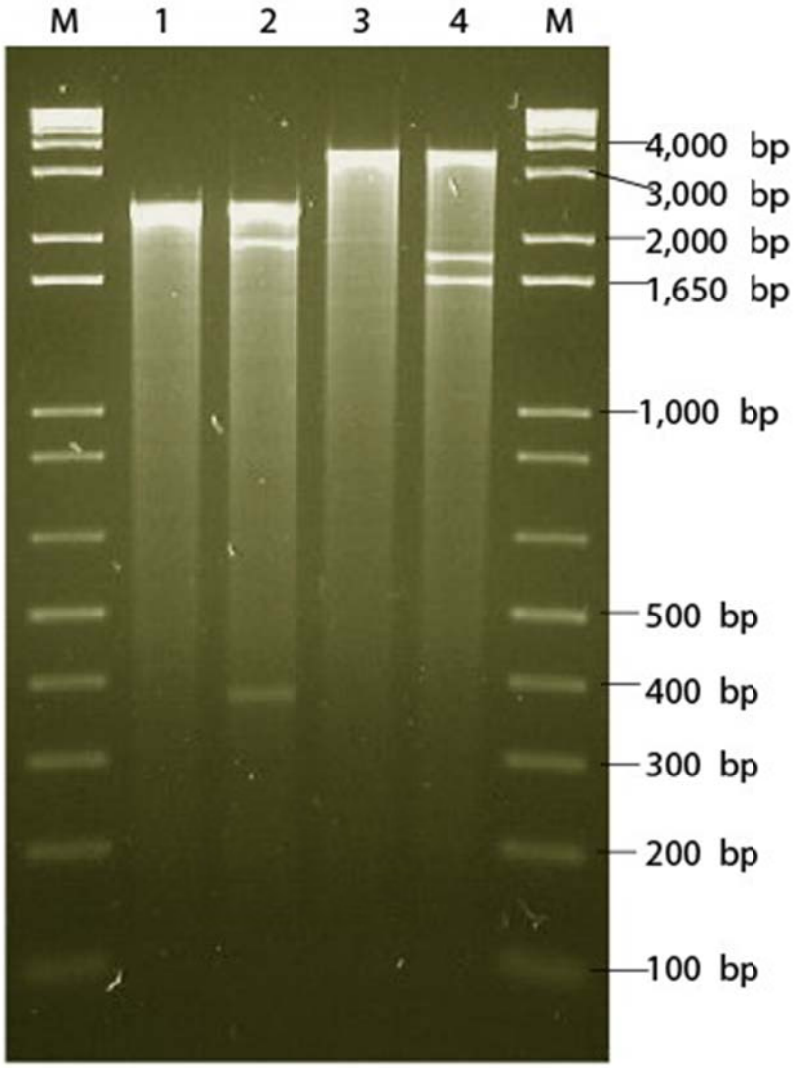


Figure 3. Surveyor Nuclease digestion products of amplicons derived from two mutants and the wild type sequence of the *E. coli rpsL* gene. The 2.3 kb and 3.5 kb PCR products were generated using Maximase™ Polymerase (Transgenomic). Mutant amplicons were annealed either alone, or in combination with the appropriate wild type amplicon. Approximately 200 ng of the unpurified amplicon DNA were digested by adding 0.5 µL Surveyor Nuclease S directly to the PCR product mix and incubating this digestion reaction for 20 min at 42°C. Digestion products were analyzed by standard gel electrophoresis using a 1.5% agarose gel run in 1X TAE. Mutant #1 (lane 1, homoduplex; lane 2, hetero/homoduplex) had a single mutation revealed by the presence of two cleavage products, 371 and 1966 bp, respectively. Mutant #2 (lane 3, homoduplex; lane 4, hetero/homoduplex) also had one mutation as shown by two cleavage products, 1622 and 1846 bp long. Lane M shows a 1 Kb Plus DNA Ladder (Life technologies, Inc., Carlsbad, CA).

Step-by-Step Instructions—Detecting Mutations with Surveyor Nuclease

This section provides detailed instructions for the detection of mutations using the Surveyor Mutation Detection Kit for standard agarose or polyacrylamide gel electrophoresis.

In general, processing of samples should be carried out from start to finish as described in this User Guide. If processing of a sample is stopped before completion of all steps, the DNA should be stored at –20°C until the next step is carried out. Exposure of any frozen sample to repeated freeze-thaw cycles should be avoided and storage at –20°C of PCR amplified DNA or Surveyor Nuclease digestion products for extended periods (>1 week) should be avoided.

Step 1—PCR Amplification of Reference and Test Samples

 **This step is critical to the success of the surveyor nuclease digestion. Do not proceed until:**

- **Your PCR yield is sufficiently high (>25 ng/µL).**
- **Your PCR product has low background (preferably a single species of the correct size).**
- **Your PCR product is free of primer-dimer artifacts.**

Several factors must be considered carefully in preparing PCR amplified DNA to be used as substrate for Surveyor Nuclease analysis. **Primer placement and amplified product quality and yield are crucial to obtaining good results**.

