Introduction

1. This protocol outlines the use of the Applied Biosystems™ RapidHIT™ ID System in conjunction with the Applied Biosystems™ INTEL GlobalFiler™ Express STR chemistry (GFE) sample cartridges for Rapid DNA analysis.
2. The methods listed in this protocol have been validated to be used with specific crime scene samples assumed to be from a single donor.
3. Each INTEL GlobalFiler™ Express sample cartridge contains an internal size standard.
4. An allelic ladder is included with each instrument run.
5. A Negative and Positive control is run with each new Primary Cartridge.
6. New lots of reagents with be Quality Control tested.
7. The RapidHit ID instruments must be kept in a secure location with limited access.
8. All operators will have their own log-in profile.
9. Only individuals who are trained will be able to use the Rapid Hit ID instruments.

RapidHIT™ ID System Specifications for INTEL GlobalFiler™ Express

1. INTEL GlobalFiler™ Express sample cartridges are single sample cartridges used for sample introduction, extraction, and polymerase chain reaction (PCR). The GlobalFiler™ Express chemistry amplifies 24 short tandem repeat (STR) loci using a six-dye system. The genetic loci amplified are: D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, DYS391, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338, and the sex-determining markers, Y indel and Amelogenin.
2. Table 1 shows the components used to perform the validation studies of this system.
3. Tables 2 and 3 list the RapidHIT™ ID System and the INTEL GlobalFiler™ Express sample cartridge run specifications.
4. The RapidHIT™ ID System consists of the Applied Biosystems™ RapidHIT™ ID Instrument using Applied Biosystems™ RapidHIT™ ID System Software. The adjunct RapidLINK™ Software is used for data management and allows for DNA

profile analysis with its embedded GeneMarker™ HID v.2.95.

Table 1.

Systems and Software	
System	Applied Biosystems™ RapidHIT™ ID System (RHID): SN RHID-0438 – Validation SN RHID-0435 – Performance Check
Software	Applied Biosystems™ RapidHIT™ ID System Software v1.1.3
Cartridges	
Sample Cartridge	RapidINTEL™ sample cartridge
Positive Control	Applied Biosystems™ RapidINTEL™ Positive Control Cartridge
Negative Control	Applied Biosystems™ RapidINTEL™ Negative Control Cartridge
Primary Cartridge	Applied Biosystems™ RapidHIT™ ID Primary Cartridge GlobalFiler™ Express Kit
Data Analysis	
Analysis Software	GeneMarker™ HID Software v2.9.5

Table 2. Applied Biosystems™ RapidHIT™ ID System specifications for GFE INTEL Cartridge

System specification	GFE INTEL Cartridge
Lysis buffer volume	300 ul
Thermal cycling	95°C for 60 seconds, 94°C for 3 seconds, 61°C for 30 seconds, 61.5°C for 30 seconds, 60°C for 480 seconds
Number of cycles	32
Injection	8 seconds, 5kV

Dye Channel	Marker	Analytical Threshold (RFU)	Minimum Heterozygous Peak Intensity Threshold (RFU)	Stochastic Threshold (RFU)	Stutter Percentage	Peak Height Ratio
Blue (6-FAM)	D3S1358	50	640	1600	27	40
	vWA	50	640	1600	25	40
	D16S539	50	640	1600	25	40
	CSF1PO	50	640	1600	22	40
	TPOX	50	640	1600	16	40
Green (VIC)	Yindel	50	640	50	21	99
	AMEL	50	640	1600	21	40
	D8S1179	50	640	1600	20	40
	D21S11	50	640	1600	25	40
	D18S51	50	640	1600	28	40
	DYS391	50	640	50	18	99
Yellow (NED)	D2S441	50	640	1600	16	40
	D19S433	50	640	1600	29	40
	TH01	50	640	1600	18	40
	FGA	50	640	1600	27	40
Red (TAZ)	D22S1045	50	640	1600	34	40
	D5S818	50	640	1600	26	40
	D13S317	50	640	1600	18	40
	D7S820	50	640	1600	18	40
	SE33	50	640	1600	30	40
Purple (SID)	D10S1248	50	640	1600	29	40
	D1S1656	50	640	1600	26	40
	D12S391	50	640	1600	30	40
	D2S1338	50	640	1600	31	40
Orange (LIZ)	-	30	30	30	30	30

Table 3. RapidHIT™ ID System v1.1.3 thresholds for INTEL GlobalFiler™ Express Sample Cartridges

RapidHIT™ ID Positive Control Cartridge

1. The RapidHIT™ ID positive control cartridge contains DNA control 007.
2. Table 4 shows the DNA profile for DNA control 007.

Table 4. DNA control 007 profile typed with GlobalFiler™ Express

Marker	GFE Positive Control	
D3S1358	15	16
vWA	14	16
D16S539	9	10
CSF1PO	11	12
TPOX	8	-
Yindel	2	-
AMEL	X	Y
D8S1179	12	13
D21S11	28	31
D18S51	12	15
DYS391	11	-
D2S441	14	15
D19S433	14	15
TH01	7	9.3
FGA	24	26
D22S1045	11	16
D5S818	11	-
D13S317	11	-
D7S820	7	12
SE33	17	25.2
D10S1248	12	15
D1S1656	13	16
D12S391	18	19
D2S1338	20	23

Viewing samples

Main Navigation Screen

1. Instrument list for managing instruments
2. DNA profile library for viewing results
3. Match, Familial, Kinship, and SED Applications
4. Instrument and user management tools
5. Run and consumable panes

1. The run data may be viewed directly from the RapidLINK™ software via two methods. From the main screen, select the DNA profile library icon (Figure 1). A list of all of the runs performed by the instrument(s) will be displayed. Double click the Sample ID to open GeneMarker™ HID. After selection, the runs are processed automatically by the GeneMarker™ HID Software to be viewed and analyzed.

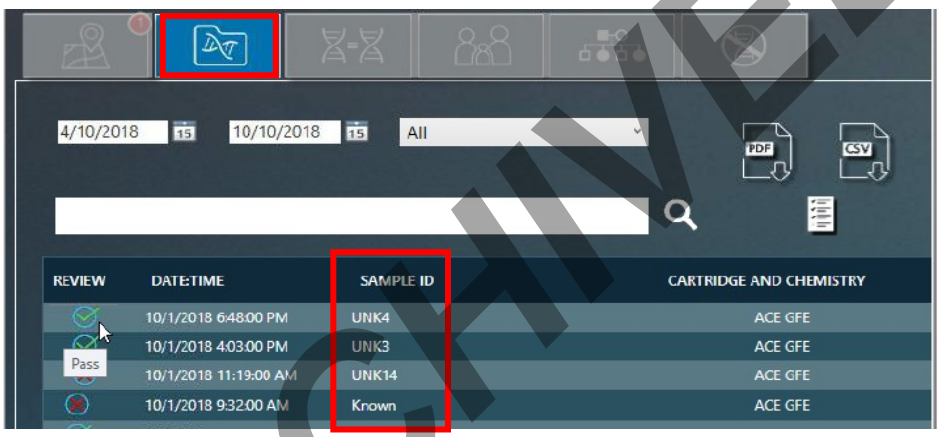


Figure 1. Selecting the DNA profile library from the RapidLINK™ software to view a sample in GeneMarker™ HID

2. To display the list of runs from a specific instrument, select the managing instrument icon, click a site pin (see arrow), and then select the instrument name (Figure 2). Once opened, a list of all of the runs performed by the instrument will be displayed (Figure 3).

