smaRT-LAMP method overview

Pre-run: **Set-up** your smaRT-LAMP apparatus

Step 1: Generate a **standard** curve for your pathogen and patient sample type (e.g. SARS-CoV-2 in saliva)

Step 2: Run patient **samples**

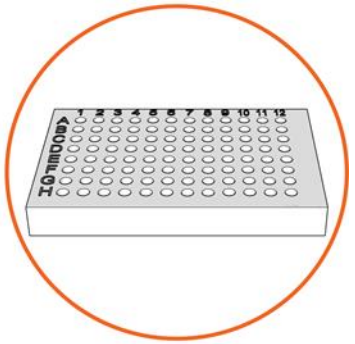
Step 3: **Analyze** samples using standard curve

for additional information on the smaRT-LAMP method, please refer to our journal article or website

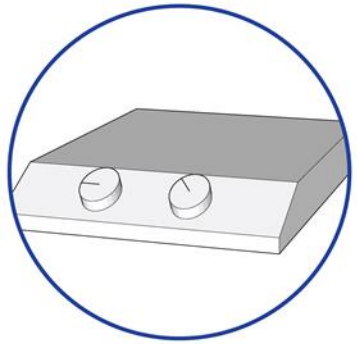
SET-UP: getting started

smartphone with app

520 nm optical filter

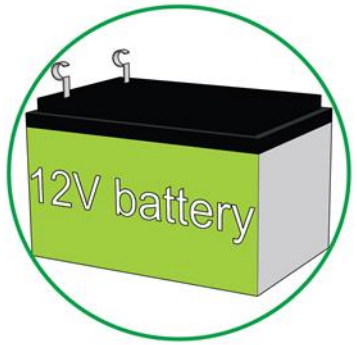


aluminum tube holder

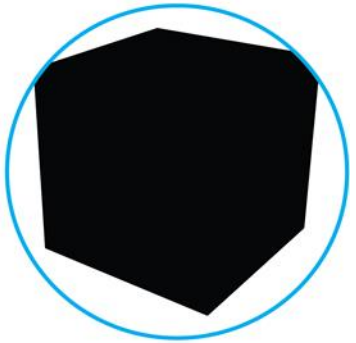


heat block

blue LED lights



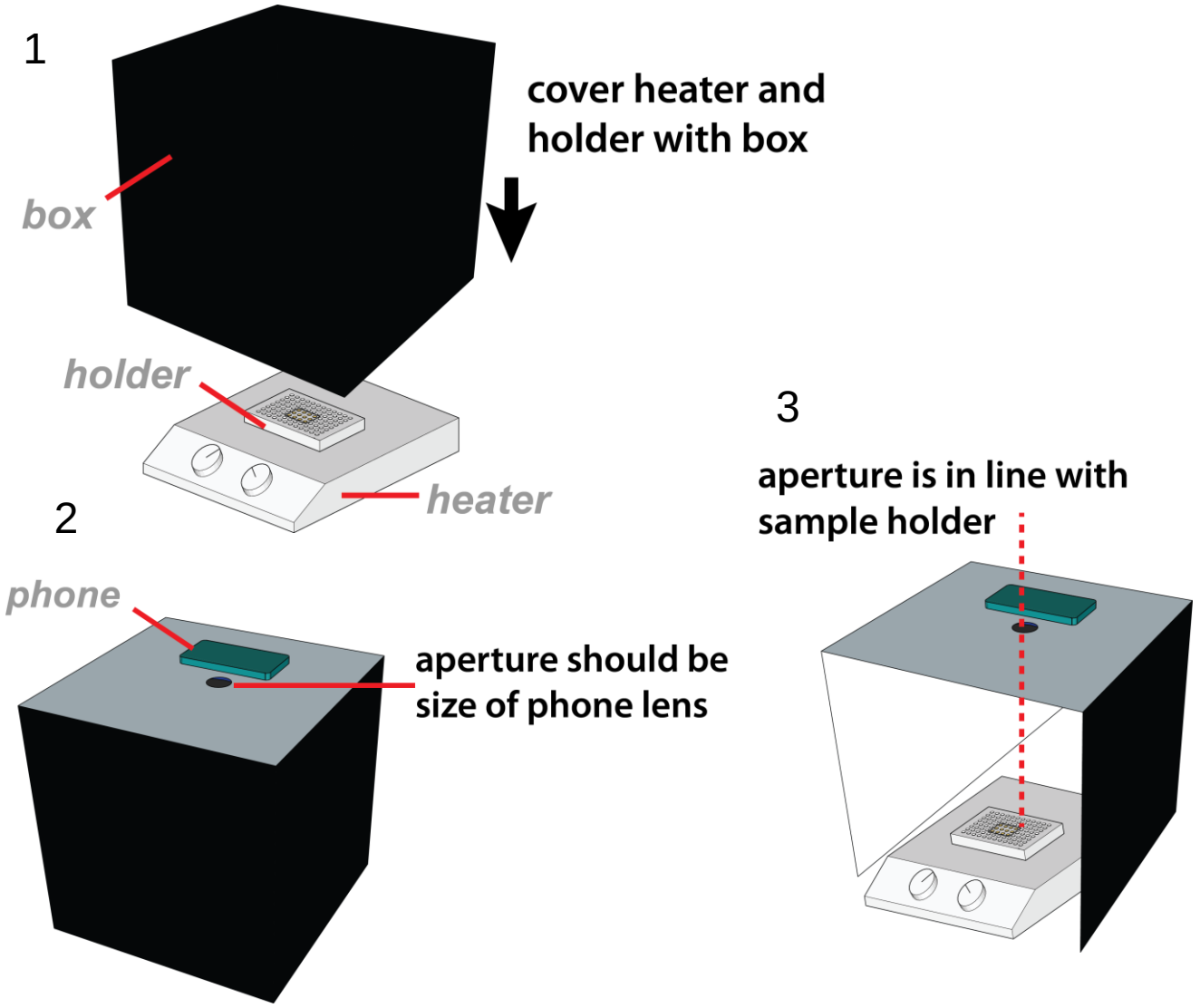
DC power source



blackout box

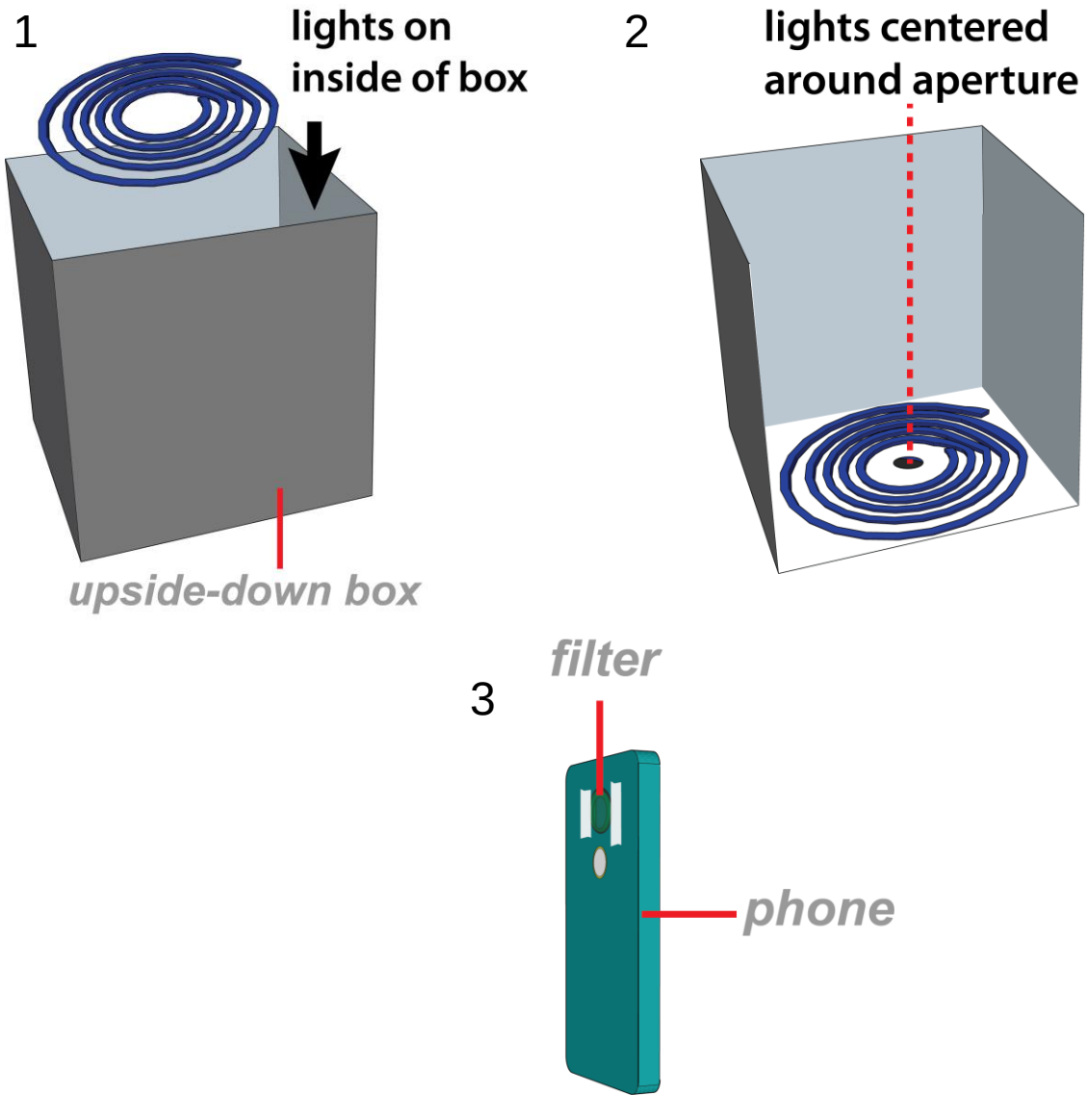
- gather the materials shown above
- close all other apps and your put phone in “Airplane mode”

