Addgene - Minimal ARP Construct List

Antibiotic Class	Antibiotic	Resistance Gene	Plasmid	Promoter	Catalog No.
Aminoglycosides	Streptomycin	*aph(3'')-Ia*	pGDP3	Pbla	112879
	2- Deoxystreptamine	*rmtB*	pGDP3	Pbla	112880
	Apramycin	*apmA*	pGDP3	Pbla	112881
	Spectinomycin	*aph(9)-Ia*	pGDP3	Pbla	112882
β-lactams	Penicillin	*NDM-1*	pGDP1	Pbla	112883
	Cephalosporin				
	Carbapenam				
Lincosamides	Lincosamides	*ermC*	pGDP4	Plac	112884
Macrolides	Macrolides	*ermC*	pGDP4	Plac	
Type B Streptogramins	Type B Streptogramins	*ermC*	pGDP4	Plac	
Type A Streptogramins	Type A Streptogramins	*vatD*	pGDP3	Pbla	112885
Streptothricin	Streptothricin	*STAT*	pGDP1	Pbla	112886
Tetracyclines	Tetracycline	*tet*(A)	pGDP4	Plac	112887
Chloramphenicols	Chloramphenicols	*CAT*	pGDP3	Pbla	112888
Fosfomycins	Fosfomycins	*fosA*	pGDP1	Pbla	112889
Rifamycins	Rifamycins	*arr*	pGDP3	Pbla	112890
Polymyxins	Polymyxins	*MCR-1*	pGDP1	Pbla	112891
Echinomycins	Echinomycins	*uvrA*	pGDP1	Pbla	112892
Tuberactinomycins	Viomycin	*vph*	pGDP1	Pbla	112893

Addgene - Dereplication SOP

1. Streak plates of LB agar containing the right antibiotic for each of the strains (pGDP1/2 – kanamycin at 50ug/mL, pGDP3/4 – ampicillin at 100 ug/mL). Grow overnight at 37°C. **Note:** LB with no selection marker should be used for the sensitive control strain that contains no resistance element.

2. Pick a single colony from each plate to inoculate a 3 mL overnight of Mueller Hinton broth per strain. Make sure to add antibiotic to the respective tubes.

3. From the overnight, make glycerol stocks of each individual strain by adding 800 µL of cell culture and 200 µL of 80% glycerol to a cryogenic vial. Mix by inverting the tube gently and store at -80 °C.

4. Create a Minimal ARP frozen stock template by pipetting 100 µL of each overnight culture into the appropriate well of a 96-well plate according to the Minimal ARP Map. Lay a sterile aluminum seal across the top of the wells and place the 96-well plate lid over the seal. This prevents contamination across wells. Store at -80°C.

5. Grow a 3 mL seed culture of the producing strain you wish to dereplicate in *Streptomyces* Antibiotic Activity Media (SAM), containing one sterile glass bead in the test tube. Grow the culture at the desired temperature (30 °C) until turbid (up to 6 days).

6. Pour 20 mL of Bennett's agar into sterile rectangular Omni Trays with lids (86 x 128 mm, Thermo Fisher Scientific).

7. Pipette 200 µL of the seed culture onto the Omni Tray plate and use a sterile cotton swab to evenly spread the culture across the entire plate.

8. Place a sheet of autoclaved Nitrocellulose membrane (cut-out to fit the Omni Trays) over the evenly spread culture on the plate. Ensure that the membrane is flush to the plate.

9. Incubate the plate for 6 days at 30 °C (upside down in a plastic bag).

10. Remove the membrane with sterile tweezers and pour 20 mL of Mueller Hinton agar (MHA) over the plate to create an overlay. After solidifying, store the plate upside down at 4°C in a plastic bag over night.

11. On the same day that the MHA overlay is poured, inoculate a 96-well plate that contains 100 µL of Mueller Hinton broth in each well with the Minimal ARP frozen stock template that is stored at -80°C (pinning tools may be used). Replace the aluminum seal on the template plate after each use. Grow the newly inoculated

plate inside of a bag at 37°C, shaking overnight. Always dereplicate using a template that is fresh, never frozen! **Note:** New templates can be made by pinning from the frozen stock. Just add 50% glycerol after growth as described in step 4 and follow the same storage procedure.

12. Using pinning tools, pin the freshly grown minimal ARP plate onto the MHA overlay of the Omni Tray plate, which contains the secondary metabolites of the strain of interest. Be careful not to pierce the agar. Incubate the pinned plate at 37°C overnight while upside down and in a plastic bag.

13. Analyze the results by comparing growth on the Omni Tray to the Minimal ARP Map.

Media:

Bennett's

- 10g potato starch
- 2g casamino acids
- 1.8g yeast extract
- 2mL Czapek mineral mix (see below)
- ddH_2O to 1L
- 15g agar (add after adjusting pH)

- pH to 6.80 (using a pH meter)
- Autoclave (45min exposure at 121°C) before use

Czapek Mineral Mix

- 10g KCl
- 10g $MgSO_4 \cdot 7H_2O$
- 12g $NaNO_3$
- 0.2g $FeSO_4 \cdot 7H_2O$
- 200µL concentrated HCl
- ddH_20 to 100mL

SAM (*Streptomyces* Antibiotic Activity Media)

- 15g glucose
- 15g soytone (soya peptone)
- 5g NaCl
- 1g yeast extract
- 1g $CaCO_3$
- 2.5mL glycerol
- ddH_2O to 1L

- pH to 6.80 (using a pH meter)
- Autoclave (45min exposure at 121°C) before use