Instructions for Use

Specimen Control Size Ladder

For use as a polymerase chain reaction (PCR) template amplification control

This product is a General Purpose Reagent (GPR).

invivoscribe

Storage Conditions: **-85ºC to -65ºC**

Catalog#		Products		Quantity
REF	20960020	Specimen Control Size Ladder – Unlabeled	(for Gel Detection)	33 Reactions
REF	20960021	Specimen Control Size Ladder – 6FAM	(for ABI Fluorescence Detection)	33 Reactions

Table of Contents

1. ASSAY USE 3

2. SUMMARY AND EXPLANATION OF THE TEST 3

2.1. Background 3
2.2. Summary 3

3. PRINCIPLES OF THE PROCEDURE 4

3.1. Polymerase chain reaction (PCR) 4
3.2. Gel Detection 4
3.3. Differential Fluorescence Detection 4

4. REAGENTS 5

4.1. Available Reagents 5
4.2. Warnings and Precautions 5
4.3. Storage and Handling 5

5. INSTRUMENTS 6

5.1. Thermal cycler 6
5.2. Electrophoresis Unit 6
5.3. UV Illumination Unit 6
5.4. ABI Capillary Electrophoresis Instruments 6

6. SPECIMEN COLLECTION AND PREPARATION 7

6.1. Precautions 7
6.2. Interfering Substances 7
6.3. Specimen Requirements and Handling 7
6.4. Sample Preparation 7
6.5. Sample Storage 7

7. ASSAY PROCEDURE 8

7.1. Materials Provided 8
7.2. Materials Required (not provided) 8
7.3. Reagent Preparation 9
7.4. Amplification 9
7.5. Detection 10
7.6. Quality Control 11

8. INTERPRETATION AND REPORTING 11

9. LIMITATIONS OF PROCEDURE 11

10. EXPECTED RESULTS 12

10.1. Expected Size of Amplified Products 12
10.2. Sample Data 12

11. TECHNICAL AND CUSTOMER SERVICE 13

12. REFERENCES 13

13. SYMBOLS 13

14. LEGAL NOTICE 14

14.1. Warranty and Liability 14
14.2. Patents and Trademarks 14

15. SPECIMEN CONTROL SIZE LADDER MASTER MIX: SINGLE PAGE GUIDE 15

1. Assay Use

The General Purpose Reagent (GPR) Specimen Control Size Ladder master mix can be used to ensure that the quantity and quality of unknown sample DNA is adequate to yield a valid result during molecular testing.

2. Summary and Explanation of the Test

2.1. Background

Sample quality is critical and therefore it must be verified before the samples are used in molecular assays. Analysis of degraded DNA samples can result in poor quality data, and the presence of inhibitors differentially affects qPCR assays.[1,2] Therefore, verification of the quantity and quality of the sample is sufficient for the molecular assay is important, and one method is to employ an amplification control master mix.

Master mixes are components of complete assays and typically consist of a buffered magnesium solution, deoxynucleotides, and multiple primers that target the gene segments of interest. Multiple primers are used to ensure a more comprehensive testing approach necessary to reliably identify clonal rearrangements. This test is complete with the exception of Taq DNA polymerase, which is not provided.

2.2. Summary

The Specimen Control Size Ladder master mix is included in many of our testing kits and targets four different genes: *Tbxas* (thromboxane synthase), *RAG-1* (recombination activating gene-1), *PLZF* (promyelocytic leukemia zinc finger) and *AF4* (ALL1 fused gene from chromosome 4). Amplification of genomic DNA (gDNA) with this master mix produces products of 100, 200, 300, 400, and 600 base pairs (bp) to ensure that the quality and quantity of the sample DNA is adequate to yield a valid result.

This assay is based on the EuroClonality/BIOMED-2 Concerted Action BMH4-CT98-3936.

