

Archiving plasmids, bacterial stocks and sequencing runs in the KR lab

Database:

We use a google sheet to track lab inventories of plasmids, strains, etc:

<https://docs.google.com/spreadsheets/d/1UaiXqP70JGJfhlvsrZNK64QIL-00urmNZmacYn0plyl/edit#gid=1450776147>

The google sheet is only editable by Chris Ingle and Kim Reynolds and is readable/searchable by all lab members. It is best practice to keep your own version of this sheet to track your personal reagents and stocks.

Naming Conventions:

This document describes the numbering system you should use to track your samples so that anyone can find (and use) your plasmids, strains, etc. easily. You will maintain two types of inventory files: one set will reflect your personal stocks (kept in your own -20 and -80 space, these are typically things you are actively working with/using) and one set will be “archived” materials – kept in the locked portion of the -80. The archived set is critical – this is what the lab relies on if an outside person makes a materials request, or if someone new joins the lab and needs to pick up on an established project. It is also a failsafe for yourself, in case you need to return to an old tube of a construct. For this reason there are two important rules for the archive – archive *regularly* and *accurately*. This means your materials should be up to date, your description should be complete and correct, and don’t put any plasmids/constructs/strains in the database until they’ve been sequence verified! The locked archive should not contain constructs or strains-in-progress. Don’t wait to until you leave the lab to archive – you should archive whenever you have completed a major chunk of a project, or have completed writing up a project as a paper. When you leave the lab all essential reagents should be in the “archived” (locked) portion of the -80, and all personal stocks should be physically removed.

Each sample/stock type has an alphabetical prefix, and an associated inventory type.

p = plasmids

g = glycerol stocks associated w/ plasmids

st = strain stocks

sq = Next gen sequencing samples

o = primers/oligos (we typically keep these only for complex oligo pools or library designs)

Please pay attention to consistency in naming/numbering as you create reagents and archive: if you are creating a glycerol stock of a plasmid, the two items should have identical numbers, but different prefixes, eg: pKR 101 and gKR 101XL would indicate a plasmid from Kim Reynolds number 101, and a glycerol stock of the same plasmid in XL1 Blue cells. As you enter names in your archive spreadsheet it is important to follow the format stocktypeInitialsNumber space celltype: for example gMR96 XL. This helps with sorting and searching for entries.

Plasmids: are numbered sequentially as follows: p(Initials)(box number)(place number)

Example: pCI 201

Generated by Chris Ingle (CI)

Box #2

Place #1

You can choose to follow this Box layout ordering, or use your own sequential numbering system. Note that if you follow the Box layout, there are only 81 places in a freezer box. Thus your stocks will be numbered: 101-181 in box 1; 201-281 in box 2, and so on. The key thing is

that each number is unique, and the identifier does not include any dashes, only a space between your initials and the number.

For each plasmid, you'll create a google sheet plasmid stock inventory item, and should fill out the following fields:

- Archive Number: stock number (as above)
- Entry type: (strain, plasmid, glycerol stock, sequencing sample, oligo)
- Personal/notebook number: If you used a different name or identifier for this construct (other than the archive number) in your lab notebook, it should go here.
- Description: a complete detailed description of what the construct contains. Keep in mind that this field should contain keywords that future lab members might search for.
- Markers: any antibiotic or fluorescent markers
- Plasmid backbone
- Growth conditions: This is where you will note any growth requirements in terms of media, pir+ cells, temperature, etc
- Insert and (if relevant) restriction sites: what is cloned into the plasmid. If referring to a single guide RNA (sgRNA), please put the homology sequence here
- Creator: yourself, Addgene, or source lab
- Associated publication: if published
- Addgene: addgene catalog number, if obtained from or archived with Addgene
- Creation date: this can be a link to your Benchling notebook
- Archive date: Date it is being added to the archive
- Location of plasmid map file: These should be stored on the BioHPC in /project/greencenter/Reynolds_lab/shared/Archive/Plasmid_maps. Please put the full path to your file here. For variations-on-a-theme type constructs (say different point mutants or sgRNAs) it is sufficient to create a single backbone reference map, and use the "insert" field of the archive to specify any substitutions or variation.

Glycerol stocks: There are two types of glycerol stocks: stocks of new strains/genotypes, and stocks storing a plasmid.

For new strains: The naming convention for new strains is st(StrainName), i.e. stXL1Blue or stER2566.

For these entries, you should fill out the fields for entry type, personal or notebook number (if the strain has a nickname you often use), description, markers, growth conditions, creator, associated publication (if published), addgene (if relevant), creation date, and archive date.

For plasmid glycerol stocks: Glycerol stocks that carry a plasmid should be numbered to match the associated plasmid, with an additional two-letter strain designation.

Example: gCI 201XL

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Contains plasmid pCI 201

In XL1Blue Cells

For these entries you will fill out the google sheet similarly as for plasmids. Please note that all plasmids should be archived as either a glycerol stock in a cloning strain (e.g. XL1Blue) or as a plasmid stock (in water or EB). Ideally both should be stored.

Sequencing Runs: A portion of any next-gen sequencing sample (sufficient to permit at least one more sequencing run) should be archived as sq(Initials)(6-number date).

Example: sqCI 140502
Generated by Chris Ingle (CI)
Sequenced on May 2, 2014

For the google sheet you should complete: entry type, personal or notebook number (if you have a nickname for this experiment), description, markers (if this was a plasmid based selection), plasmid (again if a plasmid based selection) , strain (this should specify the strain used during selection), growth (this should describe conditions of the selection), creator, publication (if relevant), a lab notebook reference and the archive date.

Primers/Oligos: are numbered sequentially following the convention for plasmids, but with the prefix “o”: o(Initials)(box number)(place number)

Example: oCI 201
Generated by Chris Ingle (CI)
Box #2
Place #1

Please note that we do not keep most oligos. Only complex oligo pools or custom libraries (say for gene synthesis) should be archived.