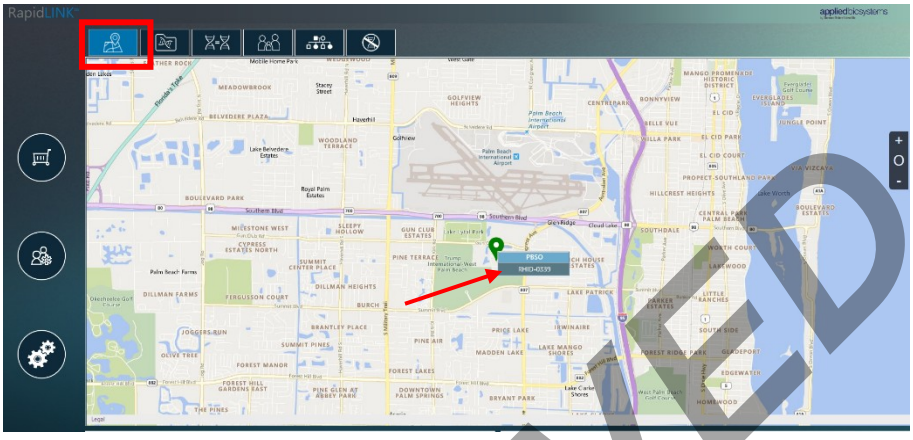


Figure 2. Selecting the specific instrument to display the list of sample runs

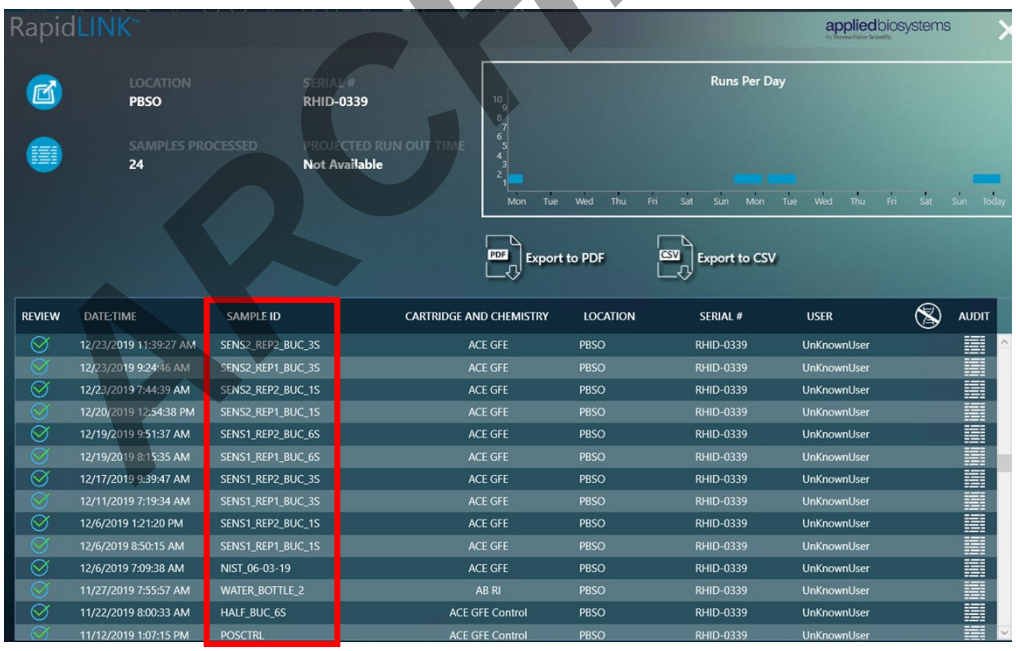


Figure 3. Selecting a sample from the RapidLINK™ software to view a sample in GeneMarker™ HID

3. Click the Sample ID to open GeneMarker™ HID. After selection, the runs are processed automatically by the GeneMarker™ HID Software to be viewed and analyzed.
4. Once opened in GeneMarker™ HID, the sample's electropherogram will be displayed. The sample name is displayed on the left hand side (Figure 4).

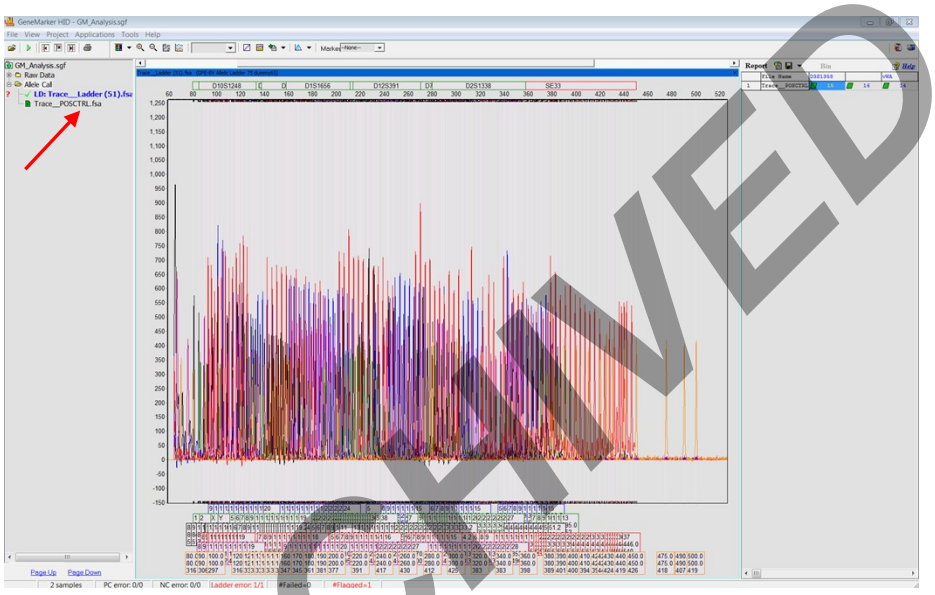
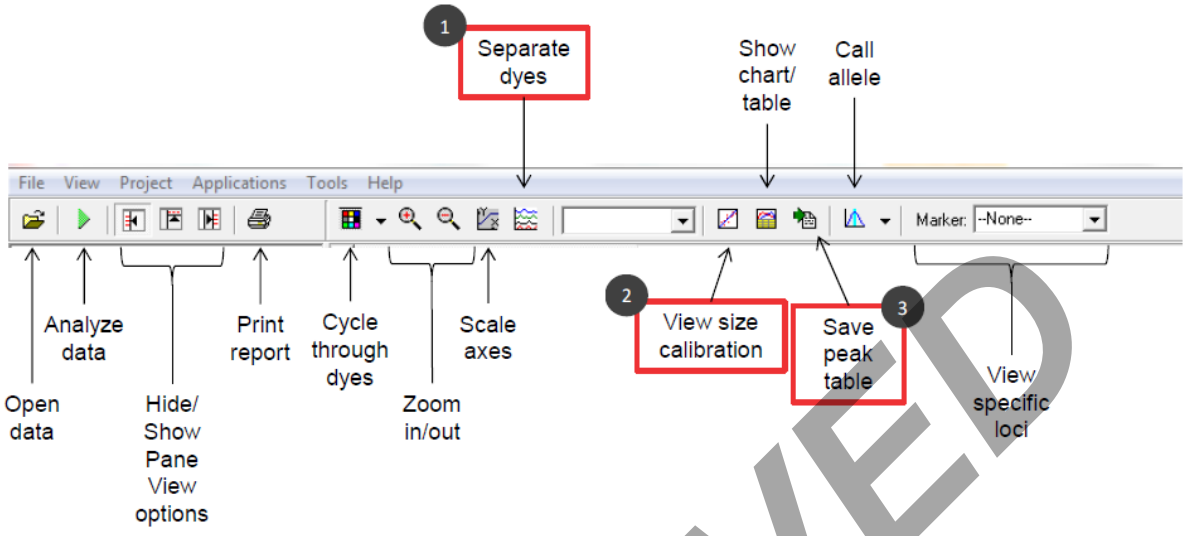


Figure 4. Sample name and electropherogram in the GeneMarker™ HID software. Note: In this figure, the profiles for the allelic ladder and sample are both opened.



1. Review electropherograms separated by dye colors and loci
2. Review the size standard (cubic spline interpolation)
3. Saving DNA profile edits to reimport into RapidLINK

5. To view and review the profile in detail, select the Browse by All Color icon from the tool bar (Figure 5).

Figure 5. Browse by all color icon from GeneMarker™ HID toolbar.

6. Use the drop-down arrow on the upper right to toggle back and forth from sample to sample (Figure 6). Within the electropherogram, left click and drag the box from upper left to lower right to zoom in and left click and drag the box from lower right to upper left to zoom out. To scroll, hold right click and move right or left.

