

Meridian²/Meridian³ operation with Kraken

! WARNING

Read and understand equipment operators manual before operating or performing maintenance. Failure to do so could result in serious injury or death.

Always follow your facility's PPE program when operating or performing maintenance on this instrument.

Meridian²/Meridian³ is a 8-channel dispensing robot capable of dispensing a range of patterns into 96-, 384- and 1536-well plates.

Meridian²/Meridian³ can be run with Kraken in three different modes:

- Single run – assays from troughs
- Single run – assays from tubes
- Multi run – assays from tubes

Software requirements:

| | Meridian version | Minimum required Kraken version |
|-----------------------|------------------|---------------------------------|
| Meridian ³ | 18.0 | 18.3.5.19931 |
| Meridian ³ | 17.23.3 | 17.10.8.19257 |
| Meridian ² | 17.23.2 | 17.8.2.18966 |
| Meridian ² | 17.22 | 17.3.5.18040 |

Required material.

- Meridian²/Meridian³ dispenser
- PC operating instrument engine software and linked to Kraken workflow manager database
- Assays for dispensing
- A suitable master mix
- 96-, 384- or 1536-well PCR plates containing template DNA

- 8-well or 16-well Meridian²/Meridian³ mixing troughs.
- Red SepraSeal removal tool (optional).
- Plate sealer (with optional Fusion³ required for 1536-well plates).
- R.O. (reverse osmosis) or DI (deionised) water.
Reverse Osmosis (R.O.) Water: 2 L for setup (approx. 2 L/day)
Standard Minimum Grade
ASTM Standard (ISO 3696) Type III
ISO Standard Grade 3
Clinical Laboratory Standards Institute

Note: Commercial/industrial R.O. water system typically meet these requirements. DI water acceptable if weekly recommended cleaning is performed.

- Vortex mixer
- Centrifuge with plate holders
- 1D barcode readers (optional but preferred)

Preparations

Thaw master mix and keep it on ice until use.

Thaw assay tubes, thoroughly vortex them and centrifuge to 1000 rpm to remove liquid from lids. Keep tubes on ice until use.

References

Protocol for Meridian² deep cleaning.

Preparations of Hydrocycler for thermal cycling of PCR plates.

Cycling plates using Hydrocycler.

Starting up

Notice

LGC Biosearch Technologies accepts no responsibility for misuse of instrument.

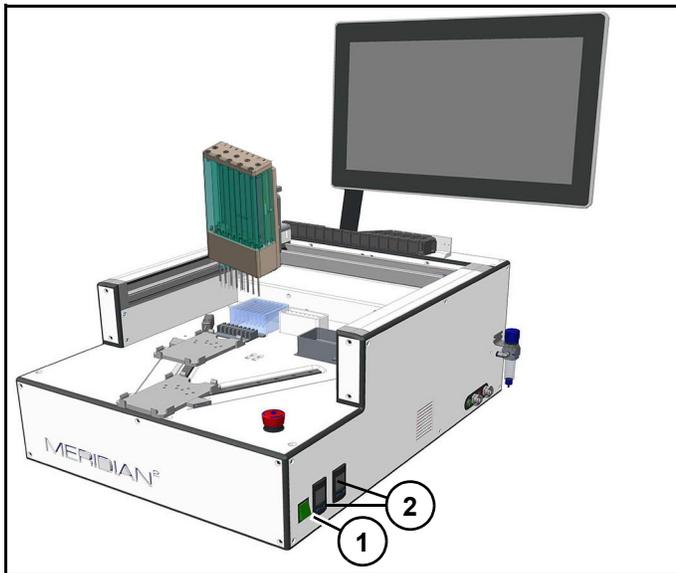


Figure 1

1. Turn on power (1) (Figure 1).
2. Turn on compressor and ensure air valve is open.
3. Ensure water bottle is filled and connected.
4. Ensure waste bottle is emptied and connected.
5. Turn on PC.



Figure 2

6. Double click "Meridian²/Meridian³" icon (1) (Figure 2).
7. Verify pressure on gauges (2) (Figure 1) are within specification range.

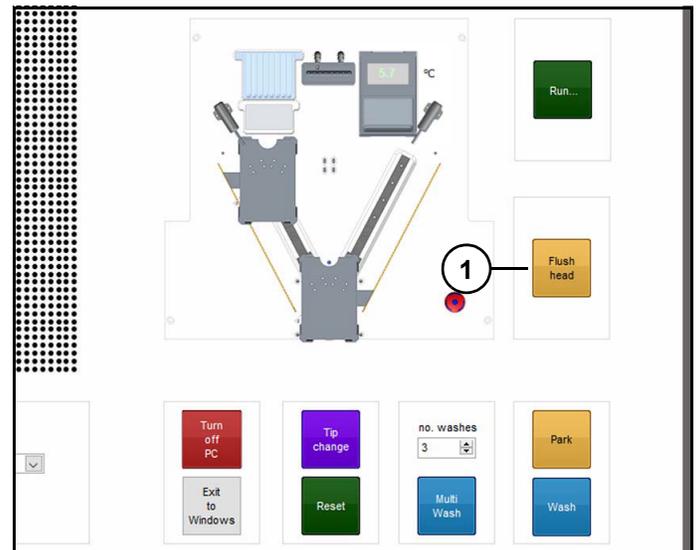


Figure 3

Dispense head will home and user will be prompted to flush dispense head.

8. Click "Flush head" (1) (Figure 3).

Flushing will blow air through dispensing head to remove any water droplets. Dispense head will stop above wash station when flush is complete.

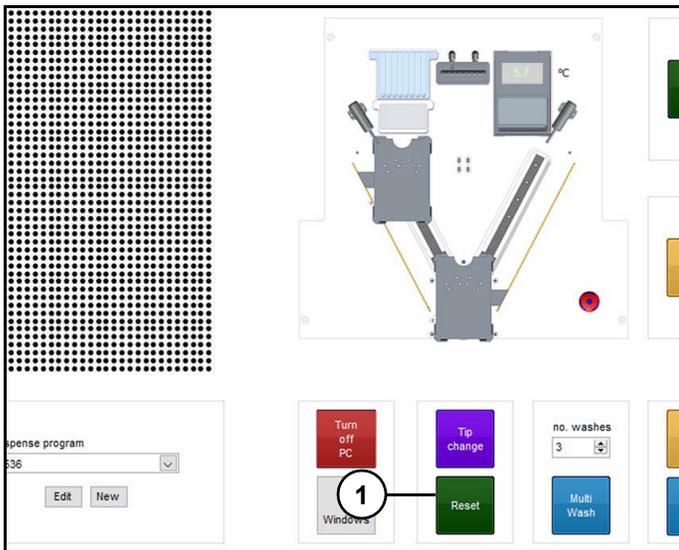


Figure 4

Refer to (Figure 4).

9. Click "Reset" (1) to home instrument. Instrument will move dispensing head to home position.

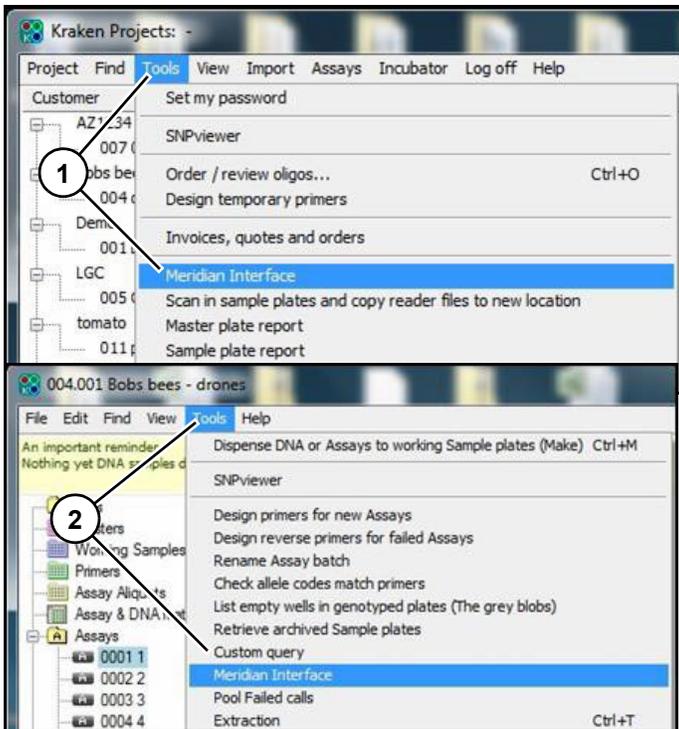


Figure 5

10. Log in to Kraken workflow manager.

11. Open interface using "Tools" drop down on "Project" window (1) or Global window (2). (Figure 5)

Note: If instrument engine software is not opened first, it will automatically open with Kraken interface.

If instrument has not been used for several hours, it is recommended to perform three wash cycles to ensure wash lines, dispensing head chamber and tips are clean.

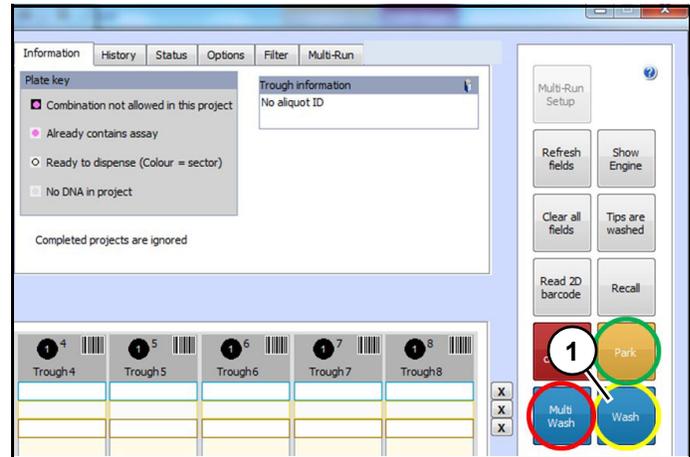


Figure 6

12. Click "Wash" (1) (Figure 6) to perform a single wash. Click "Wash" two more times to perform two more washes.

Note: Multi-wash cycle on instrument can also be used to perform these three washes.

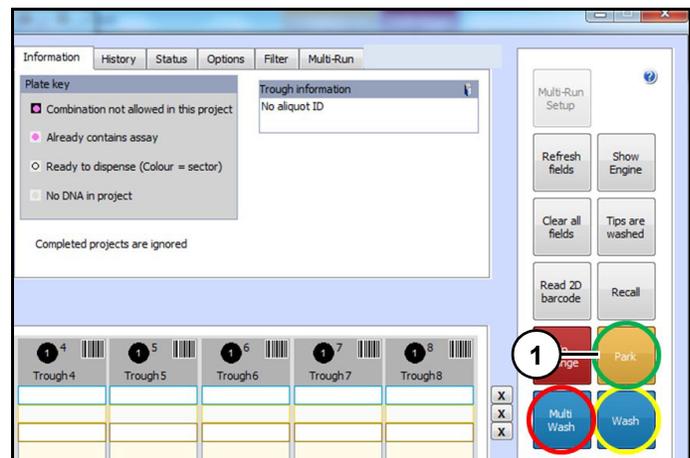


Figure 7

13. Click "Park" (1) (Figure 7) to move dispense head to parked position.

Note: This position can be taught from "Teach position" window available in instrument software. Ideally, Park position should be taught above wash station. See teaching park position in Meridian²/Meridian³ user's manual.

