T7 RNA polymerase-driven inducible cell lysis for DNA transfer from *Escherichia coli* to *Bacillus subtilis*

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**Summary**

The majority of the good DNA editing techniques have been developed in *Escherichia coli*; however, *Bacillus subtilis* is better host for a plethora of synthetic biology and biotechnology applications. Reliable and efficient systems for the transfer of synthetic DNA between *E. coli* and *B. subtilis* are therefore of the highest importance. Using synthetic biology approaches, such as streamlined lambda Red recombineering and Gibson Isothermal Assembly, we integrated genetic circuits pT7L123, Repr-ss-1 and pLT7pol encoding the lysis genes of bacteriophages MS2, ΦX174 and lambda, the thermosensitive repressor and the T7 RNA polymerase into the *E. coli* chromosome. In this system, T7 RNA polymerase regulated by the thermosensitive repressor drives the expression of the phage lysis genes. We showed that T7 RNA polymerase significantly increases efficiency of cell lysis and transfer of the plasmid and bacterial artificial chromosome-encoded DNA from the lysed *E. coli* into *B. subtilis*. The T7 RNA polymerase-driven inducible cell lysis system is suitable for the efficient cell lysis and transfer of the DNA engineered in *E. coli* to other naturally competent hosts, such as *B. subtilis*.

**Introduction**

The Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *Bacillus subtilis* are among the key cellular chassis suitable for a plethora of synthetic biology devices and biotechnology applications (Harwood and Cranenburgh, 2008; Ajikumar *et al.*, 2010; Comminchau *et al.*, 2014; Juhas and Ajioka, 2016). Consequently, both *E. coli* and *B. subtilis* have been successfully used for the production of a number of the industrially important products, such as biofuels, biosensors, pharmaceuticals and natural dyes (Ajikumar *et al.*, 2010; Yim *et al.*, 2011; Park *et al.*, 2012; Zhou *et al.*, 2012; Hao *et al.*, 2013; Manabe *et al.*, 2013; McKenzie and Ajioka, 2013). Furthermore, both *E. coli* and *B. subtilis* are considered to be promising chassis for engineering of the minimal and tailor-made cell factories (Comminchau *et al.*, 2013; Juhas *et al.*, 2014b; Juhas, 2015; Juhas and Ajioka, 2016). The majority of the good DNA editing techniques have been developed in *E. coli*; however, *B. subtilis* has many advantages. This includes formation of endospores able to withstand severe environmental insults and secretion of the industrially valuable proteins into the cultivation medium. Furthermore, natural competence allows easy transformation of *B. subtilis* with foreign DNA and its integration into the chromosome by homologous recombination (Shi *et al.*, 2013; Yadav *et al.*, 2014).

The reliable and efficient systems for transfer of synthetic DNA, particularly of the high-molecular-weight DNA, which is prone to physical breakage, between *E. coli* and *B. subtilis* chassis are therefore of the highest importance. Several methods have been developed to address this issue in the last few years. The conditionally inducible cell lysis system relying on the lambda prophage has been used successfully for the transfer of DNA released from the lysed *E. coli* cells into *B. subtilis* (Itaya and Kaneko, 2010; Kaneko and Itaya, 2010; Gao *et al.*, 2013). In this system, the co-culture method was used to transfer the high-molecular-weight DNA from *E. coli* undergoing lambda prophage-induced lysis to *B. subtilis* (Itaya and Kaneko, 2010; Kaneko and Itaya, 2010). Furthermore, transfer of genetic circuits from the lysed *E. coli* to *B. subtilis*, which does not rely on the entire phage to lyse the donor cell, has been demonstrated recently (Juhas *et al.*, 2016). This system utilizes only the individual lysis genes from multiple phages to disrupt the donor *E. coli* cell. A similar system, based on the lytic genes of the lactococcal bacteriophage φUS3, has been developed for an inducible lysis of *Lactococcus lactis* for accelerated cheese ripening (de Ruyter *et al.*, 1996, 1997).

