1. Update on Country Information:

* Only 8 out of 40 countries had more than 10 visits (pls refer to country\_count.csv), so maybe it isn’t feasible to pool DNA by country, and we should stick with region...
* Some countries have large pools of travelers, such as Japan (n=41), mainland China (n=34), Russia (n=23) and Italy (n=18). I think looking at metagenomic data for these countries should *hopefully* lead to some interesting results...
* 13 participants traveled to more than one region. I’m not sure how Kan assigned the participant’s regions because she only selected one region for these ‘multiple-region’ participants. I don’t think this is a big issue for the functional metagenomics, we can just pick the region that the participant visited more often. (e.g. One participant visited Beijing and multiple European countries, so I’ll assign them to be pooled with the EU samples for functional metagenomics)

1. Update on Functional Metagenomics Protocol

* SzeWangs protocols used a PCR purification kit after gel extraction. I think we should order it as well, should I ask quotation from Bun, and we can add it to the Gel Extraction Kit order? (<https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/dna-clean-up/qiaquick-pcr-purification-kit/?catno=28506>; cat. No. 28104). Sorry for overlooking this, I assumed that the gel extraction kit would also purify DNA.
* Also, I’ve drafted 11 antibiotics that we could use for plate screening (pls refer to ‘List of Antibiotics 1.xslx’, sheet titled ‘Draft Antibiotics’). 8/11 are listed as critical antimicrobials by WHO (<https://www.who.int/publications/i/item/9789241515528>).
* I also went on the CLSI website to find the ideal MIC breakpoints for ampicillin (because Sze Wang wrote 100ug/mL and that was really high). Since ceftiofur is mainly used for swine pathogens, CLSI didn’t establish a resistance MIC breakpoint in human isolates, so I had to use the breakpoint from the vetinary protocols. <https://clsi.org/standards/products/free-resources/access-our-free-resources/>
* What are your thoughts on this list? Should we run this list by Hein? There are a couple of antibiotics I wanted to test at first, like vancomycin and erythromycin, but according to CLSI *E. coli* is intrinsically resistant to them, and is a poor target bacteria for testing its resistance...
* Also, Sze Wang seemed to do MIC determination using the AST machine with clones after plate screening – I remember last time we said we didn’t really need to do it because we don’t really care about their MIC, but I just realized it might be a good way to see if the AMR genes isolated from one clone can confer resistance to multiple antibiotics... What do you think?

1. Plan for this week

* Thursday
  + Reculture DH5-Alpha cells to test viability and to prepare for electro-competency
  + Hilda is also coming to the lab, so she can show where the plasmid vectors are and we can culture it for extraction
  + Prepare antibiotic plates
* Friday
  + Prepare DH5-Alpha cells to be electrocompetent using Sze Wang’s protocol
  + Extract plasmid vector