Kraken interface screen identification

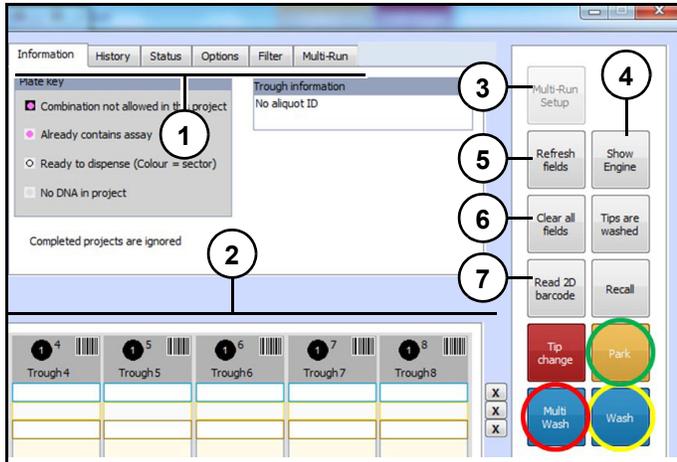


Figure 8

Refer to (Figure 8).

Kraken tabs (1): Tabs displays information and settings.

Trough displays (2): Displays trough information.

Multi-Run Setup (3): Initiates multi-run when active.

Show Engine (4): Displays instrument interface.

Refresh fields (5): Refreshes information fields.

Clear all fields (6): Clears all information fields.

Read 2D barcode (7): Initiates barcode read.

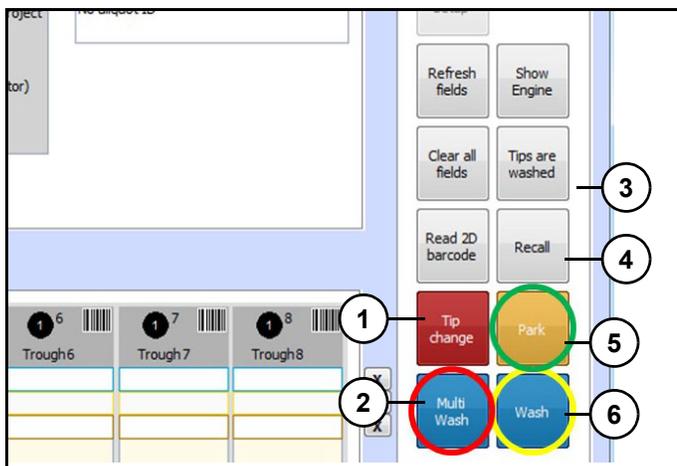


Figure 9

Refer to (Figure 9).

Tip change (1): Moves dispense head to left front of deck. So tips can be easier removed and installed.

Multi Wash (2): Initiates a multi-wash cycle.

Tips are washed (3): Sets tip status for all tips to washed.

Recall (4): Reloads aliquot barcodes and their trough positions from previous run.

Park (5): Moves dispense head to taught park position.

Wash (6): Initiates a single wash cycle.

Kraken tabs

Information.

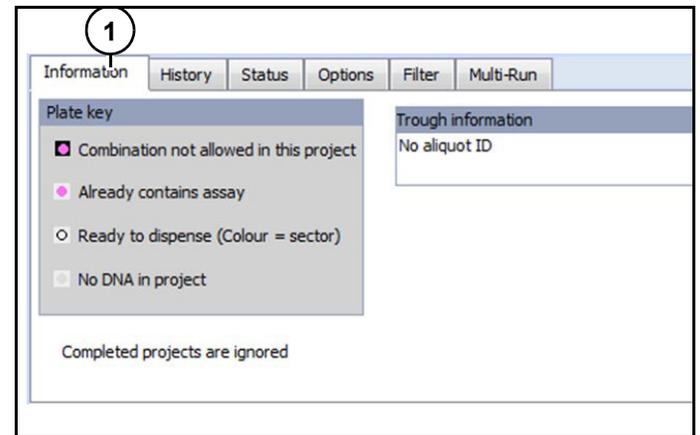


Figure 10

Displays information (1) (Figure 10) in Kraken interface.

History.



Figure 11

History (1) (Figure 11) displays information about actions performed on instrument since interface was last opened.

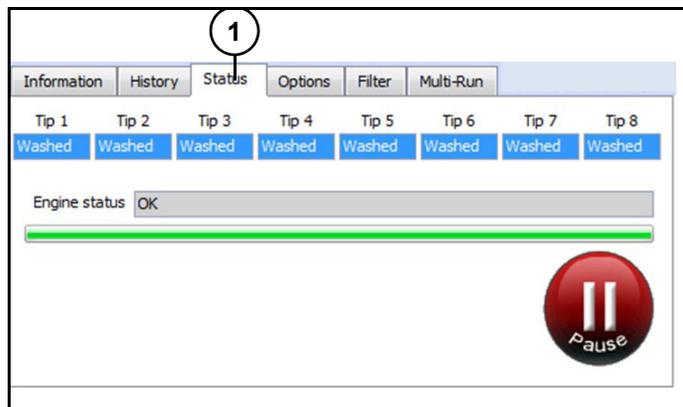
Status.

Figure 12

Status (1) (Figure 12) displays instrument and tip status (washed, full or empty).

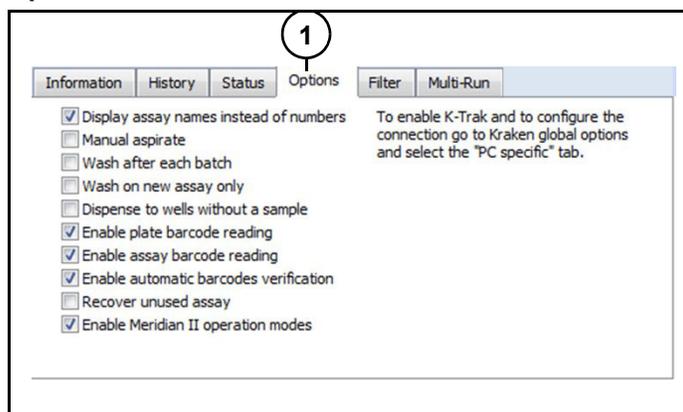
Options.

Figure 13

Options (1) (Figure 13) displays options available for instrument operation via Kraken.

- **Manual aspirate:**
User will need to activate reagent aspiration manually.
- **Wash after each batch:**
Instrument will wash tips after each dispensing batch.
- **Wash on new assay only:**
Instrument will wash tips only when a new assay is being used.
- **Dispense to wells without a sample:**
Instrument will dispense in all plate wells, even in those not having any DNA (empty wells).
- **Enable plate barcode reading:**
Instrument will automatically use plate barcode

reader integrated on side of dispensing head, to scan plate barcodes during run (no need to use an external barcode reader).

- **Enable assay barcode reading:**
Instrument will automatically use 2D barcode reader integrated below the assay tube rack, to scan the aliquot barcodes during the run (no need to use an external barcode reader).
- **Enable automatic barcodes verification:**
Instrument will automatically check that plate and assay tube barcodes scanned during run are same as barcodes scanned during run setup.

Note: "Enable plate barcode reading" and "Enable assay barcode reading" options should also be checked in order to allow automatic verification.

- **Recover unused assay:**
Reagent volume left in tips at end of plate dispensing will be dispensed back into mixing trough.
- **Enable instrument operation modes:**
If selected, an additional dropdown box will be displayed in top left corner of Kraken interface and will allow user to select between three operation modes:
Single run – assays from troughs
Single run – assays from tubes
Multi-run – assays from tubes

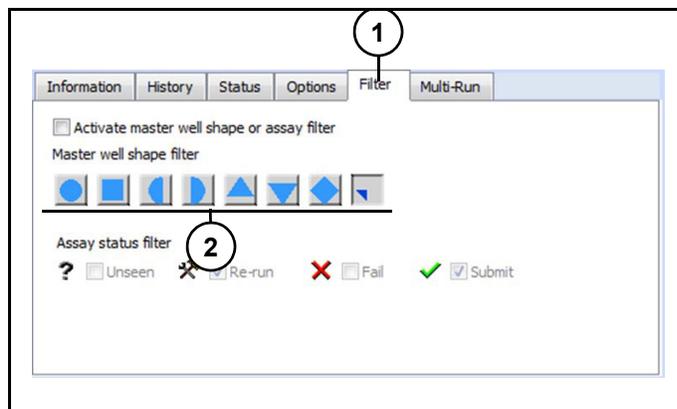
Filter.

Figure 14

Refer to (Figure 14).

Filter (1) allows selection of certain types of wells to be dispensed with certain type of assay, according to following filters:

- Master well shape:
When master plates are created in Kraken, wells can be assigned with one of shapes shown (2) . These shapes are transferred to corresponding wells in working sample plates, and can then be used as filters to select wells to be dispensed by instrument.
- Assay status:
Assay created or imported in Kraken are assigned a status (Unseen, Re-run, Fail or Submit). This status can be used as a filter to select assays to be used for dispensing plate on instrument. If an aliquot is scanned and does not correspond to selected assay status, Kraken will display an error.

Multi-Run.

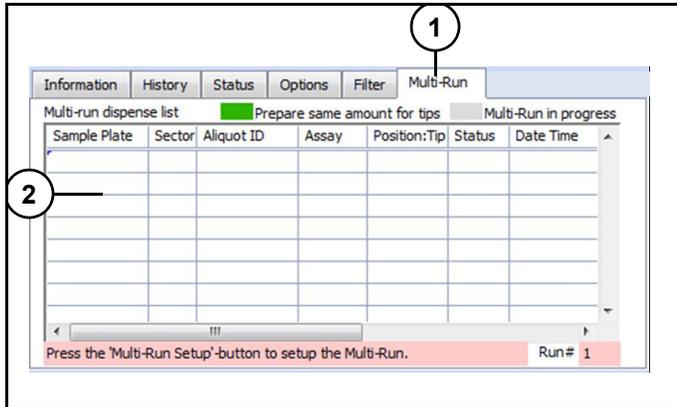


Figure 15

Refer to (Figure 15).

Multi-Run (1) will only show information if Multi-Run operations mode is selected. Table (2) lists information related to plates, assays and tips used for run.