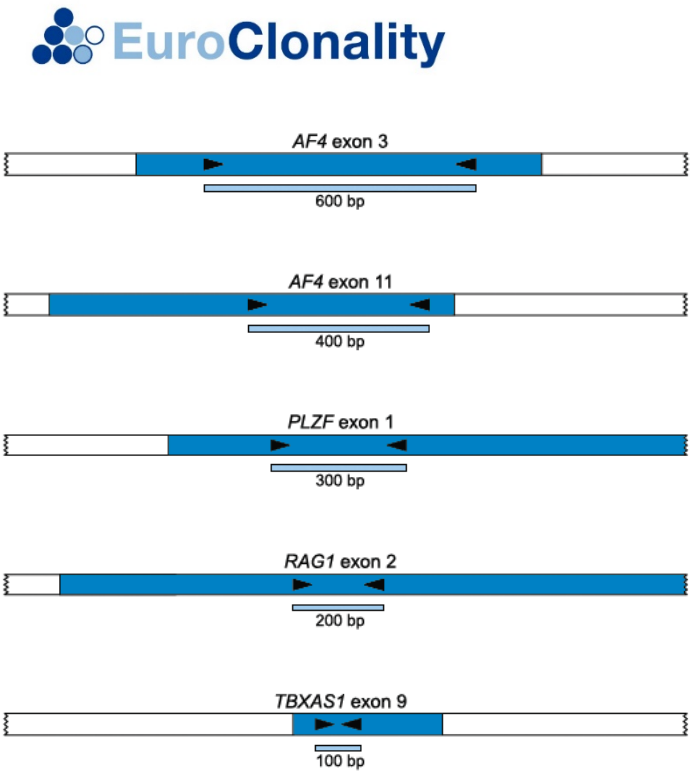

Figure 1. This figure at left is a representation of the four different genes targeted by the Specimen Control Size Ladder master mix and the site of primer binding.

3. Principles of the Procedure

3.1. Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a technology used for quick and easy amplifying DNA sequences, which is based on the principle of enzymatic replication of the nucleic acids. This method has an irreplaceable role and constitutes one of the basic methods for DNA analysis. PCR enables targeted regions of small amounts of DNA to be exponentially amplified, generating larger amounts of the target region. This reaction can be tailored in order to amplify specific sequences within the DNA, which are known as target sequences. The chosen target sequences are identified and "amplified" using primers, short oligonucleotides that are designed to recognize and flank the sequence of interest.

3.2. Gel Detection

Gel electrophoresis, such as agarose gel electrophoresis or non-denaturing polyacrylamide gel electrophoresis (PAGE), is commonly used to resolve amplicon products based on their size, charge, and conformation. Since DNA is negatively charged, when an electrical potential (voltage) is applied across the gel containing PCR products, the electrical field causes the amplicons to migrate through the gel. Smaller DNA fragments are able to easily migrate through the gel matrix, whereas larger DNA fragments migrate more slowly. This causes a separation of the amplicon products based on size. Ethidium bromide or other DNA intercalating dyes can then be used to stain and detect these products in the gel.

3.3. Differential Fluorescence Detection

Differential fluorescence detection is commonly used to resolve the different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores) so that they can produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in unsurpassed sensitivity, single nucleotide resolution, differential product detection and relative quantification. In addition, the use of agarose and polyacrylamide gels, as well as the use of carcinogens such as ethidium bromide, can virtually be eliminated.

4. Reagents

4.1. Available Reagents

Table 1. Available Products

	Catalog #	Description	Total Reactions
REF	20960020	Specimen Control Size Ladder – Unlabeled (for Gel Detection)	33 Reactions
REF	20960021	Specimen Control Size Ladder – 6FAM (for ABI Fluorescence Detection)	33 Reactions