T7 RNA polymerase expression system, based on the RNA polymerase of bacteriophage T7, is among the...
most efficient and widely used systems for the expression of recombinant proteins (Studier and Moffatt, 1986; Kortmann et al., 2015; Wei et al., 2017). T7 RNA polymerase is independent of the host auxiliary transcription factors, has high processivity and specificity towards the p77 promoter, and is able to produce long transcripts faster than the multi-subunit bacterial RNA polymerase (Kortmann et al., 2015; Wei et al., 2017). Consequently, T7 polymerase has been successfully used for regulating protein expression in a number of bacteria, including Pseudomonas aeruginosa, Pseudomonas putida, Streptomyces lividans, Streptomyces coelicolor, Rhodobacter capsulatus,Ralstonia eutropha, Bacillus megaterium, Corynebacterium acetoxidophilum and Corynebacterium glutamicum (Brunschwig and Darzins, 1992; Herrero et al., 1993; Barnard et al., 2004; Gamer et al., 2009; Lussier et al., 2010; Arvani et al., 2012; Equbal et al., 2013; Kortmann et al., 2015; Wei et al., 2017).

Here, we present an inducible cell lysis system, which utilizes the chromosomally integrated genetic circuit encoding T7 RNA polymerase, to drive the expression of the phage lysis genes for the reliable and efficient cell lysis and plasmid and bacterial artificial chromosome (BAC)-borne DNA transfer from E. coli into B. subtilis.

Results and discussion

Construction of the T7 RNA polymerase-driven inducible cell lysis system

To construct the T7 RNA polymerase-driven inducible cell lysis system for the controlled lysis of E. coli and DNA transfer from the lysed E. coli cells to B. subtilis, we first engineered genetic circuits pT7L123, Repr-ts-1 and pLT7pol (Fig. 1A and Fig. S1). The genetic circuit pT7L123 encodes lysis genes of the three bacteriophages, namely MS2, ΦX174 and lambda. We chose lysis genes of the bacteriophages MS2, ΦX174 and lambda mainly because they have different mechanism of action and are all well characterized (Young, 1992; Gründling et al., 2001; Berry et al., 2008; Shulman et al., 2012; Tanaka and Clemons, 2012; Catalão et al., 2013).

Furthermore, we showed previously that combining lysis genes of the bacteriophages MS2, ΦX174 and lambda in the same cell significantly improves lysis efficiency of E. coli cells (Juhas et al., 2016). Lysis genes of the bacteriophages MS2, ΦX174 and lambda in the engineered genetic circuit pT7L123 are located downstream of the p77 promoter, which is regulated by the T7 RNA polymerase. We incorporated a RBS upstream of the first lysis gene (MS2) of the genetic circuit pT7L123. Furthermore, we isolated the genetic circuit pT7L123 with the T0 transcription terminator on the 5’ end and with the t1 transcriptional terminator on the 3’ end obtained from the Registry of Standard Biological Parts (http://parts.igem.org/Main_Page; Juhas et al., 2014a; Fig. 1A and Fig. S1).

The genetic circuit Repr-ts-1 encodes the thermosensitive lambda repressor regulated by a strong constitutive promoter. In addition, Repr-ts-1 harbours a RBS and double terminator (B0015) from the Registry of Standard Biological Parts (Fig. 1A and Fig. S1).

The genetic circuit pLT7pol encodes T7 RNA polymerase (Wyckoff and Matthews, 2000) located downstream of the pL promoter, which is regulated by the thermosensitive lambda repressor (Fig. 1A and Fig. S1). Furthermore, pLT7pol harbours a RBS and is isolated with the T0 transcription terminator on the 5′ end and with the t1 transcriptional terminator on the 3′ end (Fig. 1A and Fig. S1).

