Leucine incorporation as a mesure of biomass production by heterotrophic bacteria

The physiological basis of the leucine (Leu) method is protein synthesis.

Leu is a constant proportion of bacterial protein (7.3%).

The Leu method consists of measuring the incorporation of radiolabeled leucine into bacterial protein over time.

Rates of Leu incorporation into protein are estimated from the appearance of radioactivity, added as ^{3}H-Leu, in the protein fraction.

Biomass production can be calculated from rates of protein synthesis because protein comprises a large, fairly constant fraction (approx. 60%) of bacterial biomass.

Measuring 3H-Leu incorporation by the microcentrifuge method (Smith and Azam 1992)

The incubation and radioassaying are both done in a 2ml microcentrifuge tube

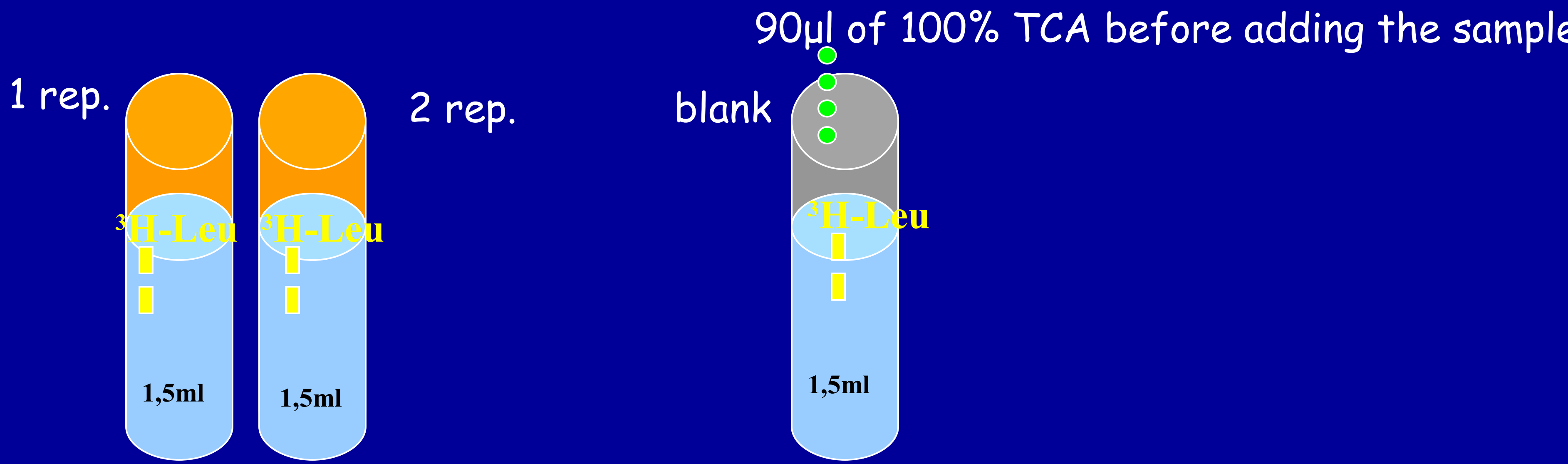
Radioactivity is collected by centrifugation

As a result the amount of radioactivity and the volume of sample and scintillation cocktail are much smaller

The processing time and variability among replicates are much better

The blank, or radioactivity in the killed controls, is usually much lower

Experimental procedure



Vortex

Incubate for (2 hours)? at *in situ* temperature

Stop incubation by adding 90µl of 100% TCA

Vortex

Storage of samples at 4°C in the dark

Laboratory activity

Centrifuge for 10min (16 000g) and eliminate SN

Add 1,5ml of **cold (4°C) TCA** to the pellet and vortex

Centrifuge for 10min (16 000g) and eliminate SN

Add 1,5ml of **cold (4°C) 80% Ethanol** to the pellet and vortex

Centrifuge for 10min (16 000g) and eliminate SN

Add 1,5 ml of scintillation liquid (with the dispenser) and vortex after 5 min

Storage of samples at 4°C in the dark until reading at the scintillation counter

Be careful……….

- Sample water using “clean techniques”. Use plastic gloves to avoid contact with sample and radioactivity.
- Acid rinse sample containers before use
- Start incubations as soon as possible
- Avoid the pellet when removing the supernatant by aspiration
- Remove the ethanol and allow the pellet to dry completely, because any traces will cause quenching during liquid scintillation counting
- After adding the scintillation liquid allow the sample to sit for as long as 2 days to maximize the dispersion of radioactivity into the cocktail.

Relating Leu incorporation to Biomass Production $\mu gC\ l^{-1}\ h^{-1}$.

Production = Leu incor * 131.2 * $(\%Leu)^{-1}$ * (C/Protein) * ID

131.2	Molecular weight of Leu	
% Leu	Fraction of Leu in protein (7.3%)	0.073
C/Protein	Ratio of cellular carbon to protein	0.86
ID	Isotope Dilution	

Leucine can be synthesized from other compounds, which leads to isotope dilution of the radiolabeled Leu. The problem is minimized by adding Leu to concentrations high enough.

Rate of Leu incorporation I (as nmol l^{-1} h^{-1})

$$I = \frac{(dpm_{sample} - dpm_{blank}) / (2.22 \times 10^{6} \text{ dpm per } \mu Ci)}{\text{Leu SA} * \text{incub time} * \text{vol}}$$

Concentration of ^{3}H- Leu to be used:

We need to verify that the concentration is saturated

No flask	volume (ml)	C leu (nM)	3H leu *	time begin	Incub time (h)	time end
C1	1.5	2,5	12.5 µl		2	
C2	1.5	5	25µl		2	
C3	1.5	10	50 µl		2	
C4	1.5	20	100 µl		2	
C5	1.5	40	200 µl		2	

Time of incubation

We need to test that we are in linear phase of the incorporation of the isotope

No flask	volume (ml)	C leu (nM)	3H leu *	time begin	Incub time (h)	time end
T1	1.5	10	50 µl		0	
T2	1.5	10	50 µl		1	
T3	1.5	10	50 µl		2	
T4	1.5	10	50 µl		4	
T5	1.5	10	50 µl		8	

Preparation of the radioactive work solution

We buy Amersham, L—(4,5-3H)leucine

If Pack size 250µCi , specific activity 162µCi/nmol and radioactive concentration 1,0mCi/ml

We want a final concentration in each sample of 10 nM leu*
10nmole/l =0,015nmol/1,5ml (for samples of 1.5 ml)

↓

2,43µCi

so 2.43 µCi in 1.5 ml

I want to introduce 50µl of leu* solution.

These 50 µl should contain 2,43 µCi

Material needed

1 calcinated, `erlen` bottle empty, to mix the leu+water

1 calcinated glass `erlen` bottle for the Milli-Q water

AUTOCLAVE

The Erlen with the Milli-Q water

Cryotubes

Tips

Mix sterile water with leu*

Distribute in sterile cryotubes in sterile conditions (max possible)