- **Amplicon size**

Fragment size (bp)	Primer placement
<1000	Primers should be placed ≥50 bp outside the region of interest. Place primers so that resulting amplicon cleavage products are ≥70 bp.
1000–4000	Primers should be placed ≥10% of the length of the substrate from the region of interest, ensuring that large digestion products can be separated from undigested substrate and small digestion products produce visible bands.

- **Optimize your PCR conditions carefully.**

  The PCR amplicon should appear as a single sharp peak or band of the expected size when analyzed by microfluidic DNA fragment analyzer or agarose gel electrophoresis. Mispriming during PCR amplification can result in the formation of spurious DNA fragments that produce increased background during Surveyor Nuclease digestion. Use primers that are at least 20 nucleotides long (oligomers 25–35 nucleotides in length are preferred), and have a G-C content of 45–60%. If possible, use a high fidelity DNA polymerase to minimize the introduction of errors that will result in higher background. If after careful design of primers and optimization of PCR conditions non-specific PCR products persist, consider using a hot-start DNA polymerase, a touchdown PCR protocol and/or a

second amplification with nested primers.

- **Amount and concentration of DNA used**

  Both the amount and concentration of DNA in a Surveyor Nuclease reaction mixture influence the efficiency and specificity of Surveyor Nuclease digestion. For the amount of enzyme recommended for use in a reaction mixture (0.5–2 µL of Surveyor Nuclease S), 200–400 ng of substrate at 50 ng/µL is optimal. If DNA product yield is <25 ng/µL, consider a second amplification with nested primers. Alternatively concentrate the DNA by ethanol precipitation and dissolve the DNA pellet in a smaller volume of 1X PCR buffer to increase DNA concentration.

Amplification of Homogeneous DNA Populations

In order to detect a homogeneous mutation in a test sample the PCR product must be hybridized with a wild-type reference PCR product to generate mismatches for Surveyor Nuclease cleavage. Both test sample and wild-type reference DNA are amplified separately with the same PCR primers. Test sample and wild-type reference PCR products are then mixed in a 1:1 ratio to maximize the formation of heteroduplexes during hybridization.

Amplification of Heterogeneous DNA Populations

A DNA sample can be heterogeneous either because it is derived from a heterozygous source or because it contains a pool of fragments derived from genetically different homozygous or heterozygous sources. Such heterogeneous samples can be PCR amplified and hybridized without mixing them with a wild-type reference DNA. The proportion of mutant to wild-type DNA in the population should be above 5–10% for analysis by gel electrophoresis. After hybridization, retain some of the hybridized PCR product as an undigested reference (control).

Sample preparation by PCR

This kit is compatible with standard DNA isolation protocols. Before PCR amplification, make sure you have enough high quality DNA starting material to amplify your gene of interest.

For PCR products <2500 bp in length, we recommend using a proofreading enzyme that is compatible with SNP detection and mutation discovery applications (e.g., Optimase Polymerase; Transgenomic Inc.).

For PCR products >2500 bp in length, use a DNA polymerase blend containing Taq DNA polymerase supplemented with a proofreading DNA polymerase (e.g., Maximase Polymerase; Transgenomic Inc.).

Perform the PCR amplification of test sample and wild-type (reference) DNA using a high fidelity, thermostable DNA polymerase according to the manufacturer's instructions, and follow the steps below.

1. Verify quality and quantity of the amplified DNA by gel electrophoresis, or using a microfluidic DNA fragment analyzer. If a single band matching the predicted amplicon size is visible in each sample proceed to the next step. If multiple bands or primer dimers are visible, do not proceed further, but optimize your PCR reaction condition or change PCR primers.

2. Use a mass DNA ladder, such as a 100 bp DNA Ladder (New England BioLabs, Beverly, MA) as a reference to estimate the concentration of the amplified DNA by visual inspection. The DNA concentration is ideally ~50 ng/µL, but should be in the range of 25 to 80 ng/µL.

3. As long as the DNA polymerase buffer is compatible with Surveyor Nuclease, the amplified DNA can be used without further purification. If the buffer conditions are not compatible, purify using a PCR cleanup kit, enzymatic cleanup kit, or ethanol precipitation.

Control Experiments—Using Control G and Control C Plasmid DNA

Two plasmid DNAs, Control G and Control C, are provided in the Surveyor Mutation Detection Kit. These two control DNAs are plasmids with inserts that differ at a single base pair. They are provided in separate vials, each at a concentration of 5 ng/µl; **forward and reverse primers needed for PCR amplification are already combined with the plasmid templates**. The sequence of the PCR product for Control G is shown below. Control C differs from Control G because it has a Cytosine base in lieu of the Guanine base (underlined). Primer sequences are underlined at the 5' and 3' end of the amplicon sequence.

