

DNA Analyst Training Laboratory Training Manual

Subject 3: Extraction and Quantitation



PRESIDENT'S
DNA
INITIATIVE



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Purpose

To instruct the trainee in the proper performance of the most commonly used DNA extraction methodologies, including Chelex® 100, organic, and FTA® procedures.

These methodologies will encompass:

- Single source samples
- Mixtures requiring a differential extraction
- Mixtures not requiring a differential extraction
- Bone
- Tissue (e.g. muscle, hair)

To instruct the trainee in the proper performance of DNA quantitation methodologies, including slot blot and real-time polymerase chain reaction (PCR).

These methodologies will encompass:

- QuantiBlot® slot blot methods, including colorimetric and chemiluminescence
- Quantitative PCR (qPCR) methods, including Quantifiler®

The trainee will also be introduced to the laboratory's procedures for quality control, to include procedures for limiting the risk of contamination.

This laboratory training manual is developed in accordance with the SWGDAM Training Guidelines ([link](#)), which recommends the analysis of at least fifty (50) samples during the course of DNA Analyst training. The laboratory should ensure that each trainee analyze a minimum of fifty (50) samples in the course of the training.

Objectives

Upon successful completion of these exercises, the trainee will be able to:

- Describe the theories and procedures of DNA extraction from bloodstains, other body fluid stains, tissues, and stain mixtures.
- Describe the controls used at the extraction stage of DNA analysis.
- Describe the reagents used for DNA extraction and their function.
- Describe the sensitivity and limitations of the extraction procedure(s) and which procedure should be used for a given circumstance.
- Explain the technique(s) used by the laboratory to overcome inhibition.
- Explain the laboratory's method(s) for using spin baskets and/or centrifugal filter units, as appropriate.
- Perform all of the laboratory's extraction processes, per the laboratory's SOPs.
- Describe the theories and procedures of DNA quantitation for slot blot and real-time PCR methods.
- Describe the controls used at the quantitation stage of DNA analysis.
- Describe the reagents used for DNA quantitation and their function.
- Describe the sensitivity and limitations of the quantitation procedures.
- Explain the techniques used by the laboratory to interpret the quantitation results.
- Describe the quality system procedures employed by the laboratory to avoid, detect, and document contamination.
- Explain the components of the qPCR instrument and their function.

- Explain the functionality of the qPCR instrument software program.
- Perform all of the laboratory's quantitation processes per the laboratory's SOP, including the calculations and dilutions.

Preparation for Exercises

Trainer Responsibilities

1. Provide documented safety practices specific to chemicals used in the extraction process, to include the pertinent MSDSs.
2. Assign required samples to be extracted and quantitated as outlined in the Individual Training Plan.
Note: It is recommended that samples extracted in the extraction section of the Laboratory Training Manual be used for all exercises. Samples should be on various substrates to mimic forensic casework. Ensure that the trainee saves samples from each exercise for subsequent exercises.
3. Demonstrate each extraction procedure, including the use of spin baskets and centrifugal devices.
4. Demonstrate each quantitation procedure.
5. Observe the trainee performing each extraction and quantitation procedure.
6. Determine the assessment criteria.
7. Review, verify, and document exercise completion.

Trainee Responsibilities

1. Review documented safety practices specific to chemicals used in the extraction and quantitation processes, to include pertinent MSDSs.
2. Observe each type of extraction procedure, including the use of spin baskets and centrifugal filter devices.
3. Perform each extraction procedure, including the use of spin baskets and centrifugal filter devices.
4. Observe each type of quantitation procedure.
5. Perform each quantitation procedure.
6. Document and submit exercise completion, as required by the trainer.

Literature

[Return to Laboratory Training Manual User Guide](#)

Exercise 1: Extraction of Single Source Samples

Purpose

To perform DNA extractions on a variety of single source samples (e.g. blood, saliva, and semen) following the laboratory's SOPs, including controls and blanks.

