

Set-up a screen using the Tecan Freedom Evo

Practical details:

- **Very viscous solutions cannot be aspirated. Dilute these stock solutions.**
 80% v/v glycerol works but not 100%
 40% w/v PEG >4000 works but not 50%
 30% w/v PEG >10 000 works but not 40%
 20% w/v PEG >20 000 works but not 30%
 100 % PEG 200-400 works
- **Always use stock solutions containing 50% more** than needed for pipetting plus an additional 5 ml since excess liquid is aspirated and some also adheres to the tips.

1) Double click on the <Tecan CrysScreen> icon on Desktop

Design screen:

- 2) Choose **Define a Screen>Define Grid Screen**
- 3) **New:**
 - a. **Select Labware Dimensions:** “8 rows by 12 columns”
 - b. **Enter New Screen Name:** “your_name_date”
 - c. Press <D>
- 4) Set up everything.
 - a. **Select Stock** (top right panel):
 - i. Mark **Buffer, Precipitant, Salt** or **Additive**
 - b. **Define Range Parameters** (top left panel):
 - i. Select your “Buffer/Precipitant/Salt/Additive” under **Stock Name**
 Note: If you do not find your solution in the lists you need to add it (go to point 5).
 Note: buffers with pre-set pH are found as Salt.
 - ii. Select “Conc. Range, Min., Max, Unit and Steps”
 Note: It is the target concentrations that should be given
 - iii. Press <>>
 - c. When all “Buffer/Precipitant/Salt/Additive” have been added press <Add>
 - i. This moves the settings to the middle panel.
 - d. Once all variations have been created and added to the middle panel, **mark and drag** them one by one to the wells below
 Note: At this point do not care about position of wells or if the screen ends up in several blocks. You will give positions below under “Design layout of screen”.
 - e. Save <Save>
 - i. Save to project “SBX” or your own project if you have one (this does not really matter because all screens are visible and usable).
- 5) Adding chemicals:
 - a. Save <Save> before leaving **Define Grid Screen** or all prior settings will be lost.
 - b. **System Configuration>Define Master Data**
 - c. Add chemical by entering it under the correct group.
 Note: Add Buffers with a pre-set pH as Salt.
 - d. Once done go back to **Define a Screen>Define Grid Screen**, but choose **Select:** “your_name_date” instead of New.

Design layout of screen in deep-well block:

- 6) Choose **Define a Screen>Define Screen Remapping**
- 7) **New:**
 - a. **Select Labware Dimensions:** “8 rows by 12 columns”
 - b. **Enter New Screen Name:** “your name_date”.remap
 - c. **Select Defined Screen:** “your name_date”
 - d. Press <D>
- 8) Choose to define screen manually by mark and drag or press <**AutoRemap**>
- 9) Save <S>
 - a. Save to project “SBX” or your own project if you have one (this does not really matter because all screens are visible and usable).

Enable communication with Gemini

- 10) Double click on the <**Shortcut to Gemini.exe**> icon on Desktop (user: “Admin” and password: “tecan24”, but since this is updated on regular basis look in pw.txt on the Desktop to find the actual password)

Pipette screen

- 11) In Tecan CrysScreen, choose **Pipette Defined Screen>Create New Instance of a Screen**
 - a. **Select a Defined Screen:** “your name_date”.remap
 - b. check/uncheck **Create Buffers On The Fly**
Note: check if you plan for the Tecan to mix your buffers from their acid/base components or uncheck if you have pre-set your pH.
 - c. **Select Gemini Script:** *C:\Program Files\Tecan\CrysScreen\CrysScreen Data.V42\Screens\Create_Screen_in_Deepwell.gem*
 - d. **Select Destination Vessel:** “Deepwell”
 - e. **Enter Final Volume:** 700-1800 µl recommended
 - f. Press <D>
- 12) Define your stock solutions in the left panel.
 - a. Check the units.
 - b. Check the positions. Give correct positions in the Pos. column. These correspond to the place on the deck of the robot where you will put your solution, i.e. if you have two salts, leave the first salt solution in position 1 and change the second solution to position 2. The positions on the deck are marked with Buffer/Percipitant/Salt/Additive/Diluent and numbers.
 - c. Press <**Check**>, to calculate required volumes of each stock solution.
Note: Use at least 1.5 times required volume + 5 ml excess as a standard rule. Always have >50 ml mH₂O as diluents, i.e. close to the maximum volume.
- 13) Wipe off the space beside the Tecan with ethanol.
- 14) Place all stock solutions and a deep-well block (with well A1 in the top left corner) in the correct positions on the deck. Check with the picture on the screen of the computer so that you get it right. Only 50 ml tubes are allowed for stock solutions and water.
Note: Use gloves. Open all solutions inside the box. Put the labelled lid on wiped-off shelf with its opening downwards.
- 15) Press <D>
- 16) Worklist(s) prepared! Continue Execution or Add to Stack: <**Continue**>

Note: If the alarm goes off during pipetting, the red lamp start blinking and it says “**Not enough liquid**” in the 50 ml tube, do one of the following:

- Check if there really is enough liquid. If not, fill more stock solution in the tube or choose to pipett nothing, make a note which well(s) and solution and pipette manually afterwards.

- If there is enough liquid, choose <**Go to the Maximum Detection Limit**> of the pipette and pipette anyway. Some solutions are not detected by the robot so it refuses to pipette. By going to the maximum detection limit you overrule this.

After pipetting

- 17) Put the lids back on the stock solutions.
- 18) Put a lid on the deep-well block (in the brown box on the floor). Be careful to close it tightly by pressing on it with a glass or metal tube.
- 19) Turn the block upside down 20 times to mix the screen solutions.
- 20) Spin the block at 200g, 20 °C for 1 min.
- 21) Remove the lid from the block inside box and dispense the liquid into crystallisation plates.
- 22) Put removable sealing tape onto crystallisation plate.
- 23) Put lid back on the deep-well block and close it tightly.
 - a. Wrap parafilm around the edges and store at 4°C.
- 24) Close the Gemini and CrysScreen programs but leave the computer on.

Good luck!

Don't hesitate to phone if you run into troubles. **Please don't break the tips!**

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