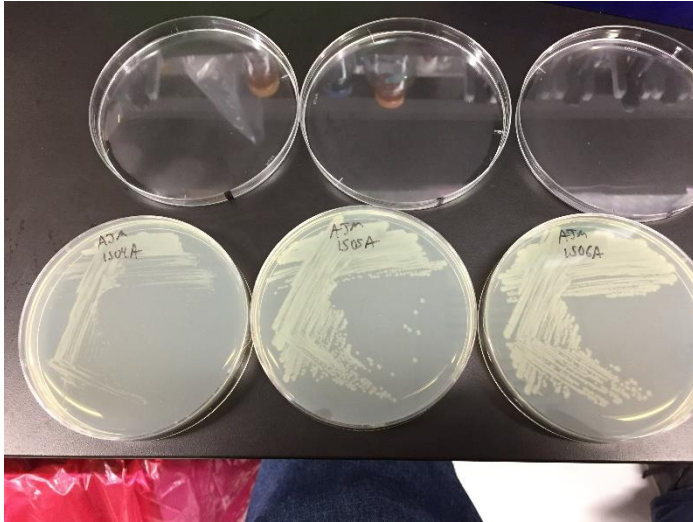


## Verification Protocol for Marionette Strains- Voigt Lab

- Inoculate 2 ml LB + 5 ug/ml Chloramphenicol with the stab and grow at 37°C until very cloudy (~8 hrs). Confirm strains grow in 5 ug/ml Chloramphenicol.
- Streak out culture on plain LB-agar plates (5 ug/ml Chloramphenicol is ok). Grow overnight at 37°C (~16 hours). Confirm size of colony (i.e., small sAJM.1504, large sAJM.1505 and 1506).



- Also use 1 ul culture in a 10 ul PCR:  
5 ul KAPA HiFi HotStart ReadyMix (2X)  
0.4 ul Forward primer (10 uM)  
0.4 ul Reverse primer (10 uM)  
1 ul culture  
3.2 ul water  
  
95°C 3min  
25 cycles of (95°C 30sec, 60°C 30sec, 72°C 60sec)  
72°C 5min
- primers to confirm Marionette insert:  
oAJM.029      CCACAGCAATGGCATCCTGGTCATCCAG  
oAJM.769      CCATGCACGTTTTGTTGTA CTGATTGCAGAAGG  
expected size 1171 bp for sAJM.1504, 1505, 1506.
- primers to confirm Marionette insert:  
oAJM.585      ATAACCAATCAGGCTTCCTACTTACAGAATTG  
oAJM.688      CAGCAGAAAATCCAGATCGGCTTTAAAGC  
expected size 526 bp for sAJM.1504, 1505, 1506.

- primers to confirm background strain identity (dcm):
  - oAJM.1083 GCGGATCGTCAAAGTCTTTCTCACC
  - oAJM.1084 GGCTGGCGTATCGAAAAAGAACTCGCexpected size of 632 bp for sAJM.1504 and 1506 (no band for sAJM.1505)
  
- primers to confirm background strain identity (araB):
  - oAJM.1085 CGCCGATTGGCCTGCTTCCAGAC
  - oAJM.1086 CAGTTCCGTCATCATCCGCGTGACexpected size of 867 bp for sAJM.1505 and 1506 (no band for sAJM.1504)