ACACCTGATCAAGCCTGTTCATTTGATTACCAGAGAGACTGTCATGATCCACATGGAGGGAAGGACATGTGT
GTTGCTGGAGCCATTCAAAATTTCACATCTCAGCTTGGCCATTTCCGCCATGGAACATCTGATCGTCGATAT
AATATGACAGAGGCTTTGTTATTTTTATCCCACTTCATGGGAGATATTCATCAGCCTATGCATGTTGGATTT
ACAAGTGATATGGGAGGAAACAGTATAGATTTGCGCTGGTTTCGCCACAAATCCAACCTGCACCATGTTTGG
GATAGAGAGATTATTCTTACAGCTGCAGCAGATTACCATGGTAAGGATATGCACTCTCTCCTACAAGACATA
CAGAGGAACTTTACAGAGGGTAGTTGGTTGCAAGATGTTGAATCCTGGAAGGAATGTGATGATATCTCTACT
AGCGCCAATAAGTATGCTAAGGAGAGTATAAAACTAGCCTGTAACTGGGGTTACAAAGATGTTGAATCTGGC
GAAACTCTGTCAGATAAATACTTCAACACAAGAATGCCAATTGTCATGAAACGGATAGCTCAGGGTGGAATC
CGTTTATCCATGATTTTGAACCGAGTTCTTGGAAGCTCCGCAGATCATTCTTTGGCG

PCR amplification of 2 µL of each DNA solution in a 50 µL reaction should produce >25 ng/µL of a 633 bp PCR product. Sufficient DNA is provided to perform ten PCR reactions with each control.

Control G and Control C plasmids can be used to troubleshoot the PCR amplification, hybridization and Surveyor Nuclease digestion steps of the Surveyor Nuclease Kit.

WE STRONGLY RECOMMEND THAT FIRST TIME USERS PERFORM THE CONTROL EXPERIMENTS OUTLINED BELOW.

Successful completion of the control experiments will give the user an appreciation for: the yield and quality of PCR product obtained with their amplification system, the amount of the user's amplified DNA that optimally matches the recommended amount of Surveyor Nuclease S in Appendix A, and the expected amount and appearance of digestion products on an agarose or polyacrylamide gel.

Use Control G and Control C as follows.

1. Amplify 2 µL of Control G and 2 x 2 µL of Control C DNA in separate 50 µL reactions using a proofreading DNA polymerase and the PCR program described in *Appendix A: Preparing PCR Products using Optimase Polymerase (Step 4)* where T_a = 65°C and the 72°C extension time is appropriate for the polymerase used, e.g. 1 min for Optimase Polymerase.
2. After amplification, analyze a 2 µL aliquot of each amplified DNA and different amounts of a DNA mass ladder [e.g., 100 bp DNA Ladder from New England BioLabs] on a 2% high resolution agarose gel or a 10% polyacrylamide gel and compare the band intensities with those of the DNA ladder to establish the DNA concentration. See *Appendix B: Agarose Gel Electrophoresis* or *Appendix C: Polyacrylamide Gel Electrophoresis* for details. The yield with Optimase Polymerase is in the range of 25–80 ng/µL.
3. Hybridize Control G- and Control C-amplified DNA in equal amounts (15 µL of each if the amplified DNA concentrations are ≥40 ng/µL) as described in ***Step 2—DNA Hybridization***. This produces a population of molecules containing 50% homoduplex, 25% heteroduplex with a C/C mismatch, and 25% heteroduplex with a G/G mismatch. Also self-anneal 30 µL of Control C homoduplex in a separate tube.
4. Digest hybridized Control G/C and Control C homoduplex with Surveyor Nuclease S. Follow the instructions in ***Step 3—Treatment with Surveyor Nuclease*** to identify the Surveyor Nuclease reaction parameters, i.e. requirements for additional MgCl2 and/or enzyme, that match the PCR

DNA polymerase/buffer formulation used to prepare the DNA substrate. Further optimization can be achieved by carrying out a pilot study matching amount of Control C and Control G/C to the recommended amount of Surveyor Nuclease. An example of this for Optimase Polymerase PCR products is shown in Table 1. Set up 6, 0.5 mL reaction tubes on ice with the indicated amounts of PCR product in Table 1 and the amount of Surveyor Nuclease and additional MgCl2 determined as described in Step 3. Incubate the tubes at 42°C for 60 min. Add 1/10th volume of Stop Solution.

5. Analyze the digested DNA in each reaction mixture as described in ***Step 4—Analysis of DNA Fragments***. Load all the samples on the gel.

Step 2—DNA Duplex Formation

In this step, sample and reference DNA are mixed and hybridized to form heteroduplexes. Make sure to take along sample and/or reference DNA alone in separate tubes to serve as controls. The use of a heated-lid thermocycler is recommended—it is important to carefully follow the protocol shown here.

If your thermocycler cannot be programmed appropriately for hybridization or if it lacks a heated lid, go to the *Performing Heteroduplex Formation without a Thermocycler* section below.

Performing Heteroduplex Formation using a Thermocycler

1. Mix equal amounts of test sample and reference PCR products in a 0.2 mL tube. Place reference DNA alone in a separate 0.2 mL tube. For efficient annealing the final volume should be at least 10 µL.