Kraken dispensing table

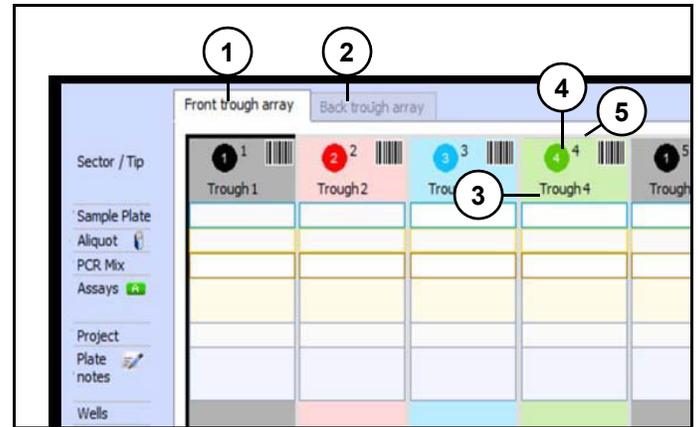


Figure 16

Refer to (Figure 16).

Front trough array (1): Refers to wells 1 to 8 of mixing trough.

Back trough array (2): Refers to wells 9 to 16 of 16-well mixing trough.

Trough well position (3): Displays well position in mixing trough.

Dispensing template sector (4): Shows color and number of sector where assay aliquot scanned will be dispensed. A sector corresponds to a set of wells in plate where a same assay is intended to be dispensed, according to dispensing template selected.

Example: "96 tx" dispensing template in which 4 assay aliquots are dispensed over a 384-well plate (96 wells per aliquot (Figure 17)).

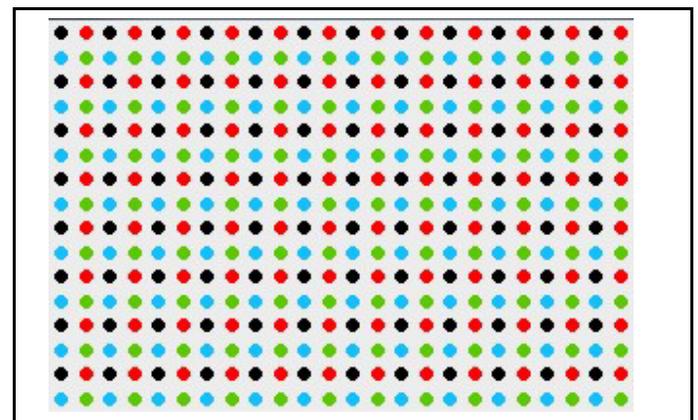


Figure 17

| Sectors with 96 tx template | |
|---|----------------------------|
|  | Sector 1 = Assay aliquot 1 |
|  | Sector 2 = Assay aliquot 2 |
|  | Sector 3 = Assay aliquot 3 |
|  | Sector 4 = Assay aliquot 4 |

Tip (5): Position of tip which will be used for dispensing designated assay aliquot in the designated sector.

Assays (4): When aliquot is scanned, Kraken automatically displays name (or KSNP number) of corresponding assay.

Project (5): Kraken project from which sample plates and assay aliquots are selected for dispensing run.

Plate notes (6): Displays where users can type notes related to plates.

Wells (7): Displays plate format (number of wells in).

Conc. (8): Displays assay aliquot concentration.

Aspirate (9): Displays volume of reagents to be aspirated for dispensing requested number of wells.

Note: This volume does not include any overage. It corresponds to [number of wells] x [volume per well].

Primer RXM (10): Displays volume of reagents to be dispensed.

Master plates (11): Kraken automatically displays name/barcode of master plate(s) from which sample plate was created.

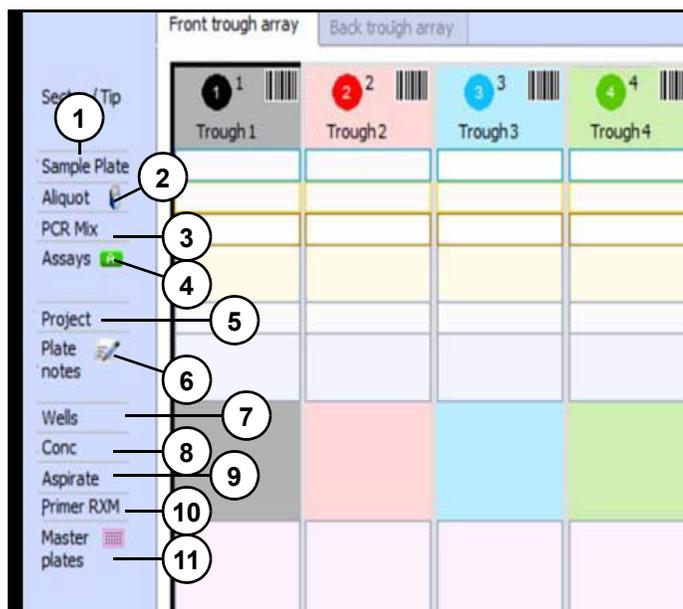


Figure 18
Refer to (Figure 18).

Sample Plate (1): Displays where sample plates to be dispensed are scanned.

Aliquot (2): Displays where assay aliquots to be dispensed are scanned.

PCR Mix (3): Should be left empty.

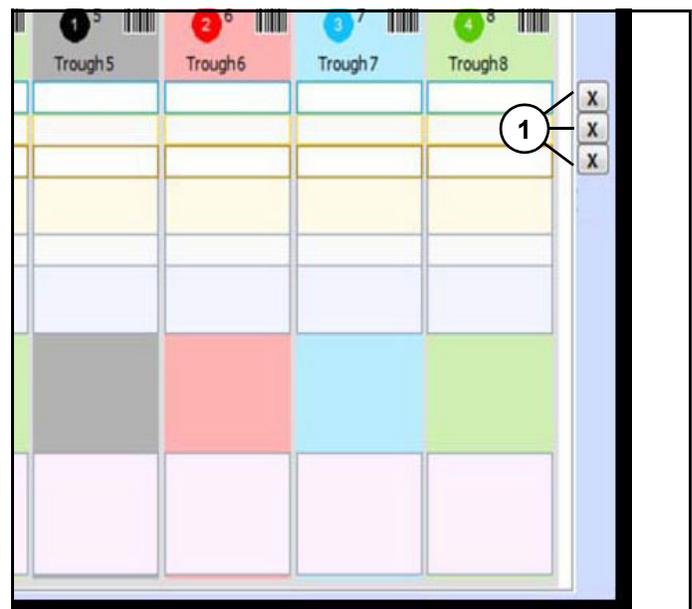


Figure 19
Refer to (Figure 19).

Cross buttons (1): Used to delete information typed in corresponding rows (Sample plate, Aliquot or PCR Mix).

Single run- assay from troughs

Single run – assays from troughs will aspirate reagents directly from Mixing trough.

User will need to manually prepare appropriate volume of reagents at correct concentration (1X or 2X) before loading them into mixing trough before starting run.

Instrument will only perform one dispensing run. Volume aspirated by each tip can only be used for dispensing over one plate.

Some typical dispensing combinations include:

- 1 tip (= 1 assay aliquots) per plate on 8 plates
Tip 1 on plate 1
Tip 2 on plate 2
And so on until Tip 8 on plate 8
- 2 tips (= 2 assay aliquots) per plate on 4 plates
Tips 1 & 2 on plate 1
Tips 3 & 4 on plate 2
And so on until Tips 7 & 8 on plate 4
- 4 tips (= 4 assay aliquots) per plate on 2 plates
Tips 1 to 4 on plate 1
Tips 5 to 8 on plate 2
- 8 tips (= 8 assay aliquots) per plate on 1 plate
- 16 assay aliquots per plate on 1 plate (8 tips will aspirate and dispense first 8 assays, wash, and then aspirate and dispense last 8 assays).

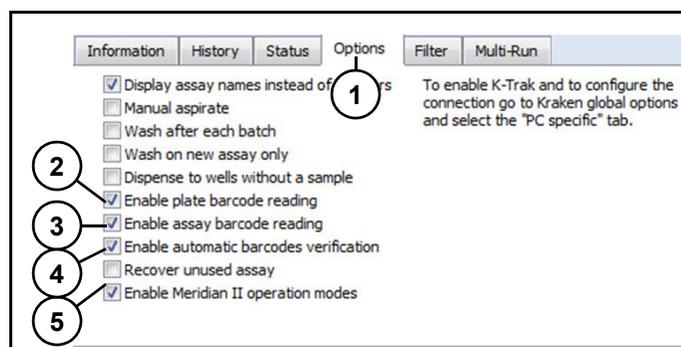


Figure 20

Refer to (Figure 20).

1. If Kraken Interface is being used for first time, select "Options" (1).
2. Check following:
Enable plate barcode reading (2).

- Enable assay barcode reading (3).
- Enable automatic barcode verification (4).
- Enable instrument operations modes (5).

These options allow instrument to automatically scan plates and assay tubes barcodes and to perform automatic barcodes verification during run.

Note: Enable plate barcode reading, enable assay barcode reading, and enable Meridian²/Meridian³ are recommended for instrument. Other displayed options are optional depending on conditions.

Note: For "Single-Runs", all options which are selected are saved by interface.

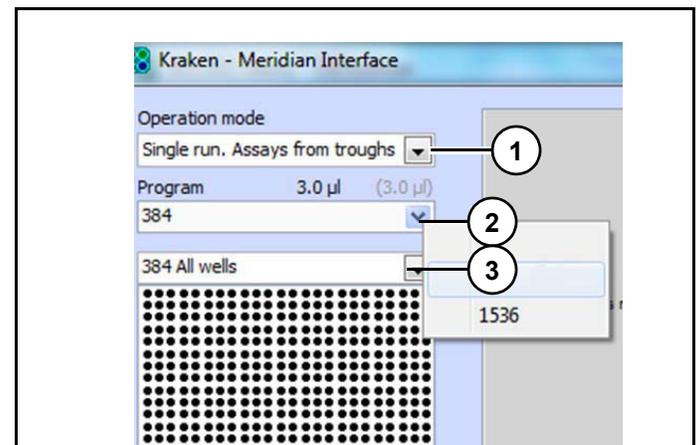


Figure 21

Refer to (Figure 21).

3. Click "Operation mode" (1) and select "Single run, Assays from troughs".
4. Click "Program" (2) and select required plate.
5. Click "Template" (3).

