DNA: Blanks and Controls

Introduction

Each DNA analytical procedure incorporates blanks and controls to evaluate contamination and ensure that the processes are working correctly.

Refer to *DNA: Contamination Control* for methods and procedures used to minimize DNA contamination of samples.

Reagent blank

The *reagent blank* is used to check for the possible contamination of the sample preparation reagents by other human DNA or by amplified DNA.

A reagent blank is evaluated by performing the extraction on the reagents without any test sample. Analysts will include at least two reagent blanks with each evidence sample extraction set.

All reagent blanks will be quantitated and the blank with the highest quantitation value will be simultaneously amplified and typed along with the evidence samples using the same instrument. The amount of reagent blank to be amplified will be the same as the least diluted test sample.

If none of the evidence reagent blanks yields a quantitation value, the analyst will select one reagent blank to amplify and type. Reagent blank(s) that are not amplified and typed will be stored with the remaining DNA evidence extracts.

For samples extracted prior to July 2009, the reagent blank must be run in at least one PCR system.

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Reagent blank with typeable signal

The appearance of typeable signals in a reagent blank indicates that

- the sample preparation reagents may have been contaminated,
- cross-contamination between samples may have occurred during preparation, or
- human DNA or amplified DNA has gotten into samples from some other source.

Steps must be taken to determine the source of contamination before further testing can be conducted, including testing of all reagents and thorough cleaning of all work surfaces and equipment.

When the signals (that is, non-background peaks) in the reagent blank are not typeable and the test samples are clearly typeable, contamination may not be serious but should be noted.

If the reagent blank yields a DNA result, the situation must be discussed with the DNA Technical Lead who will determine the appropriate action.

Reagent blank failure must be noted in the case file and the appropriate *Instances of Contamination* logbook maintained by the DNA Technical Lead.

Negative PCR control

The *negative PCR control* is a check for the possible contamination of the reagents used to prepare the PCR amplification mixture by other human DNA or by amplified DNA.

The negative PCR control will contain only the reagents used to prepare the PCR amplification mixture for each batch of samples. The negative PCR control tube may be left open during the preparation of the PCR solution as an added control to monitor the environment of the preparation area.

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Negative PCR control typeable signals

If typeable signals occur in the negative control, it should be re-run on the instrument.

If after re-running, typeable signals still appear in the negative control, the analysis may need to be repeated.

When the signals (that is, non-background peaks) in the negative control are not typeable and the test samples are clearly typeable, contamination may not be serious but should be noted.

In the case of a limited sample, the entire situation needs to be assessed before determining a DNA type.

Negative PCR control failure must be noted in the case file and the appropriate *Instances of Contamination* logbook maintained by the DNA Technical Lead.

Positive PCR control

The positive PCR control ensures that the amplification and typing process is working properly.

If the positive PCR control fails to yield a typeable signal, the positive control can be re-run on the instrument. If it is shown that the quantitation of the control DNA is low, the results may be reported as long as the QC stain control gives the correct genotype (see below).

If the positive control gives incorrect results, the analysis must be repeated.

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QC stain control

The QC stain control ensures that the extraction procedure worked properly. The stain consists of blood of a known type that acts as an internal laboratory control, since the analyst does not know the DNA type.

A QC stain control will be extracted at the same time as the reference samples.

At the end of the analysis, the QC stain control information is entered and verified within DNA Manager under *STR Analysis Review GeneMapper ID-X*.

In the event that an incorrect type is obtained for the QC stain control, the analysis must be repeated. The analyst must try to determine the underlying cause for the typing error.

Substrate control

Where appropriate, an unstained portion of the substrate can be sampled and run through at least one typing system to check for inherent background DNA.

Any contribution of DNA detected in the substrate must be taken into consideration when interpreting results.

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Contamination

Cases of contamination detected in blanks or controls must be evaluated on a case-by-case basis with the Technical Lead and Supervising Criminalist.

Corrective measures are determined to be successful when

- all the blanks and controls are free of contamination, or
- the underlying cause has been identified and any necessary changes in the procedure, policy, protocol, or analyst's laboratory practice have been performed.

Records concerning the contamination problem and appropriate corrective measures must be placed in the case file and the appropriate *Instances of Contamination* logbook maintained in the possession of the DNA Technical Lead.