June 2009

EZ1® Validation Guide

For the EZ1 DNA Investigator Kit

BioRobot® EZ1

EZ1 Advanced

EZ1 Advanced XL

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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Product Use Limitations

The EZ1 DNA Investigator Kit is intended for molecular biology applications. This product is neither intended for the diagnosis, prevention, or treatment of a disease, nor has it been validated for such use either alone or in combination with other products.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding EZ1 instruments, the EZ1 DNA Investigator Kit, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Introduction

Validation in genetic identity and forensics laboratories

Validation is the process used by the scientific community to acquire the necessary information for accessing a procedure's reliability to obtain a specific, desired result. The validation process also serves to identify critical aspects of a procedure that must be controlled and monitored, while defining the limitations of the procedure (1).

As such, validation of new equipment or methods is an important part of a forensic quality assurance system. An internal validation study must always be conducted when a new technique is introduced to a laboratory, or when an existing method is replaced by a new one. The new laboratory procedure must be shown to be robust, reliable, and reproducible by the personnel performing the test in that laboratory (2).

A validation assesses analytical performance characteristics, such as precision and reproducibility, sensitivity, and linearity. If the new method replaces, parallels, or expands an existing procedure, concordance also needs to be shown. Demonstrating the absence of (cross-) contamination events is critical. It is essential that known and nonprobative evidence samples are used for validation testing (1).

Official guidelines

A number of international and national authorities provide guidelines and recommendations for validation testing in human identity and forensic environments. These guidelines were incorporated into this guide wherever applicable to forensic preanalytical sample preparation. These include the DNA Advisory Board (DAB) Quality Assurance Standards for Forensic DNA Testing Laboratories (1), recommendations of the DNA Commission of the International Society of Forensic Genetics (ISFG) (3), Validation Guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM) (4), and validation guidance issued by the European Network of Forensic Science Institutes (ENFSI) (5, 6).

Simplifying validation

Molecular forensics requires analysis of a multitude of sample types, varying greatly with respect to source, carrier or matrix, DNA quality and quantity, presence of inhibitors, or age. In addition, validations must be conducted in agreement with guidelines or regulations issued by authorities.

As a result, there is variation among laboratories regarding design of validation studies. QIAGEN's Validation Guides are intended to make validations easier by offering standardized concepts.

About this guide

QIAGEN's Validation Guides assist with the design and practical experimental execution of a meaningful and representative validation plan, whilst considering time and other resource cost factors. This guide leads you through the validation of the EZ1 DNA Investigator Kit on the BioRobot EZ1, EZ1 Advanced, and EZ1 Advanced XL and focuses specifically on human identity and forensic preanalytical sample preparation.

The guide is divided into six parts:

- Introduction
- Definitions of performance parameters that comprise the validation
- Instructions for the experiments of the validation study
- Troubleshooting Guide
- References
- Appendix: Longitudinal Study (Optional) — DNA Integrity Over Time

All QIAGEN technical and scientific literature is available at www.qiagen.com. Refer to the web site for the most current version of the Validation Guide.

Please note that this document is a proposal of QIAGEN GmbH and does not replace consultation with your competent authority to approve the necessary individual validation steps in your laboratory.

Validation Study Performance Parameters

The recommended minimum parameters for the validation of preanalytical extraction methods for DNA profiling are sensitivity, linearity, repeatability, and reproducibility.

If existing methods will be replaced by the new method, or if the new method complements existing methods, concordance should also be demonstrated.

If the established new method will be used to process multiple samples in parallel, a study on absence of contamination is critical.

As part of a concordance study, it should be demonstrated that the DNA profiling quality of the new method at least compares to, or is better than, the existing or reference method.

Sample types typically encountered by the validating laboratory, or specific sample types that will be subject to the new method should be used for the validation study. A performance assessment with case-type samples should also investigate the influence of different cell types, different sample carrier substrates and mixtures from more than one biological source.