We first cloned all three engineered genetic circuits pT7L123, Repr-ts-1 and pLT7pol into plasmid pSB1K3 (FRTK; Juhas et al., 2014a) next to the kanamycin resistance cassette, which is flanked by FRT (flippase recombinase target) sites, employing Gibson Isothermal Assembly method (Gibson et al., 2009; Merryman and Gibson, 2012; Data S1). Then, we integrated the genetic circuits pT7L123, Repr-ts-1 and pLT7pol into the E. coli chromosome using the streamlined lambda Red recombinase-mediated approach described previously (Dat-senko and Wanner, 2000; Juhas et al., 2014a). By integrating the genetic circuits pT7L123, Repr-ts-1 and pLT7pol into the E. coli chromosome, we generated the strain Ec(RpT7L123pLT7i1). We removed the kanamycin resistance marker from the integrated modules pT7L123, Repr-ts-1 and pLT7pol in the strain Ec(RpT7L123pLT7i1) using plasmid pEP-FLP, which encodes constitutively expressed FLP recombinase (St-Pierre et al., 2013). We confirmed the correct assemblies of the genetic circuits pT7L123, Repr-ts-1 and pLT7pol and their successful integration into the E. coli chromosome by diagnostic PCR (Fig. 1B and Table S1) and sequencing.

The expression of the pLT7pol-encoded T7 RNA polymerase in the engineered strain Ec(RpT7L123pLT7i1) is inhibited by the Repr-ts-1-encoded thermosensitive repressor (Fig. 1A). T7 RNA polymerase then in turn regulates expression of the genetic circuit pT7L123, which encodes lysis genes of the bacteriophages MS2, ΦX174 and lambda. At 30°C, the expression of T7 RNA polymerase from the pL promoter in the genetic circuit pLT7pol is inhibited by the Repr-ts-1-encoded thermosensitive repressor. A simple temperature shift to 42°C alleviates this repression and leads to activation of the T7 RNA polymerase, which in turn leads to expression of the lysis genes of the bacteriophages MS2, ΦX174 and lambda from the p77 promoter and cell lysis. DNA released from the lysed E. coli cells can be subsequently taken up by other hosts, such as B. subtilis (Fig. 1A).
RNA polymerase increases lysis efficiency of the inducible cell lysis system

To test the effect of the integrated T7 RNA polymerase on the inducible cell lysis, we compared the lysis efficiency of the engineered T7 RNA polymerase-driven temperature-inducible cell lysis system in the strain Ec (RpT7L123pLT7i) with the strains Ec(Ri) and Ec (RpLL123i). The strain Ec(Ri) only harbours the genetic circuit Rep-ts-1 encoding the thermosensitive repressor integrated in the chromosome. The strain Ec(RpLL123i) has the genetic circuits Rep-ts-1 and pLL123 integrated into the chromosome, but does not harbour the genetic circuit pLT7pol. Consequently, as the strain Ec
(RpLL123i) has not been augmented with the genetic circuit pLT7pol, the expression of the lysis genes of the bacteriophages MS2, φX174 and lambda in the strain Ec(RpLL123i) is directly regulated by the thermosensitive repressor (Juhás et al., 2016) instead of through T7 RNA polymerase.

We first analysed the cell lysis efficiency by measuring absorbance over time in the investigated strains with the microplate reader (Fig. 2A). The absorbance of the strains Ec(Ri), Ec(RpLL123i) and Ec(RpT7L123pLT7i) grown at the restrictive temperature for the thermosensitive repressor for 24 h was compared with the absorbance when the same strains were grown in both at the restrictive and permissive temperature (Fig. 2A). The statistical t-test analysis revealed significant differences between the absorbance measured at induced and non-induced conditions in strains Ec(RpLL123i; $P < 0.005$) and Ec(RpT7L123pLT7i; $P < 0.005$), but not the strain Ec(Ri). To test the effect of the T7 RNA polymerase in the strain Ec(RpT7L123pLT7i) on the cell lysis, we calculated the differences between the mean absorbances of the strains Ec(RpT7L123pLT7i) and Ec(RpLL123i), which does not harbour T7 RNA polymerase, grown at restrictive and restrictive and permissive conditions (Fig. 2A). The statistical t-test analysis revealed significant differences between the degree of the cell lysis of Ec(RpT7L123pLT7i) and Ec(RpLL123i) with 11% ($P < 0.05$) increase in the differences between the mean absorbances in the strain Ec(RpT7L123pLT7i; Fig. 2A).

