Standard Operating Procedure **SOP056**

Title: SAFE USE AND MAINTENANCE OF THE APPLIED BIOSYSTEM STEPONE REALTIME POLYMERASE CHAIN REACTION (PCR) SYSTEM

Location: CBE Laboratories

1. PURPOSE

The intent of this SOP is to describe the use and maintenance of the Applied Biosystems StepOne RT-PCR Machine.

2. SCOPE

The Applied Biosystems StepOne Real-Time PCR System (StepOne System) uses fluorescent based PCR chemistries to provide:
(1) Quantitative detection of target nucleic acid sequences (targets) using real-time analysis.
(2) Qualitative detection of targets using post-PCR (endpoint) analysis.
(3) Qualitative analysis of the PCR product (achieved by melt curve analysis that occurs post-PCR).

This SOP assumes that the user knows how to handle DNA and/or RNA samples and prepare them for PCR.

Tutorials, Guides and Troubleshooting documents for first time and other users are available at http://www3.appliedbiosystems.com/AB Home/Support/TutorialsTroubleshooting/index.htm# and from http://www3.appliedbiosystems.com/AB Home/Support/TutorialsTroubleshooting/RealTimePCRTroubleshootingGuide/index.htm. An Essential Reference list is provided in Annex 1

This SOP does not cover the experimental procedures that can be run on the instrument. These should be derived on a case-by-case basis using the guidelines signposted in this SOP. Protocols for the experimental types described in this document should be derived to support the risk assessment of the individual work activity e.g. under COSHH assessment of reagent kits.

SPECIAL NOTES: HEALTH & SAFETY

(i) General Instrument Safety

Using the instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

- The instrument must be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide (SOP56/SPG). If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

- Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

(ii) Chemical Safety

Wastes produced by the StepOne instrument are potentially hazardous and can cause injury, illness, or death. Refer to local SOP039 for chemical storage, handling, and disposal procedures.

Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
The MSDS for any chemical supplied by Applied Biosystems is available from http://www3.appliedbiosystems.com/sup/msds/search.htm. NOTE: Each time you receive a new MSDS packaged with a hazardous chemical, ensure that the appropriate MSDS is replaced in the Master files.

- Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, lab coat, and gloves when handling reagent and waste bottles.

- Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, and lab coat.

- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS. Refer to SOP039 for further guidance - record spill in the Spill Record Log.

- Avoid the use of mutagenic gel staining reagent. The use of Ethidium Bromide is not permitted in the CBE Laboratories. Working concentrations of GelRed for example, which is a non-mutagenic and non-cytotoxic dye can be used for gel staining.

(iii) Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in the Laboratory Unit.

- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local SOP, COP and national regulations.

- CAUTION: Mixed chemical and biohazardous waste materials may require special handling, and disposal limitations may apply. Consult the relevant MSDS.Refer to SOP039 and SOP003 for guidance.

(iv) Electrical Safety

- Severe electrical shock can result from operating the StepOne instrument without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.
- Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in the laboratory unit.
- For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.
- Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.
- Use properly configured and approved line cords for the voltage supply in your facility. ➢ Plug the instrument into a properly grounded receptacle with adequate current capacity.

(v) LED Safety

To ensure safe LED operation:

➢ The system must be maintained by an Applied Biosystems Technical Representative.

➢ All instrument panels must be in place on the instrument while the instrument is operating. When all panels are installed, there is no detectable radiation present. If any panel is removed when the LED is operating (during service with safety interlocks disabled), you may be exposed to LED emissions in excess of the Class 3B rating.

➢ Do not remove safety labels or disable safety interlocks.

(vi) Biological Hazard Safety

Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local SOP, COP and/or national regulations.

- Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves.
- All work with viable biological materials should be performed in using physical containment devices such as a BSC. NOTE: Once the biological material has been added to the lysis buffer employed in the extraction procedure and rendered non-viable, subsequent extraction of the genomic sample can be carried out outside the BSC if necessary.

(vii) Workstation Safety

Correct ergonomic configuration of the workstation can reduce or prevent effects such as fatigue, pain, and strain.