Tasks

Extract the following:

- Five (5) blood samples
- Five (5) cells samples (a combination of buccal cells and vaginal epithelial cells and neat semen)
- Three (3) to five (5) diluted blood samples (e.g. 1:10, 1:100, 1:1,000, 1:10,000)

Suggested Samples

- Blood samples
 - Dried blood on different substrates (e.g. carpet, concrete, glass, paper, wood, cotton, nylon, stain card)
 - Liquid blood
- Cells
 - Semen, saliva, vaginal fluid
 - Diluted semen, saliva, vaginal fluid (e.g. 1:10, 1:100, 1:1,000, 1:10,000)
 - Dried semen, saliva, vaginal fluid on different substrates (e.g. carpet, concrete, glass, paper, wood, cotton, nylon, cigarette butts, envelopes, stamps)

Resources

Sample Protocols: [3.01](#), [3.03](#), [3.05](#)

Exercise 2: Differential Extraction of Mixed Samples

Purpose

To perform differential DNA extractions on a variety of mixed samples (e.g. mixtures containing spermatozoa) following the laboratory's SOPs, including controls and blanks.

Tasks

Extract the following:

- Fifteen (15) sperm positive mixed samples

Suggested Samples

- Semen mixed with vaginal fluid, saliva, blood, and urine
- Diluted semen mixed with vaginal fluid, saliva, blood, and urine
- Semen and vaginal fluid mixtures collected with varied post coital collection times

Resources

Sample Protocols: [3.01](#), [3.02](#), [3.06](#)

Exercise 3: Extraction of Compromised Samples

Purpose

To perform DNA extractions on compromised samples (e.g. low level and/or degraded samples) following the laboratory's SOPs, including controls and blanks.

Tasks

Extract the following:

- Four (4) low level samples
- Four (4) degraded samples

Suggested Samples

- Low level samples
 - Touch evidence* (swabbing from pens, pencils, weapons, etc.)
 - Samples diluted by laboratory to mimic low level samples
- Degraded samples
 - Aged samples
 - Sample subjected to ultraviolet light
 - Sample treated with DNase
 - Touch evidence* (swabbing from pens, pencils, weapons, etc.)

* May be included under both categories.

Resources

Sample Protocols: [3.01](#), [3.03](#), [3.05](#)

Exercise 4: Non-differential Extraction of Mixed Samples

Purpose

To perform non-differential DNA extractions on mixed samples (e.g. blood/blood, blood/saliva, vaginal secretions/buccal) following the laboratory's SOPs, including controls and blanks.

Tasks

Extract the following:

- Ten (10) mixed samples (no sperm)

Suggested Samples

- Mixtures of blood, saliva, urine, vaginal fluid
- Diluted mixtures of blood, saliva, urine, vaginal fluid

Resources

Sample Protocols: [3.01](#), [3.02](#), [3.03](#), [3.04](#), [3.05](#), [3.06](#)

Exercise 5: Extraction of Inhibited Samples

Purpose

To perform extractions on inhibited samples (e.g. denim, leather, bacteria, soil) following the laboratory's SOPs, including controls and blanks.

Tasks

Extract the following:

- Five (5) inhibited samples

Suggested Samples

- Stains on denim, leather, or other heavily dyed material
- Stains treated with fingerprint developing reagents (ninhydrin, superglue, amido black, commassie blue, physical developer)
- Stains treated with cleaning agents (bleach, detergent)
- Stains exposed to fire or mixed with soil

Resources

Sample Protocols: [3.01](#), [3.02](#), [3.03](#), [3.04](#), [3.05](#), [3.06](#)

Exercise 6: Extraction of Hair and Tissue Samples

Purpose

To perform extractions on hair and tissue samples (e.g. muscle, hair with root, fetal tissue) following the laboratory's SOPs, including controls and blanks.

Tasks

Extract the following:

- Five (5) hair and tissue samples

Suggested Samples

- Muscle tissue
- Fetal tissue
- Pulled hairs
- Shed hairs with root material

Resources

Sample Protocols: [3.01](#), [3.03](#), [3.06](#)

Exercise 7: Non-probative (Mock Case) Samples

Purpose

To perform extractions on non-probative and/or mock case samples following the laboratory's SOPs, including controls and blanks. The analysis data from this exercise may be used in the trainee's mock trial.