4.2. Warnings and Precautions

- Dilution, reducing amplification reaction volumes, or other deviation in this protocol may affect the performance of this master mix.
- Use glass distilled de-ionized molecular biology grade water for the preparation of specimen DNA.
- Handle specimens in approved biological safety containment facilities and open only in certified biological safety cabinets.
- Materials are stable until the labeled expiration date when stored and handled as directed. Do not use kits beyond their expiration date.
- Adherence to the protocol will assure optimal performance and reproducibility. Ensure correct thermal cycler programs are used, as other programs may provide inaccurate/faulty data such as false-positive and false-negative results.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.
- Perform all laboratory procedures with standard personal protective equipment (gloves, laboratory coats and protective eyewear). Follow good laboratory practices and universal precautions when working with specimens. Do not pipette by mouth. Do not eat, drink or smoke in laboratory work areas. Wash hands thoroughly after handling specimens and assay reagents. Handle specimens in approved biological safety containment facilities and open only in certified biological safety cabinets. Use molecular biology grade water for the preparation of specimen DNA.
- Use extreme care to avoid any contamination of reagents or amplification mixtures with samples, controls or amplified materials. Use fresh, aerosol-resistant pipette tips between samples and between dispensing reagents.
- Closely monitor all reagents for signs of contamination (*e.g.*, negative controls giving positive signals). Discard any reagents suspected of contamination.
- To minimize contamination, wear clean gloves when handling samples and reagents and routinely clean work areas and pipettes prior to setting up PCR.
- Follow uni-directional workflow between separate work areas in the PCR laboratory: begin with master mix preparation, move to specimen preparation, then to amplification and finally to detection. Autoclaving does not eliminate DNA contamination.
- Perform pre- and post-PCR steps in separate spaces. Avoid taking paper and other materials from post-PCR into the pre-PCR space.
- Dedicate all pipettes, pipette tips and any equipment used in a particular area to that area of the laboratory.
- Decontaminate non-disposable items with 10% bleach and rinse with distilled water two separate times before returning them to the starting areas.
- Use sterile, disposable plastic ware whenever possible to avoid contamination.

4.3. Storage and Handling

- **Store the master mix at -85ºC to -65ºC** until ready to use.
- All reagents and controls must be thawed and vortexed or mixed thoroughly prior to use to ensure that they are completely resuspended.
- Due to high salt concentrations, PCR master mixes are sensitive to freeze/thaw cycles. Minimize the exposure of master mixes to freeze/thaw cycles.

For any questions, please contact our technical staff. We are happy to help you determine optimal storage needs.

5. Instruments

5.1. Thermal cycler

- Use or function: Amplification of DNA samples
- Performance characteristics and specification:
  - Minimum thermal range: 15ºC to 96ºC
  - Minimum ramping speed: 0.8ºC/sec
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.
- See section 7.4. *Amplification* for thermal cycler program.

5.2. Electrophoresis Unit

- Use or function: DNA fragment separation
- Performance characteristics and specification:
  - Capable of running at 35 V to 135 V for extended times
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.

5.3. UV Illumination Unit

- Use or function: DNA detection
- Performance characteristics and specification:
  - Capable of emitting light at a wavelength of ~302 nm
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.

5.4. ABI Capillary Electrophoresis Instruments

- Use or function: Fragment detection and analysis
- Performance characteristics and specification:
  - The following capillary electrophoresis instruments will meet the performance needs for this assay:
    - ABI 310 Genetic Analyzer (1-capillary)
    - ABI 3100 Avant Genetic Analyzer (4-capillaries)
    - ABI 3100 Genetic Analyzer (16-capillaries)
    - ABI 3130 Genetic Analyzer (4-capillaries)
    - ABI 3130xL Genetic Analyzer (16-capillaries)
    - ABI 3500xL Genetic Analyzer (24-capillaries)
- Follow manufacturer's installation, operation, calibration, and maintenance procedures.

6. Specimen Collection and Preparation

6.1. Precautions

Biological specimens from humans may contain potentially infectious materials. Handle all specimens in accordance with your institute's Bloodborne Pathogen program and/or Biosafety Level 2.

6.2. Interfering Substances

The following substances are known to interfere with PCR:

- Divalent cation chelators
- Low retention pipette tips
- EDTA (not significant at low concentrations)
- Heparin

6.3. Specimen Requirements and Handling

This master mix tests genomic DNA (gDNA) from the following sources:

- 5 cc of peripheral blood, bone marrow biopsy or bone marrow aspirate anti-coagulated with heparin or EDTA. Ship at ambient temperature; OR
- Minimum 5mm cube of tissue shipped frozen; or at room temperature or on ice in RPMI 1640; OR
- 2 µg of gDNA; OR
- Formalin-fixed paraffin embedded tissue or slides

6.4. Sample Preparation

6.4.1. Using any method of DNA extraction, extract the gDNA from unknown samples.

6.4.2. Resuspend DNA to final concentration of 100 µg – 400 µg per mL in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or distilled water.

6.5. Sample Storage

Store samples using a method that prevents degradation of DNA.