- Minimize or eliminate these effects by configuring the workstation to promote neutral or relaxed working positions.
- To minimize musculoskeletal and repetitive motion risks:
  - Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
  - Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

3. RESPONSIBILITES

Laboratory Manager shall ensure that:

- All users of the instrument have received instructions in both general safety practices and specific safety practices for the instrument.
- All users have read and understood all applicable Material Safety Data Sheets (MSDSs).
- All Users have been trained and authorised to operate the instrument.
- The instrument is regularly maintained.

Operators shall ensure that:

- They have read and understood all applicable Material Safety Data Sheets (MSDSs).
- They have an approved COSHH assessment for the experimental run supported by a written protocol.

4. EQUIPMENT AND MATERIALS

4.1 Equipment and Consumables

(i) Applied Biosystems StepOne Real-Time PCR system. Serial Number 271000497.
(ii) Accuspin Micro-R centrifuge, Serial Number 0001094-01-00.
(iii) The StepOne system supports the consumables listed below. These consumables are for use with both standard and Fast reagents/protocols.

NOTE: Use only Fast consumables (reaction plates, tube strips, and tubes) with the StepOne system, even when performing an experiment with standard reagents.

- MicroAmp@ Fast Optical 48-Well Reaction Plate, Part No #4375816
- MicroAmp@ 48-Well Optical Adhesive Film, #4375323
- MicroAmp@ Fast 8-Tube strip, #4358293
- MicroAmp@ Optical 8-cap strip, #4323032
- MicroAmp@ Fast Reaction Tube with Cap, #4358297
- MicroAmp@ Fast 48-Well Tray, #4375282.
- MicroAmp@ 48-Well Base Adaptor; #4375284 ,
- MicroAmp@ 96-Well Support Base, #4379590

4.2 Reagents

IMPORTANT! The use of the following reagents in CBE Laboratories is subject to COSHH risk assessment and the provision of an experimental protocol.

The following reagent types (chemistries) can be used to detect PCR products on the Applied Biosystems Real-Time PCR System:

- TaqMan@ reagents
- SYBR@ Green reagents
- Other fluorescence-based reagents

The Reagent Guide (SOP056/RG) provides information about the reagents that can be used on the Applied Biosystems Real-Time PCR Systems, including:

- An introduction to TaqMan@ and SYBR@ Green reagents
- Descriptions and design guidelines for:
  — Quantitation experiments
  — Genotyping experiments

— Presence/absence experiments

(i) TaqMan Reagents

TaqMan reagents include Applied Biosystems TaqMan@ assays (pre-formulated mixes that contain probe and primer sets) and Applied Biosystems TaqMan@ master mixes. The assays are specific to the target of interest. The master mixes contain the remaining components needed for the PCR reaction. TaqMan reagents can be used for:

- Quantitation experiments, including:
  — Standard curve
  — Relative standard curve
  — Comparative CT
- Genotyping experiments
- Presence/absence experiments

Applied Biosystems offers two types of TaqMan@ probes:

- TaqMan@ MGB (minor groove-binder) probes with non-fluorescent quencher (NFQ)
- TaqMan@ TAMRA ™ probes with TAMRA ™ dye as quencher

(ii) SYBR Green Reagents

SYBR Green reagents include primers and master mixes that contain SYBR@ Green I dye. SYBR Green reagents can be used for the following quantitation experiments:

- Standard curve
- Relative standard curve
- Comparative CT

NOTE: Multiplex PCR cannot be performed using SYBR Green reagents.

(iii) Other Reagents

Other fluorescence-based reagents can be used on Applied Biosystems Real-Time PCR Systems. However, if you are using other reagents, ensure that the spectra of the dye(s) you select are appropriate, i.e. for the spectral range of data collection. For information on your instrument's filters or spectral range, see your instrument's installation and/or maintenance guide

5. PROCEDURE

The StepOne system collects raw fluorescence data at different points during a PCR, depending on the type of run that the instrument performs. Regardless of the run type, a data collection point or read on the StepOne ™ instrument consists of three phases:

1. Excitation — The instrument illuminates all wells of the reaction plate within the instrument, exciting the fluorophores in each reaction.

2. Emission — The instrument optics collect the residual fluorescence emitted from the wells of the reaction plate. The resulting image collected by the device consists only of light that corresponds to the range of emission wavelengths.