SET-UP: placement of components



- to perform smaRT-LAMP, you will need a heat block, insulated sample holder, blue light source, and black box
- place the opened box over the sample holder
- cut a viewing hole for the phone camera lens on the top of the box (opposite the open end)
- check that hole is directly above the holder

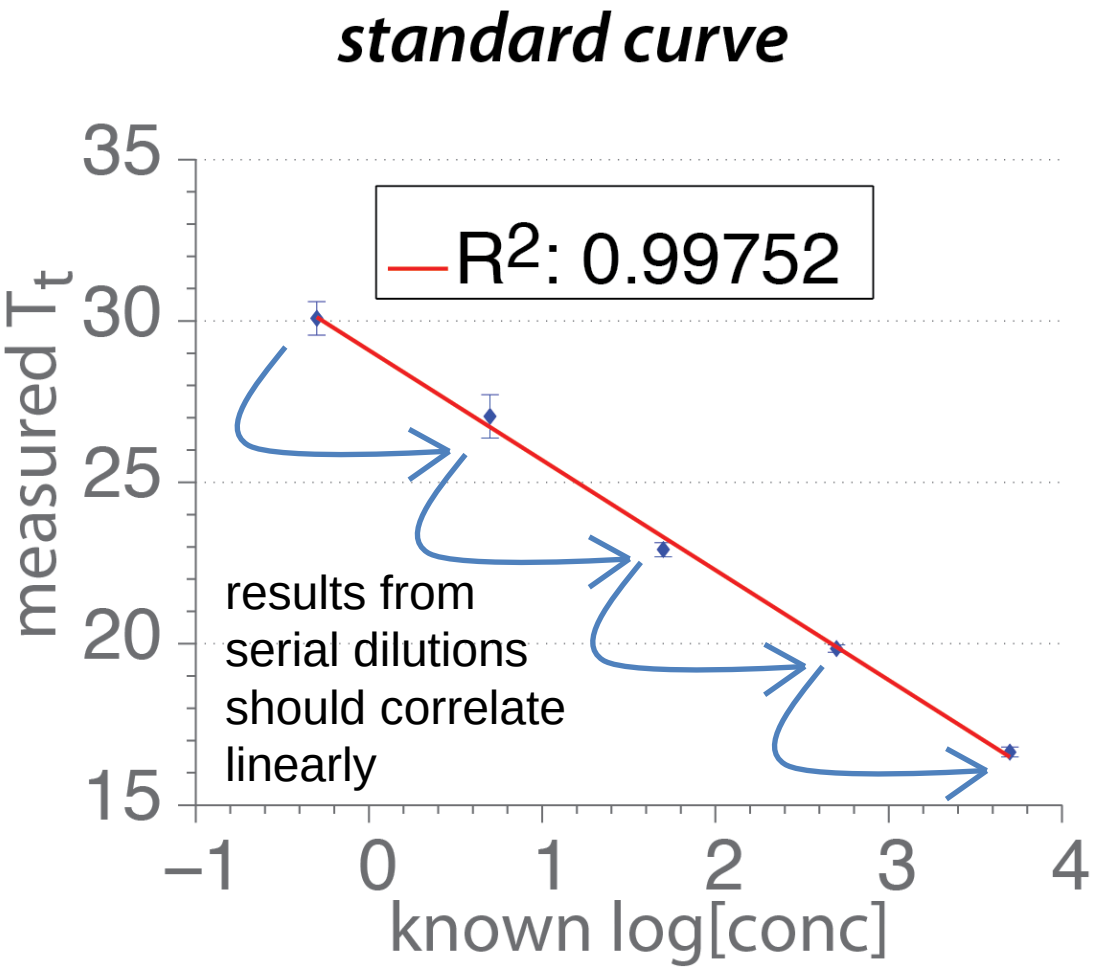
SET-UP: prepare for fluorescence imaging