This experiment suggested that the strain Ec (RpT7L123pLT7i) harbouring the chromosomally integrated T7 RNA polymerase-encoding genetic circuit had the highest proportion of the dead cells after the temperature shift from 30°C to 42°C. The means and standard errors were calculated from the three biological replicates. The statistical t-test was conducted to assess the variations in the proportion of the live/dead bacteria. Stars indicate significant difference in the proportion of the live bacteria between Ec(RpLL123i) and Ec(RpT7L123pLT7i).

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the numbers of CFUs after the induction of the cell lysis as described in the Experimental procedures section (Fig. 2B). The numbers of the viable cells in both strains Ec(RpT7L123pLT7i) and Ec(RpLL123i) were significantly lower than in the strain Ec(Ri). Furthermore, the statistical t-test analysis showed that the number of viable cells in the strain Ec(RpT7L123pLT7i) was almost four times lower ($P < 0.005$) than in the strain Ec(RpLL123i), which does not harbour T7 RNA polymerase, thus confirming the increased lysis efficiency of the engineered T7 RNA polymerase-driven inducible cell lysis system (Fig. 2B).

In addition to the above experiments, we measured the cell lysis efficiency by staining the analysed strains grown at the restrictive and permissive conditions with the BacLight Live/Dead bacterial viability stain and evaluated the stained bacterial strains by fluorescent microscopy. This analysis showed that the viability of strains Ec(RpT7L123pLT7i) and Ec(RpLL123i) was significantly lower than the viability of the strain Ec(Ri); Fig. 2C). Furthermore, the statistical t-test analysis revealed a significant difference in the proportion of the live and dead bacteria in the investigated strains Ec (RpT7L123pLT7i) and Ec(RpLL123i) with 26% ($P < 0.05$) increase in the number of the dead bacteria and bacteria with the damaged membranes in the strain Ec(RpT7L123pLT7i) after the induction of the cell lysis (Fig. 2C).

These experiments showed that chromosomally integrated T7 RNA polymerase in the engineered T7 RNA polymerase-driven temperature-inducible cell lysis system in the strain Ec(RpT7L123pLT7i) significantly increases the cell lysis efficiency compared with the strain Ec(RpLL123i) where the cell lysis is directly controlled by the thermosensitive repressor. To compare the efficiency of the engineered T7 polymerase-driven inducible cell lysis system with the chemical lysis system, we used a modified phenol–chloroform method (Song et al., 1999). To quantify the number of the viable E. coli cells, we counted the numbers of CFUs after the induction of the cell lysis by phenol–chloroform as described in the Experimental procedures section (Fig. 2B). The number of the viable cells after the chemical lysis was significantly lower than in strains Ec (RpT7L123pLT7i), Ec(RpLL123i) and Ec(Ri), thus showing that the phenol–chloroform-induced cell lysis is more efficient than the temperature-inducible cell lysis. Overall, the direct counting of the CFUs of the viable bacteria after the induction of the cell lysis provided the most significant difference in the lysis efficiency between the strains Ec(RpT7L123pLT7i) and Ec (RpLL123i), with 73% decrease in the number of the viable cells ($P < 0.005$) in the strain Ec (RpT7L123pLT7i; Fig. 2B).

**T7 polymerase-driven inducible cell lysis for DNA transfer from E. coli to B. subtilis**

We used plasmid pJScav and integrative bacterial artificial chromosome iBAC(cav)1 and iBAC(10)-borne DNA to investigate the suitability of the T7 RNA polymerase-driven inducible cell lysis system for transfer of DNA released from the lysed E. coli into B. subtilis. pJScav and iBAC(cav)1 harbour genetic circuits encoding *Bacillus*-specific chloramphenicol resistance and the yellow fluorescent protein mVenus flanked by the sequences homologous to the *amyE* target site in the *B. subtilis* chromosome. In addition to chloramphenicol resistance and mVenus, iBAC(10) harbours a 10 kb DNA fragment flanked by the sequences homologous to the *amyE* site.

To test the ability of pJScav, iBAC(cav)1 and iBAC (10) to integrate into the *amyE* site in the *B. subtilis* chromosome, we transformed equivalent amount of pJScav, iBAC(cav)1 and iBAC(10) DNA into the competent *B. subtilis* cells as described in the Experimental procedures. pJScav, iBAC(cav)1 and iBAC (10) did integrate into the *B. subtilis* chromosome, with pJScav yielding the highest number of transformants ($7.2 \times 10^4$ transformants per µg DNA; Fig. 3A).

