

## *Supplementary Material*

### **1 Text S1 The conjugation with a long-time treatment of spectinomycin**

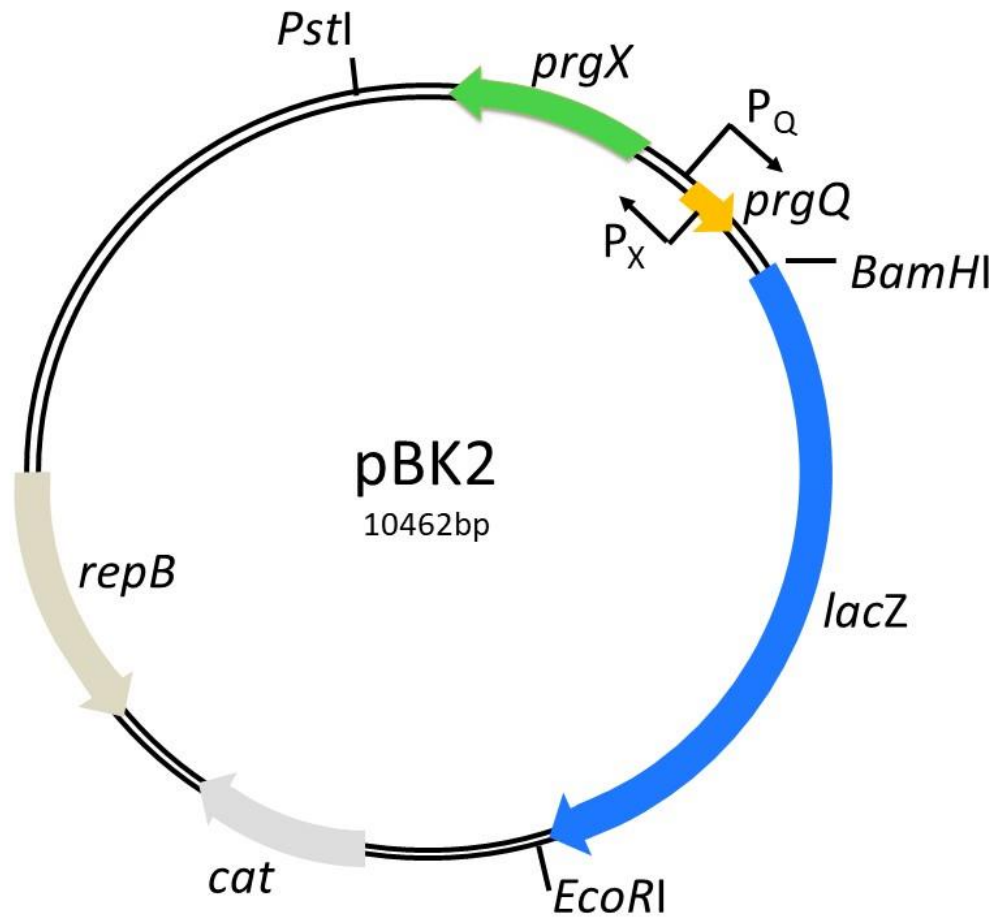
For the conjugation of pCF10 with the treatment of spectinomycin for 20 hours, we used OG1RF and OG1SSp as donor and recipient cells, respectively. We separately cultured overnight donor cells and recipient cells in Todd-Hewitt Broth (THB) at 37°C with the treatment of 25 µg/ml spectinomycin. We then washed cells twice and made a 1:10 dilution. We maintained the concentration of spectinomycin at 25 µg/ml and incubated cells for 60 min. Then, we mixed one volume of recipients with ten volume of donors in liquid mating for 3 hours. Serial dilutions of liquid samples were plated on agar. The results are in Fig. S2. The exposures to antibiotics notably increased the conjugation.

### **2 Text S2 The response of pBK2 with a short-time treatment of streptomycin**

In Fig. 3a, the overnight culture of cells was treated by antibiotics. Here, we examined the cellular behaviors under a short-time treatment with spectinomycin. We did a 1:10 dilution to the overnight culture of JRC101(pBK2) and then administrated 5 µg/ml spectinomycin. Cells were exposed to spectinomycin for 2 hours incubation followed by 1.5 hours of induction by 30 ng/ml cCF10. The results are in Fig. S3. Administration spectinomycin increased beta-galactosidase activity.

