

Pulse/Chase Experimental Protocol

4/21/99

Specific for labelling nascent tailspike polypeptide chains (and, as a control, Salmonella proteins)

Materials needed

biological: *Salmonella* 7136 fresh O/N in minimal media
Hi-titer (1×10^{11} pfu) phage stock (5-/13-, or N110 for another control)
minimal media
casamino acids (10%)
Buffer R + OG [ribosome buffer (50 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 150 mM KCl) plus 80 mM octyl-gluco-pyranoside]
radioactive: 2ml of ¹⁴C-labeled L-amino acid mixture (per phage used)
radioactive hood clear for work
geiger counter
hot pipetmen
radiation badge, safety glasses, lab coat
other: SS34 tubes (4) and rotor
liq. N₂ dewer
ice tub

Detailed protocol

1. Start (2) 50 ml cultures of 7136, using 1 ml of O/N culture, in 125 ml baffle flasks, at 30°C.
2. Grow until cell density reaches 2×10^8 cells/ml. For steps 3-10, the cultures will be treated differently:

	labeling cellular stuff (R)	labeling nascent tailspike (T)
3.	Pulse with 1 ml ¹⁴ C-labeled aa (100 μCi).	Wait 15 min.
4.	Culture 10 min.	
5.	Chase with 10 ml <i>ice cold</i> casamino acids	
6.	Infect at MOI=10 (vol=60 ml)	Infect at MOI=10 (vol=50 ml)
7.	Culture 90 min.	Culture 75 min.
8.		Pulse 1 ml ¹⁴ C-aa (100 μCi).
9.		Culture 10 min.
10.		Chase with 10 ml cold cas-aa.

11. Chill both cultures on ice.
12. Pour into SS34 tubes (two per culture) and spin at 11K rpm for 5 min.
13. Drain pellets and resuspend in 0.25 ml Buffer R + OG.
14. Transfer suspended cells to microfuge tubes, and freeze in liquid N₂. Store at -30°C.