President's DNA Initiative – Analyst Training

Laboratory Training Manual

Subject 4: DNA Amplification

Colorado Bureau of Investigation 2015

PRESIDENT'S DNA INITIATIVE

Advancing Justice Through DNA Technology

Laboratory Training Manual

Subject 4: DNA Amplification

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Laboratory Training Manual

Subject 4: DNA Amplification

Purpose

To instruct the trainee on the amplification process, including the proper techniques for amplification set-up and thermal cycler operation and maintenance.

The following subjects are interdependent:

- Subject 4: DNA Amplification
- Subject 5: Amplified DNA Product Separation
- Subject 6: STR Data Analysis and Interpretation

Upon the sequential completion of these subject areas, the trainee will be able to interpret the data produced in these laboratory exercises.

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

Objectives

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

- Describe the theories and procedures of the amplification process.
- Describe the controls used in the amplification process.
- Describe the reagents and their functions in the amplification process.
- Demonstrate the operation of the thermal cycler, including maintenance procedures.
- Explain the conditions under which mineral oil is used in the amplification process.
- Describe the quality system procedures employed by the laboratory to avoid, detect, and document contamination.
- Perform the laboratory's amplification process, per laboratory SOPs.

Preparation for Exercises

Technical Leader Responsibilities

1. Provide documented safety practices specific to chemicals used in the amplification process, including the pertinent MSDSs.

Trainer Responsibilities

1. Assign required samples to be amplified as outlined in the Individual Training Plan.
   Note: Ensure that the trainee saves samples from each exercise for subsequent exercises.
2. Demonstrate each amplification procedure, including modifications.
3. Demonstrate the proper use of a thermal cycler.
4. Demonstrate and discuss the proper calibration and maintenance of a thermal cycler.
5. Observe the trainee perform the proper calibration and maintenance of a thermal cycler.
6. Observe the trainee performing each step in the amplification procedure.
7. Determine the assessment criteria.

8. Review, verify, and document exercise completion.

Trainee Responsibilities

1. Review documented safety practices specific to chemicals used in the amplification process, to include pertinent MSDSs.
2. Observe each step of the amplification procedure(s), including modifications.
3. Perform each amplification exercise.
4. Perform the calibration and maintenance of a thermal cycler.
5. Document and submit exercise completion, as required by the trainer.

Literature-see link in portal

Exercise 1: Thermal Cyclers

Purpose

To observe and perform the proper maintenance, calibration, and operation of each type of thermal cycler per the laboratory's SOP's. This demonstration should include the establishment of cycling parameters, operation, and proper documentation of the maintenance performed on the thermal cycler(s). (Proper documentation may include logbooks and/or worksheets.)

Tasks

- Identify and explain the functions of the various components of the instrument
- Establish and verify the cycling parameters
- Calibrate the thermal cycler, as per laboratory SOPs
- Demonstrate proper operation of the thermal cycler(s)

Resources

Laboratory Manuals

User Manuals: Applied Biosystems

Exercise 2: Amplification of Dilution Series

Purpose

To familiarize the trainee with the amplification process and assess the trainee's skills. Following the laboratory's SOPs (including controls and blanks), perform the laboratory's amplification methods on a dilution series from a known sample. *Note for the Y-STR kit, use only male DNA.*

Tasks

Using the table below, make dilutions of a single source known sample and amplify:

Dilution Factor	Concentration in ng/µl	Amplification volume in µl	Total Target of DNA in ng
Neat sample	0.5	10	5
1:1 of 0.5 ng/µl	0.25	10	2.5
1:1 of 0.25 ng/µl	0.125	10	1.25
1:1 of 0.125 ng/µl	0.0625	10	0.625
1:1 of 0.0625 ng/µl	0.03125	10	0.3125
1:1 of 0.03125 ng/µl	0.015625	10	0.15625
1:1 of 0.015625 ng/µl	0.0078125	10	0.078125
1:1 of 0.0078125 ng/µl	0.0039062	10	0.039062

Note: Values noted above show the dilutions; the use of significant figures was not taken into account.

Resources

Laboratory Manuals

User Manuals: Applied Biosystems, Promega

Exercise 3: Amplification of Two Donor Mixed Samples

Purpose

To amplify and collect data from two donor mixed samples for use in Subject 6: STR Data Analysis and Interpretation. This data will be used to determine the percent contribution necessary to detect a minor contributor in two donor mixture samples. *Note for the Y-STR kit, use only male DNA.*

Tasks

Using the table below, make dilutions from two single source known samples and amplify:

Sample Number	Donor #1 (ng)	Donor #2 (ng)
1	.1	.9
2	.3	.7
3	.5	.5
4	.7	.3
5	.9	.1

Resources

Laboratory Manuals

User Manuals: Applied Biosystems, Promega

Exercise 4: Amplification of Previously Extracted Known Samples

Purpose

To perform the laboratory's amplification procedure(s) on all previously extracted known samples, following the laboratory's SOPs (including controls and blanks).

Tasks

Amplify the following:

- All samples extracted in the training program

Resources

Laboratory Manuals

User Manuals: Applied Biosystems, Promega

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Subject 4: DNA Amplification

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 during amplification set-up?
- What is the purpose of controls, to include positive, negative, and reagent blanks?
- What are the components of each amplification kit? What is the purpose or function of each component?
- Why do most commercial kits utilize Taq Gold versus Taq?
- What are the steps in the PCR process? What occurs during each step?
- What is the expected effect of decreasing the annealing temperature? Of increasing the annealing temperature?
- What is the expected effect on decreasing the extension time? Of increasing the extension time?
- What is multiplexing?
- How does multiplexing make DNA analysis more efficient?
- What factors must be taken into consideration when designing a multiplex PCR reaction?
- What is the specific chemistry of the multiplexing kit used?
- What is the effect of a mutation in a primer binding site?
- What is the laboratory's current target concentration for amplification of single source samples?
- What is the expected result at the higher DNA template concentration? At the lower DNA template concentration?
- What is preferential amplification?
- What is differential amplification?
- What are the conditions under which differential and/or preferential amplification occurs?
- What inhibitors of amplification may co-extract with DNA.
- How do inhibitors affect amplification?
- What effect, if any, do the extraction reagents have on amplification, to include Chelex® 100 and phenol/chloroform/isoamyl alcohol?
- What procedures can be used to overcome inhibition?
- What is the effect of increasing the template DNA concentration to be amplified (e.g. 5 ng or more)?
- Is DNA amplification performed differently when a mixture is known to be present? Explain.
- What is the expected result when a minor component of a mixture is less than 10%of the total concentration?
- Are there any benefits to using commercial extraction kits?
- What amplification reagents are considered critical reagents per the laboratory's SOPS?
- How is it determined that a thermal cycler is functioning properly during a run?
- What maintenance is regularly performed on a thermal cycler?
- What is the expected effect of the DNAse treatment on the extracted DNA? Will this be observed in the amplified product? How?
- What mechanisms are involved with DNA degradation?
- What typing results can be expected from degraded DNA?
- What inhibitors of amplification may co-extract with DNA.
- How do inhibitors affect amplification?

- What effect, if any, do the extraction reagents have on amplification, to include Chelex® 100 and phenol/chloroform/isoamyl alcohol?
- What procedures can be used to overcome inhibition?