Station B: Bioneer Extraction Protocol

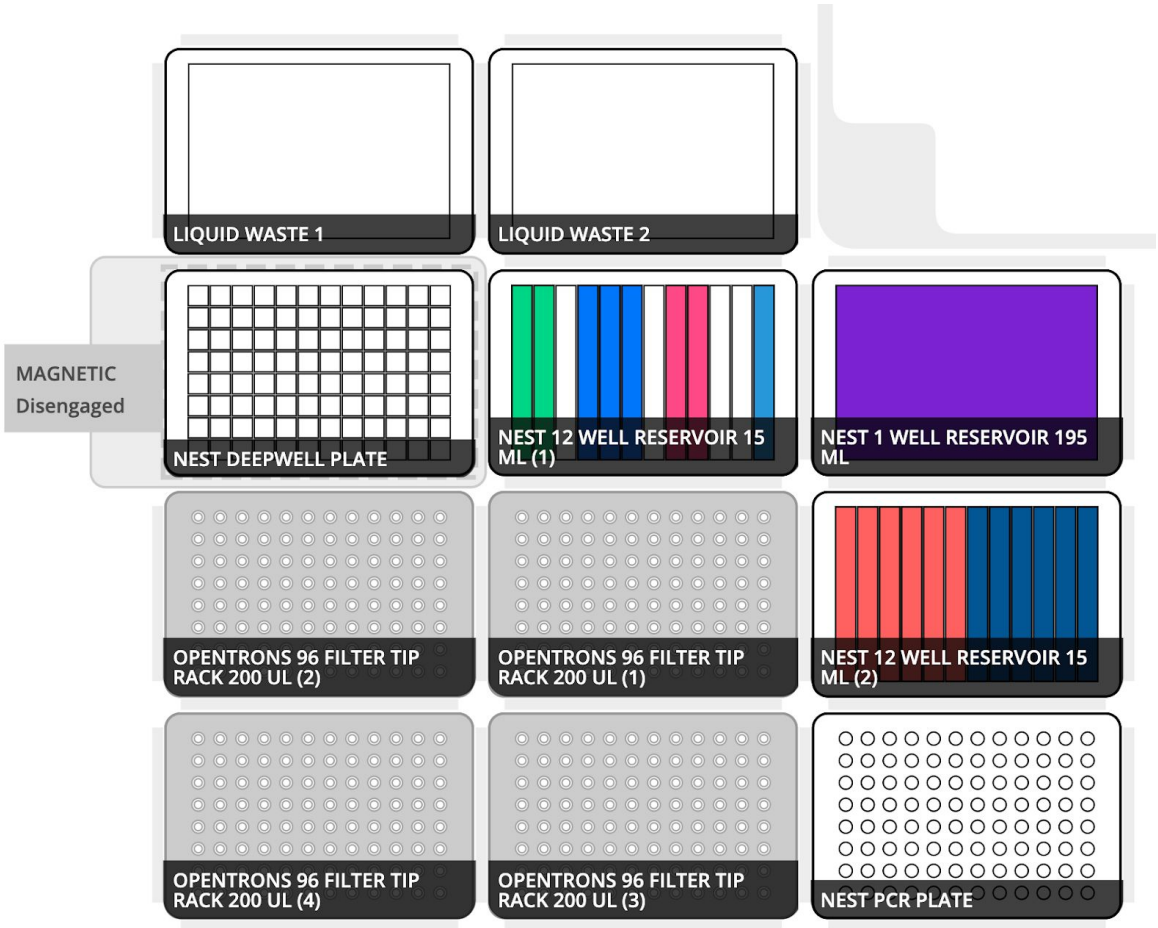
Code parameters:

- Change the sample number on line 10

Pipettes:

- P300 multi-channel on the left mount

Deck Layout (for 96 samples):