7. Assay Procedure

7.1. Materials Provided

See Table 1 for materials provided.

7.2. Materials Required (not provided)

Table 2. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Part Number	Notes
DNA Polymerase	Roche: • EagleTaq DNA Polymerase or equivalent	05206944190	N/A
Positive Control	Invivoscribe, Inc. • IVS-0000 Polyclonal Control DNA	40920010	N/A
Molecular Biology Grade or USP Water	N/A	N/A	DNase / RNase free
Calibrated Pipettes	N/A	N/A	Capable of accurately measuring volumes between 0.5 µL and 1000 µL.
Vortex Mixer	N/A	N/A	N/A
PCR plates or tubes	N/A	N/A	Sterile
Filter barrier pipette tips	N/A	N/A	Sterile, RNase/DNase/ Pyrogen-free
Microcentrifuge tubes	N/A	N/A	Sterile
Gel Detection			
Ethidium Bromide	Thermo Fisher Scientific: • UltraPure™ 10 mg/mL Ethidium Bromide	15585-011	N/A
Agarose Gels	Thermo Fisher Scientific: • MetaPhor™ Agarose, 125 g or • Lonza™ NuSieve™ 3:1 Agarose	BMA50180 or BMA50090	N/A
6% Polyacrylamide Gels	Thermo Fisher Scientific: • Novex® TBE Gels (6%, 12 well)	EC62652Box	N/A
TBE Running Buffer	Thermo Fisher Scientific: • Novex TBE Running Buffer (5X)	LC6675	Dilute 1:5 prior to use.
Gel Loading Buffer	Thermo Fisher Scientific: • 10X BlueJuice™ Gel Loading Buffer • Novex Hi-Density TBE Sample Buffer (5X)	10816-015 LC6678	N/A
ABI Fluorescence Detection			
Hi-Di Formamide	Thermo Fisher Scientific: • Hi-Di™ Formamide	4311320	N/A
Size Standards	Invivoscribe, Inc.: • Hi-Di Formamide w/ROX size standards for ABI 310 • Hi-Di Formamide w/ROX size standards for ABI 3100 Thermo Fisher Scientific: • For ABI 3500 instruments: ○ GeneScan - 600 [LIZ]™ v2.0 • For ABI 3100 or 3130 instruments: ○ GeneScan™ - 400HD [ROX]™	60980051 60980061 4408399 402985	N/A

Table 2. Materials Required (not provided)

Reagent/Material	Recommended Reagents/Materials and Suppliers	Part Number	Notes
Spectral Calibration Dye Sets	Thermo Fisher Scientific: • For ABI 310 instruments: ○ NED Matrix Standard ○ And Fluorescent Amidite Matrix Standards [6FAM, TET, HEX, TAMRA, ROX] • For ABI 3100 and 3130 instruments: ○ DS-30 Matrix Standard Kit (Dye Set D) • For ABI 3500 instruments: ○ GeneScan - 600 [LIZ]™ v2.0	 402996 401546 4345827 4345833	N/A
Polymer	Thermo fisher Scientific: • POP-4™ Polymer: ○ POP-4 for 310 Genetic Analyzers ○ POP-4 for 3100/3100-Avant Genetic Analyzers ○ POP-4 for 3130/3130xL Genetic Analyzers • POP-7™ Polymer: ○ POP-7 for 3130/3130xL Genetic Analyzers ○ POP-7 for 3500/3500xL Genetic Analyzers	 402838 4316355 4352755 4352759 4393714	N/A

7.3. Reagent Preparation

- Test all samples in **singlicate**.
- **Test positive** and **no template** controls with the master mix.

7.3.1. Using gloved hands, remove the master mix from the freezer. Allow the tube to thaw, then gently vortex to mix.

7.3.2. In containment hood or dead air box, remove an appropriate aliquot to clean, sterile microfuge tube.

- The aliquot volume is 45 µL for each sample + 135 µL (3 x 45 µL) for the positive, negative and no template controls.
- Include an additional 20 µL to correct for pipetting errors.

7.3.3. Add the appropriate amount of Taq DNA polymerase (0.25 µL @5 U/µL per 50 µL total reaction volume) to the master mix and gently mix by inverting several times or gently vortexing.

