Ettan Spot Picker

Every time you use the spot picker, you MUST sign the log book.
Rinse tray after use.
Put camera cap back on.
Purge with H_2O

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Setup

1. Take cap off of camera
2. Liquid Supply. You need either ddH2O or a wash/destain buffer connected to the picker head, via the syringe pump. The liquid is used for aspirating gel plugs and dispensing them into the wells of the microtiter plates. The picker head is also rinsed in liquid between each pick.
3. Rinse buffer bottle thoroughly with ddH20, fill with ddH2O, and re-insert line. Place container below waste outlet
4. Waste. The waste tube from the rinse station should be connected to a waste bottle placed beneath the instrument.
5. Gel Tray. Rinse gel tray thoroughly with ddH20 between use; use Kimwipe to scrub. See below for gel tray setup.
6. Racks and microtiter plates. The gel plugs are collected in microtiter plates, which are placed in 2 racks to the right of the locator plate. Install the racks aligning the holes on the underside with the pins on the locator plate. The rack label should face the front of the Spot Picker. Place up to 4 microtiter plates in the racks (1 and 2 are in the right hand rack, with 1 to the front; 3 and 4 are in the left hand rack. With 3 to the front). Note that well A1 should face the front of the instrument and on the left. The plate has a little play in the holder.
7. Selecting a picker head. Decide what size picker head you need. See below for installing a different head and camera alignment.
8. Start Spot Picker. Usually on (no power drain on instrument and camera is off until you open software and the instrument Homes). Switch on the power at the rear of the instrument.
9. Turn on computer. Open Ettan Spot Picker Instrument Control software. Icon on desktop or Start/Programs/Ettan Spot Picker/Ettan Spot Picker.
10. The picker head will be moved to the home position when the software starts. Select OK to start homing. This takes about 20 sec.
11. Prime the system. When the picklist is loaded, the software will tell you how much volume of the buffer is required to pick all the spots. Priming removes air bubbles in the spot picker tubing and also rinses the tubing with fresh solution.
    a. Tools/Prime Syringe

b. In the Prime Syringe window, enter the # of priming strokes - 5. Press Prime.
c. When the priming is completed, press Exit. (you can select the Stop button if you want to interrupt the priming).

12. See below for System setup. You have to set the Z coordinate for every gel. X and Y should be fine unless you change the picker head. Camera calibration, moving and setting the XYZ of the picker head, Calibrating the scanner and maintenance are also below.

Setting System Parameters

1. System/System Setup. At any stage, the user can decide to abandon the System Setup by pressing the Cancel button. If the Cancel button is pressed, any changes made to the System Setup will be lost. To retain new System Setup parameters, press the Save and Exit button. This screen allows the user to set the position of the picker head according to the microplates and rinse station, the height of the picker head versus the gel backing material, the parameters used for picking the gel plugs and the reference markers area ranges, which are used by the detection software.
2. Press the Go to Home button.
3. Moving the picker head around. The blue arrows visible on the right hand side of the screen are used to move the picker head to various positions. By pushing the buttons on the arrows, the head will move stepwise in the corresponding directions. As long as a button is pushed, the head will keep moving.
    a. Move X/Y. Usually don't need to do unless you change the picker head. Move the picker head horizontally; the outermost buttons will move the head in 10 mm steps, the middle buttons in 1 mm steps and the innermost buttons in 0.1 mm steps.
    b. Move Z. Have to do this one with every gel. See step #5. Move the picker head vertically in 5, 1 and 0.1 mm steps, respectively.
    c. Be careful moving the picker head because if the head hits an obstacle, the motor will shut off and the instrument will be reinitialized. So be careful lowering the picker head in the Z axis also rapid moving in the X/Y axis when the picker head is very low (only use the innermost arrows).
    d. Once the picker head is placed appropriately, press the Set button.
4. Picker head versus gel Z position. This value must be checked before every picking run. This value indicates the Z-height at which the picker head meets the gel backing. This must be correctly set for maximum picking efficiency.

a. Use the Move X/Y blue arrows to move the picker head to an area where there is gel backing, but no gel, or not an important area of the gel.
b. Use the Move Z arrows to carefully lower the picker head. Using the innermost button will move the picker head in 0.1 mm steps.
c. When the tip of the picker head touches the backing, you will see a gap between the holder and the picker head piston.
d. If a gap does not form, the tip of the picker head is not touching the gel backing. This will result in the gel plugs being left in the gel or picked partially.
e. If a gap greater than 2 mm can be seen, the picker head is pressing down too hard on the gel backing. This will result in a shorter life span for the picker head and has the potential to scratch the glass plates.
f. The correct position for the picker head is with a 1 –2 mm gap showing at the top of the piston.
g. Press the Set Gel-Z Coordinate when the Z position of the picker head is correct.

