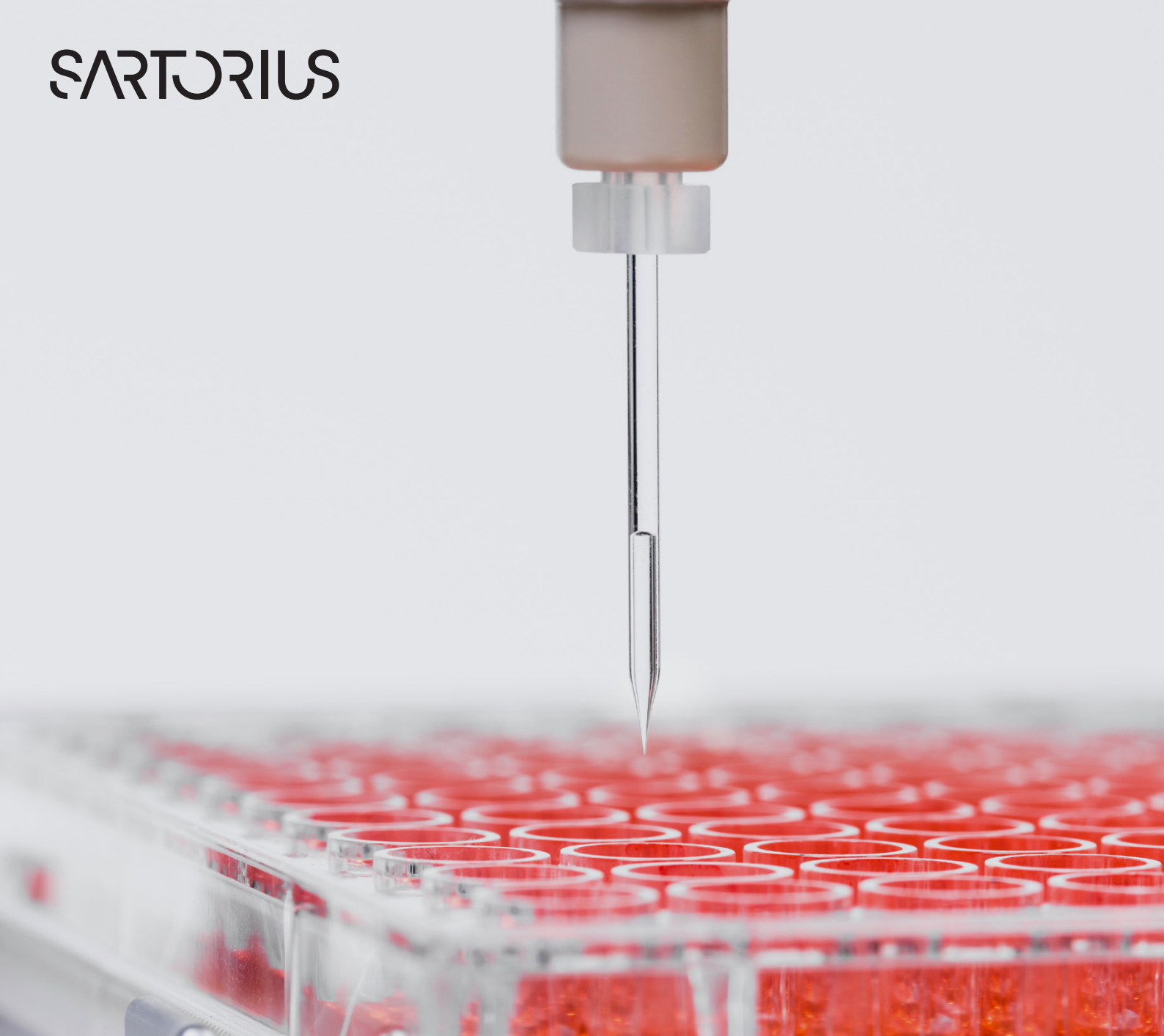


SARTORIUS



Cell Selection and Retrieval

CellCelector Picking and Transfer

Accurate and Gentle Cell and Colony Picking

- Single cell, adherent colony and semi-solid media picking modules for different cell types and research applications
- Unique features for enhanced cell handling, including autofocussing and automated tool sensors
- Particle movement correction for low adherence suspension cells
- Before and after picking confirmation

CellCelector Picking Modules

Picking Modules

The CellCelector has three different modules which can be selected depending on the properties of the object to be picked.