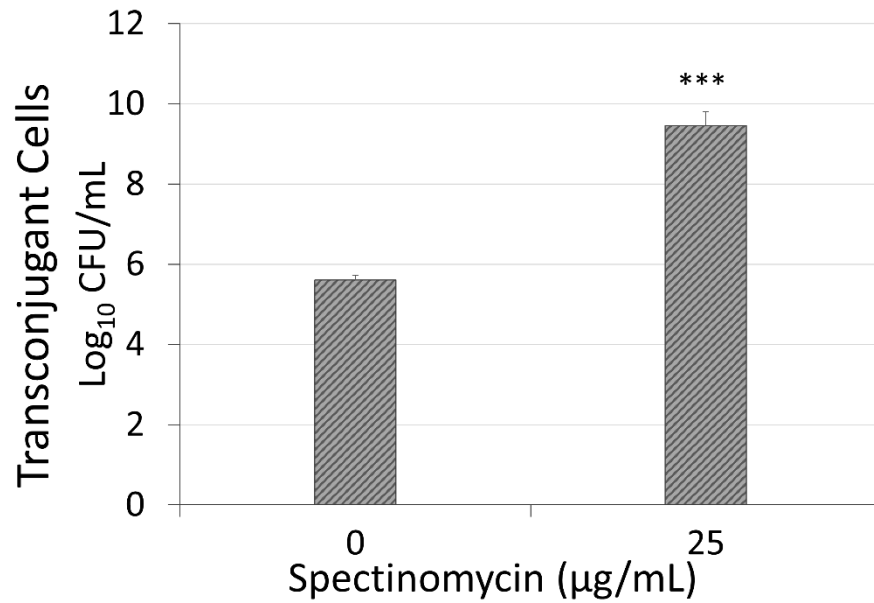
## 3 Figures and Tables

## 3.1 Supplementary Figures

**Supplementary Figure S1. The gene map of pBK2.**

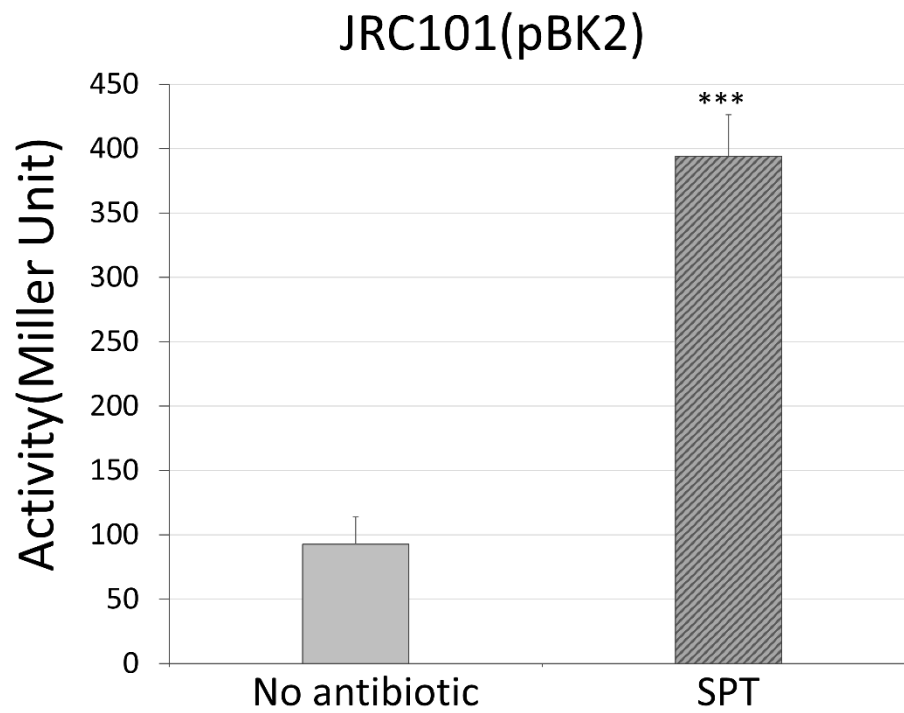
The backbone of pBK2 is pCI3340, a plasmid in *Lactococcus lactis*. Genes *prgX* and *prgQ* are from the plasmid pCF10. The codon of gene *prgQ* encoding  $Q_L$  RNA, which is the conjugal determinant, was replaced by the *lacZ* in pBK2.

