Switch on epMotion, wait until the third beep. Switch on computer. Wait until Windows load fully.

Connect to Biokemia.

In LIBRA, generate CSV segments 1-10.

For choosing lysates: i) first filter according to site of origin (i.e. VRNZH); ii) for rack #1, filter for only HEALTHY, and for rack #2 only TUMOR or STROMA; iii) filter so that the concentration of each lysate is greater or equal than 2.05 mg/ml; iv) sort ascending according to solubilization date; v) filter so that all lysates end with 1; vi) filter out all used lysates (used=0); *vii) FOR THE FIRST PLATE ONLY (OUT OF THE 15 FOR EACH PROJECT): POSITIONS 14, 15 AND 16 OF RACK #1 SHOULD BE HEK293, HeLa AND HepG2 respectively, AND FOR rack #2 POSITIONS 14, 15 AND 16 SHOULD BE HepG2, K562 and MCF7. Remember that these are from OBI, and not from another site of origin.* Save and finalize the FRR. Export to excel the chosen lysates; sort the lysates by ascending in UF position column.

Segment1: Buffer from ResRack --> WP96 #1 for normalizing lysates 1-16 to 2 g/l

Segment2: Lysates 1-16 --> WP96 #1

Segment3: Buffer from ResRack --> WP96 #2 for normalizing lysates 17-32 to 2 g/l

Segment4: Lysates 17-32 --> WP96 #2

Segment5: Buffer from ResRack --> WP96 #1 for accepting lysates from same plate for dilution purposes

Segment6: in WP96 #1 from left column to right column for dilution of lysates to 2, 4, 8 and 16x

Segment7: Buffer from ResRack --> WP96 #2 for accepting lysates from same plate for dilution purposes

Segment8: in WP96 #2 from left column to right column for dilution of lysates to 2, 4, 8 and 16x

Segment9: WP96 #1--> left side of WP384 #1

Segment10: WP96 #2--> right side of WP384 #1

Generate excel sheet with selected lysates.

Remove lysates from UF, thaw them while rocking and place them in their respective rack position (uncapped).

Disconnect from Biokemia.

Double click “epBlue 40.6”.

Login with your credentials.

Single click “Application Editor”.

Navigate to christos2 > FINAL VERSIONS OF PROTOCOLS > Lysates load to two plates\_normalizations\_buffer transfers\_dilutions.

Make sure that you have placed all racks, plates, reload tips and reservoirs on worktable correctly. Fill up ResRack with minimum 25 ml of buffer (Benz+Laemmli, no need for benzonase, protease and phosphatase inhibitors, but include DTT in Laemmli). Place reload tips so that the three holes are on the left side.

Click on “Switch to procedure”.

Import CSV files **from BOTTOM to TOP**:

Segment8 --> step 16

Segment 7 --> step 14

Segment 6 --> step 12

Segment 5 --> step 10

Segment 4 --> step 8

Segment 3 --> step 6

Segment 2 --> step 4

Segment 1 --> step 2

Copy steps 1-108.

Click on step 108.

Paste steps 1-108. This will perform all operations twice, and is needed because the pipetting tool cannot exceed 50 l. Like this, 100 or 50 l will be in each WP96 well, from where 12.5 l can be comfortably taken twice for generating the WP384 (see below).

Check for errors.

Save the protocol and change its name by adding the date as prefix in YYYYMMDD format, followed by FRR.

Make sure that the lysates have thawn (do NOT vortex or shake them). Start the procedure. Press OK on all confirmation requests regarding number of samples. **Do NOT change any numbers during this step**. **Highlight ONLY “Use required minimum volumes”**. Remain nearby, replace reload tips when indicated. The whole procedure (both this and next epMotion protocol) requires 10 reloads per WP384. Place reload tips so that the three holes are on the left side. The process “Lysates load to two plates\_normalizations\_buffer transfers\_dilutions” is ~150 min long.

After the process is finished, remove racks with lysates; recap lysates and put them back to the UF.

Navigate to christos2 > FINAL VERSIONS OF PROTOCOLS >2 WP96 to 1\_WP384

Fill the two leftmost and two rightmost columns of a WP384 with buffer, manually (12.5 l in each well). Place it on the worktable, where indicated (bottom right).

Make sure that you have placed all racks, plates, reload tips and reservoirs on worktable correctly.

Click on “Switch to procedure”.

Import CSV files **from BOTTOM to TOP**:

Segment10 --> step 4

Segment9 --> step 2

Check for errors.

Save the protocol and change its name by adding the date as prefix in YYYYMMDD format, followed by FRR2.

Start the procedure. Press OK on all confirmation requests regarding number of samples. **Do NOT change any numbers during this step**. **Highlight ONLY “Use required minimum volumes”**. Remain nearby, replace reload tips when indicated. Place reload tips so that the three holes are on the left side. The whole procedure (both this and previous epMotion protocol) requires 10 reloads per WP384. The process “2 WP96 to 1\_WP384“ is ~30 min long.

After the process is finished, barcode WP384 as indicated by LIBRA. Stick the barcode on the top, left side; it does not matter if the sticker covers the row letters. Seal and store in UF in its dedicated position. Discard the two WP96s.