PROTOCOL FOR PJAZZ® CLONES

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Simplifying Genomics

METHOD FOR HIGH THROUGHPUT SEQUENCING OF PJAZZ CLONES

Protocol courtesy of Laboratory for Genomics and Bioinformatics, University of Oklahoma Health Sciences Center, OK
http://www.microgen.ouhsc.edu

1 Prep DNA using Millipore Montage Plasmid Miniprep 96 well kit.

2 Set up the sequencing reactions using Phenix 384-well FrameStar PCR plates.

- **On ice, make a 2X Master Mix for 100 rxns:**
  - 16.50 µl Big Dye V3.1
  - 0.78 ul Primer @ 320 pmol/µl (2.5 pmol/rxn)
  - 118.00 µl 5X ABI sequencing buffer
  - 164.72 µl DIUF water
  - 300.00 µl Total
- ***Use 3µl of 2X Master Mix per rxn***
- ***Add.3ul of template (60ng-180ng) per rxn***

3 Cycle.

- ***95 ºC for 4 min***
- ***then 25 cycles of:***
  - 95 ºC for 15 sec
  - 55 ºC for 5 sec
  - 60 ºC for 2 min

4 Hold at 4 ºC until ready for use.

5 Clean up with ethanol precipitation or Sephadex (G-50).