Forensic molecular biology evidence needs to be archived for extended periods of time, often for many years. This requires that the preanalytical extraction method purifies biological evidence to minimize potential for degradation over time. Incomplete removal of nucleases or unfavorable physicochemical buffer compositions may lead to degradation. These effects will be invisible at the time of the initial validation study start but can be surveyed by an extended archiving study set. It is therefore recommended to initiate a longitudinal archiving study at the time of the validation.

Sensitivity[1] and linearity

The sensitivity study assesses the range of DNA quantities able to produce reliable typing results.

Linearity evaluates how output signal intensity varies in proportion to DNA input. Usually, the regression line for the linear section is calculated by regression analysis and deviations of the curve's linearity are indicated as endpoints.

Both studies can be combined into one experimental setup.

Reproducibility[2] and repeatability

Reproducibility is defined as the variation in measurements obtained when two or more people or two or more different laboratories measure the same unit (sample) using the same measuring technique. It confirms that consistent results are achieved with the same sample types.

Repeatability is the variation in measurements when one person measures the same unit (sample) with the same measuring equipment.

Concordance

The concordance study evaluates the quality of results of the new method in comparison to the current or reference method.

Contamination[3]

The contamination study demonstrates the absence of contamination events that would compromise the integrity of results.

Case-type samples[4]

Three representative sample types are investigated in the concordance study. If desired, more sample categories typically processed at the validating laboratory can be added to an optional extended case-type samples study.

Matrix dependence assesses the influence of various carrier substrates – or matrices – on analytical results. The influence of different cell types, carrier substrates and the performance with mixtures (i.e. samples consisting of biological material from more than one contributor) is more broadly documented.

[1] For the US: Corresponds to SWGDAM Revised Validation Guidelines 2.3.

[2] For the US: Corresponds to SWGDAM Revised Validation Guidelines 2.5.

[3] For the US: Corresponds to SWGDAM Revised Validation Guidelines 3.6.

[4] For the US: Corresponds to SWGDAM Revised Validation Guidelines 2.6.

Validation Study

In this section, instructions are provided for the validation. The section starts with an overview of the validation study to allow planning of resources and time. The guide then covers the preparation and execution of the validation study experiments.

General considerations

Sample numbers

Unless otherwise indicated, dilution series should include 5 to 10 samples and be run in triplicates. At least five independent samples per experiment should be tested for the validation of individual sample types. Use of negative controls may be considered, but is not required.

Homogenous experimental conditions

In order to reduce variability, use the same lot of materials and the same downstream instrumentation within each of the validation study modules.

Ensure identical treatment and run conditions for comparison samples. Comparison samples should be run in parallel if possible.

Analytical parameters

The intended analytical result of the process for which the new extraction technology is validated should be central to the evaluation. Normally this is a short tandem repeat (STR) profile.

Other than in the linearity study, it is strongly advised not to make DNA quantification results the primary success criteria of this validation study. Quantification results do not fully predict whether a downstream analysis will be successful, and the results of different quantification assays may not be fully comparable.[1]

All downstream methods used in the validating laboratory should be verified and included to the minimum criteria: sensitivity, linearity, repeatability and reproducibility – these could include conventional autosomal-, gonosomal-, or mini-STR analysis, as well as any other sequence or SNP-based chromosomal and mitochondrial DNA (mtDNA) genotyping techniques.

[1] While quantification provides necessary information for method normalization and documentation, the predictive value of a quantification result for downstream performance (e.g., the quality of a STR profile) is limited. Inhibitor removal and physicochemical adequacy of the eluate can only be fully assessed by subjecting the sample to the appropriate downstream analysis.

Assuming that (autosomal) STR analysis is the laboratory's main analytical method, meaningful combinations should be arranged for complementary methods in a case-type study: Y-STR analysis should be incorporated to sexual assault or male/female mixture samples, while mini-STR or mtDNA sequencing could be employed with bone, tooth, or specimens assessing performance with compromised DNA evidence.

Results

The correct determination of the expected genotype stands at the center of the performance assessment of the EZ1 DNA Investigator kit for a given sample type or concentration.

