Plasmids request from the human ORFeome and plasmid database Entered by Cheryl Borgman and
Janes Lab Protocols Lixin Wang 8/22/18

I. Requests from the human ORFeome

1. Determine if an ORF of interest is in Version 5.1 of the Human ORFeome (http://horfdb.dfci.harvard.edu/hv5/)
   - *The Janes Lab has a complete replica plating up to and including plate #51035.*
   - *ORFs outside of Version 5.1 can be purchased individually* (http://dharmacon.gelifesciences.com/cdnas-and-orfs/mammalian-orfs/ccsb-human-orfeome/ccsb-human-orfeome-collection/).
   - *The ORFeome was cloned into pDONR223 (5004 bp) carrying spectinomycin resistance and is stored in DH5α cells.*
   - *All ORFs lack a stop codon for C-terminal tagging.*
2. Place a targeted ORF request with Cheryl.
   - *Provide the gene symbol AND the Internal ID.*
   - *For each gene, check the GenBank Coding Sequence against the RefSeq coding sequence to see how different the clone is from the reference.*
   - *It is best to keep the number of requests to ~five so that the load is manageable.*
   - *If you would like your plasmids fastest, offer to make the agar plates with the correct selection antibiotic—spectinomycin.*
3. Cheryl will identify the ORF clone in the –80ºC storage, puncture the aluminum foil with a pipet tip to sample the bacteria expressing the clone (sealing the hole with a ToughSpot), streak out the sampled bacteria on an LB + 50 μg/ml spectinomycin agar plate, and incubate the plate at 37ºC overnight.
   - *It is not unusual for clones to grow very differently within and among ORFs.*
4. Miniprep six colonies of each ORF, sending all out for sequencing using M13(-21) forward and M13 reverse primers on pDONR223.
   - *Not all ORF ligations have been cloned out or fully maintained, but there should be at-least one sequence-verified clone among the six.*
   - *The M13(-21) forward primer ensures that the N-terminus of the insert is fully sequenced*
5. Sequence-verified pDONR223 ORF clones are suitable for PCR cloning (see Janes_PCRcloning.pdf) or LR recombination (see Janes_GatewayLRrecomb.pdf) into the pLX series of lentiviral vectors.
   - *pLX302 puro and pLX304 blast destination vectors add V5 tags to the C-terminus of the ORF.*
   - *Empty pLX vectors can not be used as negative controls, because they express CmR and ccdB genes, which are normally recombined out.*
   - *Suitable negative-control vectors for pLX are EGFP, LacZ-V5 (yielding LacZ-2$\times$V5 if recombined into pLX302 OR pLX304), luciferase, HcRed, or BFP (located in the general plasmids boxes)*

II. Requests from the plasmid database

1. Determine if the plasmid of interest is in the lab database (smb://nas.storage.virginia.edu/BME$/BME-Labs/JanesLab/PlasmidRegister.fmp12)
   - *Take a working copy of the PlasmidRegister.fmp12 and search on your own computer. Do <u>not</u> search or make changes to the official copy on the lab server.*
2. Place a plasmid request with Lixin by email.
   - *Provide the full plasmid name, box number, and position number.*
   - *If you would like your plasmids fastest, offer to make the agar plates with the correct selection antibiotic(s).*
3. Lixin will streak out the glycerol stock(s) and alert you when they are in the incubator.
4. Prep colonies as needed (miniprep, midiprep, etc.).
   - *The user should confirm plasmid database entries by restriction digest, but there is not a need to sequence unless the digest fails.*
5. Digest-verified colonies are suitable for use.