3. Collection — The instrument assembles a digital representation of the residual fluorescence collected over a fixed time interval. The StepOne ™ software stores the raw fluorescent image for analysis.

After a run, the StepOne software uses calibration data (spatial, dye, and background) to determine the location and intensity of the fluorescent signals in each read, the dye associated with each fluorescent signal, and the significance of the signal.—

5.1. General PCR Practices

Use the following precautions to minimize sample contamination and PCR product carryover:

- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves when preparing samples for PCR amplification. Change gloves whenever you suspect that they are contaminated.

- Maintain separate areas, dedicated equipment, and supplies for:
  — Sample preparation.
  — PCR setup. Never bring amplified PCR products into the PCR setup area.
  — PCR amplification.
  — Analysis of PCR products.
- Open and close all sample tubes carefully. Avoid splashing or spraying PCR samples.
- Use positive-displacement pipettors or air-displacement pipettors with filter-plugged tips. Change tips after each use.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 1% Virkon or 70% IMS.

5.2. Site Preparation and Installation of the Applied Biosystems StepOne) Real-Time PCR System

When the system is received or when the system has been moved or disconnected from the power supply the installation procedure detailed in the "Site Preparation Guide" (SOP056/SPG) and "System Installation" instructions provided on the quick Reference Card (SOP056/SI) must be followed.

(i) The site for installation of the system should be prepared according to the requirements detailed in SOP056/SPG)

(ii) Use the checklists (copies) in SOP056/SPG to ensure that all preparations for installing the system have been made. These should be retained in the Equipment File.

(iii) Follow the procedures detailed in SOP056/Sl for:

- Unpacking the StepOne system
- Setting up the StepOne instrument
- Setting up the computer
- Preparing the RNase plate
- Performing the RNase P Run
- Analysing the RNase P Run

5.3 Moving the Instrument

Perform this procedure to safely move the instrument short distances (for example, between laboratories in the same building).

(i) Materials Required: Original packing plate or a MicroAmp@ Optical Reaction Plate

(ii) Prepare the Instrument:

1. Load the packing plate or empty reaction plate into the instrument:
   a. Open the instrument drawer.
   b. Place the packing plate or empty reaction plate onto the sample block(s).
   c. Close the instrument drawer.

2. Raise the sample block(s) to secure it for transport:
   a. Touch the instrument touchscreen to awaken it, and then touch.
   b. In the Main Menu, touch Tools Menu, touch Ship Prep, then touch Ship Prep.
   c. Wait for the instrument to raise the sample block(s), then power off the instrument when prompted.

3. Disconnect the power cord from the back of the instrument.

(iii) Move and Reconnect the Instrument: Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. At least two people are required to lift the instrument.

CAUTION: Moving your instrument can create subtle changes in the alignment of the instrument optics. Applied Biosystems recommends that you run a TaqMan@ RNase P Fast Instrument Verification Plate after you move the instrument to confirm the performance of the system.

1. Move your instrument according to the guidelines (step 6 on page 16). Do not carry the instrument by the instrument drawer. Carrying the instrument by the drawer can damage the instrument optics.

2. Install the components of the system according the chosen layout. If the RNase P experiment performed at the end of the installation: Passes, then you have successfully moved the instrument. Fails, recalibrate the instrument according to the following:

a. Perform a spatial calibration (see "Perform a Spatial Calibration" on page 94).
b. Perform a background calibration (see "Perform a Background Calibration" on page 98).
c. Perform a dye calibration (see "Perform a Dye Calibration" on page 104).
d. Perform another RNase P experiment (see "Perform the RNase P Experiment" on page 41 or page 64).

5.4. Real-Time PCR Applications: Getting Started

The Real-Time PCR System supports many real-time quantitative PCR applications, including gene expression analysis, using relative standard curve and comparative CT for relative quantitation, and standard curve for absolute quantitation.

In addition, the system enables qualitative, post-PCR detection of nucleic acids for allelic discrimination (SNP genotyping) assays and presence/absence (plus/minus) assays that use internal positive controls. New applications include melt curve analysis as an independent application and real-time PCR amplification using the allelic discrimination (SNP genotyping) application.