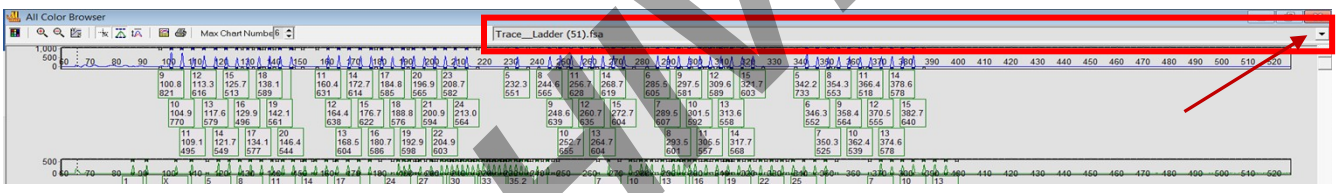


Figure 6. Toggle from sample to ladder in GeneMarker™ HID

7. To view the internal size standard (ILS) for a sample or ladder in the run file, choose the Size Calibration icon on the top toolbar (See Figure 7). Use the list on the left hand side to view the ILS for the desired sample. The name of the sample in view will appear above the orange ILS.

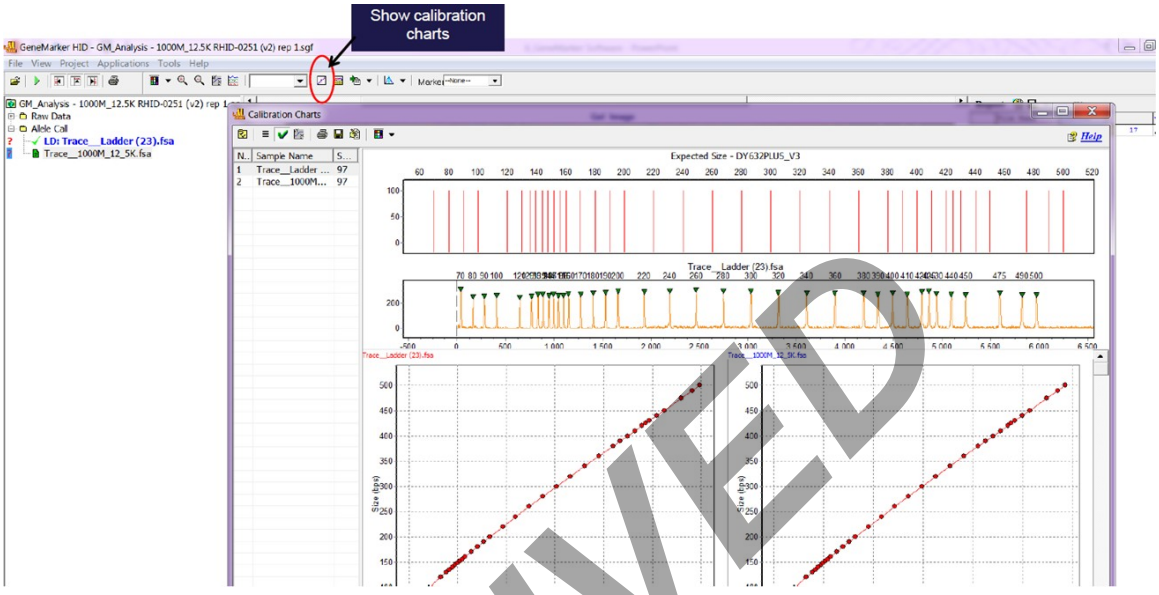


Figure 7. Size Calibration icon in GeneMarker™ HID to view ILS

8. Ensure that all required internal lane size standard peaks are called in all samples. Select the 'Size Calibration' icon in the toolbar menu. Select each sample in the list on the left to view the ILS trace for that sample. Verify that these GS600 standard peaks are present (60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, 460, 480, and 500) and the sizing quality score is ≥ 88 (the software flags the sizing quality of the ILS when it is <88).

Editing profiles in GeneMarker™ HID

1. Choose the marker to edit by using the drop-down tool bar on the upper right (See Figure 8). Click and drag the mouse to zoom in and out of the electropherogram.
2. To delete artifacts (e.g. stutter, pull up, etc.), right click on the peak and choose "Delete". The peak will appear with an "X" above it (See Figure 8). The deleted peak will be observed in the allele comments in the chart/table as

shown in Figure 8.

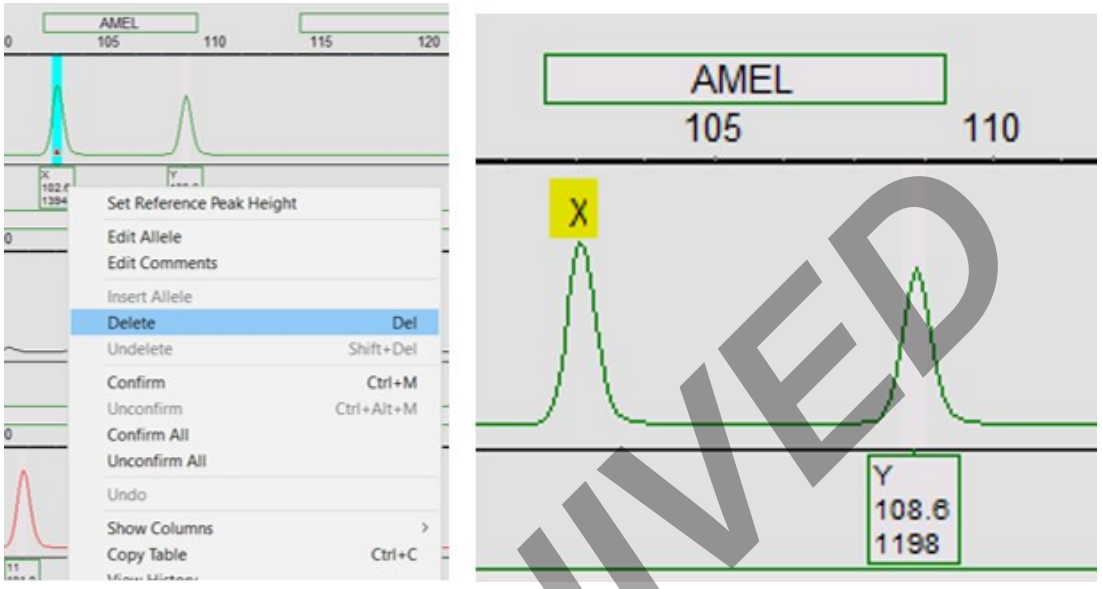


Figure 8. Deleting artifacts in GeneMarker™ HID

3. To edit a peak, right click on the peak and choose “Edit Allele”. The allele information can then be edited and the peak will appear with an “E” above it (Figure 9).

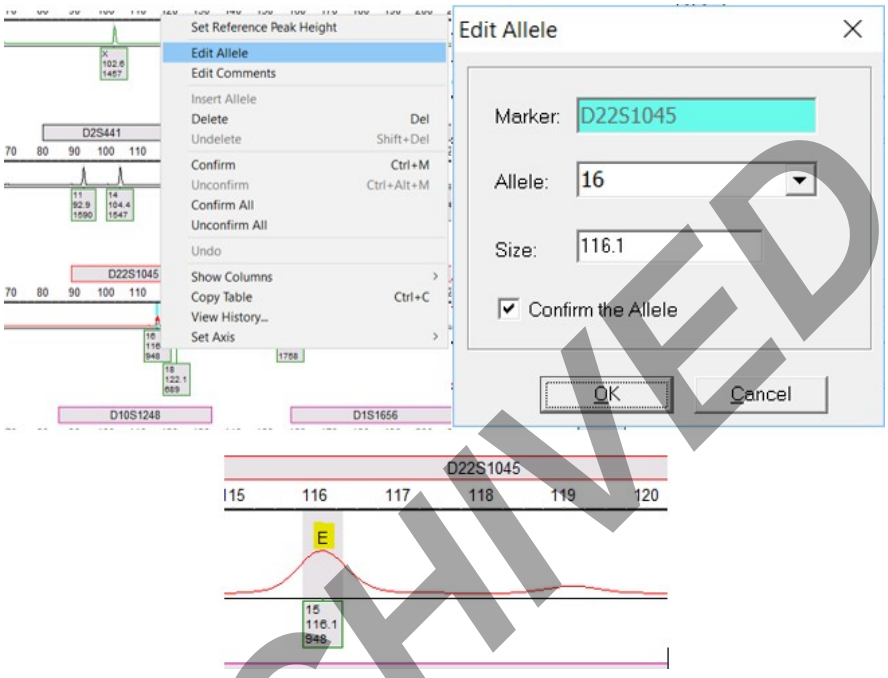


Figure 9. Editing peaks in GeneMarker™ HID

GeneMarker™ HID Quality Flags

1. To review quality flags from GeneMarker™ HID in the DNA profile, select the show chart/table icon from the tool bar (Figure 10). The table displays the allele call, size, RFU height of the peaks, and the quality reasons for flagged alleles (Figure 11). Alleles flagged will appear as yellow in the electropherogram (Figure 12).

