Plasmid deposition in lab repository Entered by Kevin Janes
Janes Lab Protocols 2/17/22

Rules for plasmid deposition:

- A plasmid that is purchased as a PCR template does not need to be deposited, provided that another template (e.g., the desired expression vector) has been deposited in the plasmid database
- All ORFeome plasmids must be put in the plasmid database by the requestor (not Cheryl); both the donor vector and the recombined vector should be deposited
- Only shRNA hairpins (purchased or cloned) that have verified knockdown should be put in the plasmid database
- If a plasmid has been cloned by a "team" (e.g., a student-postdoc and a research staff), it is the job of the student-postdoc to deposit the plasmid
- If a plasmid has been cloned by a team of multiple students or postdocs, it is the job of the student or postdoc who assembled the ligation reaction to deposit the plasmid (they have the most knowledge about how the plasmid was built)
- If a plasmid has been cloned independently by a research staff, it is the job of the research staff to deposit the plasmid
- Published plasmids should also be deposited with Addgene shortly after a publication has appeared (contact Kevin or Cheryl for login and password information)
- It is strongly recomended to deposit a plasmid as soon as possible after it has been cloned and verified; however, do not put files "in progress" on the server because they will confuse Lixin
- For specific examples, deposited plasmids entries are stored on smb://nas.storage.virginia.edu/BME$/BME-Labs/JanesLab/Plasmid database dropbox

1. Electronically fill out the Plasmid Register Data Entry Template (Janes_Plasmidregister.xls) and upload this file along with the sequencing file(s) as a subfolder to the following folder on the Janes Lab server: smb://nas.storage.virginia.edu/BME$/BME-Labs/JanesLab/Plasmid database dropbox Change the name of the entry template and sequencing file to reflect the name of the plasmid accurately. Provide the complete name of each plasmid in the file name of the entry template and use the following convention: DEPOSITOR VECTOR NTAG-INSERT (RESIDUES)-CTAG RESISTANCE [e.g., YourLastName_pBabe FLAG-Nrf2 (391-605) neo.xls]. When filling out the Plasmid Register Data Entry Template, please adhere to the following conventions for consistency:
   - Date Created:
     - Enter (roughly) when the plasmid was cloned. If unknown, enter the date when the plasmid was deposited.
     - Do not enter a date for gift plasmids that were obtained or purchased.
   - Created By:
     - Enter the name of the individual in the lab who cloned the plasmid.
     - For gift plasmids, enter the name of the PI of the lab that cloned the plasmid and indicate "gift plasmid" in parentheses.
   - Insert Name:
     - For full-length human genes, common gene names may be used (e.g., junD).
     - For non-human genes, please add a prefix that indicates the species (ms = mouse, rt = rat; e.g., msTgfbr3).
     - For truncated genes, please add a suffix that indicates the amino acids included (e.g., Nrf2 (391-605)).
     - For mutated genes, please add a suffix that indicates the amino acids mutated (e.g., FKHR (H215R))
     - For riboprobe plasmids, please enter the gene name in all caps and add a suffix that indicates the version number (if applicable) and "p" for probe (e.g., TGFBI.v1.p).
     - For shRNA plasmids, please enter the targeted gene with an "sh" prefix (e.g., shMEN1).
     - For empty vectors, enter "None", even if they have been engineered to include additional elements compared to a parent vector (this information will be included in the "Vector" field).
   - 5'/3' Site:

    - Enter the restriction enzymes used for both the insert (upper row) and the vector (bottom row).
    - For empty vectors, enter "N/A", even if they have been engineered with restriction enzymes to include additional elements compared to a parent vector (this information will be included in the "Sequence" field).
- Species:
    - Enter the species for the insert.
    - For empty vectors, enter "N/A".
- Tag:
    - Enter the epitope tag for the insert.
    - A triple tag should be indicated as "3x".
    - For untagged inserts and empty vectors, enter "N/A".
- Bacterial Strain:
    - Enter the bacterial strain from the glycerol stock that was deposited.
    - Enter "Unknown" if a plasmid was received as an agar stab or if the information is not available.
- Full Length/Amino Acids:
    - Indicate whether the insert is a full-length version of the gene.
    - If it is a truncation, indicate the amino acids included in the insert.
    - For riboprobe or shRNA plasmids, enter "N/A" in the "Amino Acids" field.
    - For empty vectors, enter "N/A" for both fields.
- Accession Number:
    - Enter the NCBI RefSeq accession number ("NM_" prefix) for the insert.
    - Do not include version numbers of the RefSeq ID (e.g. NM_005811.3 should just be NM_005811).
    - For empty vectors, leave blank.
    - For reporter inserts (EGFP, luciferase, etc.), leave blank
- Cloning Procedure:
    - Enter whether the insert was added by PCR, subcloning, oligo annealing, or LR recombination.
    - For empty vectors that were engineered, the cloning procedure may be added here.
    - For empty vectors that were not engineered, enter "N/A".
- Sequenced:
    - Indicate whether the insert was sequenced in that plasmid. (If a donor vector was sequenced but the LR recombined plasmid was not, then the donor entry should say "yes" and and the recombined plasmid should say "no".)
    - If yes, include the sequencing file(s) when depositing the plasmid. ABI files containing the raw electropherograms are the preferred format.
- Bacterial Resistance:
    - Enter the antibiotic(s) for bacterial selection (ampicillin, chloramphenicol, etc.).
    - Enter the incubation temperature if not 37ºC.
- Sequence:
    - For plasmids with inserts, enter the expected sequence of the insert or open reading frame, including any epitope tags, mutations, truncations, etc.
    - For empty vectors that were not engineered, enter the entire plasmid sequence as reported in the literature.
    - For empty vectors that were engineered, enter the sequences of the parent vector in lower case letters and the engineered segment highlighted in ALL CAPS.
    - Deviations from the RefSeq ID Accession Number due to mutation or polymorphism should be highlighted in ALL CAPS.
    - For shRNA plasmids, enter the entire stem-loop oligo with the targeting sequence highlighted in ALL CAPS.
- Notes:

- This is a free field to include any additional information that may be relevant for the plasmid (origin of the template, details on deviations from the RefSeq ID Accession Number).
- If diagnostic digest or gel electrophoresis is important to confirm lack of plasmid recombination, provide details here—make sure you have used the exact diagnostic digest to confirm lack of plasmid recombination before depositing.
- For riboprobes, enter the validated probe concentration.
- For pGEX expression plasmids, enter the induction conditions and any purification notes.
- For viral vectors, enter relevant infection conditions (number of serial infections, volumes of virus, etc.).

2. Transfer 5 μl of purified DNA to a clearly labeled microcentrifuge tube and freeze at –80°C in the "Temporary DNA stock box".
   - *Lixin will prepare glycerol stocks and a miniprep to transfer to the permanent repository after entering them in the database.*