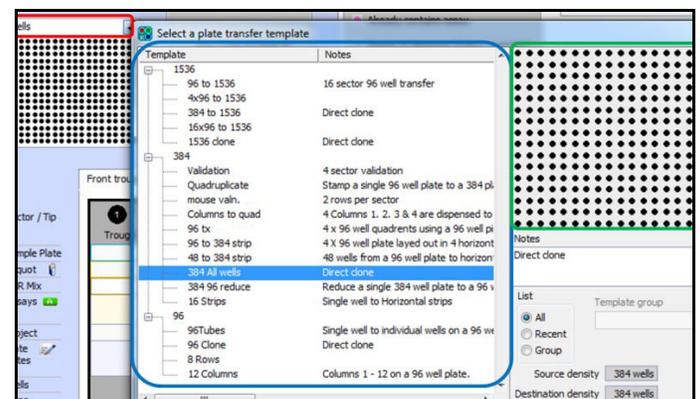


Figure 22

6. Select dispense template (Figure 22).

Scanning plates and assay aliquots

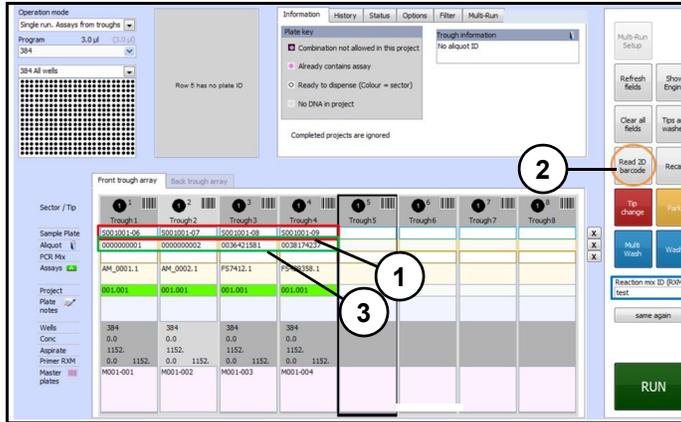


Figure 23

7. Using a handheld 1D barcode scanner (connected to instrument PC), scan barcodes of plates to be dispensed, into sample plate field (1) (Figure 23).

Note: Plates must be created within Kraken project to which Kraken interface is connected.

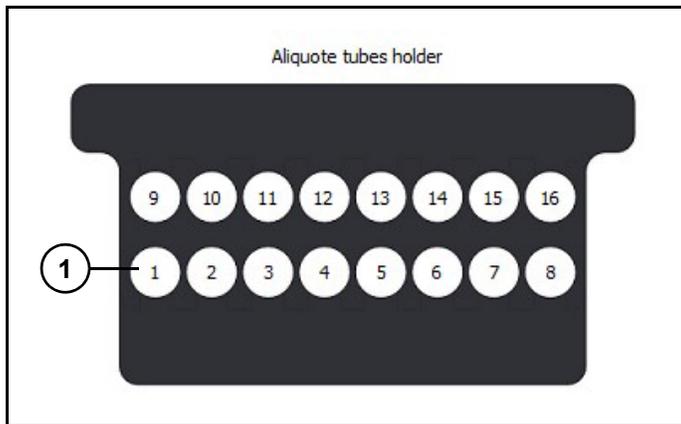


Figure 24

8. Place assay aliquot tubes to be used on assay tube holder located on instrument deck. First assay tube (1) (Figure 24) (used for tip 1) needs to be placed on left end of front rack, and following tubes should be placed in order from left to right on front rack.

9. Click "Read 2D barcode" (2). 2D barcode reader will scan tubes and corresponding barcodes will be automatically displayed in "Aliquot" (3). (Figure 23)

Note: Assay aliquots used must be created or imported in Kraken project used.

Example 1.

Four 384-well plates dispensed with "384 all wells" template, and same assay being dispensed on all plates.

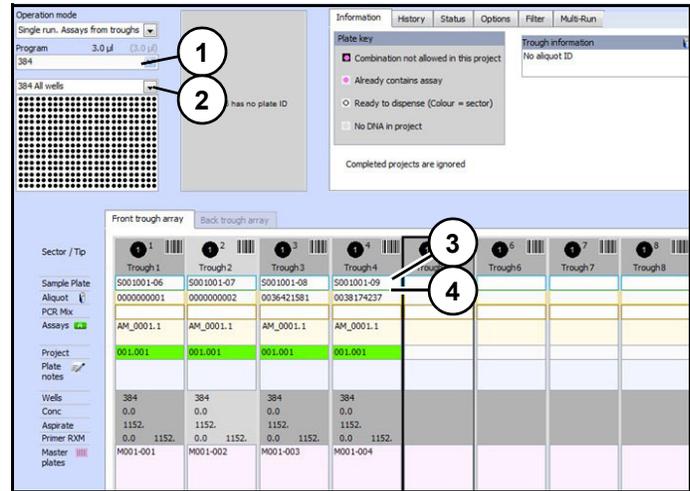


Figure 25

Refer to (Figure 25).

- Program (1): 384
- Template (2): 384 all wells
- Sample plate (3): four 384-well plates scanned
- Aliquot (4): 4 aliquots of same assay were placed on positions 1 to 4 of tube holder and scanned using "read 2D barcode"

Note: 4 different aliquots of same assay are required here because options "enable assay barcode reading" and "enable automatic barcodes verification" were activated. Although same assay will be dispensed on 4 sample plates, 4 different aliquots are required to allow 2D barcode reader to scan assay on tube holder positions 1 to 4.

Example 2.

Two 384-well plates dispensed with “96 tx” template, and same 4 assays on both plates.

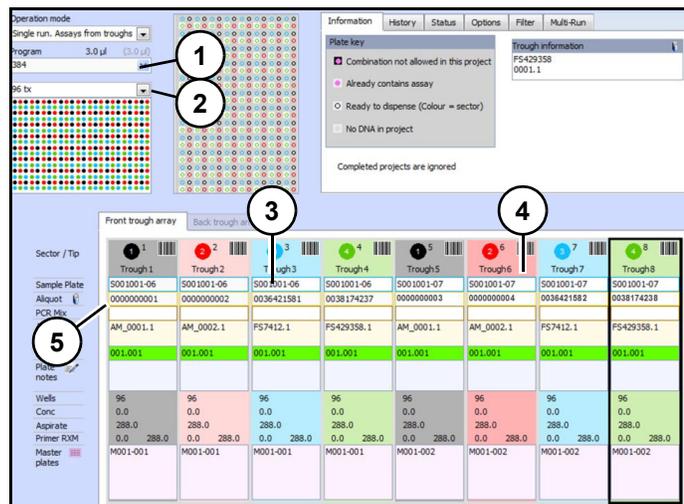


Figure 26

Refer to (Figure 26).

- Program (1): 384
- Template (2): 96 tx
- Sample plate: four 384-well plates scanned.

Plate 1 (S001001-06) (3) was scanned 4 times (once for each assay) in columns 1 to 4 (corresponding to tips 1 to 4, and to wells 1 to 4 of mixing trough).

Plate 2 (S001001-07) (4) was scanned 4 times (once for each assay) in columns 5 to 8 (corresponding and to tips 5 to 8, and to wells 5 to 8 of mixing trough)

- Aliquot (5): Since each assay will have to be dispensed in 2 plates, each assay was dispatched in 2 aliquots:

| Sector | Assay | Aliquot 1 | Aliquot 2 |
|--------|-----------|------------|------------|
| 1 | AM_0001.1 | 0000000001 | 0000000003 |
| 2 | AM_0002.1 | 0000000002 | 0000000004 |
| 3 | FS7412.1 | 0036421581 | 0036421582 |
| 4 | FS429358 | 0038174237 | 0038174238 |

- Aliquots were placed on tube holder in order to match with sectors to be dispensed on sample plates, and to be aspirated by correct tip:

| Assay/ Aliquot | Plate | Sector | Tube holder position | Tip |
|----------------------|-------|--------|----------------------------|-----|
| Assay 1 Aliquot 1 | 1 | 1 | 1 | 1 |
| Assay 2 Aliquot 1 | 1 | 2 | 2 | 2 |
| Assay 3 Aliquot 1 | 1 | 3 | 3 | 3 |
| Assay 4 Aliquot 1 | 1 | 4 | 4 | 4 |
| Assay 1 Aliquot 2 | 2 | 1 | 5 | 5 |
| Assay 2 Aliquot 2 | 2 | 2 | 6 | 6 |
| Assay 3 Aliquot 2 | 2 | 3 | 7 | 7 |
| Assay 4 Aliquot 2 | 2 | 4 | 8 | 8 |

- Aliquots were scanned using “Read 2D barcode”.

Note: As in Example 1, different aliquots of same assays are required here because options “enable assay barcode reading” and “enable automatic barcodes verification” were activated.

Preparing reagents

Prepare volume of reagent mix necessary for each well of Mixing trough. Concentration of reagent mix to be prepared (1X or 2X) depends on use on dry or wet DNA.

Use following calculation formula:

$$A_v = (V_w \times N_w) \times (1+A_f) + A_o + P_v$$

Note: These values can be found in instrument software in "Aspiration Dispense Parameters" window (Figure 27).

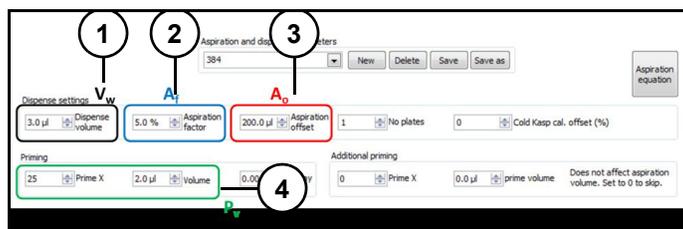


Figure 27

Refer to (Figure 27).

- A_V : Aspiration volume (reagent volume aspirated per trough).
- V_W (1): Volume of reagent to be dispensed per well (μL).
- N_W : Number of wells to be dispensed.
- A_f (2): Aspiration factor (%).
- A_o (3): Aspiration offset (μL).
- P_v (4): Priming volume (number of priming dispenses x volume per dispense in μL).

Starting run

Note: Ensure model of mixing trough placed on instrument deck is correct. If dispensing template has ≤ 8 sectors then use 8-well mixing trough only. If template has >8 sectors then use the 16-well mixing trough only.

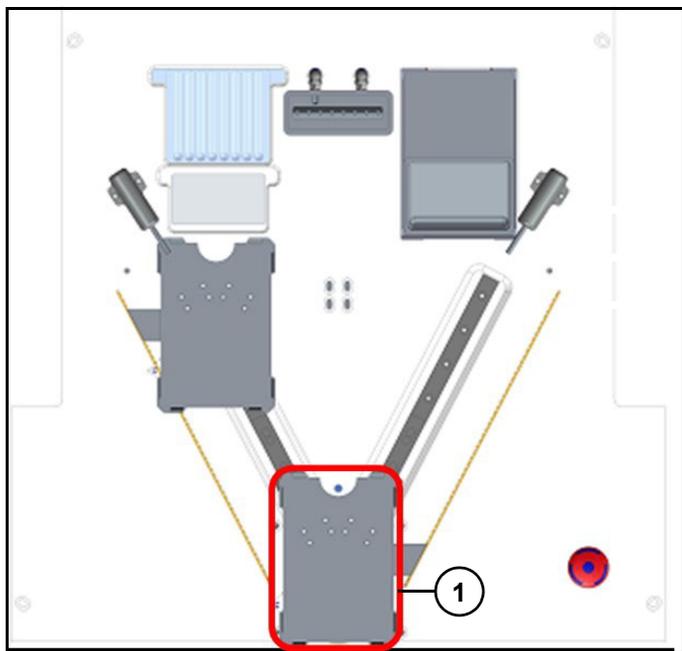


Figure 28

Refer to (Figure 28).

Place first plate to be dispensed onto plate shuttle (1). A sensor will detect plate presence.