Each module consists of a robotic arm, a spring-loaded tool head and a syringe pump, which creates a negative pressure to facilitate particle aspiration, and can be rapidly exchanged in seconds. The software automatically detects the new module and provides the relevant corresponding settings.

Modules include:

- **Single Cell Picking Module** – used for the transfer of suspension cells, adherent single cells, cell clusters, and defined colony areas
- **Adherent Colony Picking Module** – used for the careful transfer of adherent colonies or 3D structures
- **Semi-Solid Media Module** – used for the picking of cell colonies from either semi-solid or solid media, such as methylcellulose, soft agar and agar

Single Cell Picking Module

The single cell picking module uses glass capillaries with diameters ranging from 20 – 220 μm , although custom made capillaries are also available with different diameters. Cells are aspirated directly into the glass capillaries, with aspiration volumes ranging from <10 nL to 12 μL for all capillary diameters.

Accurate picking from all surfaces is facilitated using a range of modifiable options, such as:

- **Fixed Picking Heights** – predominantly used for adherent cells on the plate bottom
- **Automatic Plate Height Sensors** – enhanced well bottom detection for uneven plate surfaces (Fig. 1)
- **Sample Autofocusing** – automatic object position focusing and picking height calculation based on focal value

Other unique aspiration features include:

- **Capillary Speed** – decreasing speed when entering the source plate can help prevent movement of low adherence single cells
- **Cell and Colony Pooling** – sequential aspiration of multiple objects, cells or colonies into the same well (Fig. 3).

In certain circumstances with adherent single cells, glass capillaries can also perform an indirect scraping action, where the height of the picking tool is set so high that the cell or plate bottom cannot be touched. The capillary then moves in a similar manner to that of the adherent colony picking tool (Fig. 2), continuously applying a negative pressure to aspirate cells.

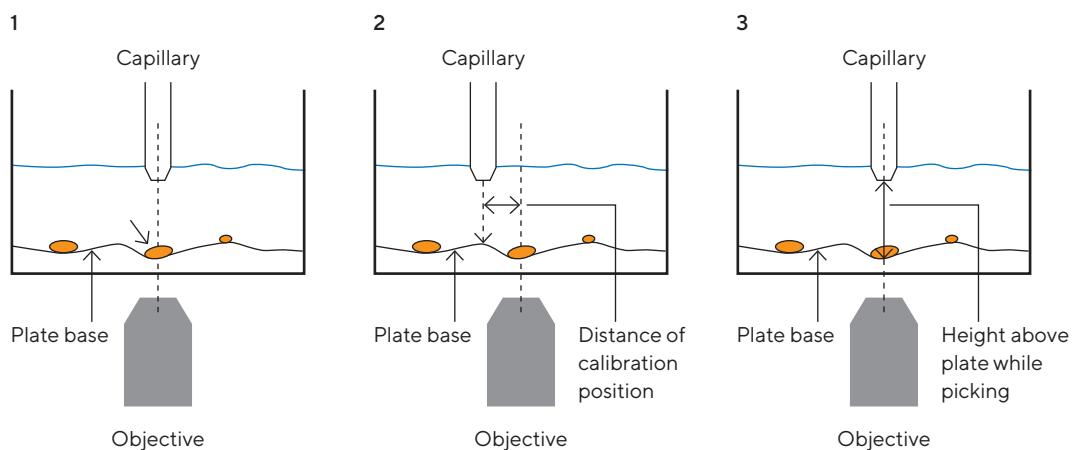


Figure 1: Tool sensor schematic and workflow. (1) robotic arm moves to the calibrated Z height of the picking position, (2) the stage deviates in the Y direction to ensure that the height measurement does not occur directly at the position of the particle, and (3) the robotic arm moves back very slowly in the direction of the plate base and the target cell, until the height sensor detects the plate bottom (dotted line), where it remains at a certain height above the measured base plate position during aspiration.