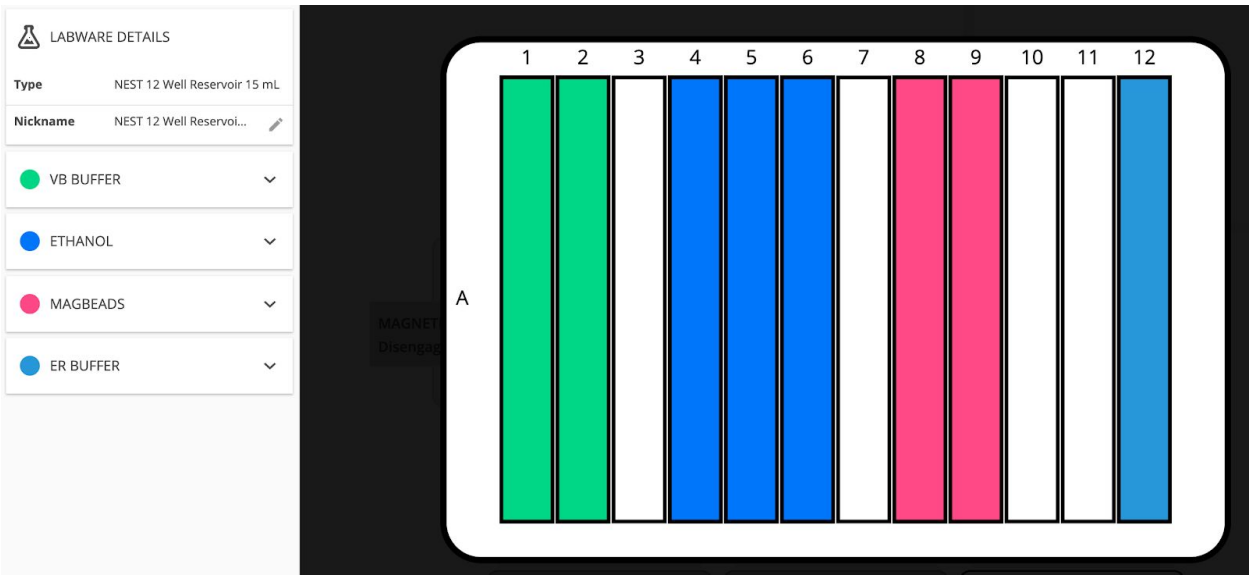
Labware and module requirements:

- 1 x Magnetic Module
- 1 x 2mL Deep Well Plate **[input with Proteinase K]**
- 10 x 200µL Filter Tip Racks (only 4 fit on the deck at a time)
- 3 x 1-Well Reservoirs **[one for VWM1 Buffer, two for liquid waste]**
- 2 x 12-Well Reservoir **[holds all other buffers]**
- 1 x 96-Well PCR Plate OR PCR strip tubes to match the number of samples **[output with eluates/extractions]**

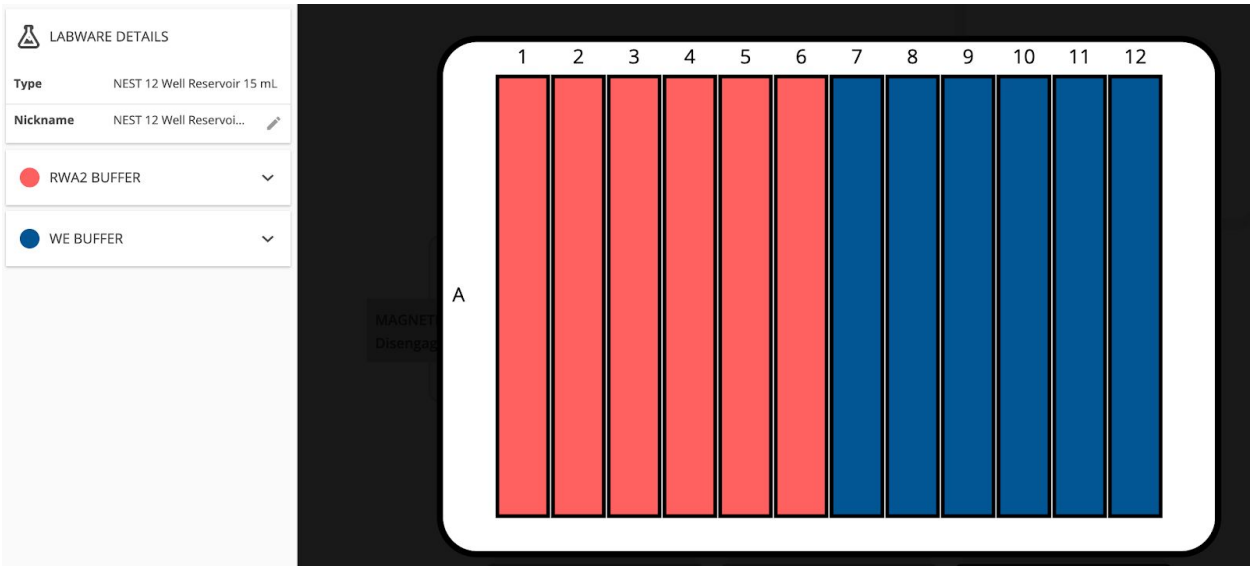
Volume requirements:
Note ~ *the below volumes account for a dead volume; the dead volume can be adjusted depending on the calibration of the pipette to the labware, but we've found it's best to have a dead volume of at least 10%*