A quantitation experiment is a real-time experiment that measures the quantity of a target nucleic acid sequence (target) during each amplification cycle of the polymerase chain reaction (PCR). The target can be DNA, cDNA, or RNA.

5.4.1 PCR Options

(i) When performing real-time PCR, you can perform a PCR reaction using either:

- Singleplex PCR —A single primer/probe set is present in the reaction tube or well. Only one target or endogenous control can be amplified per reaction.

  Or

- Multiplex PCR — Two or more primer/probe sets are present in the reaction tube or well. Each set amplifies a specific target or endogenous control. Typically, a probe labelled with FAM ™ dye detects the target and a probe labelled with VIC@ dye detects the endogenous control.

NOTE: SYBR@ Green reagents cannot be used for multiplex PCR.

NOTE: TAMRA™ dye reagents should not be used as a reporter or quencher with the StepOne system.

(ii) 1 -Step or 2-Step RT-PCR?

Reverse transcription-polymerase chain reaction (RT-PCR) is used to quantify RNA. RT-PCR can be performed as a I-step or 2-step procedure.

You can perform reverse transcription (RT) and PCR in a single reaction (I-step) or in separate reactions (2-step). The reagent configuration you use depends on whether you are performing 1or 2-step RT-PCR:

- In I-step RT-PCR, RT and PCR take place in one buffer system, which provides the convenience of a single-tube preparation for RT and PCR amplification. However, you cannot use Fast PCR master mix or the carryover prevention enzyme, AmpErase@ UNG (uracil-N-glycosylase), to perform I-step RT-PCR.

- 2-step RT-PCR is performed in two separate reactions: First, total RNA is reverse transcribed into cDNA, then the cDNA is amplified by PCR. This method is useful for detecting multiple transcripts from a single cDNA template or for storing cDNA aliquots for later use. The AmpErase@ UNG enzyme can be used to prevent carryover contamination.

5.4.2 Genotyping experiments (Guide SOP056/GE)

The guide explains how to perform genotyping experiments on the StepOne system. The guide provides a tutorial using example experimental data and provides instructions for completing your own genotyping experiment, including:

- Experimental design

➢ Preparation of PCR reactions
➢ Running the experiment on the StepOne system ➢ Analysing the experiment

(i) What Is a Genotyping Experiment?

A genotyping experiment is an endpoint experiment used to determine the genotype of unknown samples. With this experiment type, you can differentiate two alleles of a single nucleotide polymorphism (SNP).

A genotyping experiment determines if unknown samples are:

- Allele 1 homozygotes (samples having only allele 1)
- Allele 2 homozygotes (samples having only allele 2)
- Heterozygotes (samples having both allele 1 and allele 2)

(ii) Components

PCR reactions for genotyping experiments include the following components:

- Sample — The DNA sample in which the genotype of the target is unknown.
- (Optional) Replicates — Identical reactions containing identical components and volumes. • Negative Controls — Samples that contain water or buffer instead of template; also known as no template controls (NTCs). Negative controls should not amplify.
- (Optional) Positive controls — Samples that contain known genotypes (homozygotes for allele 1, homozygotes for allele 2, and heterozygotes for alleles 1 and 2). • Instruments - Genotyping experiments require two steps: thermal cycling (PCR amplification) followed by endpoint detection of the resulting fluorescence signals. You can perform the thermal cycling step (PCR amplification) on an Applied Biosystems Real-Time PCR System or on a standalone thermal cycler.

5.4.3 Presence/Absence Experiments

A presence/absence experiment -is an endpoint experiment that indicates the presence or absence of a specific nucleic acid sequence (target) in a sample. The actual quantity of target is not determined. Presence/absence experiments are commonly used to detect the presence or absence of a pathogen, such as a viral or bacterial pathogen. For example, a presence/absence experiment might be used to determine if Salmonella bacteria are present in hamburger meat. The results show if Salmonella bacteria are present or are not present; the quantity of bacteria is not determined.

The StepOne and StepOnePlus systems support the following reagents for presence/absence experiments:

- TaqMan@ reagents
- Other fluorescent-based reagents

NOTE: Presence/absence experiments are not supported for Fast master mix or Fast protocols.

