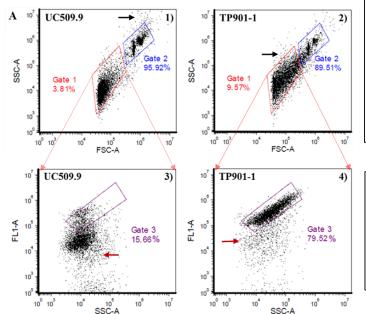


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Title	Detecting Lactococcus lactis prophages by Mitomycin C-mediated induction coupled to flow cytometry analysis				
Authors	Oliveira, Joana; Mahony, Jennifer; Hanemaaijer, Laurens; Kouwen, Thijs R.; Neve, Horst; MacSharry, John; van Sinderen, Douwe				
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Parameters

Run limits: 5000 events.ml⁻¹ Medium flow rate: 33 µl.min⁻¹

Phage particles detection: SSC (Side Scatter) versus FSC (Forward Scatter)

Purpose: Discriminate different particles based on size and complexity in the induced lysate

Analysis

Gate 1- Phage particles population (virions produced by cell lysis)

Gate 2- Microspheres (used as a fluorescence & size reference control)

Gate Outliers- Cell debris and/or background noise (dots outside of each gate; black arrow)

Parameters

Fluorescence detection: FL1 (BP Filter 530/30) *versus* SSC Purpose: Detect phage DNA particles stained with SYTO-9

Analysis

Gate 3- Phage DNA stained with SYTO-9 in our induced lysate sample (phage population detected in Gate 1)

Gate Outliers- Background noise (dots outside of each gate; red arrow)



Flow cytometry analysis

		% of events (SSC versus FSC)		% of fluorescence (FL1 versus SSC)		
		Sample population	Beads	Cell debris/ noise	Phage particles	Background noise
L. lactis strains	Features	Gate 1	Gate 2	Outliers of Gates 1 & 2	Gate 3	Outlier of Gate 3
UC509.9	No prophage released (negative control)	6.73 ± 2.07	92.93 ± 2.12	0.34 ± 0.05	16.33 ± 3.76	83.67 ± 3.76
NZ9000 (TP901-1 erm)	TP901-1 <i>erm</i> prophage (positive control)		87.97 ± 1.26	1.05 ± 0.15	80.60 ± 2.39	19.39 ± 2.39

Figure S1. Schematic representation of the flow cytometry parameters, analysis and general results for the two *L. lactis* control strains. BD Accuri TM C6 flow cytometer was used for the implementation of the correct parameters to detect and enumerate phage DNA particles stained with SYTO-9 dye. (**A1** and **A3**): Cytograms of 3 μg.ml⁻¹ MmC-treated *L. lactis* UC509.9 (prophage-free lactococcal strain used as negative control); (**A2** and **A4**): Cytogram of 3 μg.ml⁻¹ MmC-treated *L. lactis* NZ9000 TP901-1*erm* (lactococcal strain harbouring the TP901-1 prophage used as a positive control).