Tasks

Extract the following:

- Two (2) non-probative and/or mock case samples

Suggested Samples

- Establish mock case or non-probative case samples for the trainee

Resources

Sample Protocols: [3.01](#), [3.02](#), [3.03](#), [3.04](#), [3.05](#), [3.06](#)

Extraction Subject Review

After completion of the laboratory manual exercises and having previously completed the corresponding theory modules, the trainee should be able to answer the following questions:

- How is contamination limited?
- What is the purpose of reagent blanks?
- What are the components for each extraction process? What is the purpose or function of each component?
- Why are samples containing sperm subjected to differential extraction?
- When is it unnecessary to perform differential extraction on a semen stain?
- Why do many differential extraction procedures utilize DTT?
- What is the purpose of the spin basket?
- What are the effects of various substrates on DNA extraction?
- What inhibitors may interfere with the analysis?
- What methods are used to overcome inhibition?
- How does the choice of extraction method change based on the level of degradation or quantity of DNA present in the sample?
- How is the DNA extraction modified when a mixture is known to be present?
- What is the minimum contribution necessary to determine the minor donor in a two person mixture?
- How is the interpretation of mixture samples complicated when the donors are related?
- How do centrifugal filter units function?
- How does the sample source affect the quality or quantity of the DNA extracted?
- Why is it important to pre-wash hair samples?
- What are the preferred extraction methods for hair and tissue samples?

Optional Exercise 8: Extraction of Bone or Teeth

Purpose

To perform extractions on bone or teeth samples following the laboratory's SOPs, including controls and blanks.

Tasks

Extract the following:

- Two(2) to five (5) samples

Suggested Samples

- Any bone (without marrow)
- Teeth (without pulp)

Resources

Sample Protocols: [3.01](#), [3.04](#)

Exercise Review Questions

Upon completing this exercise, the trainee should be able to answer these questions:

- What additional techniques must be employed to handle these samples?
- What are the additional concerns with regard to contamination when working with these samples?
- What are the obstacles in interpreting data from these samples?
- What methods are employed for dealing with inhibition?
- What facility modifications and/or additional equipment are required to process these samples?

Optional Exercise 9: Commercial Extraction Reagents/Kits

Purpose

To learn about or utilize (depending on laboratory SOPs) commercially available systems for DNA extraction and isolation.

Tasks

Some of these extraction systems may already be in use in the trainee's laboratory. If any of these listed extractions were performed in Exercises 1 through 7, the trainee does not need to repeat the same extractions.

Using the manufacturers' protocols included in the selected kit or kits:

- The trainee may extract several samples of each type of biological material. (*Note:* These samples should be saved for further analysis.)

Suggested Samples

- Any samples as outlined by the manufacturers for the extraction systems listed below.

Resources

Sample Protocols: [3.01](#)

User Manuals:

Include, but not limited to:

- [Qiagen QIAamp®](#)
- [MO BIO UltraClean™](#)
- [Invitrogen™ -- Charge Switch® Forensic DNA Purification Kit](#)
- [Promega DNA IQ™ System](#)
- [Whatman® FTA®](#)
- [Promega Differex™ System](#)

Exercise Review Questions

Upon completing this exercise, the trainee should be able to answer these questions:

- What are the advantages of the selected commercially available DNA extraction system(s) versus organic or Chelex extractions?
- What are the components of the extraction process(es)? What is the purpose or function of each component?

Exercise 10: Sample Preparation and Instrumentation

Purpose

To observe and perform the preparation of a sample plate to include the known standards, samples, and controls.

To observe and perform the proper maintenance, calibration, and operation of each type of qPCR instrument per the laboratory's SOP's. This demonstration should include the use of the software as well as the proper documentation of the maintenance performed on the instrument(s). (Proper documentation may include logbooks and/or worksheets.)