Donor:OG1RF(pCF10)  
Recipient:OG1X



**Supplementary Figure S2. The conjugation of pCF10 with a long-time treatment of spectinomycin.**

In comparison to that of no treatment, administration of 25 µg/ml spectinomycin increases the number of transconjugant cells. (\*\*\*) indicates p-value <0.001)



**Supplementary Figure S3. The response of the conjugal gene to a short-time treatment of streptomycin.**

A 3.5-hour treatment with streptomycin increases beta-galactosidase activity. (\*\*\*) indicates p-value <0.001)

### 3.2 Supplementary Tables

**Table S1 : Equations of mathematical model**

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$[O] = \frac{[N] \cdot [I]^4}{[I]^4 + K_{DNA} \cdot [C]^4}$
$\frac{d[Q_S]}{dt} = \left( k_{P_Q,loop} [O] + k_{P_Q,unloop} ([N] - [O]) \right) \left( \frac{K_{Q-X_t} \cdot [X_t]}{1 + K_{Q-X_t} \cdot [X_t]} \right) - (\lambda_{Q_S} + \mu_D) [Q_S]$
$\frac{d[Q_L]}{dt} = \left( k_{P_Q,loop} [O] + k_{P_Q,unloop} ([N] - [O]) \right) \left( \frac{1}{1 + K_{Q-X_t} \cdot [X_t]} \right) - (\lambda_{Q_L} + \mu_D) [Q_L]$
$\frac{d[Q_t]}{dt} = [k_{Q_t,loop} [O] + k_{Q_t,unloop} ([N] - [O]) - k_t [X_t] \cdot [Q_t] - k_t [X_t] [Q_t] - (\lambda_{Q_t} + \mu_D) [Q_t]$
$\frac{d[X]}{dt} = k_{P_X,loop} [O] + k_{P_X,unloop} ([N] - [O]) - k_t [X_t] \cdot [Q_t] - (\lambda_X + \mu_D) [X]$
$\frac{d[X_t]}{dt} = k_{X_t,loop} [O] + k_{X_t,unloop} ([N] - [O]) - (k_{P_Q,loop} [O] + k_{P_Q,unloop} ([N] - [O])) \left( \frac{K_{Q-X_t} \cdot [X_t]}{1 + K_{Q-X_t} \cdot [X_t]} \right) - k_t [X_t] [Q_t] - (\lambda_{X_t} + \mu_D) [X_t]$
$\frac{d[i]}{dt} = k_i ([Q_S] + [Q_L]) \phi [D + T] - k_{T_i} \cdot ([i] - [I]) \phi [D + T] - \lambda_i \cdot [i]$
$\frac{d[I]}{dt} = k_{T_i} ([i] - [I]) - (\lambda_I + \mu_D) [I]$
$\frac{d[c]}{dt} = k_c \phi [R] - k_{T_c} ([c] - [C]) \phi [D + T] - \lambda_c [c]$
$\frac{d[C]}{dt} = k_{T_c} ([c] - [C]) - (\lambda_C + \mu_D) [C]$
$\frac{d[PrG]}{dt} = k_{PrG} [Q_L] - (\lambda_{PrG} + \mu_D) [PrG]$
$\frac{d[D]}{dt} = \mu_D [D]$
$\frac{d[R]}{dt} = \mu_R [R] - k_{conju} ([D] + [T]) \cdot \left( \frac{[R]}{[D] + [T] + [R]} \right) \cdot [PrG]$
$\frac{d[T]}{dt} = \mu_D [T] + k_{conju} ([D] + [T]) \cdot \left( \frac{[R]}{[D] + [T] + [R]} \right) \cdot [PrG]$

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**Table S2: List of variables**

<b>variable</b>	<b>Description</b>
$O$	The ratio of the DNA bound by (PrgX-iCF10) <sub>4</sub>
$Q_S$	$Q_S$ RNA
$Q_L$	$Q_L$ RNA
$Q_t$	truncated RNA in prgQ direction
$X$	mRNA producing PrgX
$X_t$	Anti-Q RNA
$i$	extracellular iCF10
$I$	intracellular iCF10
$c$	extracellular cCF10
$C$	intracellular cCF10
$Prg$	conjugation proteins
$D$	donor cells
$R$	recipient cells
$T$	transconjugant cells

