Checklist for a new microbiome sequencing project

Projects usually fall into three phases: 1. Planning, 2. Sampling & Sequencing, 3. Data analysis. Each phase may include the following steps.

1. Planning
   - Decide whether the project theme is discovery based or hypothesis driven
   - Decide what sample metadata to collect (e.g., clinical information, location) and how it will be stored.
   - Begin to obtain research compliances and approvals for human and animal use, biosafety, etc. (Visit this handy guide: http://medicine.emory.edu/hitchhikers).
   - Decide whether to use an amplicon based sequencing (e.g. 16S) or shotgun metagenomics approach
   - How much sequence data per sample (this will depend on the sample type, sequence technology, aims of experiment, budget)
   - Consider which sequencing technology is most appropriate and which software will likely be used for analysis
   - Plan logistics of sample extraction and storage.
   - Perform power calculations to estimate the number of samples necessary to achieve stated objectives
   - Develop budget. Costs can include sampling & DNA extraction supplies, sequencing, sample and DNA storage, data storage and processing, labor for taking samples, DNA extraction and sequencing, data analysis

2. Sampling & Sequencing
   - Collect samples
   - Store before DNA extraction, if necessary
   - DNA extraction
   - Sequencing
   - Long term storage of DNA and/or samples post-sequencing

3. Data analysis
   - Sequence quality assessment
   - Human sequence removal (if necessary)
   - Data analysis pipeline (operational taxonomic unit assignment, metabolic reconstruction etc) OTU definition link: http://drive5.com/usearch/manual/otu_definition.html
   - Exploratory data analysis
   - Inferential analysis
   - Hypothesis testing
   - Public database submission
   - Long term data storage
   - Manuscript writing

Credit: Dr. Timothy Read, 2014