Figure 10. Show chart/table icon in GeneMarker™ HID.

No.	Dye	Size	Height	Ht_Ratio	Marker	Allele	Difference	Quality	Score	Allele Comments	Sample Comments	Quality Reasons
1	Blue	125.9	70	1.00	D3S1358		0.10	Check	1.8			IHO
2	Blue	176.7	66	0.50	vWA		0.00	Pass	1.4			
3	Blue	188.8	133	1.00	vWA		0.00	Pass	5.0			
4	Blue	301.5	51	1.00	CSF1PO		0.10	Check	0.9			IHO
1	Green	91.5	119	1.00	Yindel		0.00	Pass	3.8			
2	Green	217.3	91	1.00	D21S11		0.00	Check	2.7			IHO
1	Yellow	92.8	175	1.00	D2S441		0.00	Pass	9.8			
2	Yellow	151.1	95	1.00	D19S433		0.10	Check	2.6			IHO
1	Red	113.0	91	1.00	D22S1045		0.00	Pass	2.0			
2	Red	119.1	52	0.57	D22S1045		0.00	Pass	0.7			
3	Red	157.9	158	1.00	D5S818		0.10	Pass	6.7			
4	Red	161.9	72	0.46	D5S818		0.10	Check	1.6			IMB
5	Red	221.9	90	1.00	D13S317		0.00	Pass	2.2			
6	Red	230.2	69	0.77	D13S317		0.00	Pass	1.5			
7	Red	281.7	52	1.00	D7S820		0.20	Check	0.9			IHO
1	Purple	112.9	83	1.00	D10S1248		0.00	Pass	2.1			
2	Purple	117.0	51	0.61	D10S1248		0.00	Pass	0.9			
3	Purple	179.0	94	1.00	D1S1656		0.10	Check	2.8			IHO
4	Purple	246.3	63	1.00	D12S391		0.00	Check	1.2			IHO
2	Purple	187.0	709	1.19	D1S1656		0.00	Pass	81.0	[<Deleted>]		

Figure 11. Chart/table in GeneMarker™ HID.

Figure 12. Electropherogram in GeneMarker™ HID displaying quality flags

2. Refer to Figure 13 and Figure 14 for the different quality flag abbreviations used in the GeneMarker™ HID software.

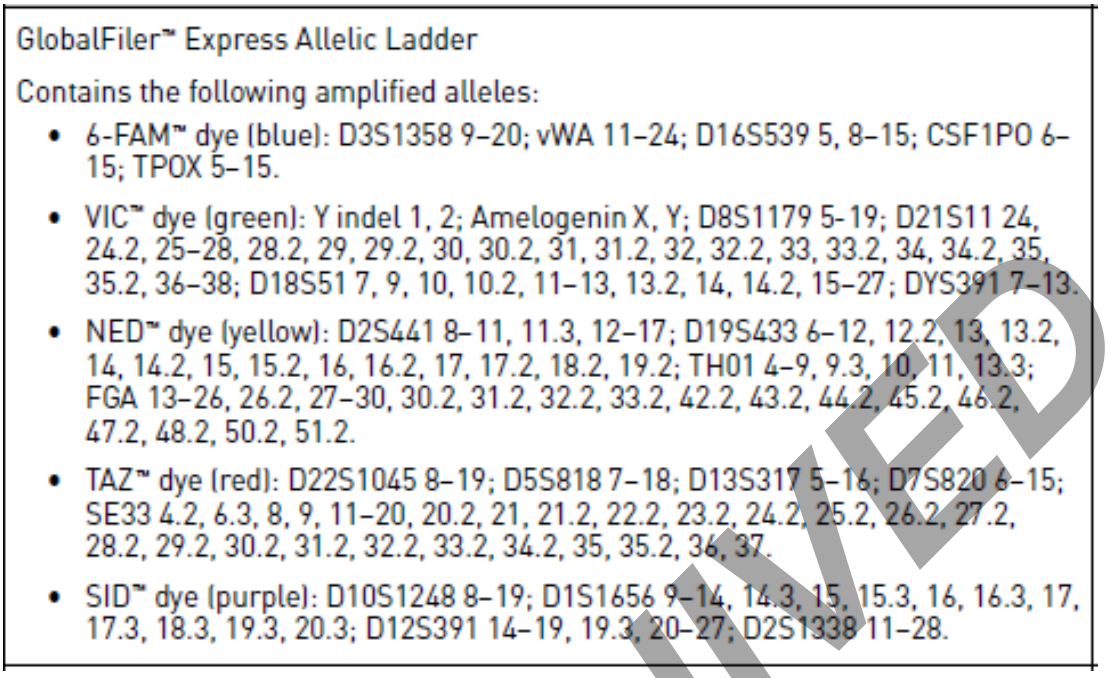
Quality Rank	Reason	Description
OL	Off ladder	Peak is outside of the marker range
OB	Out of bin	Peak is within the marker range but outside of a bin
IHO	**Inconclusive homozygous**	Peak intensity is within the homozygous inconclusive range set for this locus
IMB	**Heterozygote Imbalance**	Peak intensity does not exceed the minimum percentage of the major peak within the marker
HI	High intensity	Peak intensity approaches and/or exceeds the maximum peak intensity filter
BC	Bin conflict	More than one called peak present within a bin
SR	Saturated (repaired)	Intense peaks with characteristic morphology are identified and "repaired" for allele calling
SP	Saturated (pull-up)	Intense peaks may cause "pull-up" or additional peaks to appear in other dye colors
PL	**Beyond ploidy**	When the number of peaks identified within a marker exceeds the maximum number of peaks expected

Figure 13. GeneMarker™ HID quality flag abbreviations

Analysis and interpretation

Analyzing Allelic Ladders

1. Ensure a ladder from the library is associated with the project run in the GeneMarker™ HID Software.
2. Check the project run to make sure the ladder has typed correctly.
3. The Global Filer Express allelic ladders are represented in Figures 14 and the allelic ladders may have artifact peaks that appear in virtual bins. With the correct settings, these peaks will appear with an “X” (see Figure 16).



GlobalFiler™ Express Allelic Ladder

Contains the following amplified alleles:

- 6-FAM™ dye (blue): D3S1358 9–20; vWA 11–24; D16S539 5, 8–15; CSF1PO 6–15; TPOX 5–15.
- VIC™ dye (green): Y indel 1, 2; Amelogenin X, Y; D8S1179 5-19; D21S11 24, 24.2, 25–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38; D18S51 7, 9, 10, 10.2, 11–13, 13.2, 14, 14.2, 15–27; DYS391 7–13.
- NED™ dye (yellow): D2S441 8–11, 11.3, 12–17; D19S433 6–12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2; TH01 4–9, 9.3, 10, 11, 13.3; FGA 13–26, 26.2, 27–30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2.
- TAZ™ dye (red): D22S1045 8–19; D5S818 7–18; D13S317 5–16; D7S820 6–15; SE33 4.2, 6.3, 8, 9, 11–20, 20.2, 21, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35, 35.2, 36, 37.
- SID™ dye (purple): D10S1248 8–19; D1S1656 9–14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3; D12S391 14–19, 19.3, 20–27; D2S1338 11–28.