   Note the following:

   - The concentration of test sample DNA and wild-type reference DNA should be in the range 25–80 ng/µL (**ideally 50 ng/µL**). About 200–400 ng of hybridized DNA is recommended for treatment with Surveyor Nuclease S, so that **each tube should contain at ≥200 ng total DNA**.
   - Heterogeneous test sample DNA that does not require the addition of wild-type, or reference DNA should still undergo the following hybridization procedure to ensure the formation of cleavable mismatches.

2. Place the tube in a thermocycler and run the following program:

Temperature	Time	Temperature ramp
95°C	10 min	
95°C to 85°C		(–2.0°C/s)
85°C	1 min	
85°C to 75°C		(–0.3°C/s)
75°C	1 min	
75°C to 65°C		(–0.3°C/s)
65°C	1 min	
65°C to 55°C		(–0.3°C/s)
55°C	1 min	
55°C to 45°C		(–0.3°C/s)
45°C	1 min	
45°C to 35°C		(–0.3°C/s)
35°C	1 min	
35°C to 25°C		(–0.3°C/s)
25°C	1 min	
4°C	Hold ∞	

The product is now ready to be treated with Surveyor Nuclease for heteroduplex analysis. Continue with *Step 3—Treatment with Surveyor Nuclease*.

Performing Heteroduplex Formation without a Thermocycler

To perform heteroduplex formation without a thermocycler:

1. Mix in equal quantities of the two PCR products to generate the heteroduplex. Set up reference DNA in a separate tube as above.
2. Incubate the mixture at 95°C for 5 min in a 1-liter beaker filled with 800 mL of water and then allow the water to come to <30°C.

Note:

Because of evaporation of liquid at the tube bottom and condensation under the tube lid, the volume in a tube should be ≥20 µL so that sufficient volume is present to prevent the concentrations of constituents in the mixture from changing substantially during the hybridization step.

3. Spin the tube contents to the bottom of the tube and mix.

The product is now ready to be treated with Surveyor Nuclease for heteroduplex analysis.

Continue with *Step 3—Treatment with Surveyor Nuclease*.

Step 3—Treatment with Surveyor Nuclease

In this step, the heteroduplex test sample DNA from Step 2 is cleaved by the Surveyor Nuclease, along with necessary reference DNA and controls.

Optimized Mg^{2+} concentration is essential to Surveyor Nuclease function. If a different PCR DNA polymerase is used to amplify the DNA, consult the table in Appendix A for appropriate Surveyor Nuclease reaction conditions to digest the DNA. If the DNA polymerase you are using is not listed in the Appendix A table, consult the instructions in Appendix A to select the appropriate reaction conditions.

Digest the hetero/homoduplex DNA experimental samples and any reference DNA in separate tubes.

Set up Surveyor Nuclease reactions for Control G and Control C duplexes as follows:

Tube No.	Volume Required (µL)					
	Hybridized Control G/C	Hybridized Control C	0.15 M $MgCl_2$	Surveyor Enhancer S	Surveyor Nuclease S	Stop Solution (Add after incubation)
1	-	6	0.6	1	1	0.9
2	-	12	1.2	1	1	1.5
3	-	18	1.8	1	1	2.2
4	6	-	0.6	1	1	0.9
5	12	-	1.2	1	1	1.5
6	18	-	1.8	1	1	2.2

Set up Surveyor Nuclease reactions for experimental sample DNA as follows:

1. The volumes of 0.15 M $MgCl_2$ Solution required are calculated based upon the volume (V) of the PCR product used.

2. For each digestion, add the following components in the order shown to a nuclease-free 0.2 mL tube (kept on ice):

Component	**Amount**
Hybridized DNA[1]	200–400 ng
0.15 M $MgCl_2$ Solution[2]	$1/10^{th}$ volume
Surveyor Enhancer S	1 µL
Surveyor Nuclease S	1 µL

[1]DNA volume should be 8–40 µL

[2]This additional magnesium is required even if PCR buffer also contains some magnesium

3. Mix by vortexing gently, by agitation or by aspiration/expulsion in a pipette tip using a micro-

pipetter.

4. Incubate at 42°C for 60 min.

    - **We recommend staring with a 60 min incubation time at 42C. In some cases, reaction time can be reduced to 20 minutes. Increasing reaction time beyond 60 min will increase DNHA degradation due to exonuclease activity of the Surveyor enzyme**

6. Add 1/10th volume of Stop Solution and mix. Store the digestion products at –20°C if not analyzed immediately.

Note:

- DNA prepared with some DNA polymerase/buffer formulations requires double the amount of Surveyor Nuclease S (2 µL; see APPENDIX A table). The amount of Enhancer S used should be kept at 1 µL in all cases.

- To reduce the number of manipulations, MgCl2, Surveyor Enhancer S and Nuclease S can be mixed and a single pipetting can be done. This mixture should be used immediately after preparation and should not be stored, since reducing agent in the Enhancer storage buffer will inactivate Surveyor Nuclease over time.

