Materials

*Bacteria*

*E. coli MFD*λ*pir* with pSAM\_Am

*A. muciniphila ATCC BAA-835*

*Media*

LB Media

BHI + 0.25% mucin (BHIM)

*Antibiotics*

Tetracycline (5 mg/mL stock in 50% EtOH)

Ampicillin (50 mg/mL stock)

Kanamycin (50 mg/mL stock)

*Miscellaneous*

Diaminopimelic acid (100 mM stock)

Disposable Cell Spreaders (Fisher 14-665)

Day -1:

1. Streak -80**°**C glycerol stocks of donor (*E. coli MFD*λ*pir* with pSAM\_Am) onto LB agar with tetracycline (10 μg/mL), ampicillin (50 μg/mL), and DAP (0.3 mM). Incubate at 37**°**C under aerobic conditions overnight.
2. Streak -80**°**C glycerol stocks of recipient (*A. muciniphila ATCC BAA-835*) onto BHIM agar plates, pre-reduced overnight in anaerobic chamber (pre-reducing plates is optional). Incubate at 37**°**C under anaerobic conditions for 24-48 hours.

Day 0:

1. Inoculate an isolated colony of *A. muciniphila* into 5 mL pre-reduced liquid BHIM media 24 hours prior to harvest (doubling time is approximately 1.5 hours). Incubate at 37**°**C under anaerobic conditions (no shaking necessary).
2. Inoculate an isolated colony of *E. coli* donor into 5 mL liquid LB media with tet + amp + dap approximately 16 hours before harvest. Incubate at 37**°**C with shaking at 225 rpm aerobically.

Day 1 (coordinating *E. coli* and *A. muciniphila* growth):

1. Dilute *E. coli* cultures 1:100 and *A. muciniphila* cultures 1:20 in 10 mL fresh media and incubate under same conditions as previously. Target OD600 for both cultures is 0.5. Once cultures have reached mid-log-phase (OD600 of between 0.3 and 0.6), it is time for harvesting cells (usually you will need to adjust down the *E. coli* culture to get a 1:1 ratio of *E. coli* to *A. muciniphila*).
2. Transferring *A. muciniphila* out of the anaerobic chamber, spin down *E. coli* and *A. muciniphila* at 3,000 x g for 5 min. Decant the supernatant from both. Resuspend only *A. muciniphila* in 100 uL BHIM.
3. Wash the *E. coli* cells with 10 mL fresh pre-reduced BHIM. Spin down *E. coli* one additional time, and resuspend in 100 μL BHIM.
4. Combine concentrated cultures and mix thoroughly by pipetting gently up and down.
5. Spot 2 BHIM + 0.3 mM DAP with 10 x 10 μL of cell mixture. Dry plates under laminar flow, then invert and incubate for 5 hours at 37**°**C under aerobic conditions.
6. After 5 hours, add 2 mL liquid BHIM to plates and resuspend mating spots. Aspirate the resuspended cells and dilute 10X in fresh BHIM (between 10-20 mL)
7. Spread 500 μL onto BHIM+ 2 μg/mL tetracycline and 25 μg/mL kanamycin (optional) 15 mm agar plates. (optional step) To calculate conjugation efficiency, prepare 10-fold serial dilutions in BHIM, and transfer to selective BHIM plates (with 2 μg/mL tet) and non-selective BHIM plates (we routinely get an efficiency around 1e-4 to 1e-5). Transfer plates into the anaerobic chamber, incubate at 37**°**C and examine after 24-48 hours for growth of transconjugants.

Day 2/3:

1. Transconjugants will appear as small translucent colonies on BHI + 0.25% mucin media.
2. Harvest transconjugants in BHIM. (optional) Wash transconjugants by centrifugation to remove solid agar debris and dead cells. Adjust OD600 to 2 with BHIM and mix with an equal volume of 50% glycerol. Aliquot 1 mL into cryotubes.

NOTE: We spot check colonies to verify the presence of the tetracycline resistance gene and an Akkermansia-specific sequence.