Integration of pPL2 derivatives into *L. monocytogenes* 10403S

adapted from Lauer et al. (2002)

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Required Materials:

sterile 47mm diameter 0.45μm HA-type filters (Millipore) (1)
- sterilize filters by autoclaving wrapped in foil

BHI broth (~30ml)

BHI plates (1)

BHI plates + 7.5μg/ml chloramphenicol & 200μg/ml streptomycin (~4) – prewarm to 30˚C

LB top agar (6ml) – temper to 46˚C

LB broth + 25μg/ml chloramphenicol (5ml)

Protocol:

1. Grow *E. coli* SM10 containing pPL2-derived plasmid (donor culture) and *L .monocytogenes* 10403S overnight in 5ml broth. SM10 donor culture is grown in LB/Cm(25) and 10403S is grown in BHI.
2. Inoculate 5ml of appropriate fresh broth with 100μl of each overnight culture.
3. Grow *E. coli* donor culture and *L .monocytogenes* 10403S to OD600 = 0.5 – 0.6 at 30°C with shaking (approximately 3.5 – 4.5 hours). If you give the *Listeria* a half-hour head start, the cultures will be ready at about the same time.
4. Mix 2.5ml of donor culture with 1.5ml of 10403S culture.
5. Filter mixture onto sterile 47mm diameter 0.45μm HA-type filter (Millipore).
6. Wash filter with 10ml BHI.
7. Place filter on a BHI plate and incubate for 2 hours at 30°C.
8. Resuspend cells gently in 2.5ml BHI.
9. Plate 25μl and 50μl aliquots in 3ml LB top agar on BHI/Cm(7.5), Sm(200) plates.
10. Incubate plates overnight at 30°C.
11. Transfer plates to 37°C and incubate overnight.
12. Pick individual colonies and streak for isolation on BHI/Cm(7.5), Sm(200) plates.
13. Make lysates and screen for integration with PCR:
    primers: NC16, PL95, 2003.4.1A
    expected products: NC16 – PL95 = 499bp
    PL95 – 2003.4.1A = 700bp (if multiple integration has occurred)
    conditions: 30 cycles, annealing temperature = 55°C
14. You can also PCR the promoter region of the integrated plasmid to confirm the presence of the desired construct.
    primers: JUPR, 2003.2.3A
    conditions: 25 cycles, annealing temperature = 58°C