Adherent Colony Module

Designed for the isolation and transfer of adherent cell colonies using stainless steel capillaries. Objects are loosened by light scraping movements around the desired particle to ensure that object morphology is unaffected, with simultaneous capillary aspiration facilitating the most gentle cellular detachment possible. To ensure that the cellular morphology of the target object remains unaffected by the scraping process, the scraping distance around the colony can be modified depending on the size of the specific object.

Using a 0.5 mm setting, for example, this involves:

- Horizontal scraping by 0.5 mm to the left of the object, 1 mm to the right, and then 0.5 mm back to the center
- Vertically scraping in a similar manner
- Simultaneous or subsequent aspiration to gently decrease object adherence (Fig. 2)

In areas of increased confluency, adherent cells, colonies or clusters may have numerous points of contact with other cellular objects. To accurately transfer only the desired object, the scraping speed can be decreased, with lower speeds used to loosen bonds between adjacent cells, thus ensuring maximum viability and the most gentle transfer possible.

The maximum aspiration volumes for each capillary are:

- 300 μm stainless steel capillary: 22 μL
- 500 μm stainless steel capillary: 28 μL
- 800 μm stainless steel capillary: 30 μL
- 1000 μm stainless steel capillary: 32 μL

Semi-Solid Media Module

This module is equipped with special disposable tips and is used for the picking of cell colonies (such as bacteria, hybridomas and CHO cells) from semi-solid (e.g. methylcellulose and soft agar) and solid (e.g. agar) media.

The maximum aspiration volumes for each plastic tip are:

- 500 μm plastic tip: 20 μL
- 1200 μm plastic tip: 15 μL

The semi-solid media module has a number of inbuilt user-defined options and functionality to enhance object transfer and viability, which include:

- **Automatic Plate Sensors** – enhanced well bottom detection for uneven plate surfaces (Fig. 1)
- **Variable Diameter Pick-Up** – manual definition of object areas for transfer
- **Vertical Movement Aspiration** – capillaries are moved vertically upwards during aspiration to loosen stickier cells or colonies that are not positioned directly on the plate bottom
- **Speed of Vertical Movement** – the extent to which increasing under-pressure can loosen non-adherent, stickier cells from the plate bottom

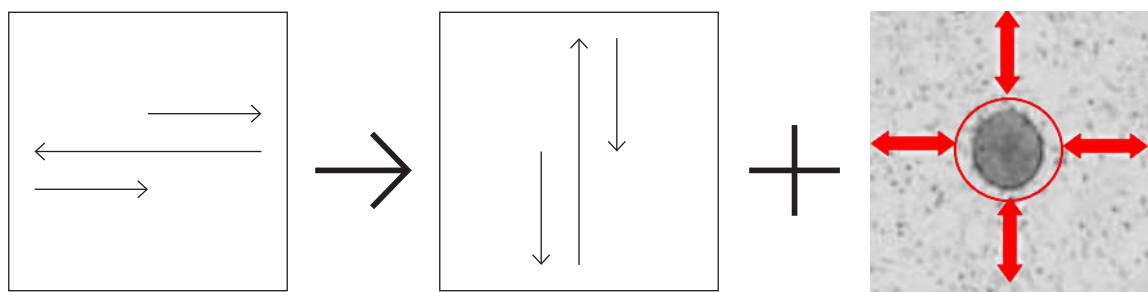


Figure 2: Adherent colony module scraping procedure, outlining (A) horizontal scraping, (B) vertical scraping and (C) cell aspiration to gently remove a 3D structure.

User Defined Aspiration Properties

Capillary Aspiration For Every Research Application

Capillary aspiration consists of 6 user-defined variables (Fig. 3), all of which can be individually adjusted depending on research application or specific assay requirements.

