Verification of Sample Integrity for the Tecan Freedom EVO®- HSM Workstation

A ReliaPrep™ Large Volume HT gDNA Isolation System Application Note

Promega Corporation

Sample Types

- Human male and female whole blood

Instrument Requirements

- Tecan Freedom EVO®- HSM Workstation

Promega Reagents and Consumables

- ReliaPrep™ Large Volume HT gDNA Isolation System (Cat.# A1751, A2751)
- 2.2ml, Square-Well Deep Well Plate (Cat.# V6781)
- Water, Amplification Grade (Cat.# DW0991)
- Human Genomic DNA: Male (Cat.# G1471)

Tecan Consumables

- LiHa Disposable Tips, 1,000µl, Filtered (Cat.# 30 000 631)
- Six Disposable Troughs for Reagents, Grey (Cat.# 10 613 049)
- Three 25ml Disposable Troughs (Cat.# 30 055 743)

Tecan Consumables

- TaqMan® Universal PCR Master Mix (Applied Biosystems Cat.# 4326708)
- PrimeTime® XL qPCR Assay for SRY (Integrated DNA Technologies, Inc.)

The Tecan Freedom EVO®- HSM Workstation provides an integrated solution for isolation of genomic DNA (gDNA) from 1–10ml of whole blood using the ReliaPrep™ Large Volume HT gDNA Isolation System. The customized liquid handler uses bulk reagent delivery through fixed tips to deliver large volumes of reagent to the deck, a vacuum manifold to aspirate waste from sample processing tubes and disposable tips for small volume transfers. This integrated system uses a series of cleaning steps to ensure that shared hardware components in contact with samples are not a cross-contamination risk.

Here we describe a method that can be used to verify sample integrity on the Tecan Freedom EVO®- HSM Workstation. We also present data from our own experiments performed during automated method development.

The methods presented here evaluate potential cross-contamination across a collection of whole blood samples from male and female donors. The resulting DNA is evaluated using quantitative PCR (qPCR) to detect the presence of the SRY gene, a single-copy Y-chromosome-encoded locus. These methods are easily adapted for users' laboratories.

Automated Processing

All processing should be performed using the standard ReliaPrep™ scripts provided with your system. We recommend regular decontamination of your Tecan Freedom EVO®- HSM Workstation before sample processing to minimize the risk of sample-sample and operator-sample contamination.

Sample Preparation: For a single test, you will need 32 × 10ml whole blood samples evenly divided between male and female donors. Samples may originate from single or multiple donors, but we recommend that no samples should originate from lab personnel who have regular contact with the ReliaPrep™ Large Volume HT gDNA Isolation System. Any potential contamination events may be cross-checked through Y-STR testing (e.g., PowerPlex® Y23 System). Samples may be fresh or previously frozen. Refer to the *ReliaPrep™ Large Volume HT gDNA Isolation System Technical Manual* #TM341 for guidance regarding compatible anticoagulants.

We recommend using only female whole blood for the negative sample. In our hands, water is a poor negative control. Sample volume and quantity may be modified to better reflect an individual lab's workflow. For assistance adapting the methods described here to other sample types, please contact Promega Technical Services at: **techserv@promega.com**

This procedure relies on the integrity of the initial sample. Therefore, take care when preparing the samples for extraction. Male and female samples should be prepared separately. Ideally, no samples should be drawn from individuals who frequently use the ReliaPrep™ Large Volume HT gDNA Isolation System.

Standard Sample Layout: The standard Tecan Freedom EVO®- HSM Workstation includes an 8-channel vacuum manifold attached to the LiHa arm. For this configuration, we recommend a checkerboard pattern of alternating samples derived from male and female donors (Table 1). This pattern ensures that the vacuum manifold tips contact alternating male and female samples.

Table 1. Standard Checkerboard Sample Layout for Cross-Contamination Testing.

	1	2	3	4
A	Male	Female	Male	Female
B	Female	Male	Female	Male
C	Male	Female	Male	Female
D	Female	Male	Female	Male
E	Male	Female	Male	Female
F	Female	Male	Female	Male
G	Male	Female	Male	Female
H	Female	Male	Female	Male

Alternative Configurations: For systems installed without an 8-channel vacuum manifold, we recommend a modified sample layout for cross-contamination testing (Table 2). In this way, the 4 vacuum tips alternate between male and female samples. All other processing steps in the method should remain unchanged.

Table 2. Modified Sample Layout for Systems Without an 8-Channel Manifold.

	1	2	3	4
A	Male	Male	Female	Female
B	Female	Female	Male	Male
C	Male	Male	Female	Female
D	Female	Female	Male	Male
E	Female	Female	Male	Male
F	Male	Male	Female	Female
G	Female	Female	Male	Male
H	Male	Male	Female	Female

qPCR Analysis of Eluates: Each sample should be evaluated for the presence of male DNA using qPCR. Below is an example of a model TaqMan® assay for the male SRY locus. This assay was used during development of the automated ReliaPrep™ methods. The reagents and supplies required for this assay are listed on the first page. Other qPCR assays for male-specific loci also may be considered, but Promega has not evaluated these assays

Use care during the qPCR setup to ensure that sample cross-contamination does not occur during analysis. Because the DNA is eluted at a high concentration relative to the assay sensitivity, we recommend changing your gloves frequently. All potential SRY positive results in female-derived samples should be repeated to exclude false-positive results.

