TITLE: **MICROARRAY cDNA CLONE GROWTH AND TEMPLATE MINIPREPPING**		PAGE: 1 of 3
SOP #: M001	REVISION LEVEL: 1	EFFECTIVE DATE:
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1. PURPOSE

This protocol describes clone handling, plate replication, and DNA template preparation in a 96 well format.

2. SCOPE

This procedural format is utilized by Human Colon Cancer and Mouse microarray projects under the supervision of John Quackenbush within the Eukaryotic Genomics Dept.

3. MATERIALS

3.1 Falcon 96 well U-bottom Plate (BD Biosciences; Cat # 353077)
3.2 96 well Square-Well Blocks (Qiagen; Cat # 19573)
3.3 Square-well Block lids
3.4 Costar 96 well V-bottom Plate (Corning; Cat # 3897)
3.5 Tape Pads (Qiagen; Cat # 19570)
3.6 Airpore Strips (Qiagen; Cat # 19571)
3.7 2 XYT media + Ampicillin (50 μg/mL)

Note: For growing clones a variety of media are acceptable. 2XYT media was selected for this application primarily because it filters more easily on the Eppendorf 5 Prime® (E5P) robotic miniprep system.

4. PROCEDURE

4.1 Clone Replication (Shallow Well Plate)

4.1.1 Before handling clones review proper handling techniques as outlined in SOP: T013.1

4.1.2 In Falcon U-bottom plates pipette 150 μL of 2XYT media + Ampicillin (50μg/mL).

4.1.3 Remove 96 well cDNA clone plates from –80° C freezer and allow them to thaw gradually at 4° C. If there is a tape cover on the clone plates, carefully remove the tape as soon as the plates are removed from the –80° C freezer in order to minimize cross contamination.

Note: Depending on the method of replication the degree of thaw may vary. For a robotic inoculation a liquid state is necessary to ensure complete replication; however, for a manual transfer a semi-solid state may be sufficient and can prevent the unnecessary thawing of clones.

4.1.4 For a manual inoculation using sterile disposable tips, transfer 5 µL of inoculum from the original clone plate to the Falcon U-bottom plate containing 150 µL of 2XYT media + Amp (50 µg/mL). The transfer can also be completed manually by using a 96 prong replicator. For high-throughput applications a robotic system (i.e. Genetix GeneTAC® G^3) is faster and more consistent.

4.1.5 Once the inoculation is complete original clone plates shall be covered with a tape sheet and immediately returned to –80°C or placed on dry ice until they are returned. The newly inoculated copy plates are placed in 37°C for 8 hours.

Note: Some replica sets have clones that grow at very different rates. The day growth is used to unify rates and provide more consistent growth. If the replica set you are working with has been replicated several times or is already yielding very uniform growth then the day growth may be omitted.

4.2 Clone Replication (Square-well Blocks)

4.2.1 Fill Qiagen Square-well blocks with 1250 µL of 2XYT media + Amp (50µL/mL) per well.

4.2.2 Inoculate Square-well Blocks.

- After the eight hours of day growth inoculate the deep well blocks manually either by pipette (~5 µL) or with 96 well replicating prong.

- One can also use the Genetix Q-bot® (or comparable robotic replicator) to replicate from shallow well plates to square-well blocks. (see SOP: T007.2 for Q-bot operation)

- If day growth is not necessary then inoculate directly from a selected source plate into a square-well block.

4.2.3 Cover square-well blocks with Airpore strips or Square-well block lids and incubate at 37°C/200 rpm for 16 hours (overnight) in a shaking incubator.

4.3 Miniprep

4.3.1 After the overnight growth, record any wells that failed to growth, spin down the blocks at 2700 rpm for 4 minutes to pellet the cells.

4.3.2 Decant the media from the blocks into a flask containing bleach.

Note: When decanting invert block quickly and cleanly to minimize any cross contamination between wells.

4.3.3 Blot any residual media by placing an inverted block onto an absorbant towel on a sterilized surface. Use a fresh towel for each block and discard used towels in designated autoclave receptacles.

4.3.4 Cover the blocks with tape sheets and store at –20° C.

4.3.5 Label Costar 96-well V-bottom plates (which will serve as collection plates) and cover them with loose fitting lids (one can use the square-well block lids for this purpose as well)

4.3.6 If working within TIGR inform the lab technicians operating the E5P robotic miniprep station of where the blocks and collection plates are located and a reasonable completion time.

4.3.7 Once the minprep has been completed the collection plates will be returned with ~100 uL of miniprep solution colored by blue dextran. Seal each plate well with an adhesive foil lid and store at –80° C.