With presence/absence experiments, you prepare PCR reactions that contain primers and probes to amplify the target and a reagent to detect amplification of the target. You can set up the PCR reactions for the presence/absence experiments three different ways:

- IPC setup — Use an internal positive control (IPC) to monitor the PCR progress and ensure that a negative result is not caused by failed PCR in the sample. PCR reactions contain two primer/probe sets: One to detect the unknown target (unknown target primer

set and TaqMan probe to detect the unknown target) and one to detect the IPC (IPC primer set and a VIC dye-labelled TaqMan probe to detect the IPC template). With this setup, there are three well types:

  - Unknown-IPC wells — Wells contain sample template and IPC template; the presence of the target is not known.
  - Negative control-IPC wells — Wells contain IPC template and water or buffer instead of sample template in the PCR reaction. Only the IPC template should amplify in negative control-IPC wells because-the reaction contains no sample template. Also called IPC+.
  - Negative control-blocked IPC wells — Wells contain IPC blocking agent instead of sample template in the PCR reaction. No amplification should occur in negative control-blocked IPC wells because the reaction contains no sample template and amplification of the IPC is blocked. Also called no amplification control (NAC).

- No IPC, singleplex setup — Use Advanced Setup to omit the IPC from your presence/absence experiment. PCR reactions contain one primer/probe set. PCR reactions do not contain the IPC. With this setup, there are two well types:

- Unknown wells — Wells contain sample template; the presence of the target is not known.

- Negative controls —Wells contain water or buffer instead of sample template.

- No IPC, multiplex setup — Use Advanced Setup to omit the IPC from your presence/absence experiment and detect two targets in one reaction. PCR reactions contain two primer/probe sets. PCR reactions do not include the IPC. With this setup, there are two well types:

- Unknown-Unknown wells — Wells contain sample template; the presence of the target is not known.

- Negative control-Negative control wells — Wells contain water or buffer instead of sample template.

5.4.4 Standard Curve Experiments (SOP056/SC)

The standard curve method is used to determine the absolute target quantity in samples. With the standard curve method, the StepOne software measures amplification of the target in samples and in a standard dilution series. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates the absolute quantity of target in the samples.

(i) Components

The following components are required when setting up PCR reactions for standard curve experiments:

- Sample — The sample in which the quantity of the target is unknown.
- Standard — A sample that contains known standard quantities; used in quantitation experiments to generate standard curves.
- Standard dilution series — A set of standards containing a range of known quantities. The standard dilution series is prepared by serially diluting standards.
- Replicates — The total number of identical reactions containing identical samples, components, and volumes.
- Negative Controls — Wells that contain water or buffer instead of sample template. No amplification of the target should occur in negative control wells.

5.4.5 Relative Standard Curve and Comparative CT Experiments

iThe relative standard curve method

The relative standard curve method is used to determine relative target quantity in samples. With the relative standard curve method, the StepOne software measures amplification of the target and of the endogenous control in samples in a reference sample, and in a standard dilution series.

Measurements are normalized using the endogenous control. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates target quantity in the samples and in the reference sample. The software determines the relative quantity of target in each sample by comparing target quantity in each sample to target quantity in the reference sample. Relative standard curve experiments are commonly used to:

- Compare expression levels of a gene in different tissues.
- Compare expression levels of a gene in a treated sample vs. an untreated sample.
- Compare expression levels of wild-type alleles vs. mutated alleles.

Components

The following components are required when setting up PCR reactions for relative standard curve experiments:

- Sample — The sample in which the quantity of the target is unknown.

- Reference sample — The sample used as the basis for relative quantitation results. For example, in a study of drug effects on gene expression, an untreated control would be an appropriate reference sample. Also called calibrator.
- Standard —A sample that contains known standard quantities; used in quantitation experiments to generate standard curves.
- Standard dilution series — A set of standards containing a range of known quantities. The standard dilution series is prepared by serially diluting standards.
- Endogenous control — A target or gene that should be expressed at similar levels in all samples you are testing. The endogenous control is used to normalize fluorescence signals for the target you are quantifying. Housekeeping genes can be used as endogenous controls.
- Replicates — The total number of identical reactions containing identical samples, components, and volumes.
- Negative Controls — Wells that contain water or buffer instead of sample template. No amplification of the target should occur in negative control wells.