1. Prepare a 7-point standard curve with male-derived DNA by performing 1:5 serial dilutions. In our hands, acceptable results were obtained when the highest standard was 100–250ng/µl. We recommend performing duplicate standard curve and no-template control reactions.
2. Prepare enough reaction mix for 50 reactions:

	Each Reaction	50 Reactions
2X Universal Master Mix	12.5µl	625µl
Primer Probe (50X)	0.5µl	25µl
Water, Amplification-Grade	9µl	450µl

3. Dispense 23µl of reaction mix into 48 wells of the optical PCR plate.
4. Transfer 2µl of each sample, standard and no-template control into wells of the PCR plate.
5. Thermal cycling conditions:
   a. 10 minutes at 95 °C for 1 cycle
   b. 20 seconds at 95 °C and 45 seconds at 60 °C for 45 cycles

DNA Quantitation and Evaluation: All eluates should be evaluated using the quantitation method that is standard practice in your laboratory. This provides a control for the overall performance of the system. DNA quality and concentration can be determined by a variety of methods including spectrophotometry, fluorescent dye incorporation, gel electrophoresis and quantitative PCR. Different DNA quantitation methods are known to frequently return dissimilar absolute quantitation values. Users should select methods for DNA quality and quantity evaluation that best predict success for the intended downstream applications. We recommend performing this analysis after obtaining the qPCR results to reduce the possibility of user-derived cross-contamination.

Optional Reagents: If you encounter a positive SRY result in a female sample, identify the source of contamination using Y chromosome STR typing of the eluates. Promega provides multiple kits for Y-chromosome STR analysis that require use of a PCR thermal cycler and an appropriately configured capillary electrophoresis instrument. For assistance with these kits, please contact Promega Technical Services at: **techserv@promega.com**

Developmental Results

The following data are results obtained using a prototype ReliaPrep™ Large Volume HT gDNA Isolation System very similar to the standard Tecan Freedom EVO®- HSM Workstation. All of the principle components and methods are shared between our system and the standard configuration. The data described below are representative of multiple data sets obtained during development. No cross-contamination event was ever detected in those experiments. Users may adapt their DNA analysis methods to conform to their common laboratory practices and available equipment. The qPCR analysis reported below was performed on a Stratagene MX3005P® qPCR System.

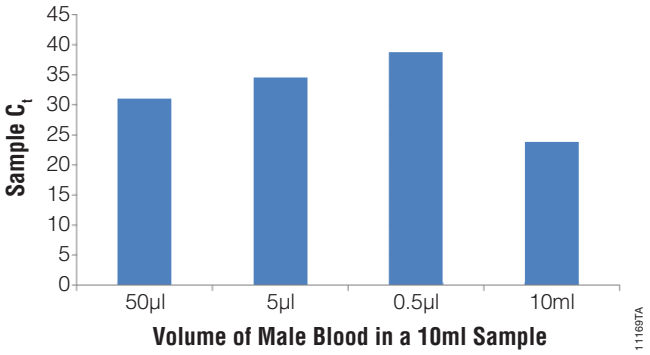

Figure 1. Verification of SRY qPCR assay efficiency. Ten milliliter blood samples with varying amounts of male whole blood in female blood were processed. Control samples of 10ml male whole blood also are shown Each bar represents the average of biological duplicates.

qPCR Analysis Efficacy

To demonstrate that the qPCR methods described in the qPCR Analysis of Eluates section would detect cross-contamination, we prepared several positive control samples by adding 50µl, 5µl or 0.5µl of male blood to 10ml of female blood. These spiked female samples alongside unspiked female blood and male blood were purified following the standard ReliaPrep™ script. The resulting DNA was analyzed by qPCR using the method described in the qPCR Analysis of Eluates section. All of the samples spiked with at least 5µl of blood returned a C_t value within the range of the standard curve (250ng/µl–16 pg/µl; Figure 1). Male DNA was detected in the sample containing 0.5µl of male blood; however, the C_t was outside of the standard curve. These data indicate that the qPCR method presented here is capable of quantitative detection of a minor cross-contamination event.

Cross-Contamination Results

A checkerboard sample array of 32 × 10ml of human male and female whole blood samples was processed according to the recommended protocols described in earlier sections. Table 3 shows the results of qPCR analysis of the resulting DNA eluates. None of the samples from female donors contained detectable levels of male DNA. While we demonstrated qualitative sensitivity outside of the standard curve in our previous results, there were no C_t values for any of the female-derived samples. Similar results were obtained over replicate experiments (data not shown).

Table 3. Concentration of Male DNA (ng/µl) Purified Using the ReliaPrep™ Large Volume HT gDNA Isolation System. Samples are displayed as they were arrayed on the HSM with female donors colored gray, and samples from male donors colored white. Samples with concentrations >250ng/µl had Ct values less than the highest standard on the standard curve.

	1	2	3	4
A	152	No C_t	175	No C_t
B	No C_t	>250	No C_t	>250
C	>250	No C_t	>250	No C_t
D	No C_t	>250	No C_t	244
E	119	No C_t	235	No C_t
F	No C_t	>250	No C_t	208
G	165	No C_t	191	No C_t
H	No C_t	185	No C_t	>250

Conclusion

This report outlines a procedure that can be used to identify cross-contamination of whole blood samples processed using the ReliaPrep™ Large Volume HT gDNA System in an automated format. The Tecan Freedom EVO®-HSM Workstation was evaluated for sample-sample cross-contamination using a sensitive qPCR-based assay. No sample contamination was detected from male-female checkerboard patterns arrayed on a sample run that was processed using the automated system. The data presented is representative of the results obtained during development of the automated scripts or methods.

Ordering Information

Product	Cat.#
ReliaPrep™ Large Volume HT gDNA Isolation System	A1751
	A2751
2.2ml, Square-Well Deep Well Plate	V6781
Water, Amplification Grade	DW0991
Human Genomic DNA: Male	G1471
PowerPlex® Y23 System*	DC2320

*Not For Medical Diagnostic Use.

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