Promega

REFERENCE MANUAL

Validation Guide for the DNA IQ™ Reference Sample Kit for Maxwell™ 16

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I. Introduction

Before a laboratory can implement any kind of automated DNA extraction system (a combination of robot and extraction chemistry) for use with reference or database samples, each laboratory needs to complete an internal validation study of the automated system. These studies are intended to show that the automated system yields DNA templates with subsequent STR genotypes that are concordant with current manual extraction procedures used in the laboratory and have no detectable levels of cross-contamination. In addition, when using the DNA IQ™ System, this validation study will demonstrate the consistency of yields obtained within the various reference or database sample types routinely extracted in the laboratory and provide the mean expected yields from these types of samples. Such information on yields can be used to determine the volume of eluted DNA added to an STR amplification reaction on a routine basis so that quantitation of purified DNA is no longer required prior to PCR. The following is a guide to suggested experiments to be performed with the DNA IQ™ Reference Sample Kit for Maxwell™ 16[(a)] for such an internal validation. A suggestion on the minimal number of samples that need to be processed is also provided at the end.

II. Concordance

Objective

The objective of the concordance study is to demonstrate that there is no difference (discordance) between the STR profile resulting from amplification of DNA purified using the DNA IQ™ Reference Sample Kit for Maxwell™ 16 and that obtained following amplification of DNA extracted using the laboratory's current standard procedure.

Testing Conditions

1. Using the DNA IQ™ Reference Sample Kit for Maxwell™ 16, extract DNA from 8–16 reference or database samples for which the STR profile has been previously determined using the laboratory's current extraction procedure.
2. Amplify the same mass of DNA template as would normally be amplified with the laboratory's choice of STR multiplex kit and reaction volume.

   Note: During validation, DNA should be quantitated using the laboratory's current method to determine mean yield from different sample types (see Section III). Input DNA template for PCR should be based on this quantitation method.
3. Analyze and type the resulting STR profile using the laboratory's standard analysis parameters and macros (for laboratories using GeneScan® and Genotyper® software) or panel and bin files (for laboratories using GeneMapper® *ID* software).

III. Consistency of Yield

Objective

Determine the mean yield of DNA obtained (using the laboratory's standard quantitation method) from each reference or database sample type routinely encountered (e.g., FTA® card punches, cotton swabs, Dacron swabs, liquid blood, etc.) and show consistency of yield within each given sample type.

Testing Conditions

- For each sample type, extract DNA from 8–16 samples in a minimum of three runs (to show consistency between runs).
- Suggestions on sample amounts to process include:
  1) Two 3mm FTA® card punches with blood
  2) ½ buccal swab
  3) 20µl of liquid blood
- For any kind on "punch" sample (e.g., from FTA® cards), ensure that the same size and number of punches are used per extraction. Take the punches in the area with the majority of the stain, not towards the card edges, as this can introduce variability in the extraction due to variability in the amount of input DNA.
- For liquid blood samples, ensure that the liquid blood is well mixed prior to removing a 20µl aliquot. This ensures that white blood cells are adequately represented in the 20µl sample.
- Determine mean and standard deviation of yield from three extractions for each sample type extracted.

IV. Cross-Contamination

Objective

Demonstrate that there is no detectable level of cross-contamination between adjacent extractions carried out using the DNA IQ™ Reference Sample Kit for Maxwell™ 16.

Testing Conditions

1. Extract 8 samples and 8 blanks (blanks can be punches from unstained FTA® cards or unused swabs) in an alternating pattern across the tray of DNA IQ™ Reference Sample Kit for Maxwell™ 16 cartridges.
2. Repeat twice for a total of three runs to demonstrate reproducibility.
3. Amplify 1µl of each eluted sample (or an appropriate amount, as determined in Section III) and 10µl of each eluted blank using the laboratory's STR multiplex kit of choice and standard cycling conditions. Assemble blank amplification reactions prior to samples to minimize contamination while setting up amplification reactions. Analyze these samples by electrophoresis using the laboratory's standard injection (or loading) and run conditions.
4. Analyze the resulting electropherograms using the laboratory's standard threshold (e.g., 100 or 150RFU).
5. Compare any partial profiles obtained with the blanks to those with samples to check for potential contamination during extraction, as opposed to contamination from another source introduced post-extraction.

V. Total Number of Samples

The previous list of experiments suggest the following number of samples per test:

Concordance:	8–16
Consistency of Yield:	24–48
Cross-Contamination:	48 [3 × (8 blanks + 8 samples)]

This is a potential total of 112 samples (including 24 blanks) if all these experiments are done separately. However, the number of samples can be significantly reduced by combining tests into one run. For example, the 8–16 samples for concordance could be the same samples used to examine consistency of yield. In addition, the three "Consistency of Yield" runs could be the same as the three "Cross-Contamination" runs". Thus, instead of 112 samples, one could reduce the number to 48 (including 24 blanks), with the total number of extraction runs required for the DNA IQ™ Reference Sample Kit for Maxwell™ 16 limited to three. These could easily be run in one day. Factoring in subsequent quantitation, amplification and analysis, it is feasible to complete the entire validation in a 5-day week or less.

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[a]U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Australian Pat. No. 732756, European Pat. No, 1 204 741 and Mexican Pat. No. 209436 have been issued to Promega Corporation for methods of isolating biological target materials using silica magnetic particles and simultaneous isolation and quantitation of DNA. Other patents are pending.

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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