

## Titration of Library

### I. Determining Correct Serial Dilution

We will select one or two “positive” TAC pools for further screening. In order to make efficient use of

Step 1. Make serial dilutions directly from the positive TAC pool by taking 20  $\mu$ l of the pool and inoculating 1.98ml of LB + Km broth (100x dilution or  $10^{-2}$ ).

Step 2. Make serial dilutions from  $10^{-2}$  to  $10^{-5}$  and plate 1ml of each dilution onto LB + Km agar plates (150 mm). Grow for 16 hours at 37C.

#### START ALTERNATIVE

Step 1. Grow 100 $\mu$ l of TAC pool in 50ml of LB + Km broth at 37C 200rpm for 8 hours. The goal is to plate a culture that is in log phase as determined by absorbance at 600nm (0.6-0.9 Abs at 600nm). If cultures are passed log-phase, transfer 1ml of the 8 hour culture to 50ml of LB + Km broth and grow at 37C 200rpm for around 4 hours until log phase is reached.

Step 2. Make serial dilutions of  $10^{-1}$  to  $10^{-8}$  and plate 1 ml onto LB + Km agar plates 150 mm and grow for 16 hours at 37C.

#### END ALTERNATIVE

Step 3. Count colonies and tabulate results.

#### Colony counts for serial dilutions

Description	Dilution Factor	# Colonies per Plate
Pool _____	$10^{-1}$	
Pool _____	$10^{-2}$	
Pool _____	$10^{-3}$	
Pool _____	$10^{-4}$	
Pool _____	$10^{-5}$	
Pool _____	$10^{-6}$	
Pool _____	$10^{-7}$	
Pool _____	$10^{-8}$	

Our Goal is to obtain a total of 9216 clones. This goal is based on a practical constraint of the Q-Pix robot that we will use to pick and grid the TAC clones. We will learn that the Q-Pix can pick approximately 3000 clones per hour. Also, when we grid to membranes, we will use the 3X3 grid pattern which allows 24 plates to be replicated into six fields. The Q-Pix can efficiently pick at a colony density of ~500 colonies/ 150 mm plate. Therefore we will need >18 plates before using the Q-Pix to pick colonies.

Step 4. Estimate the probability that we will recover the clone(s) of interest from a secondary screen of 9216 clones.

## **Reagents:**

### LB

	Per Liter
Bacto-tryptone	10 gm
Bacto-yeast extract	5 gm
NaCl	10 gm

pH to 7.5 with NaOH

### LB/ 5% Sucrose

	Per Liter
Bacto-tryptone	10 gm
Bacto-yeast extract	5 gm
NaCl	10 gm
Sucrose	50 gm

pH to 7.5 with NaOH

### Kanamycin

25 mg/ml in H<sub>2</sub>O; sterilize by filtration through 0.22 micron filter  
Use 1 ml/liter for TAC vector in DH10B E. coli  
(accepted range 1- 2 ml/liter)