For STR genotyping, recommended quantitative parameters for performance assessment and documentation are:

- Frequency of full or complete STR profiles (FP)
- Frequency of partial or incomplete STR profiles (PP)
- Frequency of no profiles detectable (NP)

The quality of the extraction chemistry impacts the quality of the STR profile which can be further assessed by:

- Locus peak balance: acceptable locus peak balance ratios are >60% for good quality samples
- Between loci peak balance: acceptable between loci peak balance ratios are >60% for good quality samples
- Peak height/area: peak height/area must be a function of DNA amount, and equivalent amounts of DNA should give equivalent peak heights/areas

Prior to genotyping, DNA yield is measured and documented:

- DNA yield (ng)

Multiple units

If the EZ1 DNA Investigator Kit is validated for use on multiple instrument units, a performance check on each of the sister instruments is sufficient. A performance check is an evaluation of a validated procedure existing in the laboratory system to ensure that it conforms to specifications (1). It should include at a minimum reproducibility, sensitivity, and a contamination assessment of each the additional instruments.

Study overview

Table 1 provides an outline of the validation study. It is recommended to reserve one day each for the sensitivity and linearity, repeatability and reproducibility, concordance, and cross-contamination studies. At least one additional day should be dedicated to extended studies on case-type samples.

Within each study category, the number of samples subject to extraction and the various analytical downstream methods employed is indicated. This allows for resource planning (time, material and equipment, personnel).

Detailed planning, documentation, and careful sample storage is recommended for the optional longitudinal or archiving study (see Appendix). For example, a 10 year study design would require keeping 10 aliquots of at least five independent original eluates archived and traceable, and the corresponding documentation accessible.

Table 1. Validation study plan, total number of samples.

Within each study category, the number of samples subject to extraction and the various analytical downstream methods employed is indicated.

Time	Study type	Comment	Sample type	Sample extractions					Analyses						
				New method		Reference method			Quantification		STR		Other*‡		
					Total		Total			Total		Total		Total	
Day 1	Sensitivity and linearity	5 dilution steps, triplicates, 3 runs of 5 samples each	Blood or cells	15	15	–	–		15	15	15	15	15	15	
Day 2	Repeatability and reproducibility	3 runs of 6 samples each	Blood or cells	18	33	–	–		18	33	18	33	18	33	
Day 3	Concordance	New and existing method: 3 sets of 6 samples each	Blood, buccal swabs, cigarette butts	18	51	18	18		36	69	36	69	36	69	
Day 4	Contamination		Blood or cells	6	57	–	–		–	–	18	87	–	–	
Day 5	Case-type samples*†	5 independent samples per sample type		15	72	–	–		15	84	15	102	15	84	

* Optional study modules or parameters.

† Assumes 3 additional case-type sample categories.

‡ Indicates number of analyses per each additional analysis parameter.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, face mask and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

For the BioRobot EZ1

- BioRobot EZ1 and disposables (cat. no. 9000705)
- EZ1 DNA Investigator Card (cat. no. 9016387)

For the EZ1 Advanced

- EZ1 Advanced (cat. no. 9001410)
- EZ1 Advanced DNA Investigator Card (cat. no. 9018302)

For documentation purposes, one of the following is required:

- EZ1 Advanced Communicator Software (supplied with the EZ1 Advanced), PC (can be connected to up to four EZ1 Advanced instruments), and monitor (cat. no. for PC and monitor 9016643)
- EZ1 Advanced Communicator Software (supplied with EZ1 Advanced) and your own PC and monitor (connection with up to four EZ Advanced instruments is not recommended)
- Printer (cat. no. 9018464) and accessory package for printer (cat. no. 9018465)

For the EZ1 Advanced XL

- EZ1 Advanced XL (cat. no. 9001492)
- EZ1 Advanced XL DNA Investigator Card (cat. no. 9018699)

For documentation purposes, one of the following is required:

