PA14NR Set PA14 Parental Transposon Insertion Library **Production Flow Chart Production Flow Chart** PA14 Tn Library plate -80°C Storage ARB PCR (wor) Culture (wor) $(280 \mu L)$ Streak PA14NR Set clones 70 µL onto LB + Antibiotic Master (mas) Add glycerol Manually pick 5,459 single/(non-SCV) Mix and Seal Supernatant (sup) colonies into 600 µL LB + antibiotic Grow in HiGro 16 hours Duplicate (dup) Robotically add glycerol (15%) ~60 Master blocks and mix Transfer 40 µL glycerol/culture mix to 10 storage plates containing 160 µL Working (wor) LB+antibiotic+glycerol (15%)- store-80°C Leftover culture/ Storage plates Glycerol Mix (10 copies) Inoculate media in deep well blocks with thawed culture from storage plates. PA14NR Set copies Grow and transfer to storage plates for Distribution as described above.