Figure 14. GlobalFiler™ Express Allelic Ladder

Figure 15. Electropherogram of Global Filer Express Allelic Ladder

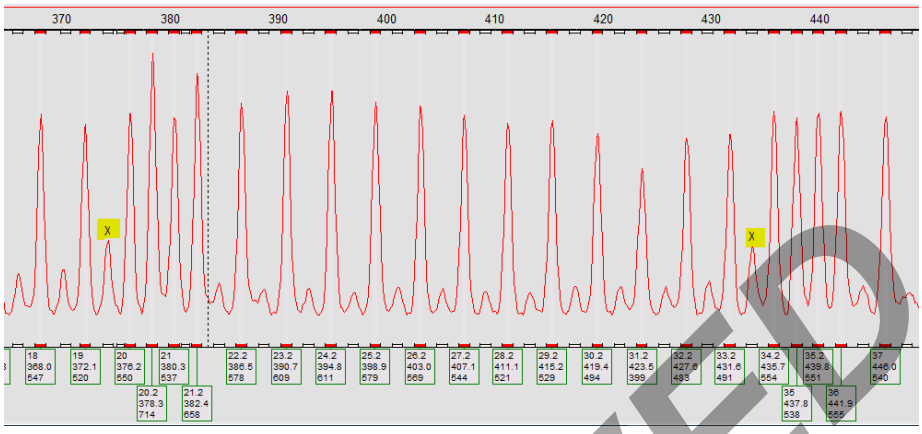


Figure 16. Electropherogram of Global Filer Express Allelic Ladder showing artifact peaks in virtual bins

Analyzing the Internal Size Standard

1. The RapidHIT™ ID will label the DY632PLUS internal size standard (ILS) fragments between 80 and 500 base pairs.
2. Check ILS to ensure that all peaks correctly called (see Figure 17).

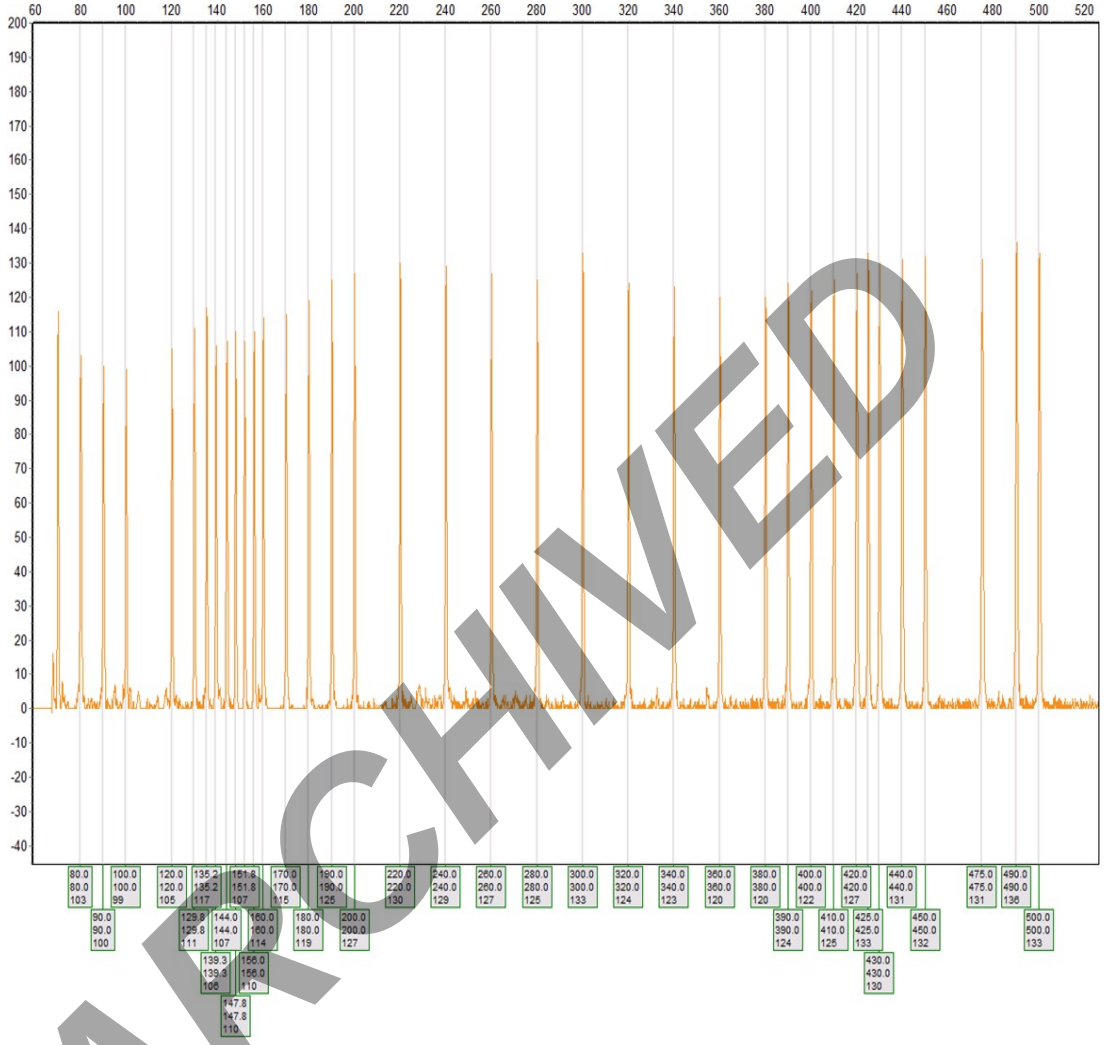


Figure 17. RapidHIT™ ID Internal Size Standard (ILS) in GeneMarker™ HID

Analyzing samples

1. All profiles generated from the RapidHIT™ ID may be manually interpreted using the GeneMarker™ HID software.
2. The INTEL GlobalFiler™ Express Sample cartridge utilizes locus specific analytical thresholds (see Table 5).
3. Stochastic effects may be evident by peak height imbalance and/or allele drop-out. To account for these effects locus specific stochastic thresholds have been established (Table 5).

4. Global and locus specific stutter filters have been established (Table 3) and are used when analyzing data with the GeneMarker™ HID software.
5. The reference sample profile must be manually interpreted in GeneMarker™ HID at each locus for the presence of alleles, elevated stutter, and artifacts. A reference sample should have no more than two alleles at each locus, with the exception of a tri-allele.
6. If there is a quality flag at a locus, the quality flag must be reviewed. Quality flags will be marked as yellow or red.
    a. Right click the flagged allele
    b. Select 'Confirm'
    c. After review and confirmation, the allele will no longer be flagged as yellow/red (see Figure 18) and will appear with an "E" (see Figure 18).
    d. When exiting, save changes to the GeneMarker™ HID project.

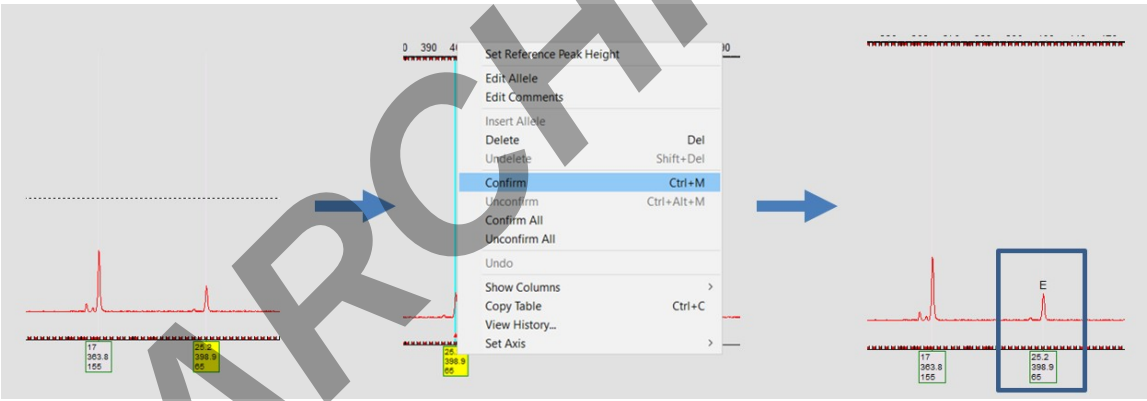


Figure 18. Reviewing a quality flag in GeneMarker™ HID

7. Save project when completed. If a profile is to be uploaded to SmallPond™ a manual export will be needed. Please see RDNA-04 SmallPond™ for manual export instructions.