These include:

- **Air Gap Volume** – the size of the airgap (in μL) aspirated before the buffer or object. The precise volume can be individually defined and serves as a separating layer between the system and the buffer
- **Air Gap Dispensed in Target Well** – air layer dispensed into the well during picking and ensures capillaries are completely emptied following cellular deposition
- **Buffer Volume** – volume of buffer | media (in μL) aspirated before the target object. Buffer can also be aspirated from a target well instead of the buffer tank to ensure that (i) no additional buffer is used, (ii) the object is directly aspirated in the target buffer, or (iii) cells are to be deposited into an entirely different media composition
- **Cell 1 Aspiration Volume** – volume occupied by the target cell following aspiration
- **Transport Air Gap (Optional)** – only defined if more than one particle should be picked per capillary, and ensures particles remain in their own droplet and do not mix when several cells are picked
- **Cell 2 Aspiration Volume** – only required if more than one particle per capillary needs to be picked

Furthermore, additional variables such as the speed of aspiration and waiting time after aspiration can be modified:

- **Aspiration Speed** – to achieve the most gentle cell transfer possible, aspiration speeds can either be minimized for extremely fragile cells, or increased for high-throughput workflows using robust cell types
- **Waiting Time** – for high viscosity media or organoid aspiration into 100% hydrogel, the waiting time after buffer aspiration and before picking can be modified depending on capillary diameter and buffer volume. Waiting times can also be modified across a variety of other processes, such as extending or shortening the capillary sterilization process, or during capillary facilitated in-situ cell lysis to prevent lysis buffer covering adjacent samples

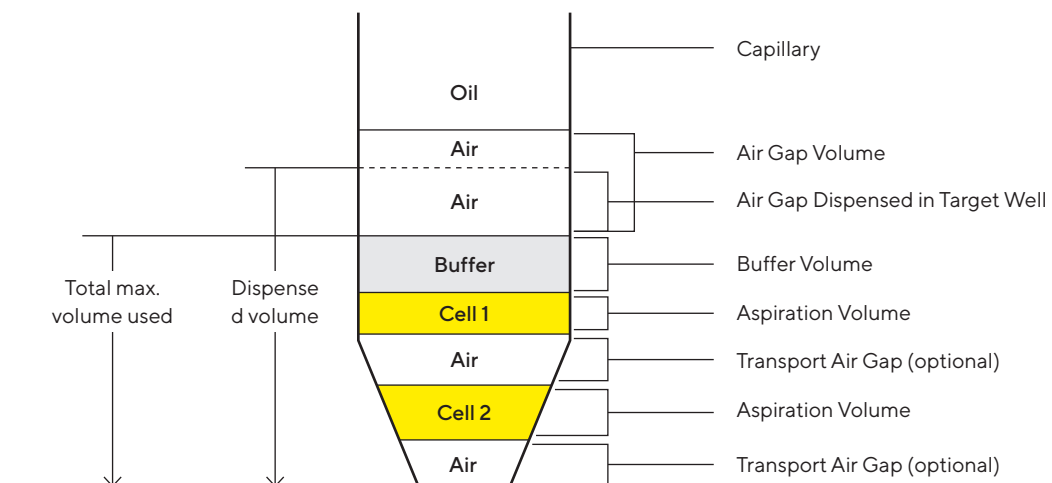


Figure 3: Schematic of the layers within a capillary which ensure hassle and contamination-free picking

Successful Picking of Free-Floating Objects

Capillary Reuse

Stainless steel adherent colony and single cell glass capillaries can either be sterilized or reused between individual wells or plates depending on cell type and research application, with the specific number of transfers per capillary individually entered, giving users full control over sterilization processes.

Accordingly, users have the option of specifying:

- **No sterilization between picks** – capillaries are not sterilized between individual transfers
- **Capillary sterilization before each picking run** – capillaries are sterilized within an experiment before each picking run
- **Capillary sterilization when the source well has changed**

In addition, both stainless steel adherent colony capillaries and semi-solid plastic tips can be discarded following each transfer, depending on user preference.