- remove the box and turn upside-down to attach the lights to the inside, on the same side as the viewing hole
- tape a green filter over the camera lens of the smartphone
- connect the lights to the battery
- preheat the entire apparatus by leaving it fully assembled with the 65 °C heat source on for 30 minutes

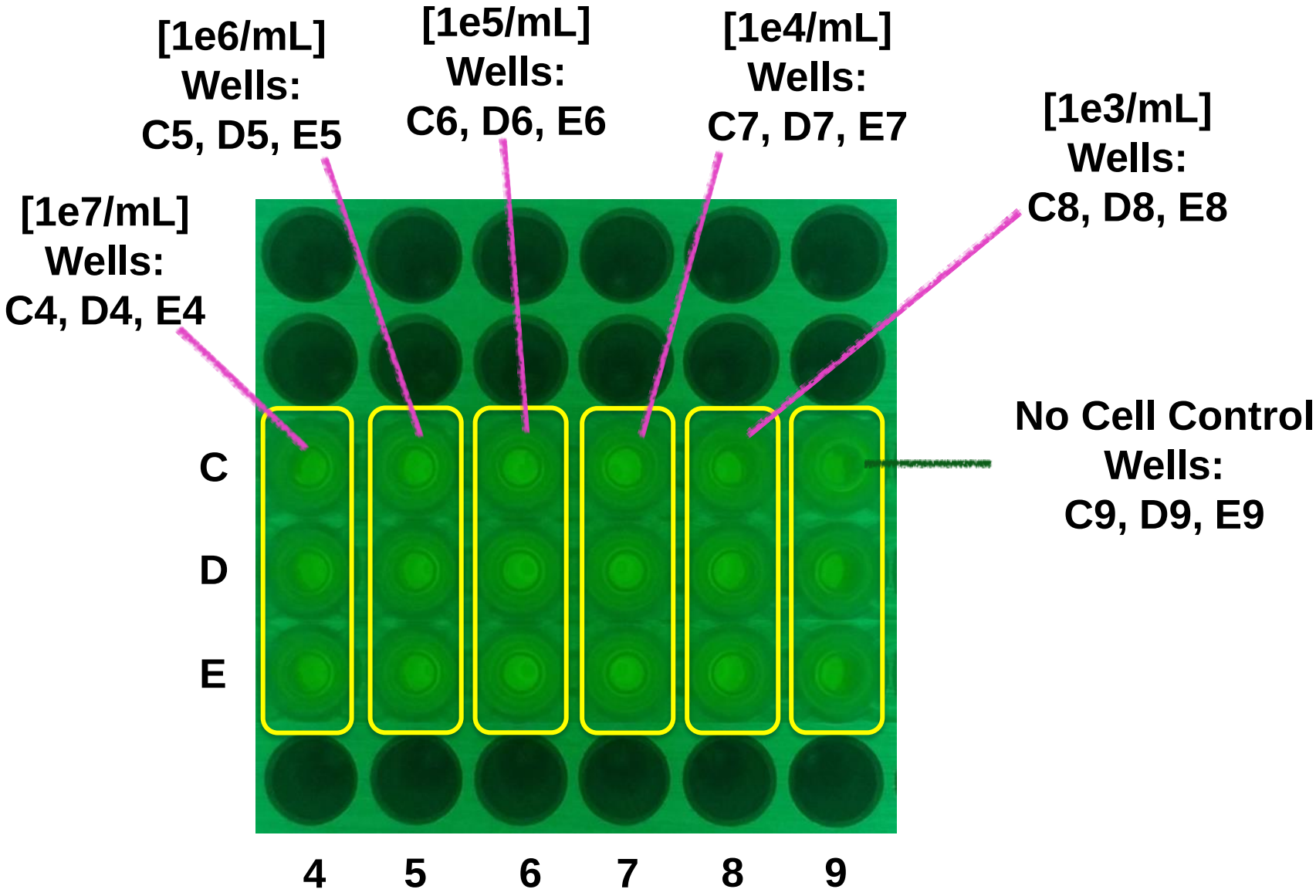
1. STANDARDS: prepare the standard curve