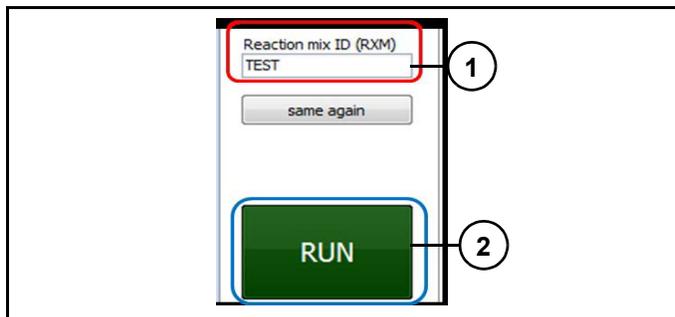


Figure 29

Refer to (Figure 29):

10. Scan Reaction Mix ID (1).

11. Click "Run" (2).

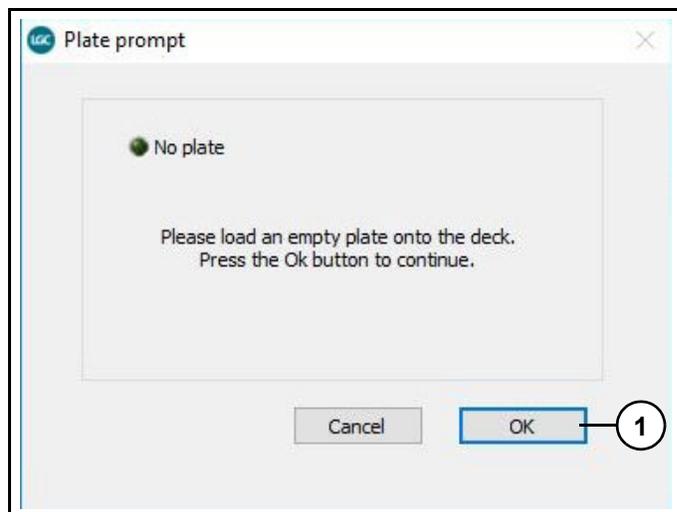


Figure 30

Refer to (Figure 30).

If a plate has not been loaded, plate sensor will detect it and a prompt will appear and request user to load an empty plate.

12. Load an empty plate.

13. Click "OK" (1).

Plate and assay barcode scanning and verification

Plate shuttle will move and bring plate to right or left position (depending on shuttle configuration before starting the run).

Instrument dispense head will home and then move above plate, scan its barcode and verify correct plate.

16. Click "Toggle shuttle" (1).
17. Click "Barcode icon" (2) to rescan plate.
18. Click "OK" (3) to resume run.

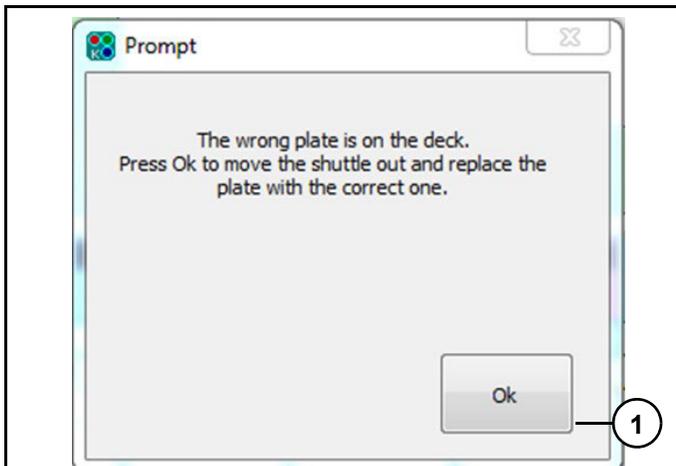


Figure 31

Refer to (Figure 31).

If barcode is wrong or scanning has failed, dispense head will park, and prompt will appear.

14. Click "Ok" (1) to bring plate to front position.
15. Check plate and replace wrong plate with correct one if necessary.

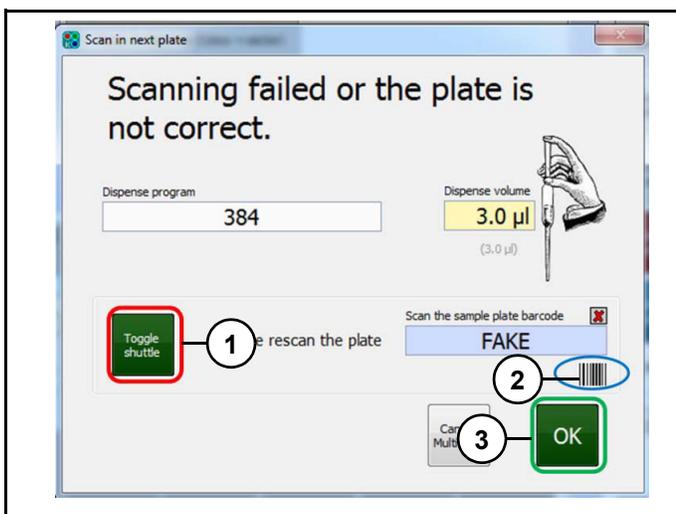


Figure 32

Refer to (Figure 32).

Reagent aspiration and dispensing

When plate and aliquot barcodes are correct, dispense head will move to wash station and execute a Wash cycle (if tips have not been washed).

Dispense head will move to mixing trough and aspirate reagents. Only tips requested for run will be activated (example, if only tips 1-4 are required, tips 5-8 will remain inactive). If number of wells to be dispensed by each tip is different (example some plates are not full with samples), each tip will have capacity to aspirate different volumes of reagents.

After aspirating dispense head will move above wash station to prime tips. Number of priming dispenses and volume dispensed each time are adjusted in "Aspiration dispense parameters" window of instrument software.

Dispense head will move above plate, dispense and move to park position when finished.

Dispense head will scan plate barcode and aliquot one more time for verification.

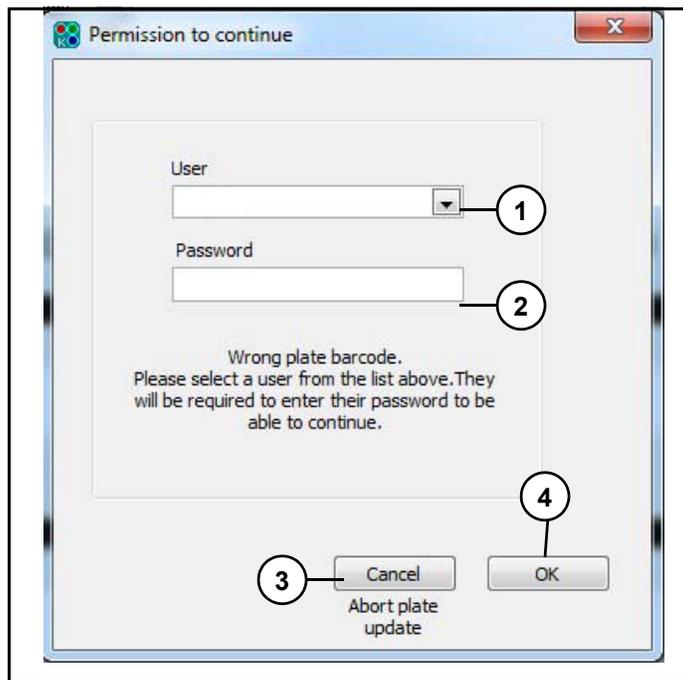


Figure 33

Refer to (Figure 33).

If scanned plate barcode is wrong, prompt will appear.

19. A Kraken user (1) will have to enter password (2) to continue run.

Note: User can click "Cancel" (3) or click "OK" (4) to modify plate.

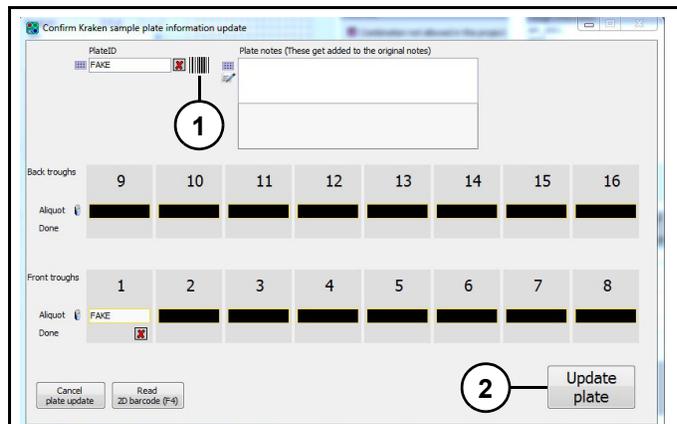


Figure 34

Refer to (Figure 34).

20. If plate barcode needs to be corrected, click "barcode icon" (1) to edit

21. Click "Update plate" (2), to save updated barcode.

Note: If scanned aliquot barcode is wrong, same prompts will appear, use same process to update.

Switching to next plate

Once barcodes are verified and first plate dispensed, dispense head will move to park position.



Figure 35

Refer to (Figure 35).

22. Place an empty plate on front position.

23. Click "OK" (1).

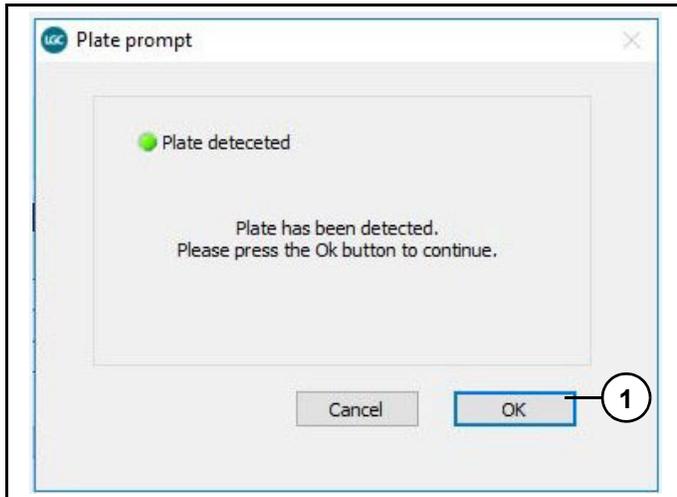


Figure 36

Refer to (Figure 36).

24. Click "OK" (1).

Plate shuttle will bring new plate to side position and dispensed plate back to front position.