Detailed information on sterilization processes can be found in the technical flyer “CellCelector Sample Deposition”.

Correction of Particle Movement Before Picking

Cells in suspension rarely remain static over extended periods of time, which means they may have already moved away from their original position between the time taken following initial scanning and prior to picking.

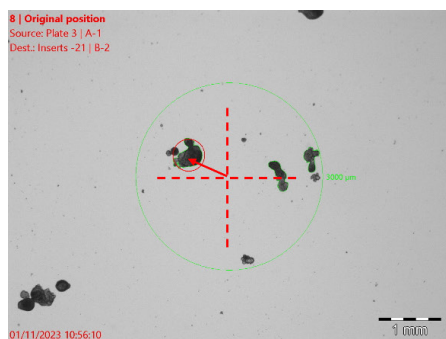
A powerful feature of the CellCelector platform allows users to automatically check and adjust the position of objects prior to transfer to increase picking accuracy (Fig. 4).

If the option for particle movement correction is activated:

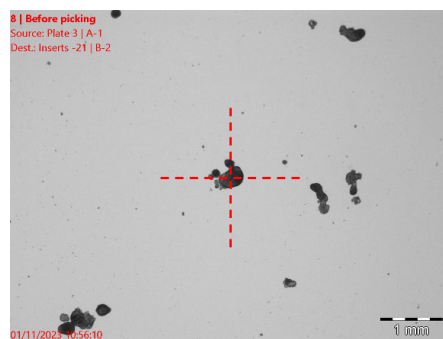
- different detection areas around the initial particle position can be pre-defined by the user
- the system takes a snapshot
- the image is analyzed
- the particle position is automatically corrected

In the case that the particle has moved into a position filled with other cells, the software will either abort picking, continue with the next particle, or identify the individual morphological features of all cells and then identify the cell with the identical characteristics as the initial target cell.

Original Position



Before Picking



After Picking

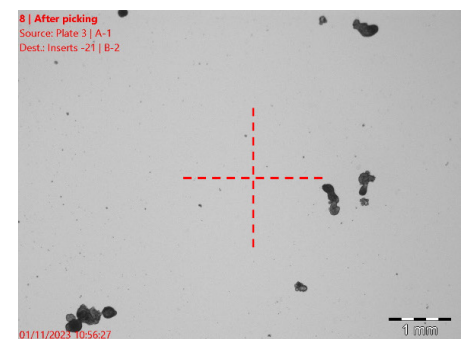


Figure 4: Correction of particle movement before picking.

In this example, organoids in suspension can easily move between the time taken from initial scanning (original position) and before picking. Activation of the correction of particle movement feature ensures that the freely moving organoid is automatically found within a pre-defined detection area (green circle, 2500 µm). After picking image confirms the successful transfer of the relevant organoid.

Particle, Picking and Results Lists

The Particle List

The Particle List outlines all the particles that were detected following scanning and provides both high level statistics of the full list, as well as detailed information on each particle.

Overview statistics include information on the total number of detected objects within the detected particle list, as well as the number of objects remaining in the particle list after implementation of the filter functions.

Individual particle statistics include parameters such as the particle identification number, X | Y coordinates, diameter, and area. In addition, information on the scan date, time and user identification during plate scanning and analysis is provided for maximum traceability.

By clicking on a specific particle in the Particle List, the object is displayed on screen either as a live or scanned image. The particle list can then be modified and filtered to remove particles which do not meet the requirements for subsequent picking.

This allows a fully automated selection of objects based on different morphological and fluorescent characteristics for subsequent picking. Identifying the top 100 antibody producing clones out of many thousands can therefore be concluded within seconds.

The Pick List

After editing the Particle List, all objects to be picked are transferred to the Pick List, which combines the detected and selected particles of all plates, wells and scans into a single list. The particles compiled within the picklist can be filtered according to a range of criteria (Fig. 5A) and objects automatically picked in sequence without any further user interaction.

Particles can also be manually selected and added to the picklist in order to be picked automatically afterwards.