- a standard curve is generated from sample “standards” with predetermined concentrations of template
- decide which template (e.g. gDNA or cells) and sample type (e.g. blood, urine, feces, saliva) best represents your patient sample and is available to you
- for example, the template could be purified genomic DNA in buffer, lysed bacteria in buffer, or lysed bacteria in blood
- once you’ve chosen your template, generate standard samples with duplicate, serially-diluted amounts of template in your sample type



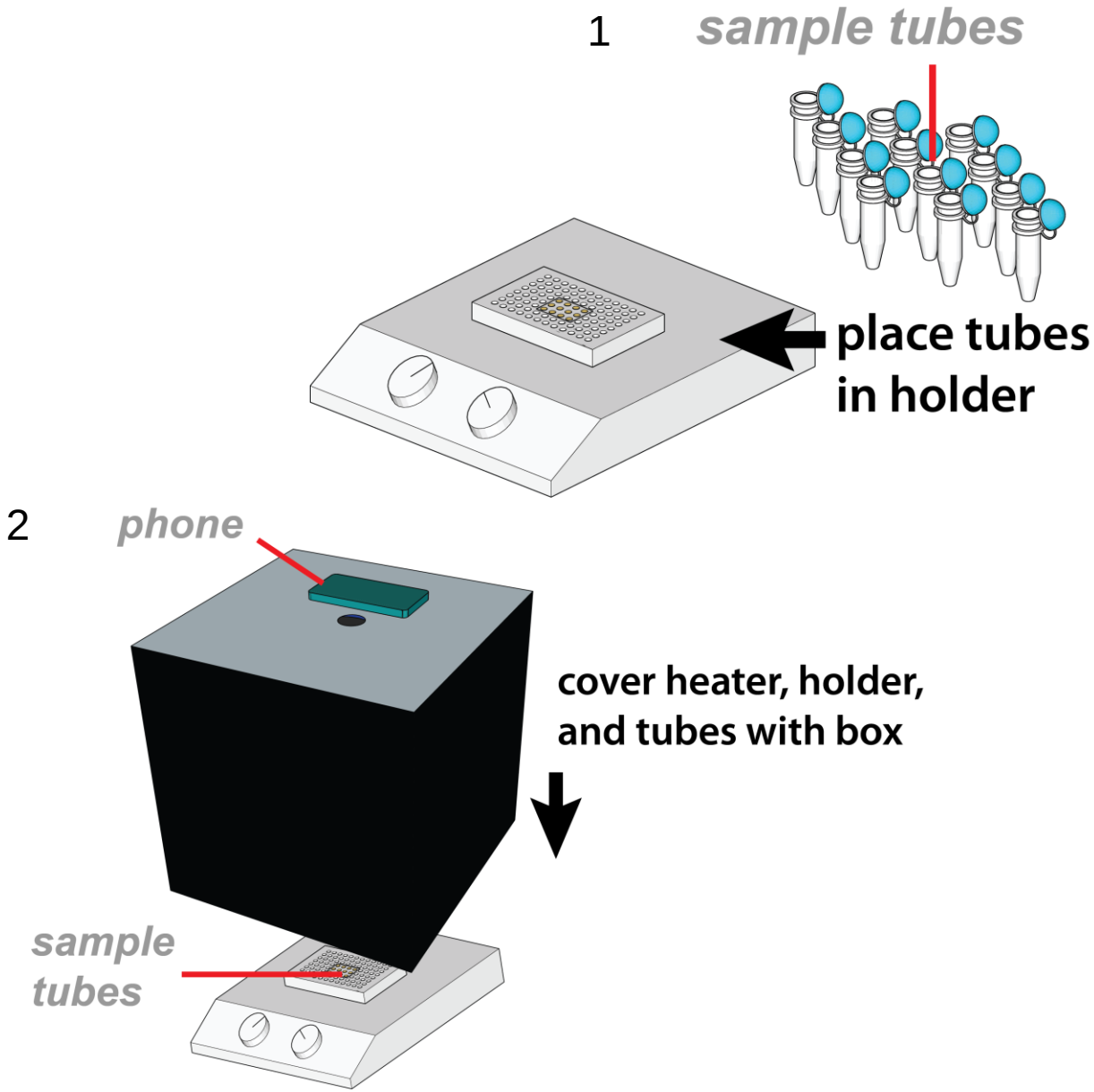
1. STANDARDS: standard curve placement map