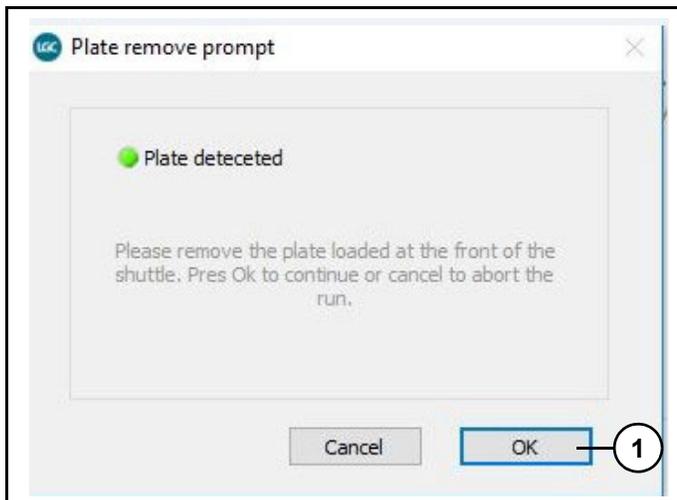


Figure 37

25. Remove dispensed plate from front position.

26. Click "OK" (1). (Figure 37).

This will activate the start of next dispensing, which will proceed similarly to steps described above, except that tips will not be washed or primed.

End of dispensing run

When last plate has been dispensed, barcodes checked and dispensed plate removed, dispense head will execute a wash cycle before stopping on park position.

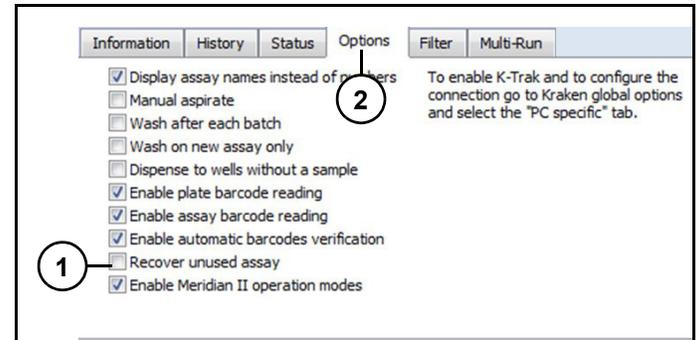


Figure 38

Note: It is possible to recover small volume of reagent remaining in dispense head at end of run. Check "Recover unused assay" (1) in Options (2) in Kraken interface before starting run. (Figure 38)

27. Remove mixing trough.

28. Rinse it with RO or DI water and dry it with a lint-free cloth.

Single run assays from tubes

Single run assays from tubes instrument tips will:

- Aspirate requested volumes of KASP Master Mix and assays from separate locations on deck and mix them automatically in mixing trough.
- Only be able to perform one dispensing run.

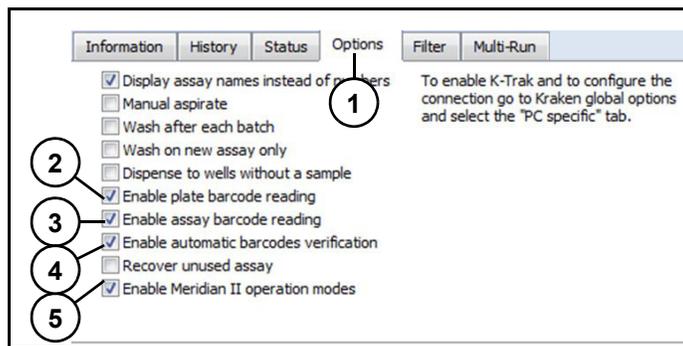


Figure 39

Refer to (Figure 39).

- If Kraken interface is being used for first time, select "Options" (1).
- Check following:
 - Enable plate barcode reading (2).
 - Enable assay barcode reading (3).
 - Enable automatic barcode verification (4).
 - Enable Meridian II operations modes (5).

These options allow instrument to automatically scan plates and assay tubes barcodes and to perform automatic barcodes verification during run.

Note: Enable plate barcode reading, enable assay barcode reading, and Enable Meridian II are recommended for instrument. Other displayed options are optional depending on conditions.

Note: For single-runs, all options that are selected are saved by interface.

- Scan plates and assays. See "Scanning plates and assay aliquots" on page 9.

Note: When instrument is aspirating from tubes, if same assay is aspirated by different tips, user will need to use as many aliquots of same assay. Example 1 and 2 still apply for this operation mode. See "Example 1" on page 9. See "Example 2" on page 10.

Preparation of reagents

Instrument will automatically prepare reagents requested for run.

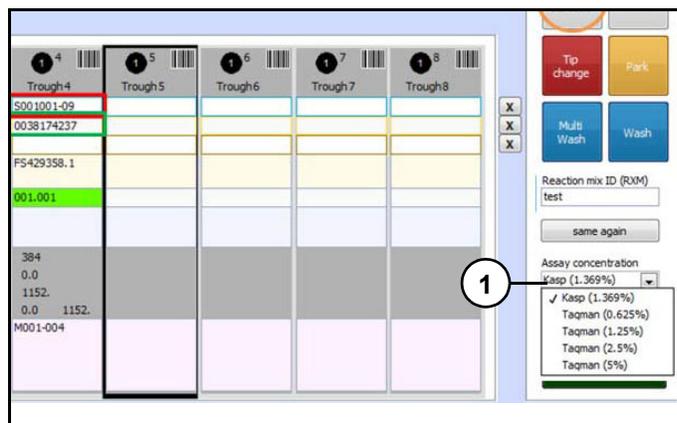


Figure 40

- Select "Assay concentration" (1) (Figure 40) to be used in reagent mix.

Note: Value of 1.369% for KASP™ corresponds to final concentration of KASP Assay in 1X KASP Master Mix.

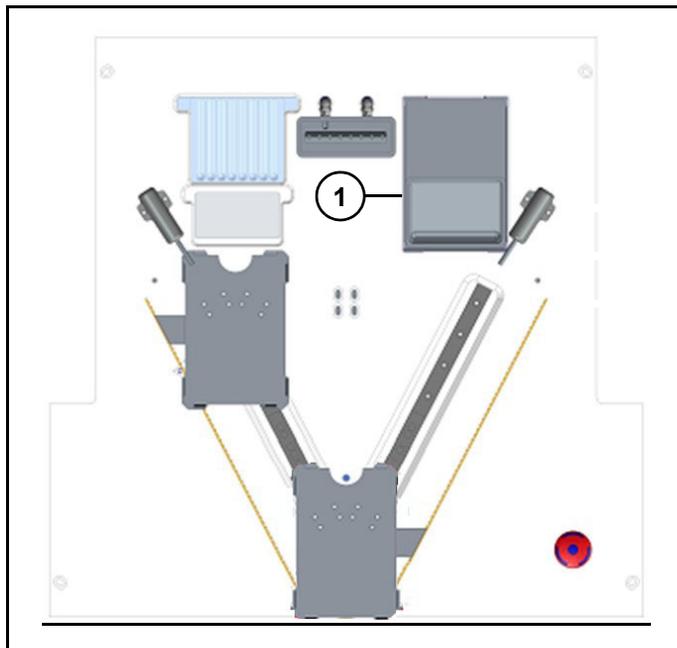


Figure 41

Refer to (Figure 41).

Transfer KASP Master Mix at required concentration in KASP trough (1).

Note: A cooling plate will keep KASP Master Mix at around 6 °C.

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- Keep assay aliquot tubes on tube holder in same positions they were scanned.
- Uncap tubes with red SeptraSeal removal tool.

To estimate how much volume of KASP Master Mix and assays will be needed for run, refer to following calculation formula:

$$A_v = [(V_w \times N_w) \times (1 + A_f) + A_o] \times (1 + M_{rf}) + M_{ro} + P_v$$

Note: These values can be found in instrument software, in "Aspiration Dispense Parameters" window. (Figure 27)

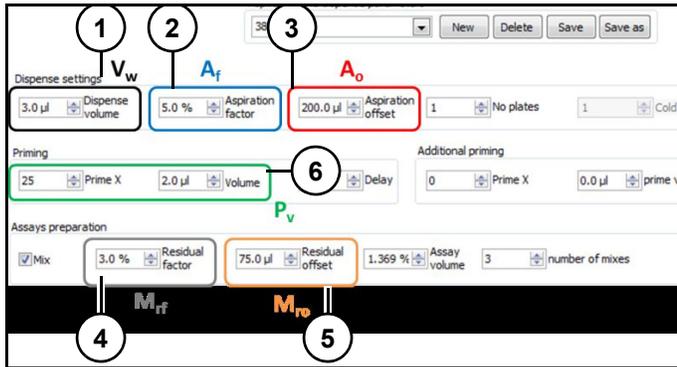


Figure 42

Refer to (Figure 42):

- A_v : Aspiration volume (reagent volume aspirated per trough).
- V_w (1): Volume of reagent to be dispensed per well (μL).
- N_w : Number of wells to be dispensed.
- A_f (2): Aspiration factor (%).
- A_o (3): Aspiration offset (μL).
- M_{rf} (4): Mixing residual factor (%).
- M_{ro} (5): Mixing residual offset (μL)
- P_v (6): Priming volume (number of priming dispenses x volume per dispense in μL).

Mixing "Residual factor" (4) and Mixing "Residual offset" (5) are related to overage that is requested for correct reagent mixing, and corresponding volume left in mixing trough after aspiration.

From Aspiration volume, it is possible to calculate volume of KASP Master Mix and of assay, depending on final assay concentration in reagent mix.

Example with KASP reagents dispensed on dry DNA:

- Plate format: 384
- Concentration of reagents dispensed in well: 1X
- Concentration of KASP Assay in reagent mix: 1.369 %
- $V_w = 3 \mu\text{L}$
- $N_w = 384$
- $A_f = 5 \%$
- $A_o = 200 \mu\text{L}$
- $M_{rf} = 3 \%$
- $M_{ro} = 75 \mu\text{L}$
- $P_v = 25 \times 2 \mu\text{L} = 50 \mu\text{L}$

Aspiration volume = $[(384 \times 3) \times (1 + 0.05) + 200] \times (1 + 0.03) + 75 + 50 = 1576.9 \mu\text{L}$.

Assay volume = $1,576.9 \times 0.01369 = 21.6 \mu\text{L}$.

1X master mix volume = $1,576.9 - 21.6 = 1,555.3 \mu\text{L}$.

It is recommended that volumes of master mix and assay placed on instrument be at least 20% higher than calculated volume.

Note: Ensure model of mixing trough placed on instrument deck is correct. If dispensing template has ≤ 8 sectors then use 8-well mixing trough only. If template has >8 sectors then use the 16-well mixing trough only.

- Start run. See "Starting run" on page 11.

Plate and assay barcode scanning and verification

See "Scanning plates and assay aliquots" on page 9.

Reagent aspiration and dispensing

Once plate and aliquot barcodes have been verified dispense head will move to wash station and execute a wash cycle (if tips have not been washed).

Dispense head will move to KASP trough and aspirate required volume of master mix. Only tips requested for run will be activated. Also, if number of wells to be dispensed by each tip is different, each tip will have capacity to aspirate different volumes of reagents.

Dispense head will move to wash station and execute a touch-off to clean external part of tips.

A prompt will appear asking user to remove cap of assay tubes.

8. Remove caps if needed.

9. Click "OK".

Dispense head will move to aliquot tubes and aspirate required volume.