- EZ1 Advanced Communicator Software (supplied with the EZ1 Advanced XL), PC (can be connected to up to four EZ1 Advanced or EZ1 Advanced XL instruments), and monitor (cat. no. for PC and monitor 9016643)
- EZ1 Advanced Communicator Software (supplied with EZ1 Advanced) and your own PC and monitor (connection with up to four EZ1 Advanced or EZ1 Advanced XL instruments is not recommended)
- Printer (cat. no. 9018464) and accessory package for printer (cat. no. 9018465)

For all instruments

- EZ1 DNA Investigator Kit (cat. no. 952034)
- Additional Buffer G2 (cat. no. 1014636)
- Study samples prepared according to the instructions provided on pages 13–14 of the *EZ1 DNA Investigator Handbook* (7).

Sensitivity and linearity

Important points before starting

- Start run 2 and 3 immediately after the previous run is finished

Things to do before starting

- Prepare a dilution series of blood in Buffer G2. Dilute 40 µl blood in 760 µl Buffer G2 and continue with four serial dilutions 1:10, using 100 µl diluted blood and 900 µl Buffer G2. Final concentrations of blood are 10 µl, 1 µl, 0.1 µl, 0.01 µl, and 0.001 µl, each in 200 µl volume. Carefully mix and vortex before each dilution step.

Runs

- Run 1: Triplicates of 10 µl and 1 µl blood samples
- Run 2: Triplicates of 0.1 µl and 0.01 µl blood samples
- Run 3: Triplicate of 0.001 µl blood samples

Procedure

1. **Transfer 200 µl of the diluted blood sample into an EZ1 sample tube (2 ml).**
2. **Continue with Protocol: DNA Purification (Trace Protocol), in the *EZ1 DNA Investigator Handbook*. Choose elution in 50 µl water in step 5 of the procedure.**

Analysis

After extraction, quantify samples using the current quantification system in use in the validating laboratory.

Following quantification, analyze samples with every STR system and any other genotyping system to be included to the validation.

Expected results

A linear relationship between sample amount input and quantitative readout (e.g. DNA yield, STR peak height/area) is demonstrated by linear regression analysis.

A quantitative range for reliable typing results and a minimal threshold (pg DNA) is determined below which the extraction does not yield amplifiable DNA.

Reproducibility and repeatability

Important points before starting

- All blood samples used must be from a single primary sample as the amount of white blood cells in the bloodstream varies from individual to individual. Prepare 3 aliquots of 100 µl blood each to be extracted in 3 individual EZ1 runs. Mix the primary sample thoroughly by vortexing before preparing aliquots

Procedure

1. **Thaw and equilibrate a 100 µl whole blood sample aliquot at room temperature (15–25°C).**
2. **Mix the blood sample thoroughly by vortexing.**
3. **Transfer 10 µl each of the blood sample into 6 EZ1 sample tubes (2 ml).**

   Note: Mix the blood sample thoroughly by vortexing immediately before transferring to the sample tubes.
4. **Add 190 µl Buffer G2 to each sample tube.**
5. **Continue with Protocol: DNA Purification (Trace Protocol), in the *EZ1 DNA Investigator Handbook*. Choose elution in 200 µl TE in step 5 of the procedure.**

Analysis

After extraction, quantify samples using the current quantification system in use in the validating laboratory.

Following quantification, analyze samples with every STR system and any other genotyping system to be included to the validation.

Expected results

Equivalent results should be generated between sample positions of the same run and between different runs.

Concordance

Run 1: Human whole blood

Important points before starting

- All blood samples used must be from a single primary sample
- Conduct extractions using the EZ1 and your current method simultaneously, if possible

Procedure

1. **Thaw and equilibrate a ≥150 µl whole blood sample at room temperature (15–25°C).**
2. **Mix the blood sample thoroughly by vortexing.**
3. **Transfer 10 µl each of the blood sample into 6 EZ1 sample tubes (2 ml).**

   Note: Mix the blood sample thoroughly by vortexing immediately before transferring to the sample tubes. Prepare 10 µl blood samples to be extracted using the reference method in a suitable vessel simultaneously.
4. **Add 190 µl Buffer G2.**
5. **Continue with Protocol: DNA Purification (Trace Protocol), in the *EZ1 DNA Investigator Handbook*. Choose elution in 50 µl water in step 5 of the procedure.**

Analysis

After extraction, quantify samples using the current quantification system in use in the validating laboratory.