- When a heterogeneous DNA sample is analyzed, a portion of the hybridized heterogeneous DNA is NOT digested with Surveyor Nuclease and is run as a control in Step 4—Analysis of DNA Fragments.

Continue with *Step 4—Analysis of DNA Fragments*

Step 4—Analysis of DNA Fragments

For DNA fragments in the 200–1500 bp range it is preferable to use 2% Agarose gels, or 10% polyacrylamide gels and a 1X TBE buffer system as shown in Figures 2A and 2B, respectively. Larger DNA fragments in the range of 1000–4000 bp can be analyzed more effectively using 1.5% Agarose gels and a 1X TAE buffer system as shown in Figure 3.

Fragment size (bp)	**Gel type**	**Buffer**
200–1000	2% Agarose, or 10% polyacrylamide	1X TBE
1000–4000	1.5% Agarose	1X TAE

Surveyor Nuclease digestion of hybridized Control G/C PCR products gives rise **to two cleavage products**, 217 and 416 bp in size, which are clearly distinguishable by agarose gel as shown in **Figure 1**. Analysis of different amounts of substrate digested with the recommended amount of Surveyor Nuclease S provides an opportunity to establish the optimal ratio of enzyme to DNA with DNA amplified with your PCR enzyme. The optimal conditions produce the maximum amount of cleavage products while maintaining low background. Keep in mind that PCR amplification of a plasmid template such as Control G or C will result in higher yields of DNA product than amplification of a comparable amount of genomic DNA template. Using 5 to 10 times more genomic DNA (50–100 ng) will give comparable yields of PCR product. This should be taken into consideration in preparing and digesting DNA amplified from genomic DNA (Figure 1).

Appendix A: SURVEYOR Nuclease Reaction Conditions

Because amplified PCR products are hybridized and digested with SURVEYOR Nuclease directly in 1X PCR buffer, careful consideration must be given to the 1X PCR buffer composition. Consult the table in this section for the SURVEYOR Nuclease reaction conditions recommended for use with a number of commercial DNA polymerase/buffer formulations. If the DNA polymerase/buffer formulation is not included in the table, follow the recommendations provided below.

Surveyor Nuclease Reaction Conditions Recommended for use with Various PCR DNA Polymerase/Buffer Formulations

Enzyme	Surveyor Nuclease S[a]	Additional 0.15 M MgCl2 (1/10th PCR volume)
Transgenomic T-Taq DNA Polymerase	1 µL	Yes
Transgenomic Optimase® Polymerase	1 µL	Yes
Transgenomic Maximase™ DNA Polymerase	1 µL	Yes
AmpliTaq® DNA Polymerase	1 µL	Yes
GeneAmp® High Fidelity PCR Kit	1 µL	
iProof™ High-Fidelity DNA Polymerase + HF Buffer	2 µL	
iProof™ High-Fidelity DNA Polymerase + GC Buffer	2 µL	
Hot Master™ Taq DNA Polymerase	1 µL	
AccuPol DNA Polymerase	1 µL	
Platinum® Taq DNA Polymerase	1 µL	Yes
Platinum® Taq DNA Polymerase High Fidelity	1 µL	
Platinum® Pfx DNA Polymerase	2 µL	
Platinum® Pfx DNA Polymerase + PCRx Enhancer	2 µL	
NEB Taq DNA Polymerase	1 µL	Yes
Phusion™ High-Fidelity DNA Polymerase + HF Buffer	2 µL	
Phusion™ High-Fidelity DNA Polymerase + GC Buffer	2 µL	
NovaTaq™ DNA Polymerase	1 µL	Yes
KOD XL DNA Polymerase	1 µL	
HotStar Taq® DNA Polymerase	1 µL	Yes
HotStar Taq® DNA Polymerase + Q-Solution	Not Recommended[b]	
ProofStart™ DNA Polymerase	Not Recommended[b]	
ProofStart™ DNA Polymerase + Q-Solution	Not Recommended[b]	
GoTaq® DNA Polymerase	2 µL	Yes
Roche Taq DNA Polymerase	1 µL	Yes
FastStart Taq DNA Polymerase	1 µL	
FastStart Taq DNA Polymerase + Q Solution	1 µL	Yes
Expand High Fidelity PCR System	1 µL	Yes
Expand High Fidelity^{PLUS} PCR System	1 µL	Yes
JumpStart™ Taq DNA Polymerase	1 µL	

Enzyme	Surveyor Nuclease S[a]	Additional 0.15 M MgCl2 (1/10th PCR volume)
AccuTaq LA™ DNA Polymerase	2 µL	
SureStart® Taq DNA Polymerase	1 µL	Yes
PfuTurbo® Hotstart DNA Polymerase	1 µL	Yes
PfuUltra™ Hotstart DNA Polymerase	1 µL	Yes
Takara Taq™ DNA Polymerase	1 µL	Yes
Takara Ex Taq™ DNA Polymerase	1 µL	Yes
Takara LA Taq™ DNA Polymerase	1 µL	

[a]All reactions contained 1 µL Enhancer S
[b]Not Recommended– reaction conditions could not be found that produced acceptable amounts of digestion products. DNA cleanup is required.

