PCR Setup

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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)

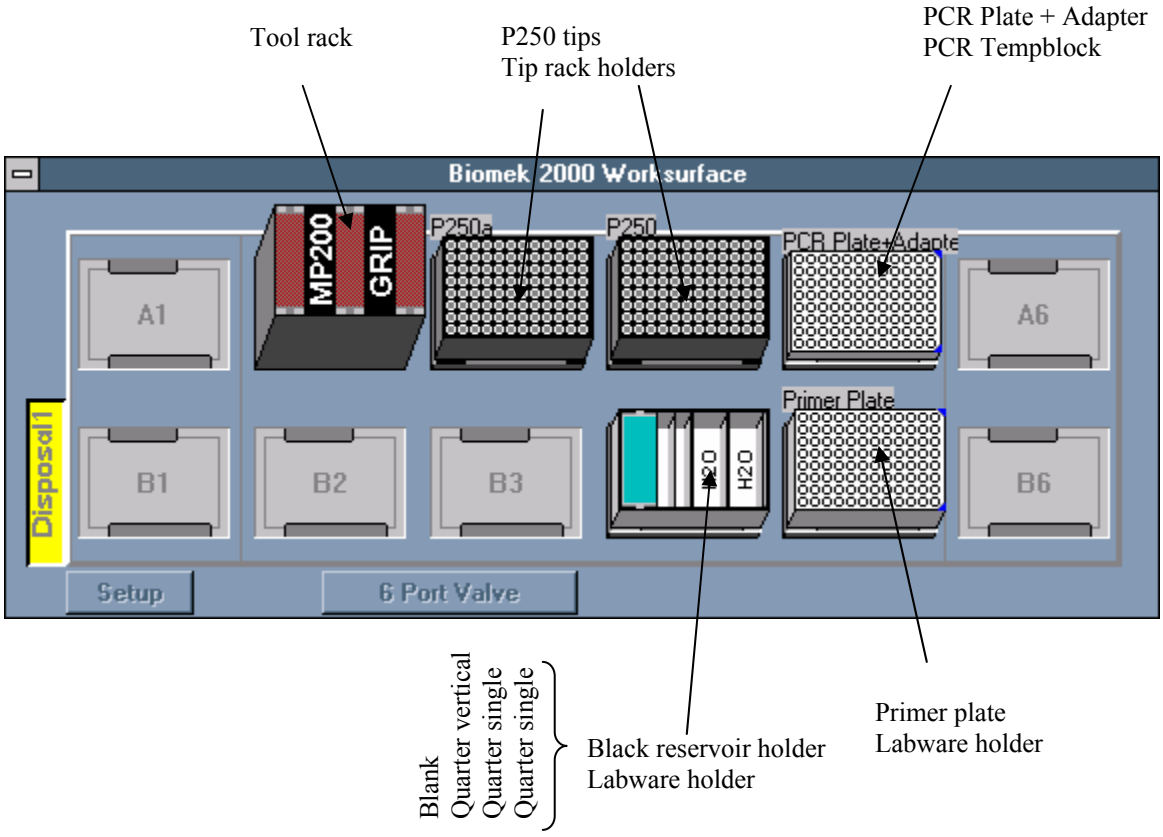
___3. Dispense 5ul of genomic DNA for each rxn in a 96-well PCR plate (on ice), according to the map (See Primer Protocol)

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_2O	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^{o}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^{o}C→ Put the prepared PCR plate into the 96-well heating block→ Close and lock the lid.