5. Camera calibration. This only needs to be done if the picker head has been replaced. See below for instructions.
6. Setting the position of the rinse station. Usually OK.
    a. Using the Move X/Y arrow, move the picker head until it is placed above the center of the rinse station.
    b. Using the Move Z arrow, slowly lower the picker head into the rinse station.
    c. If it appears that the picker head will miss the center of the rinse station, adjust the X/Y position of the picker head as appropriate and continue to lower the picker head.
    d. When the picker head is just above the rinse stations, adjust the X/Y position so that in it centrally placed.
    e. Lower the picker head gently into the rinse station.
    f. If a gap is formed at the top of the picker head piston, then the picker head has been lowered too far. Raise the picker head slightly so that the gap is no longer present, plus an additional 2 mm up.
    g. Press the Set Rinse X/Y/Z button.
7. Setting the positions of the microtiter plates. The microtiter plates are numbered. They should be placed with the A1 well facing the operator. During System Setup only this well has to be defined. Usually OK.
    a. Using the Move X/Y arrow, move the picker head until is in centrally placed above the A1 well of the microtiter plate in position 1.
    b. Using the Move Z arrow, slowly lower the picker head into well A1.

c. The current position of the picker head is continuously read back from the instrument and displayed in the corresponding text boxes.
d. If it appears that the picker head will miss the center of the well, adjust the X/Y position of the picker head as appropriate and continue to lower the picker head. When the picker head is in the A1 well, adjust it so that it is centrally placed.
e. The Z-position of the picker head should be adjusted so that ~2 mm of the tip is within the well. This will enhance the dispensing efficiency of the spot picker.
f. When the picker head is correctly positioned in the well, press the Set 1 button.
g. At the first microtiter plate, press the Set Plate Z button – this sets the Z height for all of the microtiter plates and does not need to be set for each plate individually.
h. Raise the picker head before moving to the next A1 well.
i. Repeat steps 1 –6 for the remaining microtiter plates, without pressing the Set Plate Z button.

8. Setting Picking parameters. Before picking spots from a gel, a number of Picking Parameters must be set, as well as Gel Z Position and Marker Area. The parameters are listed below, but most aren't changed.
    a. Jazz. This the wiggle that the picker head does to pick and lift the spot off the gel. Usually fine, but if you aren't picking up any spots (and you're sure the Z coordinate is fine), increase the Jazz in 0.1 increments. Default is 1 mm.
    b. Aspirate volume and Aspiration flow.
    c. Dispense volume and dispense flow.
    d. Lift.
    e. Rinse volume and Rinse strokes.
    f. Reference Marker area.
9. Save and Exit. If you don't select this, any changes you have made will not be saved.

Placing Gel on Spot Picker

1. Prep gel tray - Rinse gel tray thoroughly with ddH20; use Crewwipe to scrub.
2. Pry gel apart with BioRad tool. Place in bottom of gel tray.
3. Cover bottom of gel tray w ddH20 to avoid air bubbles. ddH2O is placed in tray after the gel is added so the reference markers don't float off. Make sure ddH2O is covering the top of the gel. If a considerable # of spots are going to be picked, you may need to have more liquid.

4. Position the gel so that the reference markers lie within the parallel lines in the center of the gel tray. The reference markers must not cross or touch the lines because this will affect the reference marker detection.
5. The gel is fastened in the gel tray using adjustable clamps (gel holders). Tighten the screws no more than finger tight. For larger gels, the gel holders should be positioned in the gel tray before the gel is placed. Otherwise, the gel could be damaged by the gel holders.
6. Place the gel tray on the left hand part of the locator plate. The guide feet on the tray should fit onto pins on the locator plate. The gel tray can only go 1 direction with the corner-mounted guide feet facing the operator (towards the front).

Picking a gel

1. Systems / systems setup / initialize instrument /Go to Home
2. Load a pick List
    a. In the Load Pick list window, Select the Load Pick List button.
    b. Browse for the pick list.
    c. Select the pick list/skip. Numbers with sample #, well # and X, Y and Z coordinates come up.
    d. Open.
    e. The pick list data will appear on screen and a representation of the spots to be picked will be drawn in the main part of the window. X and Y coordinate columns are empty, because the reference markers have not been detected yet.
    f. In the Load Pick List window, press Next.
    g. If microtiter plates have not been previously loaded, label the appropriate # of plates and load on the microplate rack. Load gel if not done already.
3. Tells you how much liquid you need for the # of spots in your list. This is liquid to expel the spot and also to rinse the picker head between spots. Next.
4. Detection of the reference Marker. You can do this Manually or automatically.
5. Automatic detection of Reference Marker. Select automatic detection button. The camera will automatically search for the marker on the left first. When if finds it, the rim of the window changes from red to green. The camera then automatically goes to the right reference marker.
6. Manual detection of Reference marker. Automatic is much easier, but sometimes the camera for some reason doesn't find the reference marker. First check for bubbles, this can mess up the camera. Following are instructions for doing manual detection and how to get a better camera image.
    a. In the Find First marker (IR1) window, press Move to First Marker.