- analysis requires a 10-fold dilution series of standards as shown for saliva samples
- Load biological triplicates of each concentration at the indicated well positions
- Use the correct concentrations for each sample type:
- **Saliva:** 1e7, 1e6, 1e5, 1e4, 1e3/mL
- **Blood:** 5e7, 5e6, 5e5, 5e4, 5e3/mL
- **Urine and Feces:** 2.5e7, 2.5e6, 2.5e5, 2.5e4, 2.5e3/mL



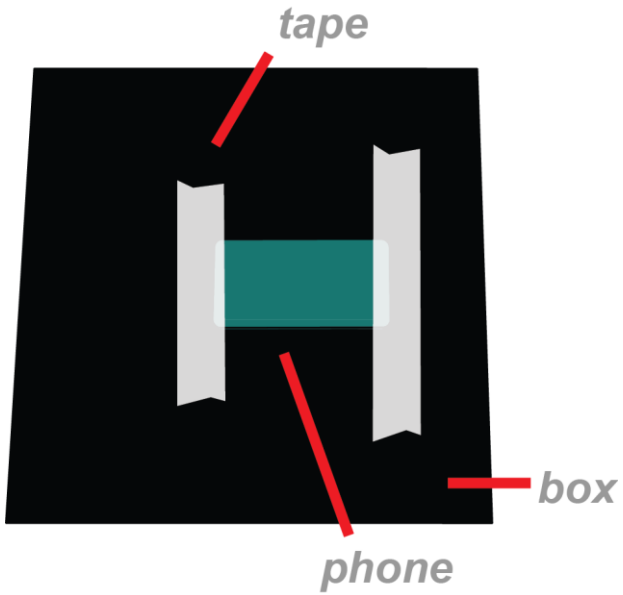
1. STANDARDS: load the standards

- following the placement map, load the tubes containing the standards into the correct positions on the holder
- place the box over the heater, holder, and tubes
- place the smartphone face-up on top of the box, making sure to align the camera lens with the viewing aperture