Following quantification, analyze samples with every STR system and any other genotyping system to be included to the validation.

Expected results

Concordant results are generated when comparing the EZ1 DNA Investigator Kit with the existing method.

Run 2: Buccal swabs

Important point before starting

- Conduct extractions using the EZ1 and your current method simultaneously, if possible

Things to do before starting

- Prepare 6 buccal swabs. Let the swab air-dry for at least 2 hours after sample collection
- Cut each swab sample in half. Use one half each for EZ1 and reference extraction
- Since swabs tend to be absorbent, it is often necessary to add a greater volume of digestion buffer to the sample in step 2. To provide sufficient digestion buffer for absorbent samples, Buffer G2 should be diluted with distilled water before use. Dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for n + 1 samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality
- Heat a thermomixer to 56°C for the proteinase K digest in step 4

Procedure

1. **Place a half swab into a 2 ml sample tube.**
2. **Add 290 µl of diluted Buffer G2.**
3. **Add 10 µl proteinase K and mix thoroughly by vortexing for 10 s.**
4. **Incubate at 56°C with shaking at 900 rpm for 15 min.**
5. **Continue with Protocol: DNA Purification (Trace Protocol), in the *EZ1 DNA Investigator Handbook*. Choose elution in 50 µl water in step 5 of the procedure.**

Analysis and expected results

See Analysis and expected results for run 1, page 16.

Run 3: Cigarette butts

Important point before starting

- Conduct extractions using the EZ1 and your current method simultaneously, if possible

Things to do before starting

- Prepare 6 cigarette butts (outer paper only). Cut each sample in half. Use one half each for EZ1 and reference extraction
- Heat a thermomixer to 56°C for the proteinase K digest in step 4

Procedure

1. **Cut the halved cigarette butt paper sample into 4 smaller pieces and place the pieces into a 2 ml sample tube.**
2. **Add 190 µl of Buffer G2.**
3. **Add 10 µl proteinase K, and mix thoroughly by vortexing for 10 s.**
4. **Incubate at 56°C with shaking at 900 rpm for 15 min.**
5. **Continue with Protocol: DNA Purification (Trace Protocol), in the *EZ1 DNA Investigator Handbook*. Choose elution in 50 µl water in step 5 of the procedure.**

Analysis and expected results

See Analysis and expected results for run 1, page 16.

Absence of contamination

Important point before starting

- All blood samples used must be from a single primary sample. A total of 6 blood samples (200 µl each) is required. Mix the primary sample thoroughly by vortexing before preparing aliquots

Runs

- Run 1: Use 3 blood samples in positions 1, 3, and 5 of the instrument. Load negative controls of 200 µl water in positions 2, 4, and 6
- Run 2: Use 3 blood samples in positions 2, 4, and 6 of the instrument. Load negative controls of 200 µl water in positions 1, 3, and 5
- Run 3: Load negative controls (200 µl water) in all positions

Procedure

1. **Thaw and equilibrate 3 x 200 µl whole blood sample aliquots at room temperature (15–25°C).**
2. **Transfer the blood samples into 3 EZ1 sample tubes (2 ml). Transfer 200 µl of water into 3 additional EZ1 sample tubes.**
3. **Continue with Protocol: DNA Purification (Trace Protocol), in the *EZ1 DNA Investigator Handbook*. Choose elution in 50 µl TE in step 5 of the procedure.**

Analysis

After extraction, quantify samples using the current quantification system in use in the validating laboratory.

Following quantification, analyze samples with every STR system and any other genotyping system to be included to the validation.

Expected results

No detectable quantities of genomic DNA should be present in blank positions.

