

	Document Number:	SOP-104-A_1
	Effective Date:	08/26/2022
	Version:	1
	Amplification Training - Run Form	

PREP

- Pen & sharpie labeled with green tape
- Separation of Post-Amp area
- Heaters on (Heat indicator lit!)
- Safety Procedures:
 - lab coat
 - gloves
 - face mask
 - face shield or goggles
- Alcohol wipe sample tubes

QR CODE	Log Link: SOP-104-B_1 {SITE}
	Location:
	Name:
	Date:
	Time:

CONTAMINATION INFRACTIONS

- PPE lapse/removal
- Use green labeled pen/marker with bare hands and not putting it to be cleaned afterwards
- Use unmarked pen/marker with clean gloves (without cleaning gloves after or changing gloves)
- Bare hands on anything improper, except in desk area
- Reuse pipette tip improperly
- Touch pipette tip to anything and then not discard it
- Touch anything dirty then go back to work (face, mask, uncleaned phone, uncleaned laptop)

NOTE

It is recommended that 1XISS and Reaction Mix be prepared before the assay run (instructions below).

DOCUMENTATION

- Check off each item after completing it
- Write legibly

GLOSSARY OF TERMS

100XIS.....	100-fold concentrated Inactivation Solution
1XISS.....	1X Inactivation Saline Solution
PGS.....	Primer Guanidine Solution
CLAMP MM.....	Colorimetric Loop-Mediated Isothermal Amplification Master Mix
RM.....	Reaction Mix (CLAMP MM and PGS)
TPC.....	Twist Positive Control

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1XISS PREP

- Record volumes of saline & 100XIS in 1XISS Prep form prior to pipetting
- Combine saline and 100XIS in a 50mL falcon tube or 30mL Chub tube, depending on volume
- Vortex 30 seconds
- Label tube with 1XISS ID (e.g., "1X_MMDD-1") and record the same ID into the 1XISS Prep Form and the table on this form

Sample Batch ID (from App):
Inactiv Heater:
1X Inactiv Saline Soln ID:
Dispense: Pipette or Manual Disp or Electric Disp

INACTIVATION

- Add 1mL of 1XISS to each sample tube
- Verify that tubes are properly sealed afterward (i.e., not cross-threaded)
- Vortex 10 sec (if individual tubes) or 30 sec (if tubes-in-rack)
- Heat 8 min in Water Bath (set to 99°C)
- Cool 10 min
- Put in refrigerator before 30 minute mark

REACTION MIX PREP

- Complete the entry in the RM Prep Form prior to pipetting
- Calc volumes correctly for PGS and CLAMP based on number of reactions
- Remove CLAMP MM and PGS from freezer to thaw, but do not allow them to warm
- Briefly spin down reagent tubes (removes reagent from the cap and tube walls)
- Pipette CLAMP MM and PGS into a 1.5mL tube using calculated volumes
- Promptly return PGS and CLAMP source tubes to freezer
- Vortex and spin down the RM tube
- Aliquot 23uL of RM into each reaction tube (plate or strips-of-8)
 - Pipetting at the bottom of tubes
 - No blowout while dispensing reaction mix (to prevent bubbles in bottom of well)
- Visually check the volumes in each reaction tube (that they appear to be even)
- If using a plate, cover filled tubes with rack lid to prevent dust
- At this stage, RM reaction tubes can be capped and placed in the freezer for storage if they are not to be used fresh.



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Amplification Training - Run Form**AMPLIFICATION REACTION**

- Remove PCR cold block from freezer ~10 min before adding tubes
- Prep RM plate/strips (thawed, not warm)
- Set up tips to align with reactions
- Use a lookup rack to keep track of sample order
 - Tip layout aligns with arrangement of sample tubes in lookup rack & aligns with layout of reaction plate/strips
- Add negative control first (2uL 1XISS)
- Add 2uL of each inactivated sample to reaction tubes, visually checking the volume in the tip each time. Pipette up & down 5X, blow out in liquid, tip touch.
- With each add, verify the correct position (tip vs sample tube vs reaction tube)
- Transfer plate/strip to PCR rack (not cold block - rxns don't go to amp cold)
- Retrieve TPC from freezer, touching only with one hand (consider this hand dirty)
- Add 2uL TPC to pos. ctrl, return TPC to freezer (IMMEDIATELY change glove)
- Cap reaction tubes / seal plate
- Change gloves again after loading tubes/plate onto amp heater
- Set main timer for 25 min
- Set secondary timer for 24 min
- Put sample tubes in fridge
- Remove rxns from amp promptly, wait 1 minute before imaging
- Take photo in lightbox
- For photo apply crop and 'Vivid' filter (or increase saturation 70% for android)
- Log amp run sheet with QR code
- On app: Intake, Process and Result tubes

Amp Heater:	Strip8 Tubes or Plates
Reaction Mix: Frozen or Fresh	
Reaction Mix ID:	
Num Reactions (including controls):	
Pos Ctrl ID:	
Neg Ctrl ID (today's 1XISS):	
Initial Inconclusives?:	
Notes:	

FloodLAMP BIOTECHNOLOGIES 	Document Number:	SOP-104-A_1
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	1	2	3	4	5	6
A 1						
B 2						
C 3						
D 4						
E 5						
F 6						
G 7						
H 8						

	7	8	9	10	11	12
A 1						
B 2						
C 3						
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