b. Move the camera with the blue arrows to a position over the first reference marker (IR1) on the gel.
c. If the reference marker is underneath the camera, but not visible in the Camera View window, use the Contrast and Threshold sliding bars to find an image of good quality. If the image appears blurry, adjust the camera aperture and focus until the marker image is sharp in the Camera view.
d. If the marker is visible in the Camera view window, but there is no image in the Marker detection window, first adjust the positions of the Contract and Threshold sliding bars until an image is visible in the Marker detection window. If adjusting the sliding bars does not lead to an image becoming visible in the Marker detection window, set the Contrast and Threshold sliding bars to their central positions. Then adjust the camera aperture until the marker is visible in the Marker detection window.
e. When the majority (or all) of the reference marker is displayed in the Marker detection window, the perimeter of the window will change from red to green. This indicates that there is a stable detection of the reference marker. If the window doesn't change to green – see user manual for some other things to try.
f. Below the Marker detection window, there are 2 boxes which show the offset of the center of the marker from the center of the camera.
g. Press the Adjust button and the reference marker will be centered in the Marker detection window. The marker is assumed to be centered when the values in both offset boxes are in the range –0.1 to +0.1. The procedure may need to be repeated until the offset values fall within the acceptable range. Achieving 0.0 offset values is not required – the software accounts the offset values so that the picking procedure will not be affected.
h. When the offset values are in the range –0.1 to +0.1, press the Move to First Marker button. It is only when the Move to First Marker button is pressed that the picker records the position of the marker.
i. Press Next.
j. In the Find Second Marker (IR2) window, press Move to Second Marker.
k. Move the camera with the blue arrows to a position over the second reference marker (IR2) on the gel.
l. When the perimeter of the Marker detection window is green, the picker has detected a stable image of the reference marker.
m. If the reference marker is not visible in any of the windows or the perimeter is red, see steps c – e.

n. Press the Adjust button and the reference marker will be centered in the Marker detection window. The marker is assumed to be centered when the values in BOTH offset boxes are in the range -0.1 to +0.1, press the Move to Second marker button.
o. In the Find Second Marker (IR2) window, press Next.
p. The software will now calculate the coordinates for the spots to be picked and the pick list table on the screen will be updated. Damaged reference markers and impurities in the gel image can result in incorrect detections. Also, reflections, shadows and incorrect set contrasts, thresholds and camera aperture can result in similar errors. This will result in imprecise picking.

7. Pick Spots.
    a. Press Pick after reference marker detection has been successful.
    b. In the Result File Location window, select the location for the picking results file. This is a file that matches a master # to a well. Create Directory.
    c. Enter output directory name and user name. The results will be saved as a folder in the selected location. Each plate filled (even if only partially) during the picking run is given a unique results file.
    d. Start Batch.
    e. The picking procedure can be paused by pressing the Pause button. The pause will occur at the end of a specific operation. Press continue to resume picker movement.
    f. The picking procedure can be stopped by pressing the Stop button. The stop will occur immediately. To continue, when the picking is stopped an Error message appears. Press Ok. In the Load Pick List window, press Load Pick List. Browse for the result of the interrupted picking run (extension .mfl). Select the result file. Do not use the correction function again! Click on Open. The result table will be shown on the screen. Ticked positions mean picked gel plugs. Filled circles represent unpicked gel plugs. The detection of reference markers has to be done again. The Picker X and Y values are missing in the table. Perform detection of reference marker, see step 5. Press Pick. The user will be informed by the software to place the microtiter plates. The partially dispensed microtiter plate and the next undispensed plate must be places in their original positions. Depending on exactly when picking was interrupted, the last gel plug being picked may be lost.
8. After picking
    a. Remove the gel from the gel tray and store/discard as appropriate.
    b. If the gel is to be discarded, scrape the gel off the glass plate with a plastic spacer. Follow cleaning instructions in 2-D electrophoresis guide.

c. Rinse the buffer lines with ddH2O using the prime syringe tool.
d. Rinse the liquid out of the gel tray with ddH2O and leave the gel tray to dry.
e. Exit the software and turn the computer and the Spot Picker off.

Picking a gel without a pick list.

The gel does not have to be scanned and no pick list must be prepared. The picking will not produce a result file, so the user must keep track of the spots. Use the white sheet as a background to obtain a better contrast of the gel image in the camera view.