Tasks

- Prepare a sample plate
- Complete the software set-up for a run
- Run a sample plate
- Evaluate the standard curve
- Identify and explain the functions of the various components of the instrument
- Demonstrate the proper use of the software as it pertains to maintenance, calibration, and operation

Resources

Sample Protocols: [3.11](#)

User Manuals: [Applied Biosystems](#), [Corbett Rotor-Gene](#)

Exercise 11: Reproducibility of the Quantitation Method

Purpose

To assess pipetting skills and to familiarize the trainee with the quantitation method. Following the laboratory's SOPs (including controls and blanks), perform the laboratory's quantitation method on five samples that vary in quantity spanning the dynamic range of the method. Run each sample three times.

Tasks

Quantitate the following:

- Five samples of varying concentration, repeat to produce three data sets for each sample
- Determine the reproducibility of each sample

Resources

Sample Protocols: [3.07](#), [3.08](#), [3.09](#), [3.10](#), [3.11](#)

User Manuals: [Applied Biosystems](#)

Exercise 12: Quantitation of Previously Extracted Samples

Purpose

To perform the laboratory's quantitation method on all samples (including the non-probative (mock case) samples) extracted in the extraction section of the Laboratory Training Manual, following the laboratory's SOPs (including controls and blanks).

Tasks

Quantitate the following:

- All samples extracted in the extraction section of the Laboratory Training Manual.

Resources

Sample Protocols: [3.07](#), [3.08](#), [3.09](#), [3.10](#), [3.11](#)

User Manuals: [Applied Biosystems](#)

Exercise 13: Calculations and Dilutions

Purpose

To determine the quantity of DNA in each sample. Determine which samples require dilution prior to amplification. Dilute the identified samples.

Tasks

Perform the following:

- Determine the quantity (ng / μ l) of DNA in each extracted sample.
- Identify the samples that require dilution and perform dilution.

Resources

Sample Protocols: [3.07](#), [3.08](#), [3.09](#), [3.10](#), [3.11](#)

User Manuals: [Applied Biosystems](#)

Quantitation Subject Review

After completion of the laboratory manual exercises and having previously completed the corresponding theory modules, the trainee should be able to answer the following questions:

General

- How much DNA should be amplified and under what circumstances can the amount be altered?
- What is the effect of too much or too little DNA being amplified?
- How is contamination limited during quantitation?
- What is the purpose of reagent blanks used in quantitation?
- What are the components for each quantitation process? What is the purpose or function of each component?
- What is the interpretation process for the quantitation method(s)?

Slot blot (QuantiBlot®)

- How does the slot blot apparatus work?
- What calibration standards are used?
- What is the purpose of the spotting solution and how does it work?
- What probe is used and why?
- What is the origin of the probe?
- Why does the membrane need to be washed after it is hybridized?
- What is the purpose of using 30% hydrogen peroxide?
- What is the purpose of using enzyme conjugate?
- How do the ECL solutions work?
- What are the advantages and disadvantages of using chemiluminescence?
- Once a slot blot is developed, what can be the cause of high levels of background?
- What is meant by ramp time as applied to chemiluminescence? How does it work?
- Is the probe specific to human DNA? If so, how is this determined?
- How are results interpreted?

Real-time PCR (Quantifiler®)

- How does qPCR on an Applied Biosystems (AB) Prism Sequence Detection System (using the Quantifiler Human and Y Human Male kits) work?
- What are the advantages of qPCR over slot blot methods?
- What two assays does the DNA quantitation assay combine? What is the purpose of each? What does each assay consist of?
- What is the purpose of the minor groove binder (MGB)?
- What does the acronym SDS stand for?
- How does the fluorescence detection on the AB Prism Sequence Detection System work?
- What is multicomponent transformation, and how does the process work?
- What are the phases of amplification? Which phase does qPCR use in the quantitation when using the Quantifiler Human and/or Y Human Male kits on an AB Prism Sequence Detection System? Why?
- What is threshold as applied to qPCR quantitation methods? What is the designation used to identify the threshold?

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- What is a standard curve? What does the R² value represent? What does the slope represent?
- In order to obtain accurate and reliable results, what should the values of R² and the slope be and why?
- How many amplification cycles are completed in qPCR on the AB Prism Sequence Detection System?
- How are results interpreted?

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