Next, to analyse our engineered T7 RNA polymerase-driven inducible cell lysis system, we used culture mix method (Itaya and Kaneko, 2010; Kaneko and Itaya, 2010) to transfer pJScav, iBAC(cav)1 and iBAC(10) from the lysed *E. coli* cells into *B. subtilis*. Following transformation of pJScav, iBAC(cav)1 or iBAC(10) into the *E. coli* strains Ec(Ri), Ec(RpT7L123pLT7i) and Ec (RpLL123i), and the temperature-induced cell lysis, pJScav, iBAC(cav)1 and iBAC(10), DNA was taken up by *B. subtilis* cultivated in the same test tube. Sequences homologous to *amyE* target site in the *B. subtilis* chromosome flanking chloramphenicol resistance and mVenus genetic circuits in pJScav and iBAC (cav)1 and 10 kb DNA fragment in iBAC(10) facilitated integration of the transferred DNA into the *B. subtilis* chromosome. As a consequence, *B. subtilis* cells, which successfully integrated transferred pJScav, iBAC(cav)1 and iBAC(10) DNA into their chromosome emitted fluorescent light and grew on chloramphenicol (Fig. 3B). Furthermore, we confirmed integrations by diagnostic PCR using flanking primers and sequencing (Fig. 3C and Table S1). We verified that the DNA transfer occurred via stable DNA in the medium by the addition of DNase I, which inhibited pJScav and iBAC(cav)1 and iBAC(10)-borne DNA transfer from *E. coli* to *B. subtilis* (Fig. 3B).

pJScav, iBAC(cav)1 and iBAC(10) transferred into the recipient *B. subtilis* with various efficiency for the donor *E. coli* strains Ec(Ri), Ec(RpT7L123pLT7i) and Ec (RpLL123i). When using strain Ec(Ri), the low-efficiency
transfer of pJScav and iBAC(cav)1 and iBAC(10) from *E. coli* to *B. subtilis* occurred due to spontaneous lysis of *E. coli* Ec(Ri) cells (Corchero et al., 2001; Juhas et al., 2016). Both donor strains Ec(Rp7L123pLT7i) and Ec(RpLL123i) transferred pJScav and iBAC(cav)1 and iBAC(10) to *B. subtilis* with higher efficiency than the strain Ec(Rc). The statistical t-test analysis revealed that there was a significant difference in the transfer of pJScav, iBAC(cav) and iBAC(10) from *E. coli* to *B. subtilis* when using strains Ec(RpLL123i) and Ec(Rp7L123pLT7i). pJScav transfer using strain Ec(Rp7L123pLT7i) led to 112% (P < 0.01) increase in the number of the positive *B. subtilis* recipients when compared with the strain Ec(RpLL123i) 6 h after the induction of cell lysis (Fig. 3D). Similarly, compared with the strain Ec(RpLL123i), iBAC(cav)1 and iBAC(10), transfer using *E. coli* strain Ec(Rp7L123pLT7i) led to 51% (P < 0.01) and 46% (P < 0.05) increase in the number of the positive *B. subtilis* recipients respectively (Fig. 3E). This showed that integration of the T7 RNA polymerase-encoding genetic circuit into the *E. coli* chromosome in our T7 RNA polymerase-driven inducible cell lysis system in the strain Ec(Rp7L123pLT7i) strongly improves DNA transfer from *E. coli* to *B. subtilis*.
Conclusions

As both *E. coli* and *B. subtilis* are among the main chassis for a number of synthetic biology and biotechnology applications, it is important to develop reliable and efficient systems for the transfer of synthetic DNA (especially the high-molecular-weight DNA, which is prone to physical breakage) between these two cell types. The previously engineered cell lysis systems either required the chemical inducer, enzyme or mechanical force or utilized the entire prophages to disrupt the donor *E. coli* cell, or their efficiency of lysis was not very high (Itaya and Kaneko, 2010; Kaneko and Itaya, 2010; Gao et al., 2013; Juhas et al., 2016).

