

Protocol for Clean Lab Practice When Setting Up PCR.

Every individual user of the MNCF should obtain a drawer for their personal use. No one other than the individual who is assigned this drawer may open it. It will be the responsibility of the individual occupying this drawer to clean and maintain their drawer.

Cleaning the drawer:

Clean with EtOH to remove any dust, dirt, or debris
Sprayed with RNase OUT and re-cleaned.

Contents of your drawer:

Tips to fit the P10, 100, 200, 1000 pipettors (label them with your name)
1.5ml tubes (autoclaved)
200µl tubes (autoclaved)
PCR plates
PCR grade water
Notebook

When setting up PCR reactions everyone is to follow the enclosed procedure to ensure continuity and reproducibility while minimizing the possibility of error or contamination.

1. In your notebook, write out your reaction conditions
 - include the calculations for the number of reactions
 - include a positive and a negative control for each primer pair
 - record the reaction profile in your note book
 - save space for a photograph of the gel analysis of your PCR experiment

Example:			X10 reactions
	H ₂ O	11.0µl	110µl
	SYBR green Mix	12.5µl	125µl
	5'-primer (10µM)	0.25µl	2.5µl
	3'-primer (10µM)	0.25µl	2.5µl
	Template	1.00µl	10µl
	Total	25.0µl	250µl

Profile: 95°C | 95°C|65°C | 4°C
 1:30min 1:00min hold
 50cycles

2. Clean bench with bleach
3. Clean pipettors with bleach
4. Clean rack(s) with bleach
5. Remove frozen primers/mixes/template(s) and place in the cleaned rack to thaw
6. While thawing, turn on thermocycler and program the desired profile
7. Fill ice bucket with ice
8. Once thawed, immediately place all reaction components on ice

9. Set-up the PCR reaction “on-ice” according to the calculations written in your notebook; **USE MASTER MIXES TO REDUCE ERROR IN PIPETTING**
10. Dispense master mixes into PCR tubes or plates and complete reaction set-up
11. Walk the reactions to the thermocycler on ice and place into thermocycler block as quickly as possible
12. Close lid and begin reaction
13. Return all reaction components to their original positions (-20°C freezer)
14. Return ice bucket to sink
15. Place tips, notebook, unused tubes/plates, and water back into drawer
16. Clean pipettors and bench top with bleach