Clear Major with minor(s)

Mixtures with a "clear" single source major may be added manually to Small Pond by a Rapid DNA Administrator. Minor profiles are not typically uploaded to Small Pond. A "clear" major is determined by training and experience of Forensic-Lab Qualified DNA Analysts. The qualified DNA analyst must have competence in DNA mixture analysis.

Quality Control of Reagents

1. All new lots of sample cartridges and the installation of a new primary cartridge will be Quality Control (QC) tested prior to use for samples.
2. The sample cartridge lots will be quarantined until the QC check has been successfully performed. For new primary cartridge, the instrument will be tagged "out of service" until installation and necessary QC testing is successfully completed.
3. Documentation of the new sample cartridge lot and/or primary cartridge will be made on the Reagent QC document (RDNA QR-01).
4. A successful test (for either sample or primary cartridge) will be expected results for a positive and negative control. No peaks detected in the negative control, and the expected genotype for the positive control.
    a. A positive sample control is a sample that the DNA profile is known.
    b. A negative control is an empty Rapid DNA cartridge.
5. Once QC passed, the sample cartridges will be moved to the kiosk refrigerator for use by agencies. The primary cartridge passing QC will allow the instrument to be used again.
6. All Rapid DNA runs are kept on the RapidLink Software and able to be viewed through Genemarker HID analysis software.

Maintenance and Routine Performance Checks

Routine maintenance

1. At a minimum one sample must be run on the RapidHIT™ ID System weekly.
2. Weekly runs for maintenance or casework runs must be documented on the RDNA – 01 Rapid DNA Maintenance Log.
3. Maintenance tasks and frequency of task will be conducted as outlined in Table 6.

Table 6. Maintenance tasks and frequency for the RapidHIT™ ID System

Task	Frequency
Run a sample cartridge if the instrument is not in use daily	Weekly
Clean the touchscreen. 1. Power off the internal computer (button on the front), hold for several seconds. 2. Power off the main power switch (button on the back). 3. Spray with a non-abrasive glass cleaner, then gently wipe the screen with lint-free lab tissues. 4. Power on the main power switch and the internal computer.	As needed

4. If needed, the lot numbers associated with each run can be found in the sample project data storyboard.
5. The main power switch at the back of the instrument should always be kept on to keep the gel cool in the primary cartridge. If the instrument is powered off for a prolonged period of time, the primary cartridge and gel cartridge may need to be replaced.

Performance Checks

1. If the RapidHIT™ ID System is idle longer than a week (7 days)**,** an internal performance check must be conducted.
2. A performance check consists of running a positive sample control and negative control.

    a. A positive sample control is a sample that the DNA profile is known.
    b. A negative control is an empty Rapid DNA cartridge.

3. Acceptable performance results for the positive sample control is a concordant DNA profile.
4. Acceptable performance check results for the negative control is a negative DNA profile.
5. Unacceptable performance check results for the positive sample control are a negative DNA profile, a partial DNA profile, or a non-concordant DNA profile,
6. Unacceptable performance check results for the negative control are obtained when DNA is present.
7. If an unacceptable performance check result is obtained for the positive sample control or negative control, the RapidHit Administrator must be notified, the appropriate root cause analysis will be conducted, and the performance check will be repeated.
8. All performance check test results must be recorded on the RDNA – 01 Rapid DNA Maintenance Log.
9. If the RapidHIT™ ID System fails to produce acceptable performance check results, the manufacturer must be contacted in to order attempt to resolve the unacceptable performance check results and/or request instrument service.
10. Failure of the performance check will be recorded on RDNA – 01 Rapid DNA Maintenance Log or can be viewed electronically on the RapidLink Software and the associated electropherograms will be stored as either hardcopy or

electronically.

11. The performance check data will be stored as either hardcopy or electronically.

Maintenance, Service and Repair

1. The vendor will be notified of maintenance, service, or repair as needed.
2. Following maintenance, service or repair a performance check must be run prior to performing Rapid DNA analysis on casework reference samples. A positive sample control and negative control must be run.
    a. A positive sample control is a sample that the DNA profile is known.
    b. A negative control is an empty Rapid DNA cartridge.
3. Acceptable performance results for the positive sample control is a concordant DNA profile.
4. Acceptable performance check results for the negative control is a negative DNA profile.
5. Unacceptable performance check results for the positive sample control are a negative DNA profile, a partial DNA profile, or a non-concordant DNA profile,
6. Unacceptable performance check results for the negative control are obtained when DNA is present.
7. If an unacceptable performance check result is obtained for the positive sample control or negative control the Rapid Administrator must be notified, the appropriate root cause analysis will be conducted, and the performance check will be repeated.
8. All performance check test results must be recorded on the RDNA – 01 Rapid DNA Maintenance Log.
9. If the RapidHIT™ ID System fails to produce acceptable performance check results, the manufacturer must be contacted in to order attempt to resolve the unacceptable performance check results and/or request instrument service.

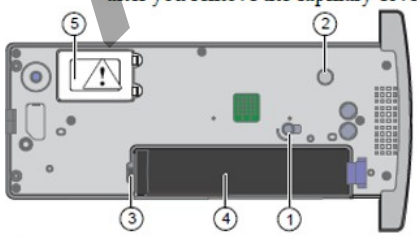
10. The date of the maintenance, service, or repair will be noted on the RDNA – 01 Rapid DNA Maintenance Log.

Replacing the Primary and/or Gel Cartridge

1. Replacement of the primary cartridge is necessary once the primary cartridge or gel cartridge has reached its expiration date or maximum run of 100, if there is a broken capillary, or if there are any other issues with the primary cartridge (e.g., internal RFID error). Note: The reagent expiration dates will be monitored by the instrument.
2. Each time the primary cartridge or gel cartridge is replaced, the RDNA – 01 Rapid DNA Maintenance Log must be completed.
3. Replacement of the primary cartridge and gel cartridge requires approximately 5 hours to complete and may only be performed when logged into the RapidHIT™ ID System as an Administrator or Supervisor.
4. The components needed for replacing the primary cartridge and gel cartridge are a new primary cartridge, gel cartridge, GFE control cartridges and utility cartridge.
5. Figure 19 illustrates the parts and of components of the primary and gel cartridge.

① Shipping plug on check valve
② Shipping plug on cathode block
③ Gel cartridge inlet
④ Gel cartridge slot
⑤ Shipping cover on capillary

⚠ **CAUTION!** The capillary is fragile. Handle the primary cartridge with care after you remove the capillary cover.



Figure

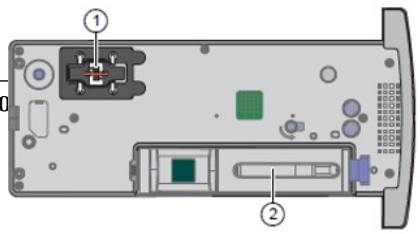


Figure
① Capillary
② Gel cartridge

Figure 19. Primary and Gel Cartridge components

6. Sign into the instrument as an Administrator or a Supervisor.
7. Touch the icon on the lower left side of the screen (Figure 20).

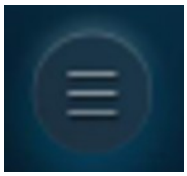


Figure 20. Login icon on the RapidHIT™ ID System

8. Touch the primary cartridge icon on the main screen (Figure 21).

Figure 21. Primary cartridge icon on the RapidHIT™ ID System

9. Choose "Yes" to confirm that you want to remove the primary cartridge (Figure 22):

Figure 22. Primary Cartridge Replacement screen on the RapidHIT™ ID System

10. The software will give step by step instruction on replacing the primary cartridge. Figure 23 shows the steps for replacement and the location on the primary cartridge.

