Constructing functional metagenomics protocol

Protocol (Penders):

1. Penders generated 21 functional metagenomic libraries and screened for antibiotic resistance
   * Each library contained 10 samples from 4 different travel regions (Northern Africa, Eastern Africa, Southern Asia, Southeastern Asia) were pooled together for each library
   * Partial restriction digestion w/ BamHI, BglII, BstYI
2. Select fragments >700bp
3. Linearize vector pZE21-MCS w/ BamHI and ligate with 100-150ng of selected DNA fragments
4. Electroporation of *E. coli*
5. Extract plasmid DNA w/ functionally screened fragments w/ kit
6. Sequence plasmids
7. Quantify relative abundance of AMR genes

Modifications to Pender’s protocol:

1. Pool samples based on more specific regions (ideally by country)
2. Use different host cells (thermal transformation rather than electroporation)
3. Screen w/ different range of antibiotics, and also includes those used on animals
   1. Significance: usage of animal antibiotics drives selection and acquisition of such resistance even in hosts?
4. Screen w/ alcohol and one bacteriocin (refer to ref 4)
   1. Significance: hygiene, alcohol consumption drive changes in resistome
   2. Significance: non-native bacteria producing bacteriocin drive changes in resistome
5. Screen w/ heavy metals
   1. Significance: heavy metal resistance co-selected with resistance genes, may provide understanding on mechanisms of transmission
      1. Coselection shown in wastewater, agricultural soil, livestock
      2. Links found between zinc/copper susceptibility and pig Salmonella isolates, which are foodborne pathogens
      3. Lots of evidence show link lab setting, but less so in farm and field obervsations
      4. No evidence showing direct link in human gut
      5. Refer to ref 1, 2

Relevant readings:

1. <https://www-sciencedirect-com.eproxy.lib.hku.hk/science/article/pii/S0048969720362318>
   1. Coselection of AMR/MGEs when heavy metals are around in poultry farm isolates
2. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4790313/>
   1. Review on coselection of heavy metal & antibiotic resistance (refer to section 4.2-6), biocides discussed in section 4.1
3. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8182900/>
   1. Dutch functional metagenomic protocol
4. <https://link-springer-com.eproxy.lib.hku.hk/article/10.1007/s00253-014-6004-0>
   1. Ethanol stress & tolerance test protocol
   2. Ethanol tolerance and growth test under ethanol stress were both measured