FloodLAMP BIOTECHNOLOGIES	Document Number:	SOP201-A
	Effective Date:	02/26/2022
	Version:	2
	PGS48 Manufacturing - Run Form	

PREP

- Safety Procedures:
  - Work in PCR Box or BSC
  - **Designated CLEAN** Lab coat, gloves, face mask, and face shield/goggles
- Wipe down all work surfaces, tube racks, and all items used with 10% bleach solution followed by ethanol (as needed)
- Turn on UV lamp for 15 minutes in PCR Box or BSC (at start or end of prev run)
- Check to ensure you have **sufficient amounts of all materials** for number of lots to be created (Calculator)
- With Scale (USSolid1), **measure 28.2mL of NF Water** in 50mL Falcon Tube, leave **in the refrigerator > 1hr** (prep ahead)**.**
- **Ensure Primer Resuspension NF Water is also in refrigerator > 1 hr** (prep ahead)**.**
- **Do Startup** of the Opentrons (last page).
- **Load Opentrons software**, open protocol "iPGS48_x68_SOP-201-B_2.json"

MAKE PGS

- **Thaw three tubes of each primer set for 10-30min after pulling from freezer**
- Spin down primer tubes after thawing
- Get 50mL Falcon Tube of NF water (at least 10mL), put in rack in hood.
- Add 1000uL of Primer Resuspension NF Water to each tube of primers
  - Do NOT stick pipette tip into tube, primers will stick to tip
  - Air drop (no contact) dispense into tubes, no tip change
- **Vortex for 10s** to dissolve
- **Let sit 1min**, then vortex again 10s
- **Centrifuge** tubes briefly

	PGS48 Manuf SOP Log Form Link ☐ Logged
Location:	
Name:	
Date and Time:	
PGS48 Lots Created:	
Materials	
50mL Falcon Tube (1) • Confirm Product ID & Lot in Log Form	
1.5mL S.C. Tubes (67) • Confirm Product ID & Lot in Log Form	
Nuclease-free Water (2) • Confirm Product ID & Lot in Log Form	
FloodLAMP Primer Tubes (9) • Confirm Product ID & Lot in Log Form	
6M Guanidine HCl • Confirm Product ID & Lot in Log Form	
Pipette ID • Confirm Product ID & Lot in Log Form	
1000uL micropipette tips • Confirm Product ID & Lot in Log Form	
Robot tips (Opentrons 1000uL Filter Tip)	

- Retrieve NF water aliquot from fridge, check that volume appears ~28.2mL
- **Add 600uL of 6M Guanidine-HCl** to NF water in 50mL Falcon Tube
- **Mix** by vortexing 10s
- **Add the full volume of all nine tubes of Primer sets** to the 50mL Falcon Tube
  - Careful to get last bit of liquid (look)
- **Mix well** by vortexing 30s
- Remove cap and pipet any liquid in the cap and add back into the tube
- **Keep PGS mix in refrigerator** until aliquoting or aliquot within 5min

Equipment in AirClean Box

- Mini-centrifuge
- Vortex
- Timer
- 50mL & 1.5mL tube racks
- Tip waste bin

ALIQUOT ON OPENTRONS

- **Arrange the robot deck** according to deck layout shown below
  - Remove lid from tip box, **verify that there is a tip in the back-left position**
  - Fill the back and front row of each of the six tube racks with 1.5mL SC tubes, uncapped [1]
  - Place the 50mL PGS reservoir tube in the plate at position “8” [2]
- **Click** “Proceed to Calibrate” button [3]
- **Follow on-screen instructions** to complete calibration procedure [3]
- **Click** “Start Run”
- When protocol has finished, **cap all tubes** inside the Opentrons hood [4]
- **Transfer tubes** to cryobox, store at -20C.
- Label crybox lid with "PGS48" & Lot#.
- **Click** “Reset Run” on OpenTrons software

[1] Make sure the caps are not obstructing the openings of the neighboring tubes.

[2] Ensure that the 50mL tube is fully seated in the holder with 10mL mark just showing.

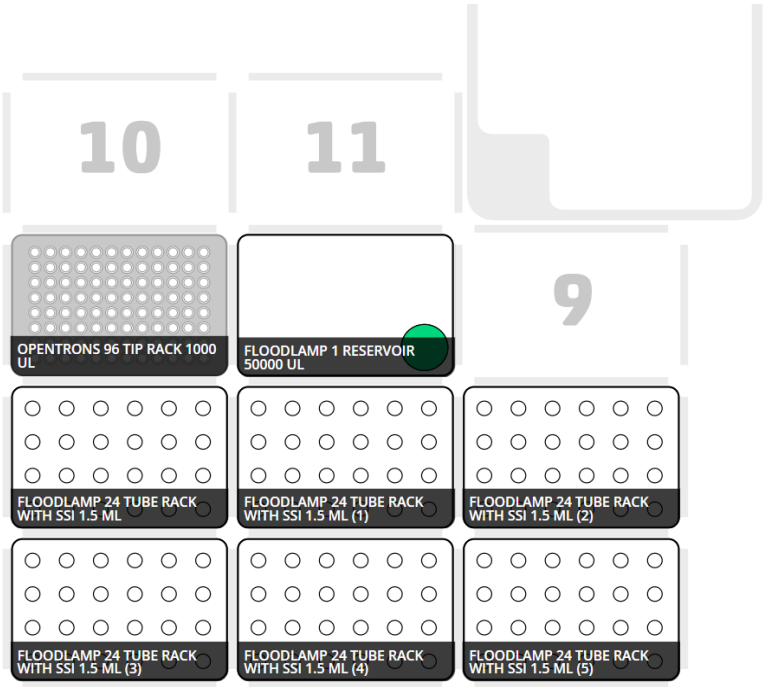
[3] Calibration procedure necessary only on the first run. Subsequent runs won’t require re-calibration until the robot is disconnected / reconnected or a different program is run. Calibrate 1.5mL tube with tube out and center XY with respect to rack hole.

[4] Note that the final 1-2 tubes may be short on volume. These should be either used to do QC verification of the batch or discarded. Write with a marker a "•" on the top of any short tubes.

Measure remaining
volume in Falcon tube:
if outside 450uL =/- 10uL, weigh all tubes indiv
spec is X to Y g, reject any tubes outside spec

Notes:

DECK LAYOUT



OpenTrons Startup

1) Turn on rocker power switch in back left (-- mark).
2) Press flashing blue button on front, wait until solid.
3) If not already open, launch OpenTrons application with shortcut on desktop, should show .
4) Start Hepa unit and put at setpoint.
5) Take lid tipbox cover off.
6) Ensure tip is in back left position of tipbox.
7) Open json protocol.
8) Run calibration.
9) Leave front panel closed (down) when running.

OpenTrons Shutdown

1) Place lid tipbox cover on.
2) Turn off Hepa unit.
3) Turn off OpenTrons rocker power switch (O mark).
4) Close front panel.