- The master mix is now ready for distribution to reaction tubes or plate and addition of sample.

7.4. Amplification

7.4.1. Aliquot 45 µL of the master mix/enzyme solution into individual tubes or wells of a PCR plate.

7.4.2. Add 5 µL of sample or control DNA to the individual tubes or wells containing the respective master mix reactions.

- Pipette up and down several times to mix.

7.4.3. Amplify the reactions using the following PCR program:

NOTE: Use the **calculated** option for temperature measurement with the PTC instruments.

Table 3. Thermal cycling conditions

Step	Temperature	Duration	Cycles
1	95ºC	7 minutes	1
2	95ºC	45 seconds	35
3	60ºC	45 seconds	
4	72ºC	90 seconds	
5	72ºC	10 minutes	1
6	15ºC	∝	1

7.4.4. Once the thermal cycling program is complete, remove the amplification plate from the thermal cycler.

7.5. Detection

- The **Specimen Control Size Ladder** master mix for ABI fluorescence detection contain primers labeled with a fluorescent dye (6-FAM). This label is detected as **BLUE** using the differential fluorescence software. The amplicons produced with this master mix are 100, 200, 300, 400, and 600 bp.

Gel Detection – Agarose TBE Gels

7.5.1. Prepare a 2% MetaPhor or NuSieve 3:1 agarose/TBE gel.

7.5.2. Individually mix 20 µL of each PCR product with 4 µL of 6X gel loading buffer.

7.5.3. Load 20 µL of this mixture into separate wells of the gel, flanked by DNA size standards.

7.5.4. Detect products using ethidium bromide or an equivalent dye.

7.5.5. Photograph the gel and interpret resulting data.

Gel Detection – Polyacrylamide TBE Gels

NOTE: Do not heteroduplex Specimen Control Size Ladder PCR products.

7.5.6. Assemble electrophoresis unit using a 6% non-denaturing polyacrylamide TBE gel and 0.5X TBE running buffer.

7.5.7. Add 5 µL of ice-cold non-denaturing bromophenol blue loading buffer to samples.

7.5.8. Load 20 µL of mixture into wells of the gel.

7.5.9. Run gel at 110V for 2-3 hours or 40-50V overnight.

- Voltage and electrophoresis time depend on the PCR amplicon size, acrylamide gel thickness and type of PCR equipment.
- Voltage and run time can be adapted accordingly.

7.5.10. Stain gels in 0.5 µg/mL Ethidium Bromide (in water or 0.5X TBE Buffer) for 5 - 10 minutes.

7.5.11. Destain gels 2X in water for 5 - 10 minutes.

7.5.12. Visualize the gel using UV illumination.

7.5.13. Photograph the gel and interpret the resulting data.

ABI Fluorescence Detection with ABI 310, 3100 & 3130 instruments

7.5.14. In a new microcentrifuge tube, mix an appropriate amount (for a total of 10 µL per reaction) of Hi-Di Formamide with ROX Size Standards. Vortex well.

7.5.15. In a new 96-well PCR plate, add 10 µL of Hi-Di Formamide with ROX size standards to individual wells for each reaction.

7.5.16. Transfer 1 µL of each PCR reaction to the wells containing Hi-Di Formamide with ROX size standards.

- Add only one sample per well.
- Pipette up and down to mix.

7.5.17. Cap or cover the PCR plate or tubes.

7.5.18. Heat the mixture to 95ºC for 2 minutes then snap chill on ice for 5 minutes.

7.5.19. Create a **sample sheet** and **injection list** for the samples.

7.5.20. Load samples and run.

- Data are automatically displayed as size and color specific peaks.

7.5.21. Review profile and controls, report results.

ABI Fluorescence Detection with ABI 3500 instruments:

NOTE: Due to instrument to instrument variation in the performance of the ABI 3500 platform, the amount of formamide, sample and size standard listed in the protocol is intended to be a starting point. The protocol may need to be optimized for specific ABI 3500 platforms.

7.5.22. In a new microcentrifuge tube, mix an appropriate amount (9.5 µL per reaction) of Hi-Di Formamide with LIZ Size Standards. Vortex well.

7.5.23. In a new 96-well PCR plate, add 9.5 µL of Hi-Di Formamide with LIZ size standards to individual wells for each reaction.