(ii) The comparative CT method

The comparative CT method is used to determine the relative target quantity in samples. With the comparative CT method, the StepOne software measures amplification of the target and of the endogenous control in samples and in a reference sample. Measurements are normalized using the endogenous control. The software determines the relative quantity of target in each sample by comparing normalized target quantity in each sample to normalized target quantity in the reference sample. Comparative CT experiments are commonly used to:

- Compare expression levels of a gene in different tissues.
- Compare expression levels of a gene in a treated sample vs. an untreated sample. • Compare expression levels of wild-type alleles vs. mutated alleles.

Components

The following components are required when setting up PCR reactions for comparative CT experiments:

- Sample The sample in which the quantity of the target is unknown.
- Reference sample — The sample used as the basis for relative quantitation results. For example, in a study of drug effects on gene expression, an untreated control would be an appropriate reference sample. Also called calibrator.
- Endogenous control —A target or gene that should be expressed at similar levels in all samples you are testing. The endogenous control is used to normalize fluorescence signals for the target you are quantifying. Housekeeping genes can be used as endogenous controls.

- Replicates — The total number of identical reactions containing identical samples, components, and volumes.
- Negative Controls —Wells that contain water or buffer instead of sample template. No amplification of the target should occur in negative control wells.

A 1% Virkon solution should be used for resolving fluorescent contamination on the StepOne ™ instrument sample block. However, excessive use of the solution can corrode the block material.

To prevent degradation of the block, observe the following guidelines when using the Virkon solution:

- Decontaminate the sample block using 1% Virkon solution only as a last resort! CAUTION: Contact time MUST be minimised to less than 10 minutes. Rinse the sample block with Virkon only after treatments of deionized water and 70% IMS (or 95% ethanol) fail to remove the contamination.

- Rinse the sample block thoroughly using deionized water after treating the block with 1% Virkon solution. Thoroughly removing residual Virkon from metal surfaces with water will minimize the long-term effects of Virkon.

5.6 Regular Maintenance of the System (refer to Guide SOP056/INM)

This guide covers:

5.6.1 Maintenance Schedule

Routine maintenance of the Applied Biosystems StepOne ™ Real-Time PCR Instrument and computer must be performed to ensure proper operation. The following is the recommended maintenance schedule (TABLE 1) of the tasks performed should be completed (see Section 8).

TABLE 1: Recommended Maintenance Schedule

Perform every	Task	See Page.....of the Guide
WEEK or before each use	1. Check the computer disk space. If necessary, archive or back u our experimental file	117
	2. Power of the computer controlling the instrument; after 30 seconds power on the computer	
	3. Clean the surface of the instrument with a lint-free cloth	
	4. Defragment the computer hard drive	
MONTH	1. Perform a background calibration*	98
1.5 YEARS	1. Perform a spatial calibration	94
	2. Perform a d e calibration	104

*NOTE: You should run a background calibration to if contamination is suspected.

**NOTE: You must run a background calibration and a dye calibration if any part of the instrument optics are replaced or moved.

(i) Connection Required for Maintenance

The spatial, background, and dye calibrations must be performed while the system is in a colocated layout. To set up the system for maintenance, connect the yellow StepOne system cable between the:

- Yellow Ethernet port of the instrument, and
- Ethernet port of the computer running the StepOne ™ software

NOTE: Do not connect the StepOne system cable to the blue LAN Port, which is reserved for a network connection.

(ii) Maintenance Notifications

When a required maintenance task such as a calibration is overdue, the StepOne ™ software and instrument display messages that prompt you to perform the necessary procedure.

You can use the StepOne ™ software to view a summary of maintenance data for the instrument. To view the maintenance information, select Instrument Maintenance Manager in the StepOne ™ software.

For each calibration (spatial, background, and dye), the software lists the following information:

- Status — Condition of the current calibration where
  _ Current indicates that the calibration is up to date.
  _Expired indicates that the calibration must be performed at the earliest possible convenience.
  _Not Run indicates that the calibration has not been run (RNase P only).

