Instructions for Use

Amplification Control

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	20960010	Amplification Control – Unlabeled	(for Gel Detection)	30 Reactions
REF	20960011	Amplification Control – 6FAM	(for ABI Fluorescence Detection)	30 Reactions

Table of Contents

1. Assay Use

The General Purpose Reagent (GPR).Amplification Control 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 Amplification Control master mix is included in many of our testing kits and targets the *HLA-DQα* locus; the Major Histocompatibility Complex or Human Leukocyte Antigen (HLA), class II, DQ alpha 1. Amplification of genomic DNA (gDNA) with this master mix produces a product of 235 base pairs (bp) to ensure that the quantity of the sample DNA is adequate to yield a valid result.

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.

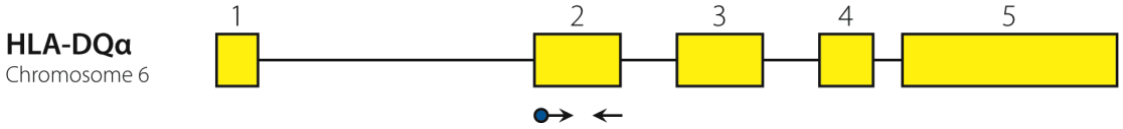

Figure 1. The figure above is a representation of the *HLA-DQα* locus and the site of primer binding.

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	20960010	Amplification Control – Unlabeled (for Gel Detection)	30 Reactions
REF	20960011	Amplification Control – 6FAM (for ABI Fluorescence Detection)	30 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)
- 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 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 3100 or 3130 instruments: ○ GeneScan™ - 400HD [ROX]™	60980051 60980061 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**, **negative**, and **no template** controls with each 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 50 µL for each sample + 150 µL (3 x 50 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 55 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 50 µL of the master mix/enzyme solution into individual PCR wells or tubes.

7.4.2. Add 5 µL 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:

- 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	94ºC	30 seconds	35
3	55ºC	30 seconds	
4	72ºC	1 minute	
5	72ºC	10 minutes	1
6	4ºC	∝	1

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

7.5. Detection

The **Amplification Control** 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 at 235 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 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

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 ice-cold non-denaturing bromophenol blue loading buffer to each PCR product.

7.5.8. Load 20 µL of the mixture into individual 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 resulting data.

ABI Fluorescence Detection with ABI 310, 3100 & 3130 instruments

7.5.14. Add 1 µL PCR product to 10 µL HI-Deionized Formamide containing ROX size standards and mix well.

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

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

7.5.17. Load samples and run.

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

7.5.18. Review profile and controls, report results.

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 gDNA of sufficient quality and quantity for analysis with polymerase chain reaction (PCR)**".

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

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 amplified Products

Master Mix	Target	Color	Control DNA	Catalog #	Product Size (bp)
Amplification Control	*HLA-DQα*	**Blue**	Any Human DNA	---	235

10.2. Sample Data

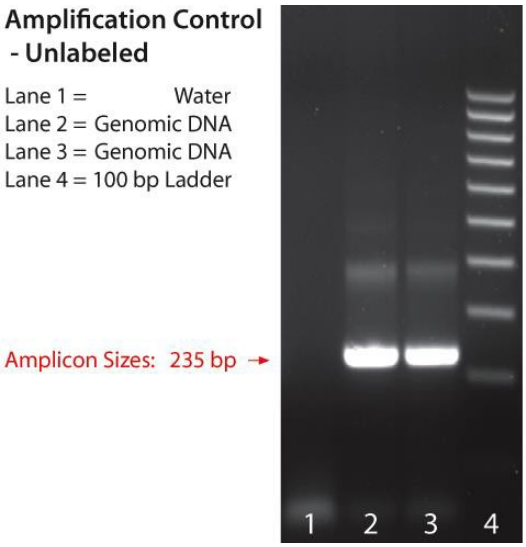

Figure 2. *Gel Detection with Agarose Gels.* This photograph at left contains data generated from two different gDNA samples (lanes 2 and 3). Both samples generate a bright band at 235 bp.

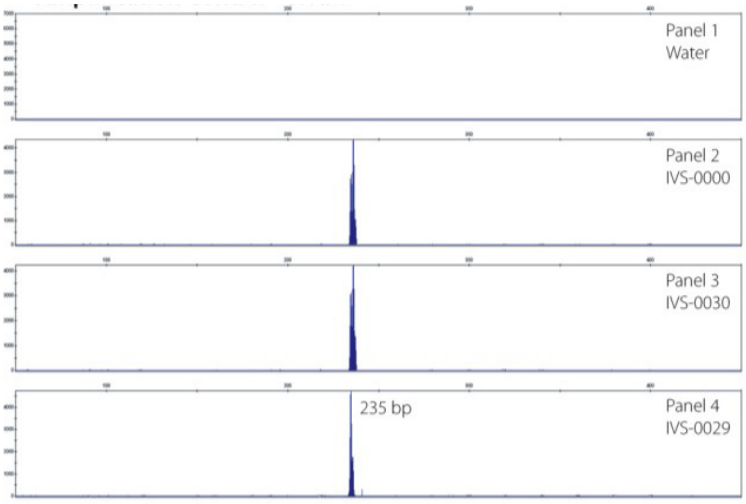

Figure 3. *ABI Fluorescence Detection.* The panels at right display differential fluorescence data generated by running water (panel 1), IVS-0000 Polyclonal Control DNA (panel 2), IVS-0030 Clonal Control DNA (panel 3) and IVS-0029 Clonal Control DNA (panel 4).

11. Technical and Customer Service

Thank you for purchasing our **Amplification Control** 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.

Contact Information

Invivoscribe, Inc.
10222 Barnes Canyon Road, Bldg. 1
San Diego, California 92121-2711
USA

Phone:	+1 858 224-6600
Fax:	+1 858 224-6601
Technical Service:	support@invivoscribe.com
Customer Service	sales@invivoscribe.com
Website:	www.invivoscribe.com
Business Hours:	7:00AM – 5:00PM PST/PDT

Authorized Representative and EU Technical Assistance

EC REP Invivoscribe Technologies, SARL
Le Forum – Bât B
515 Avenue de la Tramontane
ZI Athélia IV
13600 La Ciotat, France

Phone:	+33 (0)4 42 01 78 10
Fax:	+33 (0)4 88 56 22 89
Technical Service:	support@invivoscribe.com
Customer Service	sales-eu@invivoscribe.com
Website:	www.invivoscribe.com
Business Hours:	9:00AM – 5:00PM CET/CEST

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.

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. Amplification Control 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 50 µL for each sample + 150 µL for the positive, negative and no template controls.
- Include an additional 20 µL to correct for pipetting errors.

15.3. Add the appropriate amount of Taq DNA polymerase (0.25 µL @5 U/ µL per 55 µL total reaction volume) to the master mix and gently mix by inverting several times or gently vortexing.

15.4. Aliquot 50 µL of the master mix/enzyme mixture to individual tubes or wells of a PCR plate.

15.5. Add 5 µL DNA from the unknown and control samples to individual tubes or wells containing the respective master mix reactions, and pipette up and down several times to mix. 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 of 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 each PCR product.

15.13. Load 20 µL of the mixture into individual 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 the gel using UV illumination.

15.18. Photograph the gel interpret the 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. Add 1 µL of each PCR product to 10 µL HI-Deionized Formamide containing ROX size standards. Mix well.

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

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

15.26. Load samples and run.

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

15.27. Review profile and controls, report results.