7.5.24. Transfer 0.5 µL of each reaction to the wells containing Hi-Di Formamide with LIZ size standards.

- Add only one sample per well.
- Pipette up and down to mix.

7.5.25. Cap or cover the PCR plate.

7.5.26. Heat denature the samples at 95ºC for 3 minutes, then snap chill on ice for 5 minutes.

7.5.27. Prepare a sample sheet and injection list for the samples.

7.5.28. Run the samples on an ABI 3500 capillary electrophoresis instrument according to the user manual.

- Data are automatically displayed as size and color specific peaks.

7.5.29. Review profile and controls, report results. (See sections 8*: Interpretation of Results* and 10*: Expected Values.*)

7.6. Quality Control

Controls are not provided with this product. IVS-0000 Polyclonal Control DNA (REF 40920010) can be used as a positive control, and sterile or molecular biology grade water can be used as a no template control.

8. Interpretation and Reporting

8.1. Report samples that fail to amplify following repeat testing as "**DNA of insufficient quantity or quality for analysis with polymerase chain reaction (PCR)**".

8.2. It is acceptable to call a sample "**Positive**" when a product is generated in the valid size range. Results can be reported as "**Positive for the detection of human genomic DNA of sufficient quality and quantity for analysis with polymerase chain reaction (PCR)**".

8.3. All assay controls must be examined prior to interpretation of sample results. If the controls do not yield the correct results, the assay is not valid and the samples cannot be interpreted.

9. Limitations of Procedure

The master mix is subject to interference by degradation of DNA or inhibition of PCR due to heparin or other agents.

10. Expected Results

The size range for this master mix has been determined testing positive control samples and gDNA. For accurate and meaningful interpretation, ignore peaks that occur outside of the expected size range. Peaks that are outside of the range cannot be assumed to be valid.

NOTE: "Color" indicates the color of products generated with the master mix when using differential fluorescence detection format (*e.g.*, ABI instruments).

10.1. Expected Size of Amplified Products

Table 4. Expected Size of Amplifed Products

Master Mix	Target	Color	Control DNA	Catalog #	Product Size (bp)
Specimen Control Size Ladder	*TBXAS1* exon 9 *RAG1* exon 2 *PLZF* exon 1 *AF4* exon 11 *AF4* exon 3	**Blue**	Any Human DNA	---	100 200 300 400 600

Note: For all detection formats the 600 bp amplicon band intensity and amplitude is quite diminished compared to the other amplicons produced. This is due to competitive amplification with the smaller amplicons. For ABI fluorescence detection, the 600 bp fragment does not appear during normal run times. Extended run times with larger size standards are required for the resolution of this peak. The preceding peak at 84 bp is due to the detection method ABI platforms use and may not always be observed.

10.2. Sample Data

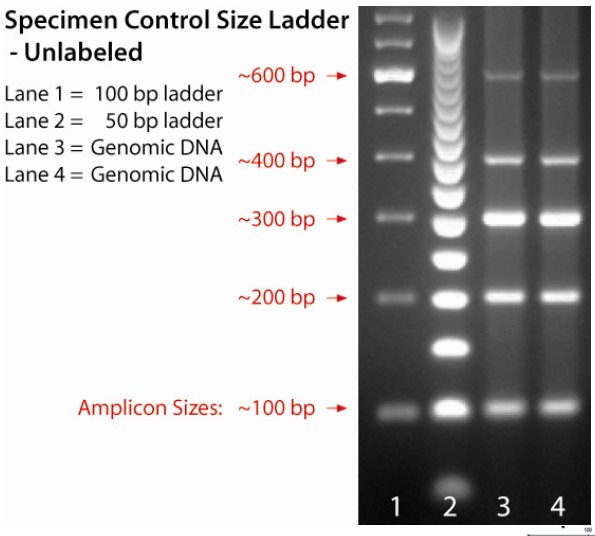

Figure 2. *Gel Detection with Agarose Gels.* This is a photograph of a gel with data generated from two different gDNA samples (lanes 3 and 4). In the first lane is a 100 bp DNA ladder and in the second lane is a 50 bp DNA ladder. Products of 100, 200, 300, 400, and 600 bp are seen in lanes 3 and 4.

