

Sequencing MegaBACE

Gather:

- Water tray, full with clean water
- Buffer plate with 100uL 1X LPA Buffer
- 6 tubes with LPA Matrix, spun 2,000rpm, 3 mins (to get rid of bubbles)
- 6 tubes with 1X LPA Buffer
- 6 tubes of clean water
- Sample plate with sequences denatured in loading buffer
- Sample sheet filled out and named as “.psd” : **Species_Plate**
#_Region.psd e.g. **Den4_01_NS1A.psd**

Some steps have a time limit. It is not critical if you miss these: **you just have to select “retry.”**

1. Open ICM. Click on PLATE SETUP tab (bottom left).
2. Click on “New.” Type in plate name, see above, but without “.psd” extension.
3. Click on INSTRUMENT CONTROL tab (bottom left). Click Start when on “Matrix Fill and Prerun.” (If it’s the first run of the day, it will start and “rinse Tips”) Notice the window below the start button tells you what you will need. (The MB has two small screens at each bottom end; the right side tells you to add things and when, the left side tells you a general overview of what’s happening; sometimes the right side asks you to add things)
4. MB will request the new loading buffer tray for the **left drawer** (and thank you when you have added it). Remove old “buffer plate,” a 96-well plate that is used for each run and collects LPA Matrix. DO NOT discard it but place it somewhere where 1) you do not mistakenly re-use it and 2) you can rinse 5x with hot water and re-use buffer plate. Place cleaned buffer plates in designed box.
5. MB will then ask you to load the 6 LPA Matrix tubes in the **right drawer** (and again thank you). MB first fills the capillaries for 3 minutes, prompt to load 6 buffer tubes, and finally does a 5 minute prerun.
6. After Prerun, MB will go to “Inject Samples and Run.”

7. MB will ask you to load “water tray” in the **right drawer** (and thank you). **save the buffer tray!!!!**
8. After tips are rinsed, MB will ask you to select your plate setup, select and click continue.
9. MB will ask you to load your sample plate “quickly.” Add it, if you wait too long just place your sample in the drawer, tell MB to “retry”, open drawer all the way, and reshut.
10. Load water tray for second rinse tip.
11. Load saved buffer plate
12. All hands on work is finished until next run.
13. **Note** that if no one else will be running sequences next, check “Sleep after this run” box before you begin. (This allows the capillaries to cool down and extends their running life) **Make sure you leave 2 hours for warm-up next day.**