

PCR Setup

Version Number: 1

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Edited by:

Materials/Reagents/Equipment	Vendor
Disposables	
96-well PCR plates	E&K (Cat#: 489096)
Pierce Mat	E&K (Cat#: 402096)
15ml conical tube	
Reagents	
Oligonucleotides (primers)—5nm	Integrated DNA Technologies (IDT), 96-well plate in Freezer
Genomic DNA working stock (4ng/ul)	ATCC, box labeled Working Stock in Freezer
dNTPs (25mM)	Promega (Cat#: U1240), Freezer
Deep Vent Polymerase, ThermalPol Buffer	New England Biolab (NEB), (Cat#: 0258L), Freezer
Equipment	
Biomek 2000	Beckman Coulter
PCR machine	Applied Biosystem
Ice bucket	

Reminder:

1. **TURN ON THE WATER BATH AT LEAST 30 MINUTES BEFORE STARTING THE EXPERIMENT.**
2. **PUT BLACK RESERVOIR HOLDER INTO THE FREEZER.**

Note: For primer ordering, please refer to **Primer Order** protocol.

PROCEDURE:

I. Preparing the genomic plate:

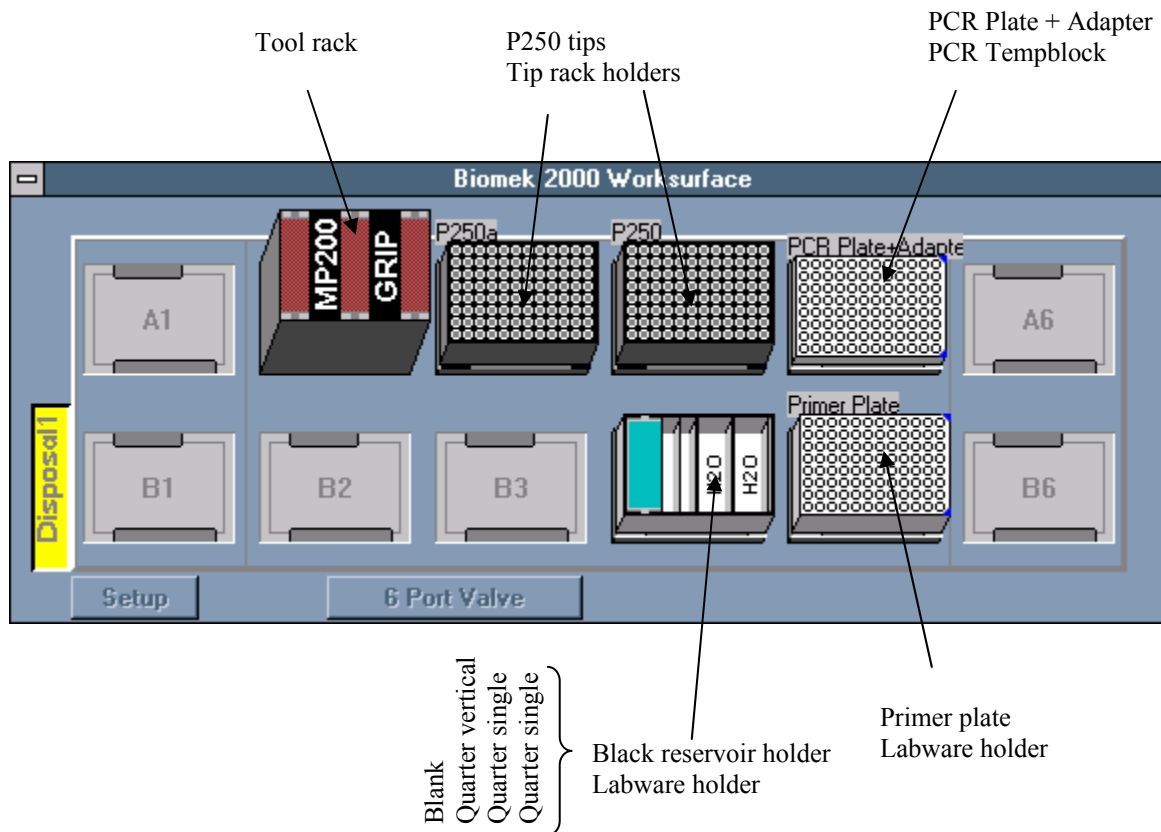
- ___ 1. Referring to the primer map to select the genomic DNA working stock (4ng/ul) from the 318 Freezer.
- ___ 2. Thaw them in an ice bucket. (Note: Dilute the stock DNA to 4ng/ul if necessary)
- ___ 3. Dispense 5ul of genomic DNA for each rxn in a 96-well PCR plate (on ice), according to the map (See Primer Protocol)

II. Preparing the rxn master mix:

- ___ 1. Prepare the following PCR rxn mix in a 15ml conical tube on ice: (Volume = 75ul / rxn mix)

Reagents	Volume (ul), n= # of samples
ThermalPol Buffer	10ul x (n +5) =
dNTPs (thawed on ice)	1ul x (n +5) =
Deep Vent Polymerase	2ul x (n +5) =
H ₂ O	62ul x (n +5) =

III. Setting up the Biomek worksurface:



IV. Preparing the reagents:

- ___ 1. Place the genomic plate on the PCR Adapter at position A5.
- ___ 2. Place the primer plate at position B5.
- ___ 3. Fill the quarter single reservoirs with autoclaved water.
- ___ 4. Fill left section of the quarter vertical with rxn master mix.

V. Running Biomek method:

- ___ 1. Double click on Biomek Lab Book Manager icon on the desktop.
- ___ 2. Select the **PCR SET UP** folder and click on **Set as Current Lab Book**.
- ___ 3. Click **Close**.
- ___ 4. Double click on Biomek Edit icon on the desktop.
- ___ 5. Click **Method** → **Open** → Select **PCR Setup 080803**.
- ___ 6. Click on **Edit** → **Patterns** → Select **PCR Plate Pattern** → **Allow Changes** → Set the pattern to match the pattern on genomic plate → Click OK to confirm the pattern.
- ___ 7. Click on **Edit** → **Patterns** → Select **N-Primers Pattern** → **Allow Changes** → Set the pattern to match the N-pattern on the primer plate → Click OK to confirm the pattern.
- ___ 8. Click on **Edit** → **Patterns** → Select **C-Primers Pattern** → **Allow Changes** → Set the pattern to match the C-pattern on the primer plate → Click OK to confirm the pattern.
- ___ 9. Click on the running man button to start the method. (Note: Save all the settings and click **Accept All** to confirm the configuration.)

After running the method:

- Cover the primer plate and store unused primers in Freezer 318.
- Cover PCR plate with mat and keep cold until running the PCR rxn.
- Turn off the water bath and clean up after use.

VI. PCR machine Setup:

- ___ 1. Turn on the PCR machine at least 5 minutes before use.
- ___ 2. Select Create → Use arrow keys to change from 25 to 35 cycles → Change the time under the first 72°C from 0:30 to 2:30 → Press Start → Use arrow keys to move the highlight down to 9600 → Press Max → Use arrow keys to move the highlight back to 50 and change it to 100 → Press Start → Wait until the temperature reaches 103°C → Put the prepared PCR plate into the 96-well heating block → Close and lock the lid.