

6/20/13

- Steven Allen

MCF = microcentrifuge

### Preparing Competent Cells

- From seed stock...
- fill ice bucket
  - pre-chill 12 MCF tubes
- transfer culture evenly into the 12 MCF tubes
- centrifuge (the smaller one) for 10 minutes
  - + lower speed setting
- decant supernatant into bleach
- resus. in 800  $\mu$ l cold CCMB80 buffer
  - add 400  $\mu$ l --- ~~in~~ in/out on cell wall w/ pipet!!
  - then, add another 400  $\mu$ l
  - do NOT vortex
  - swirl cells at end by hand
- incubate on ice --- 20 mins.
- Lent. 10 min. (same as above)
- decant supernatant 175
- resuspend in ~~400~~  $\mu$ l cold CCMB80 buffer
  - combine 4 tubes into 1
- incubate on ice --- 20 mins.
- pre-chill ice ~~bag~~ 3 MCF tubes
- store in large freezer

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Started Plasmid Preps of K824012 (probably contaminated),  
Nitrate Detector A, J044 50-1, and K824008.