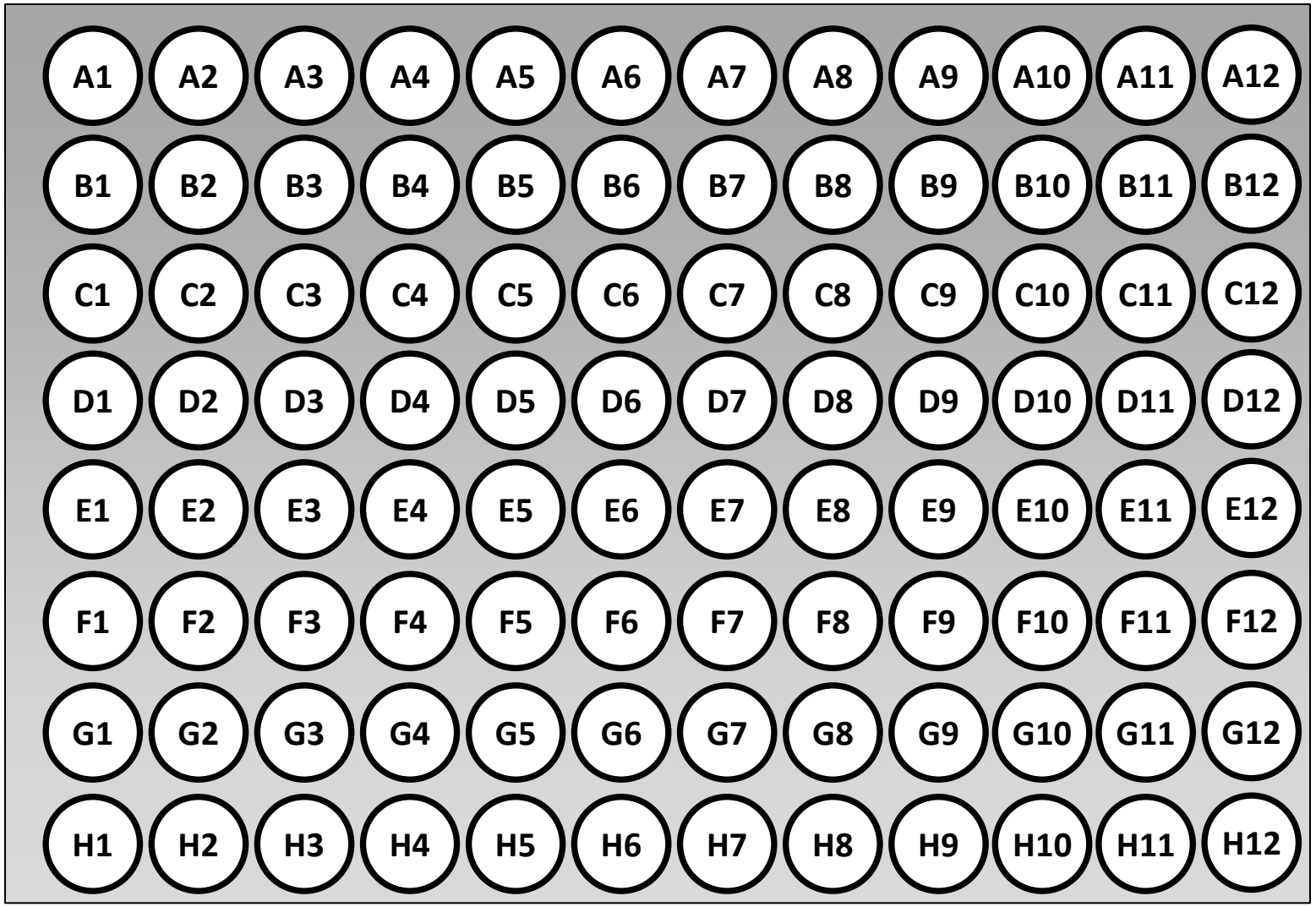
1. STANDARDS: run standard reactions

1. launch the BACTICOUNT app
2. from the home page, choose “Start Bacterial Analysis”
3. select the sample type (blood, urine, feces, or saliva)
4. click “1. Record Standard Curve”
5. type a name for your standard curve (you can use this curve file again for the same pathogen and sample type)
6. load standards, and ***immediately*** after, press “OK” (this begins the reaction timer)
7. place the box over the samples, and the phone on the box
8. touch the “Tap To Focus” button to precisely align the sample tubes within the map on the viewing screen
9. you can ensure that the phone and system components will not move by taping them into place on a stable surface
10. when the camera position is set, hit the “Begin Recording Amplification” button to start the recording process



