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Isolation and isotopic labeling of bacteria V.1

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Abstract

This protocol describes how to isolate bacteria in order to label its biomass using ^{13}C-glucose and ^{15}N-ammonia. The first steps aim to isolate candidate bacteria that are able of growing on the chosen substrates for isotopic labeling.

The protocol was applied to the isolation of bacetria from freshwater algal cultures, although it can be applied to natural samples.

The M9 media used in the protocol is based on the media from Marley, Lu and Bracken (2001).

References:

Marley, J., Lu, M., & Bracken, C. (2001). A method for efficient isotopic labeling of recombinant proteins. *Journal of Biomolecular Nmr*, *20*(1), 71–75.

Troubleshooting

Safety warnings

Use sterile/autoclaved materials through all the protocol to avoid contaminations.

1

Prepare M9 media stocks

The recipe for 1L of minimal growth medium (M9) is as follows:

		Volume
	5x M9 salts (autoclaved)	200 mL
	D-glucose stock (20%, 0.2µm filter sterilized)[a]	20 mL
	Vitamins stock (0.2µm filter sterilized)[b]	0.5 mL
	Trace Metals stock (0.2µm filter sterilized)[b]	1 mL
	1 M $MgSO_4$ (autoclaved)	2 mL
	1 M $CaCl_2$ (autoclaved)	0.1 mL
	MQ H_2O	(complete to 1L)

Mix all components labeled as autoclaved, and autoclave them to ensure sterility. After cooling of the autoclaved mix, add the rest of the components by 0.2 µm filter sterilization.

In order to prepare **solid media** for plating, add 1.5% agar (15 gr agar per 1 L of media) prior to autoclaving. Allow cooling of the media prior to add the 0.2µm filter sterilization components. Pour the media in Petri dishes and allow solidification of agar.

To prepare 1 L of the 5x M9 salts stock (autoclaved):

		Weight
	KH_2PO_4	15 g
	Na_2HPO_4 (anhydrous)	34 g

	NaCl	2.5 g
	NH_4Cl[a]	5 g

[a] **For isotopic labeling, use D-glucose-1-^{13}C (99% atom ^{13}C) and ammonium-^{15}N chloride (98% atom ^{15}N)**

[b] Vitamins and trace metals stock used were the same as for algal DY-V media (see https://ncma.bigelow.org/media/wysiwyg/Algal_recipes/NCMA_algal_medium_DY-V.pdf)

2

Bacterial plating

Use a small volume (i.e. 0.1 mL) of the sample you want to isolate your bacteria to inoculate M9 agar plates. Ensure correct striking and dispersal of the inoculum to facilitate picking of individual colonies afterwards.

3

Bacterial growth

Incubate bacteria at a temperature of choice. Bacteria isolated from the algal cultures were chosen to grow at 30 ºC.

Inspect plates after 24h until big enough colonies are visible to pick.

4

Picking and transfer of colonies

Using sterile picks, transfer a single colony of bacteria into M9 liquid media.

Recommended to do a first transfer into a small volume (~ 25 - 50 mL).

Prepare a battery of tubes beforehand with M9 liquid media and pick a variety of colonies.

5

Production of labeled bacterial biomass

In order to produce a large ammount of biomass, transfer some of the bacteria from the M9 cultures tubes to a larger volume (i.e. 1L)
Let bacteria grow until media is completely cloudy and a maximum yield is achieved of biomass (can be 3-4 days until C and N is consummed).

NOTE: for isotopic labeling, M9 media used in this step will contain $^{15}NH_4Cl$ and ^{13}C D-glucose.

6 **Production of HKB**

Once you have enough biomass, follow up with the **protocol for making heat killed bacteria** (start at the 3rd step).