**Table S3: List of parameters values**

<b>Parameter</b>	<b>Description</b>	<b>Value</b>
<b>N</b>	Plasmid copy number	5
$\mu_D$	Specific growth rate of donor/transconjugant cells	$2.58 \times 10^{-4} \text{ s}^{-1}$
$\mu_R$	Specific growth rate of recipient cells	$3.35 \times 10^{-4} \text{ s}^{-1}$
$k_{P_Q,loop}$	Transcription rate of full-length P <sub>Q</sub> RNA in looped state	$7.23 \times 10^{-3} \text{ s}^{-1}$
$k_{P_Q,unloop}$	Transcription rate of full-length P <sub>Q</sub> RNA in unlooped state	$8.87 \times 10^{-2} \text{ s}^{-1}$
$K_{X_i,loop}$	Transcription rate of X <sub>t</sub> in looped state	$1.02 \times 10^{-2} \text{ s}^{-1}$
$k_{X_i,unloop}$	Transcription rate of X <sub>t</sub> in unlooped state	$1.2 \times 10^{-3} \text{ s}^{-1}$
$k_{P_X,loop}$	Transcription rate of X in looped state	$8.23 \times 10^{-3} \text{ s}^{-1}$
$k_{P_X,unloop}$	Transcription rate of X in unlooped state	$1.21 \times 10^{-2} \text{ s}^{-1}$
$k_{Q_i,loop}$	Transcription rate of Q <sub>t</sub> in looped state	$1.80 \times 10^{-3} \text{ s}^{-1}$
$k_{Q_i,unloop}$	Transcription rate of Q <sub>t</sub> in unlooped state	$1.08 \times 10^{-2} \text{ s}^{-1}$
$k_{conju}$	Conjugation rate constant	$2 \times 10^{-4} (\text{nM})^{-1} \text{ s}^{-1}$
$k_i$	Generation rate of extracellular iCF10	$0.5 \text{ s}^{-1}$
$\phi$	Volume conversion factor	$1 \times 10^{-13} \text{ s}^{-1}$
$k_{T_i}$	Transport rate constant of iCF10	$1 \times 10^{-4} \text{ s}^{-1}$
$k_{T_c}$	Transport rate constant of cCF10	$1 \times 10^{-3} \text{ s}^{-1}$
$k_c$	Generation rate of extracellular cCF10	$0.6 \text{ s}^{-1}$
$k_i$	Rate constant of interaction between X RNA and Q <sub>t</sub> RNA	$1 \times 10^{-3} (\text{nM})^{-1} \text{ s}^{-1}$
$K_{Q-X_i}$	Equilibrium constant of Q and X <sub>t</sub> interaction	$4.43 (\text{nM})^{-1}$
$K_{DNA}$	Ratio of equilibrium binding constant of cCF10 to PrgX and iCF10 to PrgX	$1 \times 10^6 \text{ s}^{-1}$
$k_{PRG}$	Generation rate of membrane protein PrgB	$1 \times 10^{-3} \text{ s}^{-1}$
$\lambda_{Q_s}$	Degradation rate of Q <sub>s</sub> RNA	$1 \times 10^{-4} \text{ s}^{-1}$
$\lambda_{Q_L}$	Degradation rate of Q <sub>L</sub> RNA	$1 \times 10^{-1} \text{ s}^{-1}$
$\lambda_{X_i}$	Degradation rate of X <sub>t</sub> RNA	$3.851 \times 10^{-4} \text{ s}^{-1}$
$\lambda_{Q_t}$	Degradation rate of Q <sub>t</sub> RNA	$1 \times 10^{-3} \text{ s}^{-1}$
$\lambda_X$	Degradation rate of X RNA	$1 \times 10^{-4} \text{ s}^{-1}$
$\lambda_i$	Degradation rate of intracellular iCF10	$1 \times 10^{-6} \text{ s}^{-1}$
$\lambda_C$	Degradation rate of intracellular cCF10	$1 \times 10^{-6} \text{ s}^{-1}$
$\lambda_i$	Degradation rate of extracellular iCF10	$1 \times 10^{-6} \text{ s}^{-1}$
$\lambda_c$	Degradation rate of extracellular cCF10	$1 \times 10^{-6} \text{ s}^{-1}$
$\lambda_{prg}$	Degradation rate of PrgB membrane protein	$1 \times 10^{-3} \text{ s}^{-1}$

**Table S4: The blast result of *prgX* and *prgQ* genes (pCF10 bases 7029 to 8263)**

<i>Description</i>	<i>Query cover (%)</i>	<i>E value</i>	<i>Per. Identities (%)</i>
<i>Enterococcus faecalis plasmid pCF10</i>	100	0	100.00
<i>Enterococcus faecalis plasmid pMG2200</i>	100	0	99.92
<i>Enterococcus faecalis strain E512 plasmid pE512</i>	100	0	100.00
<i>Enterococcus faecalis 62 plasmid EF62pB</i>	100	0	100.00
<i>Enterococcus faecalis strain CVM N48037F plasmid pN48037F-2</i>	100	0	99.92
<i>Enterococcus faecalis strain sorialis plasmid Efsorialis-p2</i>	100	0	99.92