Read the literature associated with your polymerase to determine the concentrations of salts and other components in the 1X PCR reaction buffer before carrying out the hybridization step.

Salt (usually KCl): should be in the range of 50 to 75 mM to ensure that complete annealing of complementary DNA strands takes place. If the PCR product was prepared with a low salt PCR buffer, sufficient 0.5 M KCl should be added to adjust the final KCl concentration to be within the range of 50 to 75 mM. **KCl concentrations above 75 mM inhibit the SURVEYOR Nuclease.**

Other 1X PCR buffer constituants: The constituents of most 1X PCR buffers support efficient digestion of heteroduplex DNA by SURVEYOR Nuclease, including:

- 10 to 20 mM Tris-HCl or Tris-SO_4 (pH 8.8 to 9.3)
- 1 to 3 mM $MgCl_2$ or $MgSO_4$
- 0.1% to 1% (v/v) nonionic detergent and BSA or gelatin
- 10 to 20 mM $(NH4)_2SO_4$
- PCR additives such as DMSO (5% v/v), glycerol (10% v/v), betaine (1 M) and 1X PCR_x Enhancer (Life Technologies) **are acceptable at or below the concentrations listed. If any of these additives are present in the PCR reaction mixture at higher concentrations than those listed, Surveyor Nuclease mismatch cutting activity will be inhibited.**

If an additive is present at an inhibitory concentration, we suggest cleaning up the PCR product Using a commercial PCR clean-up column or ethanol precipitation before the hybridization step. The DNA should then be placed in a buffer compatible with the hybridization and Surveyor Nuclease digestion steps, such as 10 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, and 50 mM KCl..

If the DNA polymerase/buffer formulation is not listed in the table and the manufacturer does not reveal the contents of the PCR buffer, you may carry out pilot Surveyor Nuclease digestion studies with Controls G and C to establish empirically the best digestion conditions. Alternatively, Clean up the DNA and proceed as described above.

Please note that the information above is provided for guidance only and does not imply or constitute a recommendation or validation of any products listed.

Appendix B: Agarose Gel Electrophoresis

Appropriate preparation, electrophoresis, and photography of agarose gels are critical steps to achieving success with Surveyor Nuclease - particularly to achieving maximum detection sensitivity.

Preparing Gels

- Using Table 2 as a guide, select the percentage and type of agarose appropriate for the DNA fragment sizes to be analyzed. For DNA fragments <1.5 kb, use a high resolution agarose that is specifically formulated to resolve smaller DNA fragments at a lower percentage gel (see Table 2), and produces minimal fluorescent background during transillumination and photography. For DNA fragments >1.5 kb, use a standard agarose that can be used to resolve DNA fragments in the 300 to 5000 kb range at the appropriate percentage (see Table 2), and also produces minimal fluorescent background during gel analysis.

Table 2. Relationship of DNA Fragment Size, Agarose Type and Gel Concentration, and Running Buffer

Agarose (%)	DNA Fragment Size (kb)	Buffer
2[a]	<1.5	TBE or TAE
1.5[b]	1.5–2.5	TAE or TBE
1.5[b]	2.5–5	TAE

[a]Specifically formulated high-resolution agarose for visualizing small fragments

[b]Multi-purpose agarose formulated for separation of larger fragments

- Select the appropriate buffer to cast and run the gel. 1X TAE buffer [40 mM Tris-acetate (pH 8.3), 1 mM EDTA] is generally used for resolution of fragments >0.5 kb. For smaller fragments, particularly when analyzing digestion products in the 100 to 300 bp range, 1X TBE [89 mM Tris-borate (pH 8.3), 1 mM EDTA] should be used. 1X TBE helps to reduce diffusion of small DNA fragments during electrophoresis, giving a sharper, more focused band.

- To visualize DNA at any time during an electrophoresis run, cast the gel in a UV-transparent gel tray and incorporate a DNA intercalating dye into the gel-casting buffer. Inclusion of dye in the gel running buffer is not necessary. We recommend the use of EtBr at 0.1–0.5 µg/mL in the gel. Within this concentration range, higher concentrations of EtBr tend to give more intense staining of DNA bands at the cost of higher fluorescence background in the gel lanes.

- Before bringing the agarose into solution by boiling in a microwave oven, hydration of the solid agarose by soaking in buffer for 20–30 min will reduce melting time, foaming, and trapping of air bubbles. Weigh the container before and after bringing the agarose into solution and restore any liquid lost during heating with water. Let the agarose cool to 55–65°C before pouring the gel.

- Use a comb <1 mm thick to help sharpen bands. Make the gel only as thick as necessary to accommodate in the wells the maximum volume of sample loaded.