1. Set up gel, Spot Picker, microtiter plates and Systems setup.
2. Press Initialise Instrument and Go to Home.
3. Select Tools/Click n/Pick.
4. Select the Picker Head Size. This will alter the size of the circle in the camera view window.
5. The first gel plug will be dispensed in plate well A1. However, the position can be edited in the Next Well window.
6. Set Contrast, Brightness and camera aperture, to achieve a good image quality.
7. Move the square in the upper right window with the mouse pointer to a preferred position of the gel.
8. Move the circle in the camera view by using the mouse pointer. When a requested gel spot is detected within the picker circle, press Pick. It may be convenient to plate a pen close to the spot which will help to find it in the camera view. Do not activate the movements of the picker during this procedure! When the picker head has dispensed the gel plug into the microtiter plate, the camera will move back to the picked position.
9. If several gel plugs from a spot are wanted to be pooled in the same well, it is possible by checking the Dispense in Single Well box in the Next Well window. This has to be done before the first picking to that well.
10. When the plate is full, replace the plate with an empty plate, and press New Plate.
11. When the picking run is completed, press Exit.
12. Follow instructions for “After Picking”.

Maintenance

1. Cleaning
    a. Remove the gel tray and the microtiter plate racks from the locator plate.
    b. Wipe the instrument cover with a soft damp tissue. If needed, use a mild detergent to remove stains.
    c. Clean the gel tray and the racks using a mild detergent.
    d. Let the instrument dry completely before use.

    e. Cleaning after use – see above.
2. Removing Picker head. After changing a picker head, a camera calibration should be performed to establish the offset between the picker head and the camera lens. The gel Z-position may also have to be adjusted in the System setup.
    a. Switch off power to the Spot Picker.
    b. Move the picker head to a convenient position to work in.
    c. Hold the top of the picking piston with one hand and with the other unscrew the picker head from the base of the piston.
    d. When the picker head is completely unscrewed from the base of the piston, place it in a safe location until it is required once more. Make sure that the retaining spring is also kept safe.
3. Attaching a new picker head.
    a. Place the retaining spring over the screw thread on the top of the picker head.
    b. Screw the picker head onto the base of the piston, making sure that the screw thread is inside the piston. At the same time, hold the top of the piston steady with your other hand.
    c. Slowly screw the picker head into the piston, while holding the top of the piston still with the other hand. The picker head only needs to be tightened to finger tight.
    d. Proceed by calibrating the distance between the picker head and the camera (#4 below.)
4. Calibrating the camera
    a. You need: white sheet for calibration; camera calibration foot; double-sided adhesive tape.
    b. Before starting, check that the camera lens is focuse don the bottom of the tray.
    c. Steps c – g focus the lens. Use the Click and Pick screen for the adjustments; select Tools/Click n' Pick.
    d. Place the white calibration sheet in the tray.
    e. Place an object on the sheet, ex. A piece of paper with printed text.
    f. With the Set Contrast and Set Brightness controls in the center positions, adjust the aperture to normal brightness.
    g. Adjust the focus ring on the lens to a sharp picture.
    h. In the main application window, under Load Pick List, select marker type Black Markers.
    i. Select System/Camera Calibration.
    j. Click on Move to move the camera to the position where the calibration foot will be place (X = 150, Y = 150).
    k. Place the calibration foot onto the picker head.

l. Locate the calibration position by placing an object under the camera or drawing a line with a pencil. View the left window of the Camera Calibration screen for orientation.
m. Place a strip of double-sided tape across the calibration position.
n. Click on Place Calibration Marker. The picker head moves to the calibration position and down towards the tray. The picker head stops at the Z-coordinate shown under Calibration Marker – the default Z-height is 10, which will be above the tray. The picker attempting to locate the calibration foot.
o. Choose the Z Coordinate stepwise to lower values and click on Place Calibration Marker again. Repeat until the calibration foot is successfully attached onto the tape. The final Z-coordinate is normally 65 – 70. Check before each step that the remaining distance is not smaller than the change of Z-coordinate.
p. When the calibration foot has been placed onto the tape, and then identified by the camera, the frame around the detection window changes from red to green.
q. Repeat the procedure 4 times. When the calibration has been successfully performed, click on Save to save the new calibration values.
r. Troubleshooting camera calibration procedure. If the calibration foot is not visible in the camera view window although it is underneath the camera, adjust the aperture until the calibration foot is visible. If the calibration foot is visible in the camera view window but not visible in the detection window, adjust the Contract and Threshold sliding bars until an image is visible in the detection window. If the calibration foot is not placed on the tape, decrease the Z-coordinate to make the picker head move closer to the tray.