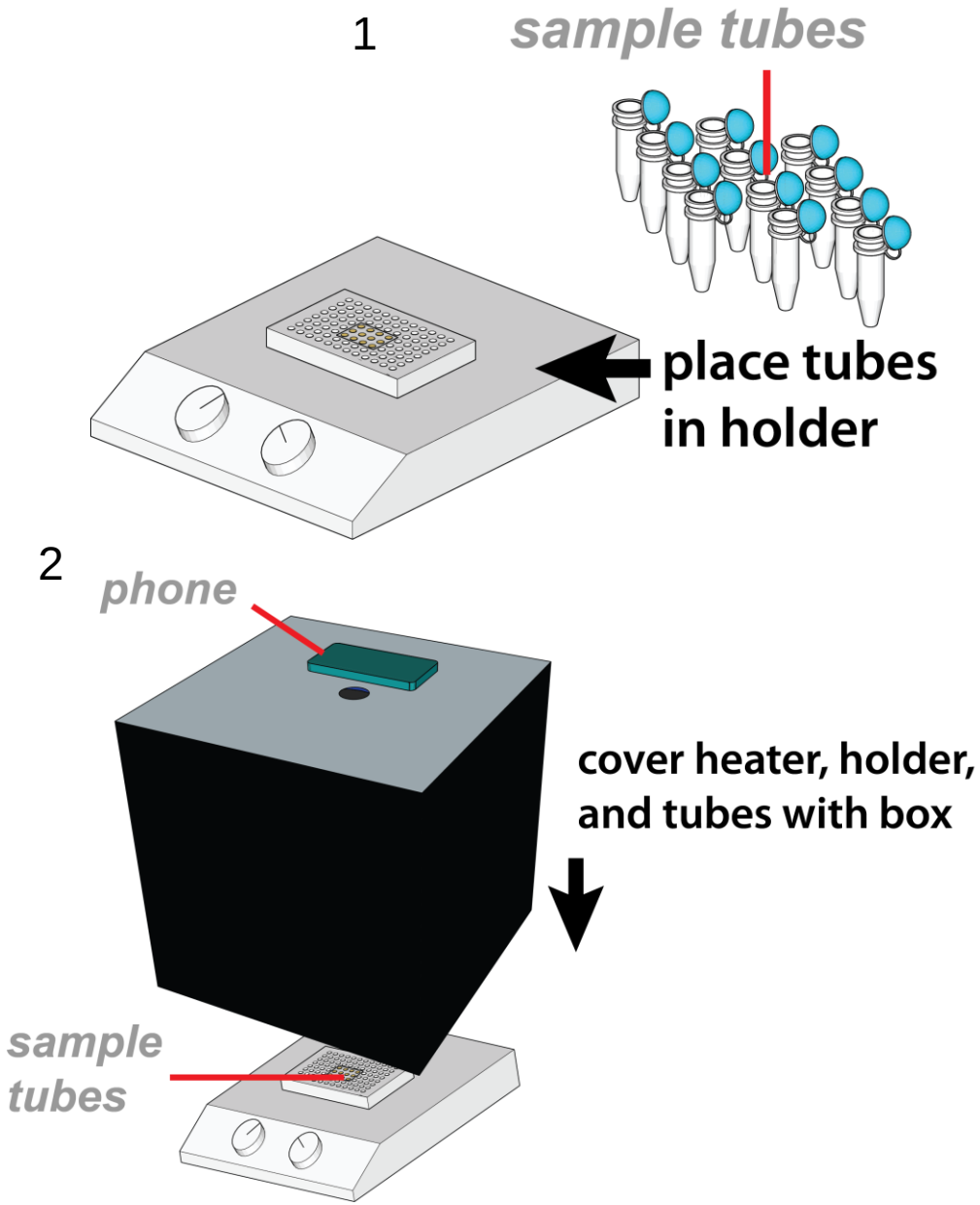
2. SAMPLES: map of unknown samples

- having a pre-recorded standard curve allows us to compute the genome concentration in up to 96 samples at once
- samples are reported as well positions “A1” to “H12” (diagramed on the right)
- **keep careful track of what sample is in each well**



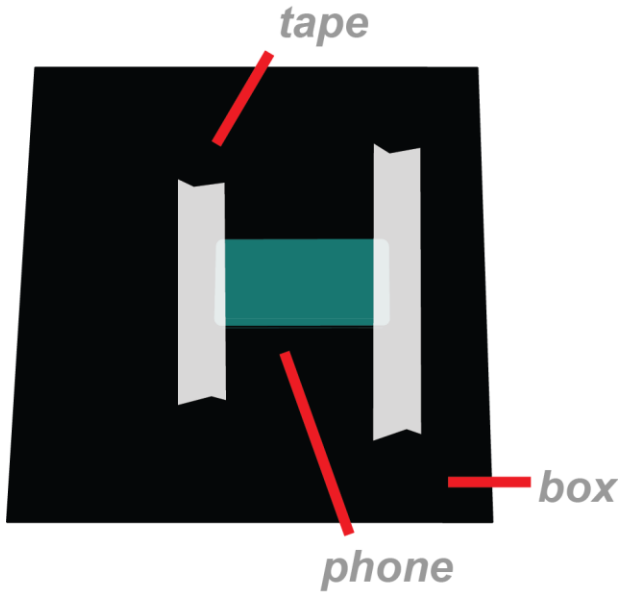
2. SAMPLES: load patient samples

- when you have completed a standard curve run with the app, you are ready to analyze patient samples
- follow the provided sample map to place the tubes containing the samples into the correct positions in the holder
- place the box over the heater, holder, and tubes
- place the smartphone face-up on top of the box, making sure to align the camera lens with the viewing aperture



2. SAMPLES: run patient sample reactions

1. launch the BACTICOUNT app
2. from the home page, choose “Start Bacterial Analysis”
3. select the sample type (blood, urine, feces, or saliva)
4. click “2. Record Sample”
5. type a name for your samples
6. load samples, and ***immediately*** after, press “OK” (this begins the reaction timer)
7. place the box over the samples, and the phone on the box
8. touch the “Tap To Focus” button to precisely align the sample tubes within the map on the viewing screen
9. you can ensure that the phone and system components will not move by taping them into place on a stable surface
10. when the camera position is set, hit the “Begin Recording Amplification” button to start the recording process



3. ANALYZE: use the app to view results

1. launch the BACTICOUNT app
2. from the home page, choose “Start Bacterial Analysis”
3. select the sample type (blood, urine, feces, or saliva)
4. click “3. Select and view results”
5. follow the prompts to select a .pasc calibration file from a standard curve run
6. likewise, select the .parr file from your sample run
7. when the two files are obtained, select “Run BACTICOUNT analysis!”
8. results are displayed in genome equivalents/mL for each of sample tested
9. for future reference, both results and raw data are exported to a folder that can be viewed offline

need help?

- for the full user guide with detailed instructions, please refer to the manual online at:

- www.bacticount.com