To address this problem, we investigated the effect of T7 RNA polymerase on the cell lysis. T7 RNA polymerase expression system is among the most efficient and widely used systems, which has been successfully applied to regulate the protein expression in a number of bacteria. We integrated three genetic circuits encoding the T7 RNA polymerase, the thermosensitive repressor and the lysis genes of three bacteriophages MS2, lambda and φX174 into the *E. coli* chromosome using streamlined lambda Red recombineering approach (Juhas et al., 2014a). The expression of T7 RNA polymerase in our inducible cell lysis system is regulated by the thermosensitive repressor. T7 RNA polymerase in turn regulates expression of the pT7 promoter, which is located upstream of the bacteriophages’ lysis genes. We showed that the chromosomally integrated T7 RNA polymerase significantly increases efficiency of *E. coli* cell lysis and transfer of the plasmid and BAC-encoded DNA from the lysed *E. coli* cells into *B. subtilis*.

The chemical lysis is even faster and more efficient; however, due to the harmful and toxic effects of the phenol–chloroform, the application of the chemical lysis approach for the direct transfer of the DNA from lysed *E. coli* cells to *B. subtilis* in a single tube would be quite problematic. Furthermore, it does not address the issue of the shearing of the high-molecular-weight DNA.

To achieve better phage lysis, other genes crucial for the phages’ life cycle could be integrated into the chromosome. It has been shown previously that only simultaneous expression of the lytic genes *lytA* and *lytH*, encoding the lysozyme and the holin proteins of bacteriophage φUS3, leads to the efficient lysis of *L. lactis*, whereas expression of holin or lysozyme alone does not cause significant lysis (de Ruyter et al., 1997). Furthermore, the genome of bacteriophage lambda has almost 50 kb and produces approximately 100 virions after each cell lysis (Gandon, 2016). Our system presented here utilizes only the single chromosomally integrated copy of the phages’ lysis genes. Further improvements of the system should undergo therefore include integration of other genes important for the phages’ life cycle. Other promoters, such as *pRac-LacI*, *pRpl18*, *pRsaA*, *pCro* and the light-inducible cl repressor can be also used to improve the efficiency of the cell lysis (Zhang et al., 2009; Gao et al., 2013; Lee et al., 2013; Juhas et al., 2016).

The possibility of utilizing other mechanisms, such as conjugative transfer of the synthetic DNA from the donor *E. coli* to the recipient *B. subtilis*, could also be investigated in more detail. Utilizing conjugative DNA transfer from *E. coli* to *Bacillus megaterium* led to the acquisition of up to 15 000 transconjugants in a single experiment (Richhardt et al., 2010). Although the efficiency of DNA transfer from *E. coli* to *B. megaterium* by conjugation is considerably lower than the DNA transfer from *E. coli* to *B. subtilis* presented in our study, conjugation is particularly important for other industrially relevant members of the genus with extremely poor natural competence, such as *B. megaterium*.

To our knowledge, this is the first study analysing the effect of T7 RNA polymerase on the inducible lysis of *E. coli* and DNA transfer and integration into the *B. subtilis* chromosome. Our engineered T7 RNA polymerase-driven inducible cell lysis system has many benefits over the previously used approaches, such as no need for chemical inducer, enzyme or entire prophage, which could be detrimental to the quality of the transferred DNA. Combined with the culture mixed method (Itaya and Kaneko, 2010; Kaneko and Itaya, 2010), our system is suitable for the reliable and efficient cell lysis and DNA transfer from the lysed *E. coli* to other naturally competent hosts, such as *B. subtilis*.