  - Last Run — Date when current calibration was run.
  - Expiry Date — Date when current calibration will expire.

(iii) Instrument Tools Menu Tests

The Tools Menu of the instrument provides several features that test the temperature of several instrument components against the product specification. Applied Biosystems recommends that you perform an annual temperature verification of the instrument depending on your usage and laboratory requirements. The temperature verification service can be performed by Applied Biosystems when the instrument is shipped for an annual service, or by an Applied Biosystems service engineer at an additional cost.

(iv) Archive and Back Up Data

- **Check Disk Space Regularly**

If you perform real-time experiments on your system, or collect real-time data for genotyping experiments, check the available space on your hard drive weekly. When the hard drive is within 20% of maximum capacity, transfer the older data to a backup storage device.

- **Archive Older Experiments**

Conserve space on the computer hard drive by using a data compression utility to archive unused or older experiments. Several commercially available compression utilities are available, such as the PKZIP and ARC archive formats common to the Microsoft@ Windows@ operating system.

- **Back Up Experiments Regularly**

Back up the experiments generated by your system to:

  - Protect against potential data loss of data caused by an unforeseen failure of the computer or its hard drive(s).
  - Conserve space on the hard drive and to optimize performance, if you remove old data after backing up.

5.7. Infrequent Maintenance (refer to Guide SOP056/INM)

Perform the following procedures as needed to resolve problems as they arise:

5.8. Equipment Malfunction

(i) If any part of the equipment fails or malfunctions, the user should contact the Laboratory Manager. With permission of the Lab Manager the user should consult the Operator Instruction Manuals to access fault finding and troubleshooting procedures.

(ii) All problems and corrective actions should be recorded in the Equipment Maintenance record.

(iii) If the equipment fails to work or malfunctions and cannot be rectified according to troubleshooting procedures detailed in the Operator and Users Manuals the Laboratory Manager must be informed and a "Do Not Use" notice should be posted on the cytometer. Contact the manufacturer for advice and coordinate with the Lab Manager for external maintenance and servicing.

(iv) External maintenance and servicing of the equipment can only be performed after it has been suitably disinfected (refer to SOP003 for further details) and a 'Decontamination Certificate' has been issued by the School/Building/Unit Safety Co-ordinator.

5.8.1 How to Troubleshoot the Installation (refer to Guide SOP056/INM)

The Guide covers:

5.8.2 How to Obtain Support

For the latest services and support information, go to http://www.appliedbiosystems.com, then click the link for Support. At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

NOTE: When you need to schedule maintenance for your StepOne ™ instrument (such as annual planned maintenance or temperature verification/calibration) contact the Applied Biosystems Care Centre. To obtain a phone number for or to send an e-mail to the centre, go to http://www.appliedbiosystems.com/support/contact.

6. DOCUMENTATION

The following records are outputs of this SOP:

FSOP056.2: Biosystems StepOne Real-Time PCR System Training Agreement

QS-FORM-009 Decontamination of Equipment Certificate

Equipment Maintenance Record

These records shall be filed in the Equipment File and stored in the CBE Office or otherwise archived for future review or retrieval.

ANNEX 1: ESSENTIAL REFERENCES

1 . Applied Biosystems StepOne™ Real-Time PCR System (manuals are kept in the PCR equipment folder):
   - Installation, Networking and Maintenance Guide (SOP056/INM)
   - Site Preparation Guide (SOP056/SPG) System Installation Guide (SOP056/Sl)
   - Reagent Guide (SOP056/RG)

- Relative Standard curve and Comparative CT Experiments Instruction Manual (SOP056/RSC)
- Standard Curve Experiments Instruction Manual (SOP056/SC)
- Genotyping Experiments Instruction Manual (SOP056/GE)

2. Documents Related to Genotyping Experiments (available from Applied Biosystems website)
3. Documents Related to Presence/Absence Experiments, to Relative Standard Curve and Comparative CT Experiments, to Standard Curve Experiments (available from Applied Biosystems website)
4. Documents Related to the Reagent Guide (available from Applied Biosystems website)

SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
1.0	**30.10.12**	Annual Review-format revised.	**2.0**
2.0	**23/06/20 JB**	Reviewed. Nothing changed	**2.0**