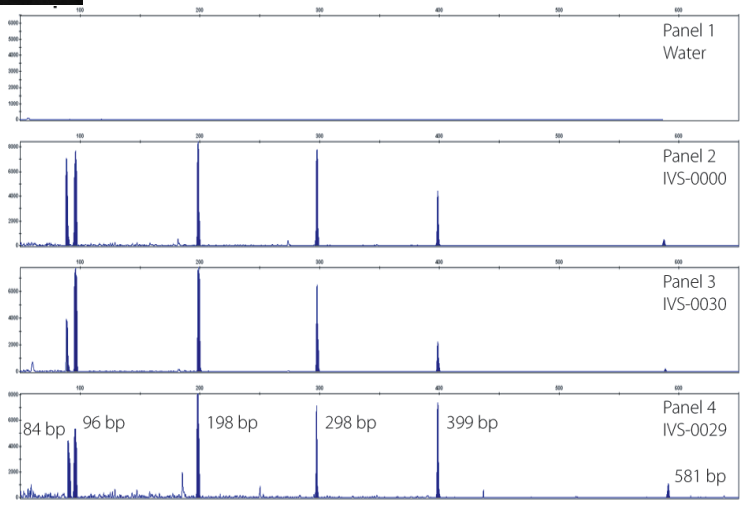

Figure 3. *ABI Fluorescence Detection*. The panels at right display differential fluorescence data generated by running water (panel 1), IVS-0000 Polyclonal Control DNA (panel 2), IVS-0030 Clonal Control DNA (panel 3) and IVS-0029 Clonal Control DNA (panel 4).

11. Technical and Customer Service

Thank you for purchasing our **Specimen Control Size Ladder** master mix. We appreciate your business. We are happy to assist you and will provide ongoing technical assistance to keep our products performing efficiently in your laboratory. Technical assistance is most rapidly obtained using our Internet site: www.invivoscribe.com or by sending an email inquiry to: support@invivoscribe.com**.**

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12. References

1. Vermeulen J, De Preter K, Lefever S, Nuytens J, De Vloed F, Derveaux S, Hellemans J, Speleman F, Vandesompele J. Measurable impact of RNA quality on gene expression results from quantitative PCR. *Nucleic Acids Res*. 2011 May;39(9):e63. Epub 2011 Feb 11.
2. Huggett JF, Novak T, Garson JA, Green C, Morris-Jones SD, Miller RF, Zumla A. Differential susceptibility of PCR reactions to inhibitors: an important and unrecognised phenomenon. *BMC Res Notes*. 2008 Aug 28;1:70.
3. JJM van Dongen, et al. *Leukemia*. 2003 Dec;17(12):2257-2317.

13. Symbols

The following symbols are used in Invivoscribe product labeling.

REF	Catalog Number		Expiration Date
VOL	Reagent Volume	EC REP	Authorized Representative in the European Community
LOT	Lot Number		Manufacturer
	Storage Conditions		Consult Instructions for Use

14. Legal Notice

14.1. Warranty and Liability

Invivoscribe, Inc. (Invivoscribe®) is committed to providing the highest quality products. Invivoscribe® warrants that the products meet or exceed the performance standards described in the Instructions For Use, as to products with such an insert. If a product is covered by product specifications and does not perform as specified, our policy is to replace the product or credit the full purchase price. No other warranties of any kind, expressed or implied, are provided by Invivoscribe®. Invivoscribe® liability shall not exceed the purchase price of the product. Invivoscribe® shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products; product efficacy under purchaser controlled conditions in purchaser's laboratory must be established and continually monitored through purchaser defined and controlled processes including but not limited to testing of positive, negative, and blank controls every time a sample is tested. Ordering, acceptance, and use of product constitutes purchaser acceptance of sole responsibility for assuring product efficacy and purchaser agreement to the limitation of liability set forth in this paragraph.

This product is a General Purpose Reagent (GPR).

