DNA Preparation

The amplification buffer was patterned after the PCR super mix by Gibco BRL. A 1.1X concentration buffer mix was made using the concentrations and the buffer described by the product sheet.
(by Mike Wilson: mwilson@niaid.nih.gov)

Reagent	[Rxn]	[1.1X]	[Stock]	X-Fold dilution	Vol./mass added
Tris/HCl pH 8.4	20mM	22mM	1M	45.45X	15.5ml
KCl	50mM	55mM	dry powder		2.89g
$MgCl_2$	1.5mM	1.65mM	1M	605X	1.162ml
dATP	200 µM	220 µM	100mM	454.5X	1.55ml
dCTP	200 µM	220 µM	100mM	454.5X	1.55ml
dGTP	200 µM	220 µM	100mM	454.5X	1.55ml
dTTP	200 µM	220 µM	100mM	454.5X	1.55ml
Acetamide (only for genomes with high G-C content)	5%	5%	dry powder		35g
H_2O					682
Total Volume					704ml

Aliquot the 1.1X Buffer and keep in –20 freezer. To this buffer add the right amounts of taq polymerase and genomic DNA to equal the final concentration indicated below. Dispense 60-100µls of this mix in each well of the PCR plate using the 50-300µls pre-PCR multi channel pipettor. Then add the primer mix. This can be stored at -20^oC until you are ready to run the PCR.

The PCR reaction is done in 96 well format with the following final concentrations per well.

- Taq Polymerase 2 units
- Genomic DNA 10ng
- PCR Buffer 1X
- Primer pairs 0.4 µM

(We receive left and right pairs of our primer sets in 2 separate 96 well. We make a separate stock of 25µM concentration for each pair. From these 25µM plates, we make a 5µM mix plate of the left and right primers, which we use as our working stock.)

PCR cycles

cycle 1:	94^o for 1:00	1 repetition
cycle 2:	94^o for 0:40"	
	52^o for 0:40"	30 repetitions
	72^o for 1:15	
cycle 3:	72^o for 5:00	1 repetition
cycle 4:	4^o hold	

(Modify temperature to optimize specific PCR product.)

Running the gel for product size

1. Make a 2.0% agarose gel in 1X TAE. (Use 150mls per tray.) Pour the gel in the tray on the bench. Use the specially made 26 well combs. (These combs are used to specially load samples from 96 well plates. Applied Scientific: genome style)
2. Make up the samples to load as follows. Transfer 2µl of the sample for each of the PCR wells into a disposable 96 well plate containing 8 µl ddH_20. Add to it 2µls of the 6X dye
3. Add the marker Phi X DNA to the first and the last well of each of the four sections of the gel.
4. To all the other wells, load 10 µl of sample. The first row of wells on the gel will contain rows A and B from the microtiter plate. The arrangement will be as follows: A1, B1, A2, B2, A3, B3..... The second row of wells will contain rows C and D etc.
5. Run the gel at 100 volts for one hour.
6. Stain the gel by shaking it for 15 min in an EtBr solution. (The EtBr solution is made up as follows: 50µls of EtBr to 500mls of water. EtBr stock is 1mg/ml). Wash with water for a few minutes.
7. Take picture and save image. We analyzed our images using the Whole Band Analyzer

Ethanol Precipitation (This is step is optional)

Currently, we do not Ethanol Precipitate our PCR products. According to our experience, EtOH precipitation increased irregularities in DNA concentrations from sample to sample by losing products in one, while concentrating in another. If you are using commercially available PCR buffer, which contain detergent or BSA, you may not be able to bypass the EtOH precipitation step. Detergent makes the spots unusually large, which causes spots to run into each other resulting in contamination. BSA may also interfere with printing and hybridization. If you need to Ethanol precipitate your PCR products, the following is our protocol.

1. Thaw PCR products and spin them down in centrifuge.
2. Add 150 µl of EtOH / sodium acetate solution into each well.
3. (3M Sodium Acetate in 95% Ethanol)
4. Cover plates and store in –20 freezer for overnight precipitation.
5. Centrifuge at 4500 rpm, which is the max speed for the Beckman Centrifuge, for 1 hr at 4 degrees.
6. Dump the solution and pat dry on a napkin.
7. Fill each well with 250 µl of 70% Ethanol, and keep in the freezer until the centrifuge is available.
8. Centrifuge again for 35 min.
9. Dump the ethanol solution
10. Dry in speed vac (located on the 4^{th} floor)
11. Set at medium heat and dry for 2 min.
12. The samples will not be completely dry at this time.
13. Resuspend in 40 µl H_20. Keep the samples at 4 degrees, then transfer to –20 freezer.