Figure 23. Steps for replacement and the location on the primary cartridge

11. Step 1: Unscrew the shipping plug in the cathode block. Save the small piece that is removed (see Figure 53).

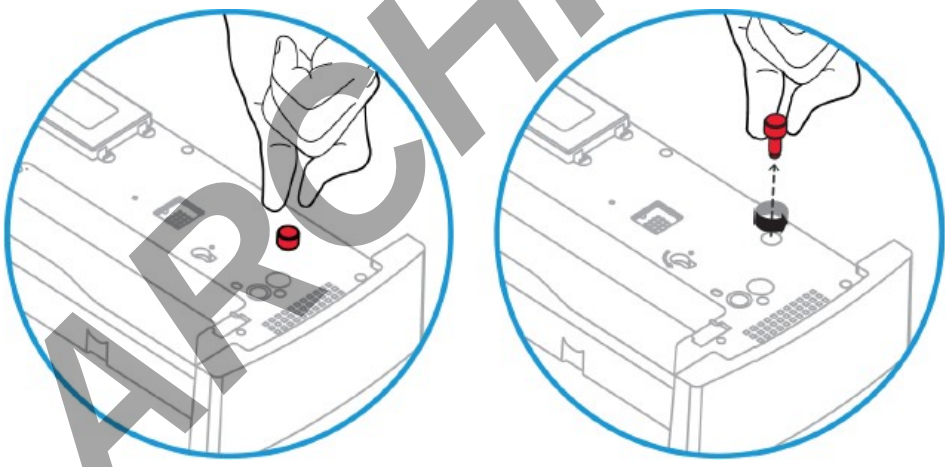


Figure 24. Step one unscrewing the shipping plug

12. Step 2: Turn the shipping plug in the check valve 90° counterclockwise, then remove it. Save the small piece that is removed (see Figure 25).

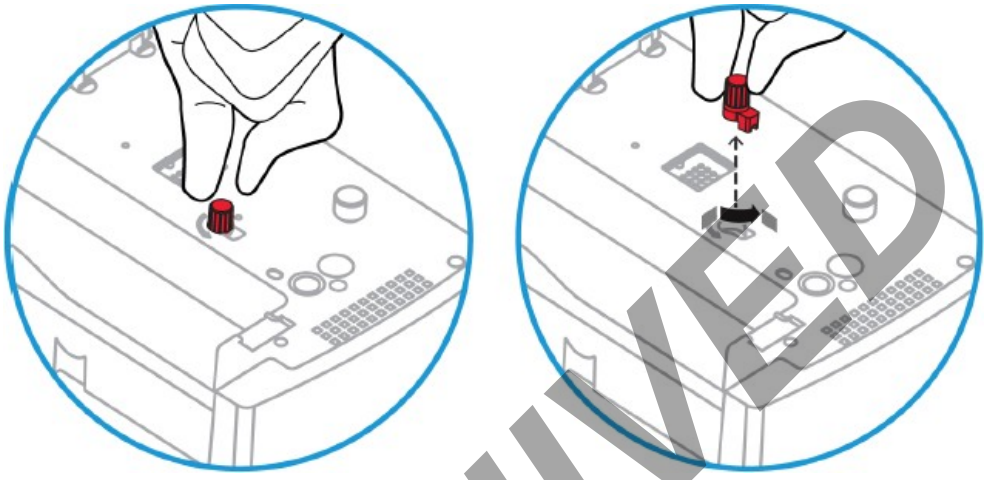


Figure 25. Step two removing the shipping plug

13. Step 3: Gel cartridge: remove the shipping plug from the gel cartridge inlet. Save the small piece that is removed (see Figure 26). Note: the foam casing surrounding the gel cartridge remains on the cartridge.

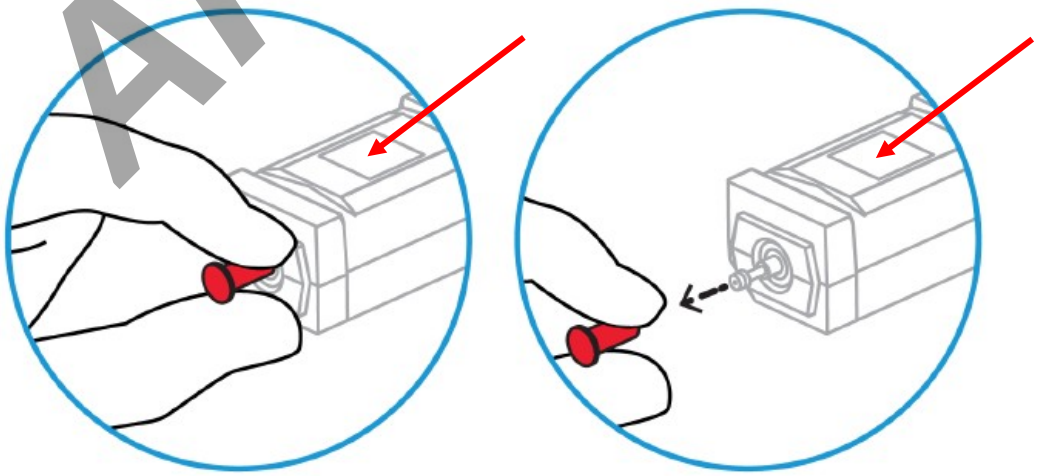


Figure 26. Step three removing the shipping plug from the gel cartridge

14. Step 4: Insert the gel cartridge into the primary cartridge with the tip of the gel cartridge facing the gel cartridge inlet (see Figure 27). Do not twist the gel cartridge. The gel cartridge clicks when it is fully inserted into the primary cartridge. The square black window should be facing up.

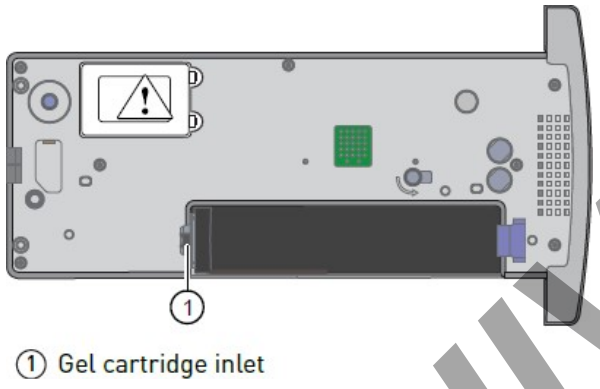


Figure 27.

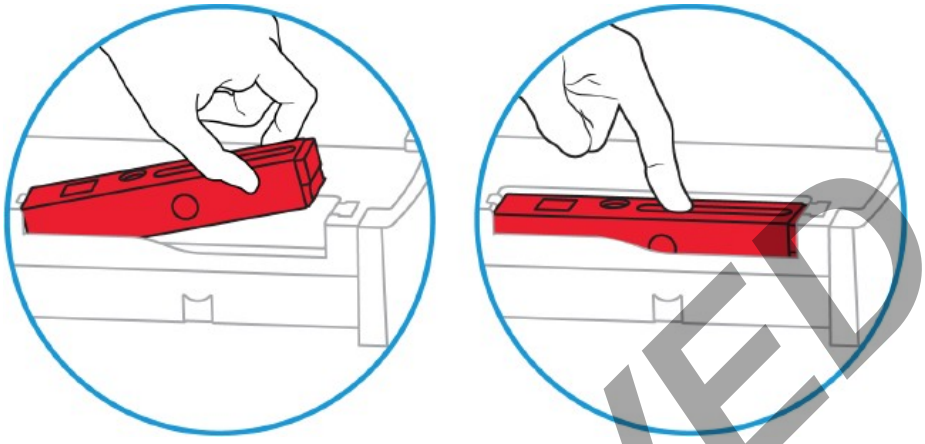


Figure 28. Step four, inserting the gel cartridge into the primary cartridge

15. Step 5: Remove the shipping cover from the capillary by pressing the brackets toward the cover, then swinging the cover up and away from the capillary. Note: The capillary is fragile. Handle with care after you remove the capillary cover. Save the cover piece that is removed (see Figure 29).