Running Gels

To analyze Surveyor Nuclease digestion products by agarose gel electrophoresis:

1. Add 1/6th volume of a 6 X loading dye buffer [10 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 50% (w/v) sucrose, 0.15% (w/v) bromophenol blue] or your own loading dye buffer of choice to each Surveyor Nuclease reaction mixture and mix the 0.2 mL tube with the Surveyor Nuclease digestion.

2. For each digestion/loading dye mixture load all the mixture into the well of an appropriate percentage agarose gel cast and run in the appropriate running buffer (see *Appendix B: Agarose Gel Electrophoresis*). Also load a DNA ladder as a size reference marker in one of the adjacent wells. Run the gel at 5 V/cm until the bromophenol blue has migrated 2/3 of the length of the gel.

3. Illuminate the gel using a UV transilluminator (250 to 300 nm) to visualize the bands and take a photograph.

Note:

Small Surveyor Nuclease digestion products (70–150 bp) are more easily detected early during a gel electrophoresis run, particularly when larger substrates are analyzed. We recommend that the electrophoresis be stopped after the dye has migrated about 1/3 the length of the gel for the purpose of photographing the gel early in the run. Then continue electrophoresis until the dye has run 2/3 of the length of the gel and photograph the gel a second time (See Figure 2)

- Carry out electrophoresis at 5–10 V/cm where distance in cm is measured between electrodes.

- Small SURVEYOR Nuclease digestion products (<100 bp) are more easily detected early during a gel electrophoresis run, particularly when larger substrates are analyzed. We recommend that the electrophoresis be stopped after the bromophenol blue has migrated about 1/3 the length of the gel for the purpose of photographing the gel early in the run. Then continue electrophoresis until the bromophenol blue has run 2/3 of the length of the gel and photograph the gel a second time.

Photographing Gels

The sensitivity of DNA fragment detection in agarose gels varies both with the wavelength of the UV transilluminator used and the sensitivity of the photography system. Most transilluminators allow selection for illumination of DNA at several wavelengths. Use illumination at the shortest wavelength possible to increase signal intensity.

Appendix C: Polyacrylamide Gel Electrophoresis

Appropriate electrophoresis and photography of polyacrylamide gels are critical steps to achieving success with SURVEYOR Nuclease - particularly to achieving maximum detection sensitivity.

Preparing Gels

We use Bio-Rad (Hercules, CA) precast 10% polyacrylamide gels with 12 wells cast and run in 1X TBE buffer. Up to 20 µL can be accommodated in each well. Alternatively, Novex 10% polyacrylamide TBE gels (Life Technologies, Carlsbad, CA) with 12 wells can be used.

To analyze Surveyor Nuclease digestion products by PAGE:

1. Add 1/6th volume of a 6 X loading dye buffer [10 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 50% (w/v) sucrose, 0.15% (w/v) bromophenol blue] or your own loading dye buffer of choice to each Surveyor Nuclease reaction mixture and mix the 0.2 mL tube with the Surveyor Nuclease digestion.

2. For each digestion/loading dye mixture load all the mixture into the well of a 10% polyacrylamide gel cast and run in 1X TBE. Also load a DNA ladder as a size reference marker in one of the adjacent wells. Run the gel at constant voltage at 125 volts until the bromophenol blue has just migrated off the gel.

3. Stain the gel in a solution of 0.5 µg/mL EtBr in 1X TBE for 20 min. Wash the gel in water for 20 min.

4. Illuminate the gel using a UV transilluminator (250–300 nm) to visualize the bands and take a photograph.

Photographing Gels

- Stain the gel for 20 min in 1X TBE containing 0.5 µg/mL EtBr. Wash the gel in water for 20 min.

The sensitivity of DNA fragment detection in polyacrylamide gels varies both with the wavelength of the UV transilluminator used and the sensitivity of the photography system. Most transilluminators allow selection for illumination of DNA at several wavelengths. Use illumination at the shortest wavelength possible to increase signal intensity.

Appendix D: Troubleshooting

Effective use of the SURVEYOR Mutation Detection Kit depends upon successful completion of a number of steps. One of the most critical is PCR amplification that must result in the production of specific, uniform-sized DNAs in sufficient quantity to be detected after hybridization and cleavage. Also critical is matching the amount of DNA and SURVEYOR Nuclease used. If you are a first-time user, you should process the control DNAs provided through all the steps as described in *Control Experiments – Using Control G and Control C Plasmid DNA*.

The Control DNAs should be used also to troubleshoot various steps in the procedure.

This appendix section covers a list of issues that you might encounter when using the SURVEYOR Mutation Detection Kit and how to resolve them.

Problem 1—Low PCR yield or no PCR product

POSSIBLE CAUSE	SOLUTION
Not enough template and/or too few cycles	Increase the template concentration and/or add more PCR cycles.
Suboptimal PCR parameters	Do one or more of the following: • Decrease the annealing temperature in increments of 2°C. • Increase the extension time. For Optimase Polymerase, use 30 sec per 250 bp. For Maximase Polymerase, use 1 min per 1 kb. • Adjust the Mg^{2+} concentration
Suboptimal DNA polymerase for target	Use a "hot-start" PCR DNA polymerase. Increase the amount of DNA polymerase.