Reagent	Volume per sample (µL)	Volume for 8 samples (µL)	Volume for 48 samples (µL)	Volume for 96 samples (µL)
VB Buffer	200	1600	9600	19200
Ethanol	400	3200	19200	38400
MagBeads	200	1600	9600	19200
VWM1 Buffer	1400	11200	67200	134400
RWA2 Buffer	700	5600	33600	67200
WE Buffer	700	5600	33600	67200
ER Buffer	100	800	4800	9600

Reservoir Setup:
Reservoir 1
VB Buffer → Slot 1 (samples 1-48); Slot 2 (samples 49-96)
Ethanol → Slot 4 (samples 1-32); Slot 5 (samples 33-64); Slot 8 (samples 65-96)
MagBeads → Slot 8 (samples 1-48); Slot 9 (samples 49-96)
ER Buffer → Slot 12 (samples 1-96)



Reservoir 2
RWA2 Buffer → Slots 1-6 (16 samples per well)
WE Buffer → Slots 7-12 (16 samples per well)

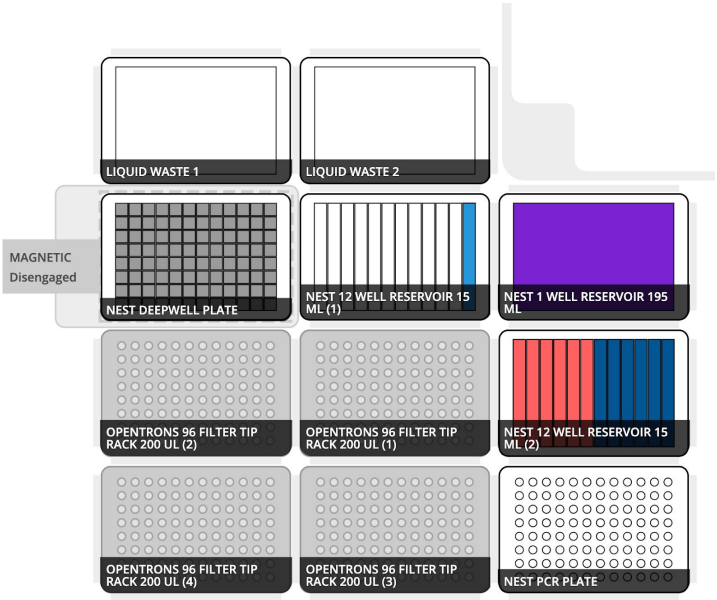


Before you begin:

1. Add the **VB Buffer**, **Ethanol**, **MagBeads**, and **ER Buffer** to the 12-Well Reservoir (in appropriate wells) and load in **slot 8**
2. Add **VWM1 Buffer** to the 1-Well Reservoir and load in **slot 9**
3. Add the **RWA2 Buffer** and **WE Buffer** to the 12-Well Reservoir (in appropriate wells) and load in **slot 6**
4. Load all other labware as designated in above and within the app.
5. Prepare for tip replenishment steps (see below)

[continued]

After you begin:
After adding the VB Buffer, Ethanol, and MagBeads, the OT-2 will aspirate off the supernatant (after the incubation). The OT-2 will then perform wash 1 with VWM1 Buffer and pause for replacing the tips. At this point, the waste bin should be emptied and the four (4) tip racks should be replaced.



After replenishing the tips and clicking resume in the app, the protocol will continue until all of the wash steps are completed. At that point, the user will be prompted to replace the tip racks in slots 1 and 2 - these tips will be used for adding the ER Buffer and transferring the elution to the final PCR Plate.