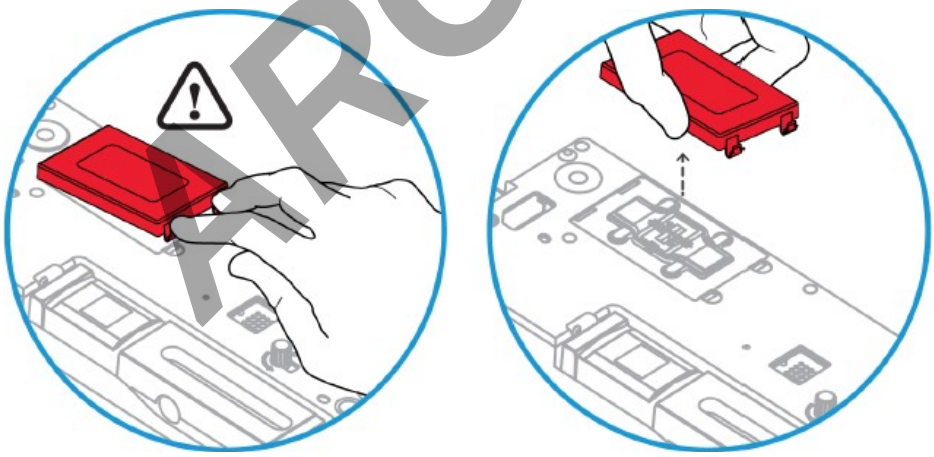


Figure 29. Step five removing the shipping cover from the capillary

16. Touch “Done” at the bottom of the primary cartridge screen. (see Figure 30). The

instrument will then prompt to insert the Utility Cartridge.

Figure 31. Selecting Done after removing the capillary cover

17. When the insert utility cartridge screen is displayed, insert the utility cartridge into the instrument (see Figure 32). The utility cartridge has a red label and is provided with the new primary cartridge. **The utility cartridge is a blank cartridge that allows for fluids to run through it. A countdown timer is displayed. This run will be approximately 5 minutes.**

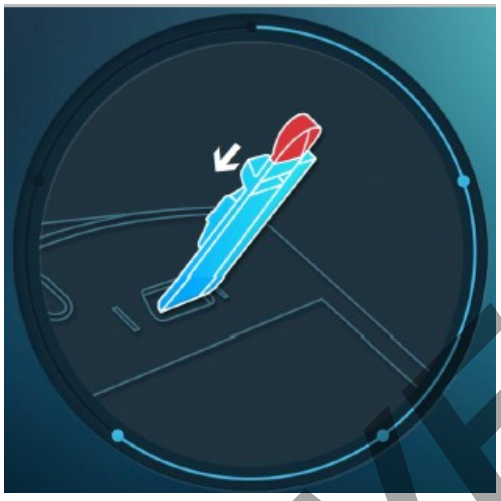


Figure 32. Inserting the utility cartridge screen

18. The instrument will prompt the operator to remove the used primary cartridge (see Figure 33A). **DO NOT** remove the utility cartridge. Pull the used primary cartridge out of the instrument and discard in the biohazard. Insert the newly prepared primary cartridge (see figure 33B).
19. **Note: The capillary is fragile. Do not let the capillary contact the instrument when you insert the prepared primary cartridge**

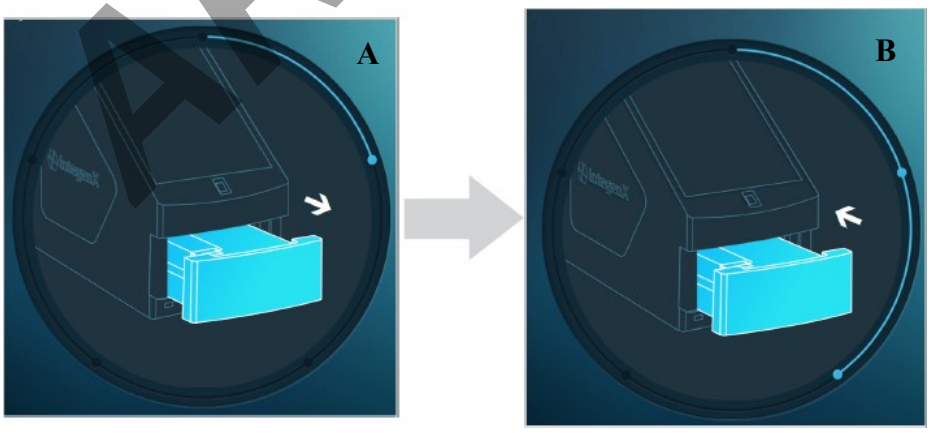


Figure 33. Removing and inserting a primary cartridge

20. A countdown timer is displayed. This run will be approximately 90 minutes.
21. When the 'remove utility cartridge' screen is displayed, remove the utility cartridge from the instrument and discard (see Figure 34).

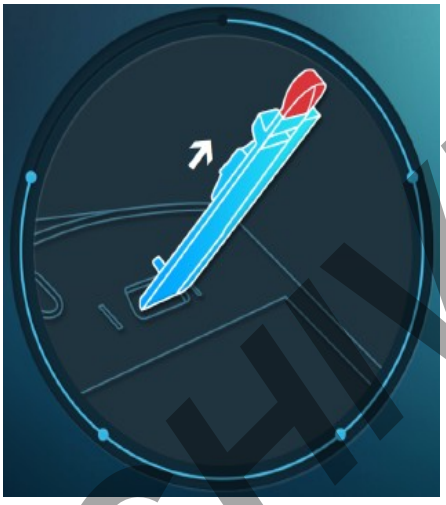


Figure 34. Removing the utility cartridge screen

22. Run the GFE Control (allelic ladder) cartridge. Insert and remove the control cartridge when the associated screen is displayed. The instrument reads the label on the cartridge and automatically assigns the sample identification as "LADDER". If you enter a name it will be overwritten in the software. The software will display ✓ for a GFE control profile that contains the expected number of alleles and ✗ for a GFE control profile that does not contain the expected number of alleles. Note: minimum run time is 60 minutes.
23. After replacing the primary cartridge, a positive sample control and negative control must be run. Running a negative control ensures that the gel is free from contamination and running a positive sample control ensures that migration is as expected. Each run has a minimum run time of 90 minutes.
24. When the runs are complete, remove the cartridge from the instrument and discard.
25. Review the status and take the appropriate action. Touch "Done" and the

instrument will automatically sign out the user and display the lock screen.

Troubleshooting

1. Troubleshooting the RapidHIT™ ID System may include the following (See Table 7). Note: When there is an error code displayed, the system will need to be shut down and re-started in order to continue processing samples.

Observation	Possible cause	Recommended action
A yellow triangle is displayed on the lock screen 	An error code has been generated.	Touch the lock screen to obtain the error code. Contact Thermo Fisher Scientific Support.
An error code is displayed when you sign in 	There is a problem with the instrument.	Record the error code. Contact Thermo Fisher Scientific Support.
is displayed on the sample identification screen	Indicates that the instrument is not connected to the RapidLINK™ Software.	See "Check the RapidLINK™ Software connection" on page 43.
You cannot easily remove the sample cartridge from the instrument	The sample cartridge is locked in the sample cartridge port.	RapidHIT™ ID system administrator or supervisor: Run the recover function (see page 42).

Observation	Possible cause	Recommended action
A power failure occurs during a run	Various causes.	If the power failure occurs early in the run, the run will stop and it will not be listed in the run log. Obtain a new swab and rerun the sample.
		If the power failure occurs late in the run but before analysis starts, the run will be listed in the run log with a red ✗.
		If the power failure occurs after analysis is complete, the run will be listed in the run log with a green ✓ if it passes or a red ✗ if it fails.
"The primary cartridge is not engaged" message is displayed on the sample identification screen 	There is a problem with the instrument or the primary cartridge.	Contact Thermo Fisher Scientific Support.

Table from RapidHIT ID System v1.0 User Guide

Table 7. Troubleshoot the RapidHIT™ ID System

2. For additional assistance, contact ThermoFisher Technical Support.

References

1. RapidHIT™ ID System v1.0 User Guide. Revision A.0. November 06, 2018.
2. RapidLINK™ Software v1.0 User Guide. Revision A.0. November 06, 2018.
3. GlobalFiler™Express PCR Amplification Kit. Revision D. October 21, 2018.
4. GeneMarkerHID Manual 2.9.5. SoftGenetics. January 2018.