10. Recap tubes.

11. Click "OK".

After aspirating assays, dispense head will move to mixing trough and mix master mix and assays.

| | Tip 1 | Tip 2 | Tip 3 | Tip 4 | Tip 5 | Tip 6 | Tip 7 | Tip 8 |
|----------------------------|--------|-------|-------|-------|-------|-------|-------|-------|
| Valve time per tip (ms) | 23.2 | 21.8 | 21.3 | 22.4 | 21.6 | 22.4 | 22.8 | 22.0 |
| Actual volume per tip (µl) | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Total volume per tip (µl) | 1576.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Figure 43

Number of mixing cycles (1) (Figure 43) can be adjusted in instrument software by editing Aspirate/Dispense parameters. Default number of mixes is set to 3. If modified, click "OK" to save changes.

After mixing reagents, dispense head will aspirate required volume, leaving a residual volume in mixing

trough, and will move above plate, dispense and move to park position.

Dispense head will scan plate barcode and aliquot for verification. See "Plate and assay barcode scanning and verification" on page 12.

12. Switch to next plate. See "Switching to next plate" on page 13.

End of dispensing run

When last plate has been dispensed, barcodes checked and dispensed plate removed, dispense head will execute a wash cycle and move to park position.

Figure 44

Note: It is possible to recover small volume of reagent remaining in dispense head at end of run. Check "Recover unused assay" (1) in "Options" (2) in Kraken interface before starting run. (Figure 44)

13. Remove mixing trough.

Rinse it with R.O. or DI water and dry it with a lint-free cloth.

If another dispensing run will follow, replace assay tubes as needed and refill KASP trough with KASP Master Mix if needed.

If no more runs are planned:

Remove assay tubes, recover any KASP Master Mix remaining in KASP trough, remove KASP trough, rinse it with R.O. or DI water and dry it with a lint-free cloth.

Multi-run assays from tubes

Multi-run assays from tubes tips will:

Aspirate requested volumes of master mix and assays from separate locations on deck and mix them automatically in mixing trough.

Be able to perform several dispensing runs with same tips and assays.

If several plates are to be dispensed with exact same template and assays, multi-run mode will allow instrument to use full capacity of tip chambers to aspirate enough reagents to dispense several plates. If tip chamber capacity is not enough to aspirate total volume requested to dispense all plates, a first aspiration will be performed to dispense a first subset of plates, and second or more aspirations will be added later to dispense remaining plates. For each aspiration, tips will dispense several plates without any wash cycle between plates allowing both time and reagent savings.

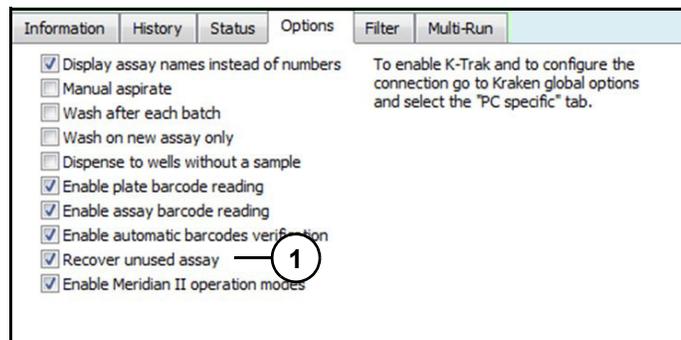


Figure 45

Kraken interface will automatically select options on "Options" tab (Figure 45), to allow instrument to automatically scan plates and assay tubes barcodes and to perform automatic barcodes verification during run.

In addition, option "Recover unused assay" (1) (Figure 45) will allow dispense head to dispense leftover reagent back into mixing trough before proceeding to next aspiration.

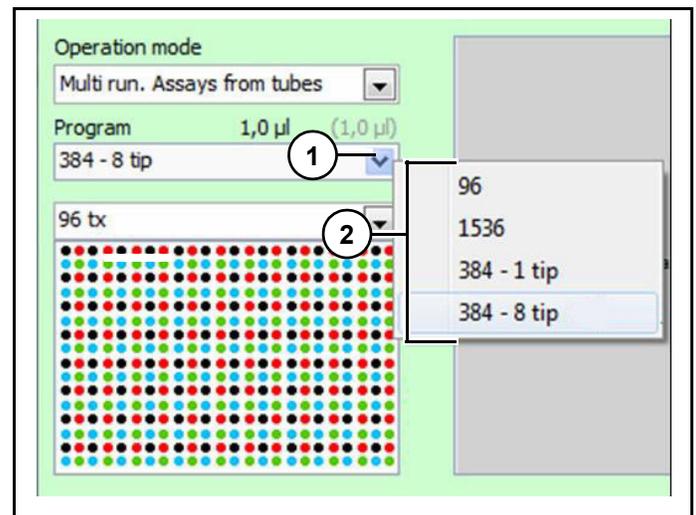


Figure 46

Refer to (Figure 46).

1. Click "Program" drop down menu (1) and select program for plate to be dispensed and number of dispensing tips available (2).

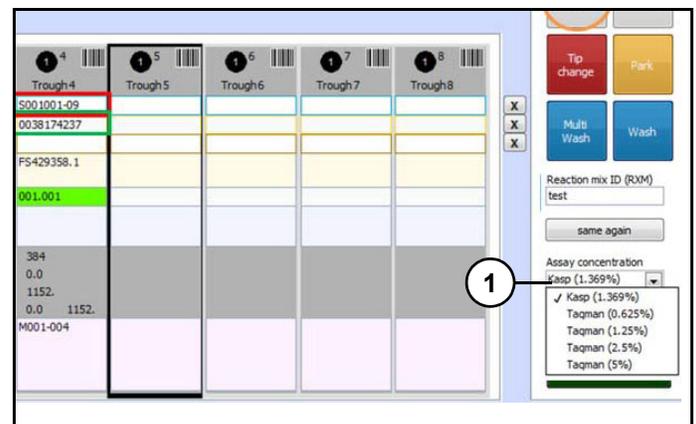


Figure 47

2. Select "Assay concentration" (1) (Figure 47) to be used in reagent mix.

Note: Value of 1.369 % for KASP corresponds to final concentration of KASP Assay in 1X KASP Master Mix.

Scanning plates and assay aliquots

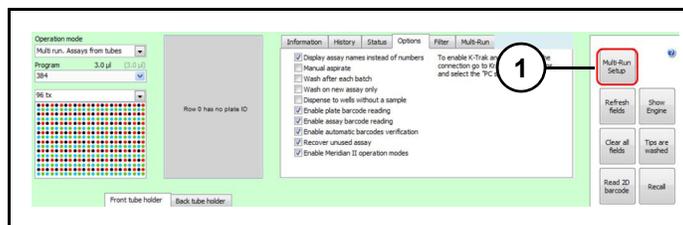


Figure 48

3. Click "Multi-Run Setup" (1) (Figure 48).

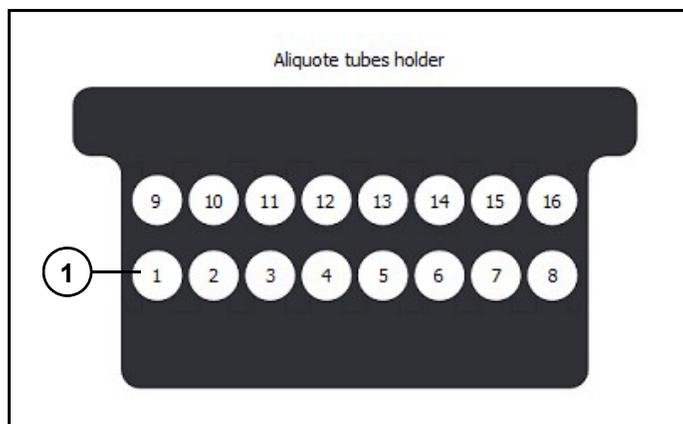


Figure 49

4. Place assay aliquot tubes to be used on assay tube holder located on instrument deck. First assay tube (1) (Figure 49) (used for tip 1) needs to be placed on left end of front rack, and following tubes should be placed in order from left to right on front rack.

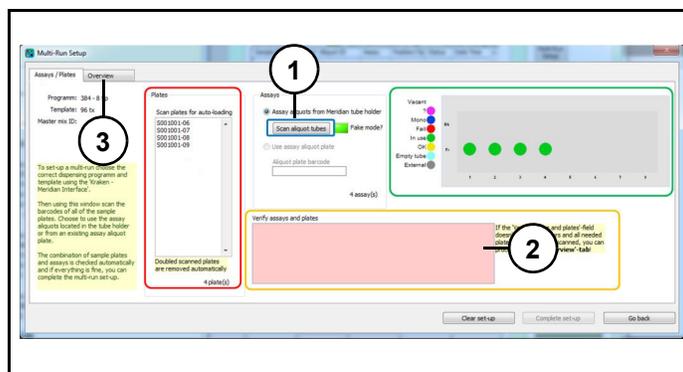


Figure 50

Refer to (Figure 50).

Scan all plates to be dispensed with pattern previously selected, using a handheld 1D barcode scanner.

5. Press "Scan aliquot tubes" (1).

Picture of tube holder will be automatically updated with position of scanned tubes and with status of the corresponding assays in Kraken ("Unknown", "Monomorphic", "Failed", "In-use", etc).

6. Check "Verify assays and plates" (2) field does not show any warning about plates and aliquots scanned. If there is a warning, correct as requested.
7. Once plates and assays have been selected, click "Overview" (3).

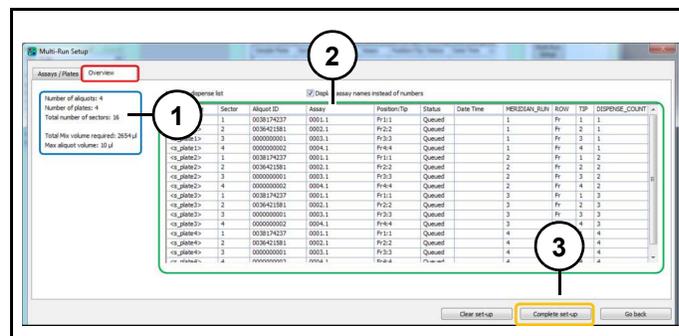


Figure 51

Refer to (Figure 51).

8. Check that number of aliquots, number of plates and total number of sectors (= Number of assays per plate x Number of plates) (1) are correct. Take note of total volume of reagents and of maximum aliquot volume required for multi-run.

Table (2) will summarise how multiple plates will be dispensed by different tips with different assays. Note: Table does not refer to any plate ID, but to place holders which are listed in sample plate column. Place holders refer to order in which random plates will be run, to allow more flexibility.

9. If setup is correct, click "Complete set-up" (3).

Preparation of reagents

Multi plate operation will:

- Automatically prepare reagents requested for run.
- Transfer KASP Master Mix at required concentration in KASP trough. Trough will keep Master Mix at around 6 °C.
 - Keep assay aliquot tubes on tube holder in same positions they were scanned.
 - Uncap tubes with red SepraSeal removal tool.

It is recommended that volumes of KASP Master Mix and assays placed on instrument be at least 20 % higher than forecast volumes displayed in Overview tab of the Multi-run setup window.