In some cases, manually selecting the relevant objects from a live image maybe quicker, especially when:

- only a few cells or colonies are to be selected
- automatic detection is difficult

The Image Gallery

During the picking process, images of the objects are shown in real time and highlighted above the pick list, with up to 25 objects displayed. Further information can be found in the technical flyer "CellSelector Documentation and Data Export".

The Results List

As soon as particles are picked, the documentation window becomes available, listing all particles that can be, or already have been, picked (Fig. 5B).

A

Source Plate	Source Well	ID	Pick Status	Scan Status	Pick / Scan Activation	
1	2016-12-08_Experiment 2	B-3	1	Unpicked	Unscanned	No
2	2016-12-08_Experiment 2	B-3	2	Unpicked	Unscanned	Yes
3	2016-12-08_Experiment 2	B-3	3	Unpicked	Unscanned	Yes
4	2016-12-08_Experiment 2	B-3	4	Unpicked	Unscanned	Yes
5	2016-12-08_Experiment 2	B-3	5	Unpicked	Unscanned	Yes
6	2016-12-08_Experiment 2	B-3	6	Unpicked	Unscanned	Yes
7	2016-12-08_Experiment 2	B-3	7	Unpicked	Unscanned	Yes
8	2016-12-08_Experiment 2	B-3	8	Unpicked	Unscanned	Yes
9	2016-12-08_Experiment 2	B-3	9	Unpicked	Unscanned	Yes
10	2016-12-08_Experiment 2	B-3	10	Unpicked	Unscanned	Yes
11	2016-12-08_Experiment 2	B-3	11	Unpicked	Unscanned	Yes
12	2016-12-08_Experiment 2	B-3	12	Unpicked	Unscanned	Yes
13	2016-12-08_Experiment 2	B-3	13	Unpicked	Unscanned	Yes
14	2016-12-08_Experiment 2	B-3	14	Unpicked	Unscanned	Yes
15	2016-12-08_Experiment 2	B-3	15	Unpicked	Unscanned	Yes

B

Source Plate	Source Well	ID	Pick Status	Scan Status	Pick / Scan Activation	Destination Plate	Destination Well	Comment
Plate 3	A-1	2	Picked	Unscanned	No	Inserts	D-1	single, 3ul
Plate 3	A-1	3	Picked	Unscanned	No	Inserts	D-2	single, 3ul
Plate 3	A-1	5	Picked	Unscanned	No	Inserts -21	A-1	array, 1.5ul
Plate 3	A-1	6	Picked	Unscanned	No	Inserts -21	A-2	array, 1.5ul
Plate 3	A-1	7	Picked	Unscanned	No	Inserts -21	B-1	array, 1.5ul
Plate 3	A-1	8	Picked	Unscanned	No	Inserts -21	B-2	array, 1.5ul
Plate 3	A-1	9	Picked	Unscanned	No	Inserts -22	A-1	array, 1.5 ul, reused cap
Plate 3	A-1	10	Picked	Unscanned	No	Inserts -22	A-2	array, 1.5 ul, reused cap
Plate 3	A-1	11	Picked	Unscanned	No	Inserts -22	B-1	array, 1.5 ul, reused cap
Plate 3	A-1	12	Picked	Unscanned	No	Inserts -22	B-2	array, 1.5 ul, reused cap
Plate 3	A-1	13	Picked	Unscanned	No	Inserts -23	A-1	array, 1.5ul
Plate 3	A-1	14	Picked	Unscanned	No	Inserts -23	A-2	array, 1.5ul
Plate 3	A-1	15	Picked	Unscanned	No	Inserts -23	B-1	array, 1.5ul
Plate 3	A-1	16	Picked	Unscanned	No	Inserts -23	B-2	array, 1.5ul
Plate 3	A-1	17	Picked	Unscanned	No	Inserts	D-6	single, 1.5ul


Figure 5: (A) The Pick List, and (B) The Results List. Numerous variables can be outlined, such as source plate, well, pick status (including unpicked, picked, failed and skipped objects), scan status, pick date, pick | scan activation, manual addition, destination plate and well information

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