Case-type samples

A case-type samples study module can be designed incorporating specimens from the following categories. Cell types typically encountered in forensic specimens are listed under "different cell types". To investigate the influence of carrier substrates ("matrix dependence"), samples of known genotype are examined after contact with a variety of matrixes. In a mixture study, known ratios of samples of known genotypes are assessed.

Refer to *the EZ1 DNA Investigator Handbook* (7) to select appropriate protocols.

Different cell types

- Saliva
- Blood or cultured cells
- Tissue
- Sperm cells
- Hair
- Teeth
- Bone

Matrix dependence

- Sperm cells, spotted on swab
- Blood, spotted on swab
- Saliva, spotted on swab
- Tape lifts
- Cells or latent fingerprints (swabbed from surface)
- Stamps on envelopes
- Cigarette butts
- Chewing gum
- Blood, spotted on noncolored fabric, colored fabric, or leather
- Saliva, spotted on noncolored fabric, colored fabric, or leather
- Sperm cells, spotted on noncolored fabric, colored fabric, or leather
- Body fluids or tissues contaminated by soil

Mixtures

Prepare and analyze mixtures of body fluids originating from two different individuals. Consider mixtures of different sample types (e.g. blood/saliva, blood/sperm, etc.) and varying mixtures ratios (e.g. 1:1, 1:2, 1:10, 1:50, 1:100).

Analysis

After extraction, quantify samples using the current quantification system in use in the validating laboratory.

Following quantification, analyze samples with every STR system and any other genotyping systems to be included to the validation.

Expected results

Fluctuations in quantitative results must be expected and are acceptable due to the variation of amount, source and types of samples.

In the mixture study, major-to-minor component peak ratios are proportional to mixture ratios. No preferential extraction from one sample type over another or from one component over the other is observed.

Troubleshooting Guide

For troubleshooting information please refer to the Troubleshooting Guide in the *EZ1 DNA Investigator Handbook* (7).

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

1. Quality Assurance Standards for Forensic DNA Testing Laboratories and for Convicted Offender DNA Databasing Laboratories. DNA Advisory Board – DAB. Forensic Science Communications, July 2000, Volume 2, Number 3. www.fbi.gov/hq/lab/fsc/backissu/july2000/codispre.htm

2. Butler, J. (2001). Laboratory Validation. In: Forensic DNA Typing: Biology and Technology Behind STR Markers. San Diego: Elsevier Academic Press, p 205.

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5. Validation and Implementation of (New) Methods. European Network of Forensic Science Institutes - ENFSI, Standing Committee for Quality and Competence (QCC). Ref. Code: QCC-VAL-001, Issue No.: 001, 4 November 2006. www.enfsi.eu/get_doc.php?uid=144

6. Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process. ENFSI DNA Working Group, 2008.

7. *EZ1 DNA Investigator Handbook*.

Appendix: Longitudinal Study — DNA Integrity Over Time

Sample archiving and DNA integrity

The internal validation is complete with studies on sensitivity and linearity, reproducibility and repeatability, concordance, absence of contamination and case-type samples (page 20).

The optional archiving study monitors the integrity of purified DNA over extended periods of time. Nondiluted validation study eluates are archived at standard storage conditions and re-assessed at $t_n = t_0 + n$ years.[1]

Ideally, the sensitivity/linearity study is utilized. DNA integrity is verified by comparing STR profiles of a given sample at $t = t_n$ to the original STR profile at $t = t_0$, or by utilizing other appropriate means, such as restriction fragment length polymorphism (RFLP).

To avoid frequent freeze–thaw cycles, aliquotted storage of the study cohort is recommended.

Repeat the analysis of the reproducibility and repeatability study annually, utilizing aliquots of the original validation cohort.

[1] i.e., $t_1 = t_0 + 1$ year; $t_2 = t_0 + 2$ years etc.

Notes

Notes

Notes

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the EZ1 DNA Investigator Kit to the following terms:

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China ■ Orders 0086-21-3865-3865 ■ Fax 0086-21-3865-3965 ■ Technical 800-988-0325, 800-988-0327

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Italy ■ Orders 02-33430-420 ■ Fax 02-33430-426 ■ Technical 800-787980

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