14.2. Patents and Trademarks

This product is covered by one or more of the following: European Patent Number 1549764, European Patent Number 2418287, European Patent Number 2460889, Japanese Patent Number 4708029, United States Patent 8859748, and related pending and future applications. All of these patents and applications are licensed exclusively to Invivoscribe®. Additional patents licensed to Invivoscribe® covering some of these products apply elsewhere. Many of these products require nucleic acid amplification methods such as Polymerase Chain Reaction (PCR). No license under these patents to use amplification processes or enzymes is conveyed expressly or by implication to the purchaser by the purchase of this product.

15. Specimen Control Size Ladder Master Mix: Single Page Guide

15.1. Using gloved hands, remove the master mixes from the freezer. Allow the tubes to thaw; then gently vortex to mix.

15.2. In a containment hood or dead air box remove an appropriate aliquot to clean, sterile microfuge tube.

- Aliquot volumes are 45 µL for each sample + 135 µL for the positive, negative and no template controls.
- Include an additional 20 µL to correct for pipetting errors.

15.2. Add the appropriate amount of Taq DNA polymerase (0.25 µL @5 U/µL per 50 µL total reaction volume) to each master mix and gently mix by inverting several times or gently vortexing.

15.3. Aliquot 45 µL of master mix to individual tubes or wells of a PCR plate.

15.4. Add 5 µL of DNA from the unknown and/or control samples to individual tubes or wells containing the respective master mix reactions and pipette up and down several times to mix.

15.5. Amplify target DNA using the universal thermal cycler program.

Gel Detection – Agarose TBE Gels

15.6. Prepare a 2% MetaPhor or NuSieve 3:1 agarose/TBE gel.

15.7. Individually mix 20 µL from each PCR product with 4 µL of 6X gel loading buffer.

15.8. Load 20 µL of this mixture into separate wells of the gel, flanked by DNA size standards.

15.9. Detect products using ethidium bromide or an equivalent dye.

15.10. Photograph the gel and interpret resulting data.

Gel Detection – Polyacrylamide TBE Gels

15.11. Assemble electrophoresis unit using a 6% non-denaturing polyacrylamide TBE gel and 0.5X TBE running buffer.

15.12. Add 5 µL of ice-cold non-denaturing bromophenol blue loading buffer to samples.

15.13. Load 20 µL of the mixture into wells of the gel.

15.14. Run gel at 110V for 2-3 hours or 40-50V overnight.

15.15. Stain gels in 0.5 µg/mL Ethidium Bromide (in water or 0.5X TBE Buffer) for 5 - 10 minutes.

15.16. Destain gels 2X in water for 5 - 10 minutes.

15.17. Visualize gels using UV illumination.

15.18. Photograph the gel and interpret resulting data.

ABI Fluorescence Detection with ABI 310, 3100 & 3130 instruments

15.19. In a new microcentrifuge tube, mix an appropriate amount (for a total of 10 µL per reaction) of Hi-Di Formamide with ROX Size Standards. Vortex well.

15.20. In a new 96-well PCR plate, add 10 µL of Hi-Di Formamide with ROX size standards to individual wells for each reaction.

15.21. Transfer 1 µL of each PCR reaction to the wells containing Hi-Di Formamide with ROX size standards.

- Add only one sample per well.
- Pipette up and down to mix.

15.22. Cap or cover the PCR plate or tubes.

15.23. Heat the mixture to 95ºC for 2 minutes then snap chill on ice for 5 minutes.

15.24. Create a sample sheet and injection list for the samples.

15.25. Load samples and run.

15.26. Review profile and controls, report results.

ABI Fluorescence Detection with ABI 3500 instruments

15.27. In a new microcentrifuge tube, mix an appropriate amount (9.5 µL per reaction) of Hi-Di Formamide with LIZ Size Standards. Vortex well.

15.28. In a new 96-well PCR plate, add 9.5 µL of Hi-Di Formamide with LIZ size standards to individual wells for each reaction.

15.29. Transfer 0.5 µL of each reaction to the wells containing Hi-Di Formamide with LIZ size standards.

- Add only one sample per well.
- Pipette up and down to mix.

15.30. Cap or cover the PCR plate.

15.31. Heat denature the samples at 95ºC for 3 minutes, then snap chill on ice for 5 minutes.

15.32. Prepare a sample sheet and injection list for the samples.

15.33. Run the samples on an ABI 3500 capillary electrophoresis instrument according to the user manual.

15.34. Review profile and controls, report results.