Note: Forecast volumes displayed on "Overview" tab are calculated using following calculation formula:

$$A_v = [(V_w \times N_w \times N_p) \times (1 + A_f) + A_o] \times (1 + M_{rf}) + M_{ro} + P_v$$

- A_v : Aspiration volume (reagent volume aspirated per trough).
- V_w : Volume of reagent to be dispensed per well (μL).
- N_w : Number of wells to be dispensed.
- N_p : Number of plates to be dispensed.
- A_f (2): Aspiration factor (%).
- A_o (3): Aspiration offset (μL)
- M_{rf} (4): Mixing residual factor (%)
- M_{ro} (5): Mixing residual offset (μL)
- P_v (6): Priming volume (number of priming dispenses x volume per dispense in μL).

These values can be found in instrument software, in Aspiration Dispense Parameters.

If more than one aspiration is required for dispensing all plates, volumes of reagents required for second and following aspirations will be calculated using following formula:

$$A_v = [(V_w \times N_w \times N_p) \times (1 + A_f)] \times (1 + M_{rf}) + P_v$$

Note: Offsets are not included to avoid cumulation to much volume in mixing trough.

Starting run

Note: Ensure model of mixing trough placed on instrument deck is correct. If dispensing template has ≤ 8 sectors then use 8-well mixing trough only. If template has >8 sectors then use the 16-well mixing trough only.

| Sector / Tip | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---------------|------------|------------|------------|------------|---|---|---|---|
| Sample Plate | <s_plate1> | <s_plate1> | <s_plate1> | <s_plate1> | | | | |
| Aliquot | 0038174237 | 0036421581 | 0000000001 | 0000000002 | | | | |
| PCR Mix | | | | | | | | |
| Assays | F5429358.1 | F57412.1 | AM_0001.1 | AM_0002.1 | | | | |
| Project | 001.001 | 001.001 | 001.001 | 001.001 | | | | |
| Plate notes | | | | | | | | |
| Wells | | | | | | | | |
| Conc | 0,0 | 0,0 | 0,0 | 0,0 | | | | |
| Aspirate | | | | | | | | |
| Primer 504 | | | | | | | | |
| Master plates | | | | | | | | |

Figure 52

Kraken will automatically update interface with inputted data (1) (Figure 52).

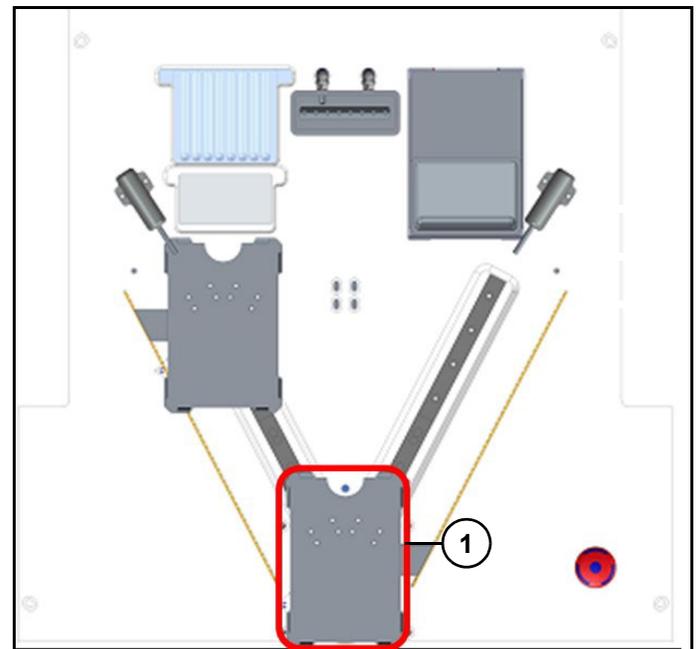


Figure 53

Refer to (Figure 53).

- Place first plate to be dispensed on plate support (1).

Note: It can be any of plates that were scanned in "Multi-Run Setup". A sensor will detect new plate when placed.

Plate and assay barcode scanning and verification

Plate shuttle will move and bring plate to right of left position (depending on shuttle configuration before starting run).

Dispense head will home and then move above plate, scan its barcode and verify plate is correct.

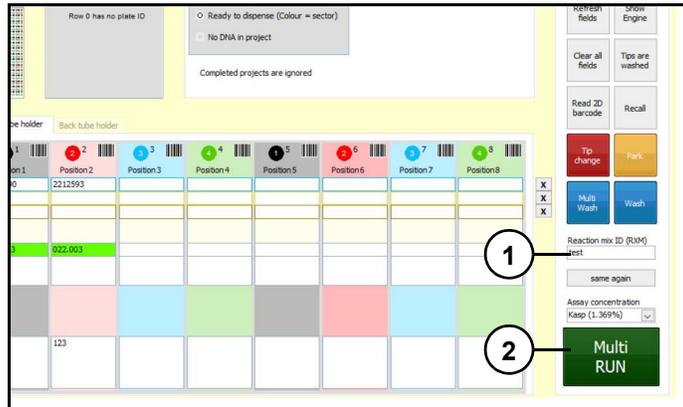


Figure 54

Refer to (Figure 54).

14. Scan "Reaction Mix ID" (1).

15. Click "Multi-Run" (2).

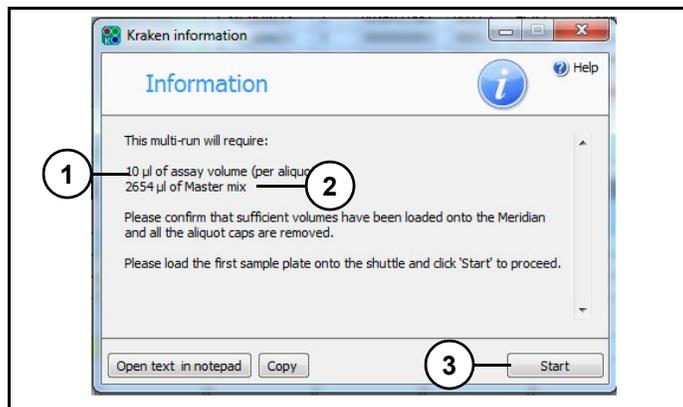


Figure 55

Refer to (Figure 55).

A prompt will appear, indicating total volume of assay per aliquot (1) and total volume of master mix (2) required for run. Ensure that enough volume of assay and master mix have been loaded. Ensure that aliquot tubes have been uncapped.

16. Click "Start" (3).

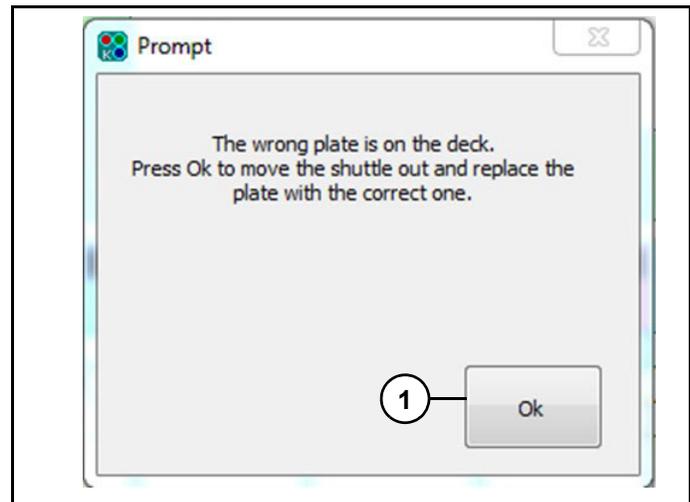


Figure 56

Refer to (Figure 56).

If plate scanned was not included in "Multi-Run Setup" or scanning has failed, dispense head will park and prompt (Figure 56) will appear.

17. Click "Ok" (1) automatically move wrong plate to front position.

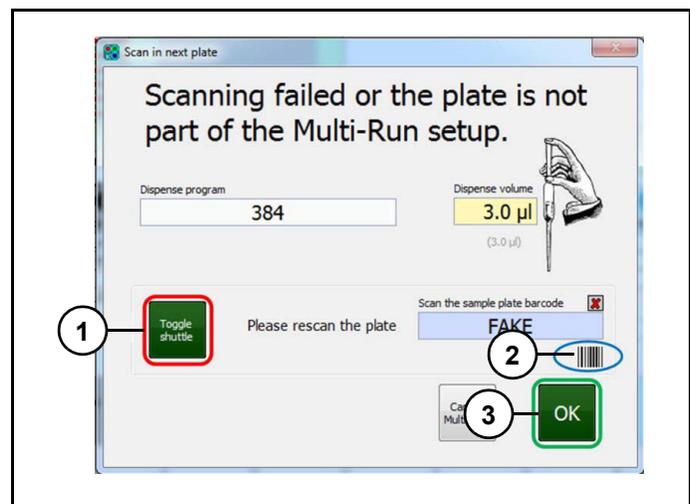


Figure 57

Refer to (Figure 57).

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18. Replace wrong plate with correct one.
19. Click "Toggle shuttle" (1) to bring correct plate to side position.
20. Click "Barcode icon" (2) to move dispense head above plate to rescan.
21. Click "OK" (3) to continue dispensing run.

Reagent aspiration and dispensing

See "Reagent aspiration and dispensing" on page 13.

Switching to next plate

See "Switching to next plate" on page 13.

Case 1:

More than one aspiration is required for multi-run.

In case more than one aspiration is required for multi-run, instrument head will dispense first set of plates, and wash tips before aspirating KASP Master Mix and assays again. These reagents will be mixed in mixing trough before Meridian² continues dispensing remaining plates.

Case 2:

If dispensing template includes more than eight assays.

1. Instrument head will aspirate reagents volumes required for dispensing assays 1 to 8 (front position of assay tube holder) to first plate and mix them in mixing trough. This first aspiration will include all overage factors and offsets.
2. Head will dispense plate 1 with assays 1-8 and then go through a wash cycle.
3. Head will aspirate reagents volumes required for dispensing assays 9-16 (from back positions of tube holder) to first plate.
4. Head will dispense assays 9-16 to plate 1 and then go through a wash process.

Steps 1-4 will repeat for all plates to be dispensed in multi-run, except that overage volume will be lower to avoid residual volume to cumulate in mixing trough.

End of dispensing run

See "End of dispensing run" on page 14.

Sealing and centrifuging plates before PCR

Once plates have been dispensed with reagents, they should be sealed either with a heat sealer such as Kube (if plates have castellated wells) or with a laser sealer such as Fusion³ (if using Biosearch Technologies flat-top black plates).

If plates are to be used for PCR in a waterbath thermocycler, seal type selected should allow permanent sealing to avoid any leakage.

For PCR applications such as KASP, which require end-point fluorescence reading, seal must be optically clear.

After sealing, plates should be centrifuged at a maximum speed of 1,400 rpm for 1 minute.

Dispensed, sealed and centrifuged plates can be stored at 4 °C for up to 1 hour before running PCR.