Problem 2—Multiple PCR products

POSSIBLE CAUSE	SOLUTION
Poor primer design	Redesign primers to improve specificity, T_m, and GC content.
Annealing temperature too low	Increase the annealing temperature in increments of 2°C.
Extension time too long	Reduce the extension time. Refer to manufacturer instructions on extension times
Cycle number too high	Reduce cycle number in increments of 2.
Suboptimal PCR conditions	Use "hot-start" PCR and/or touchdown PCR or perform nested PCR.

Problem 3—No cleavage products observed upon analysis after Surveyor Nuclease treatment of known heteroduplex

POSSIBLE CAUSE	SOLUTION
Proportion of mismatch target too low	Mix equal amounts of test and reference DNA before annealing.
Cleavage site too close to PCR product end	Redesign the primer set to move the target site away from ends.
Inactive SURVEYOR Nuclease	Perform the Control reaction to verify enzyme performance.
Too little enzyme	Increase the amount of SURVEYOR Nuclease 2-fold and repeat digestion.
Too little substrate	Concentrate the PCR products by ethanol precipitation before annealing.
Incompatible 1X PCR buffer	Consult Appendix A for suggested courses of action

Problem 4—High background after SURVEYOR Nuclease treatment

POSSIBLE CAUSE	SOLUTION
Suboptimal hybridization step	Do the following: 1. Make sure the DNA concentration is in the range of >25 ng/µL to <50 ng/µL. 2. Repeat the hybridization step, taking care to cool the annealing mixture slowly. 3. Add 1X PCR reaction buffer to precipitated products before annealing. 4. Use sufficient sample volume (>20 µL) during hybridization in a water bath. 5. Add 1/10 volume 0.5 M KCl to PCR product contained in low salt 1X PCR reaction buffer
Errors introduced by PCR enzyme	Use a high fidelity PCR enzyme, such as Optimase Polymerase.
Incompatible 1X PCR buffer	Consult Appendix A for suggested courses of action
Too much SURVEYOR Nuclease	Reduce the SURVEYOR Nuclease 2-fold and repeat digestion.
DNA amount too low	Increase the DNA amount to at least 200 ng of substrate per 1 µL of SURVEYOR Nuclease S used.
Nonspecific PCR products	Optimize the PCR parameters to increase specificity. Always use an appropriate substrate as a control to identify background.
Enhancer S has lost activity	Increase the amount of Enhancer S 2-fold and repeat digestion.

Can’t fix your problem?

Contact techsupport@idtdna.com for technical help and advice.

Be prepared to provide the following information to assist with troubleshooting Surveyor reactions:

- PCR Reaction Condition specifics
    - Source of template DNA (isolation method) for reference and mismatch
    - Specific PCR buffer and enzyme used
    - Cycling conditions (temperatures and cycle numbers)
    - Gel image of PCR product before nuclease digest, including Controls C and G
    - DNA concentration from PCR reaction
- Surveyor reaction Conditions used
    - Amount of duplex, enhancer and nuclease added
    - Length of Surveyor treatment
    - Temperature for Surveyor treatment (thermocycler or heating block?)
- Gel Image after nuclease treatment alongside Control C or G alone and C/G Heteroduplex
- Application used for (CRISPR/TALEN/ZFN, mutation screening, SNP detection etc)

Appendix E: Specific examples of use of Surveyor Nuclease

Surveyor Nuclease has been used to identify and analyze mutations in a variety of organisms and cell types. Surveyor Mutation Detection Kits also provide an easy method for confirming genome modifications using CRISPR and other methods. A short list of examples of the variety of applications of Surveyor Nuclease includes:

- CRISPR genome modification [5-9]
- germline mutations in human genes:
    - ATRX for X-linked mental retardation[10]
    - ABCC6 associated with pseudoxanthoma elasticum [11]
    - HBB gene linked to β-thalassemia [12]
- genes involved in uracil catabolism [13, 14]
- mtDNA and nuclear gene mutations associated with respiratory chain defects [15-18]
- mutations associated with kidney disease [19-21]
- somatic mutations in:
    - EGFR gene in human tumors [22-27]
    - JAK2 tyrosine kinase gene in patients with myeloproliferative disorders [28, 29]
    - a variety of genes in patients undergoing radiotherapy[30]
    - p53 gene in squamous-cell carcinomas[31] and hematological malignancies [32]
    - KIT tyrosine kinase receptor gene in various malignancies [33, 34]
    - PKC412 tyrosine kinase receptor in mast cell leukemia [35]
    - LKB1 tumor suppressor in lung cancer [36]
    - VHL gene in renal tumors [37]
    - hCDC4 gene in patients with AML [38]
- mutations in drug-resistant genes of *Mycobacterium tuberculosis* [39, 40] and in Group II introns of bacterial rRNA genes[41]
- induced point mutations in barley [42, 43] and error-free clones generated from a plant cDNA library by PCR-based cloning [44]

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