Experimental procedures

Strains, plasmids, BACs and growth conditions

All bacterial strains, BACs and plasmids used in this study are listed in Table 1. Luria-Bertani broth (LB) was used for routine growth of *B. subtilis* and *E. coli*. Growth media were supplemented when needed with the following concentrations of antibiotics: kanamycin (5 µg ml⁻¹) and chloramphenicol (5 µg ml⁻¹) for growing *B. subtilis*, and kanamycin (50 µg ml⁻¹), chloramphenicol (30 µg ml⁻¹) and ampicillin (100 µg ml⁻¹) for growing *E. coli*. Bacterial cultures were grown at 30°C, 37°C or 42°C on solid media for 24 h. Liquid bacterial cultures were incubated on a rotatory shaker at 200 r.p.m. and 30°C, 37°C or 42°C, according to requirements. To generate competent *B. subtilis* cells, *B. subtilis* was routinely grown in a starvation medium composed of 5× minimal salts solution (potassium hydrogen phosphate (75 mg ml⁻¹), potassium dihydrogen phosphate (25 mg ml⁻¹), ammonium sulfate (10 mg ml⁻¹), sodium citrate (1 mg ml⁻¹), magnesium sulfate heptahydrate (1 mg ml⁻¹)) and glucose (0.5% w/v).

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Table 1. Bacterial strains, plasmids and bacterial artificial chromosomes (BACs) used in this study.

| Characteristics | Reference |
|-----------------|-----------|
| **Strains**     |           |
| Ec              | *Escherichia coli* wild-type strain K12 MG1655 | Hayashi et al. (2006) |
| Bs              | *Bacillus subtilis* wild-type strain 168 | Laboratory collection |
| Ec(Ri)          | *E. coli*, chromosomally integr. ts repressor | Juhas et al. (2014a) |
| Ec(RpLL123i)    | *E. coli*, integr. ts repressor, MS2, φX174, λ | Juhas et al. (2016) |
| Ec(RpTL123pLT7i) | *E. coli*, ts repressor, MS2, φX174, λ, pT7 promoter, T7 polymerase pl promoter | This study |
| Bs(acavi)       | *B. subtilis*, integr. chloramphenicol, mVenus | This study |
| Bs(10i)         | *B. subtilis*, integr. 10 kb insert | This study |
| **Plasmids and BACs** |           |
| pSB1K3          | Standard assembly plasmid, BioBricks | Parts registry |
| pKM208          | IPTG-inducible lambda Red system | Murphy and Campellone (2003) |
| pE-FLP          | FLP recombinase, constitutive expression | St-Pierre et al. (2013) |
| pFRTKr          | pSB1K3 with ts repressor | Juhas et al. (2014a) |
| pTARA           | Plasmid with T7 RNA polymerase | Wycuff and Matthews (2000) |
| pJScav          | Plasmid with chloramphenicol, mVenus | Parts registry |
| pFRTpTL123      | MS2, φX174, λ with pT7 promoter in pSB1K3 | This study |
| pFRTpLT7        | T7 polymerase with pl promoter in pSB1K3 | This study |
| iBAC(10)        | BAC, amyE sites, 10 kb insert | This study |
| iBAC(cav)1      | BAC, amyE sites, chloramphenicol, mVenus | This study |

Transformation of competent *B. subtilis* cells

To induce competency, *B. subtilis* was grown first in 10 ml minimal medium composed of 5× minimal salts solution, casamino acids (0.02% w/v), tryptophan (20 μg ml⁻¹), iron ammonium citrate (2.2 μg ml⁻¹) and glucose (0.5% w/v) at 200 r.p.m. and 37°C for 18 h; 1.4 ml of this culture was inoculated into 10 ml of the fresh minimal medium and grown at 200 r.p.m. and 37°C for another 3 h. Then, 11 ml of the starvation medium composed of 5× minimal salts solution and glucose (0.5% w/v) was added and cells were incubated at 200 r.p.m. and 37°C for additional 2 h and 45 min. 0.3 ml aliquots were transformed with 15 μl of plasmid or BAC DNA in 15 ml polypropylene tubes and incubated first at 200 r.p.m. at 37°C for 1 h and then at 200 r.p.m. at 37°C for 2 h with additional 700 μl LB. Subsequently, 20–200 μl was plated onto selection plates and incubated at 37°C for 20 h. DNA integrations into the *B. subtilis* chromosome were verified by diagnostic PCR and sequencing.

DNA amplification and modification

DNA purification was performed using Qiaquick Gel Extraction kit (Qiagen, Hilden, Germany). Plasmid isolation was performed with Qiaprep Spin Miniprep kit (Qiagen). Isolation of BACs was performed using Qiaprep Spin Miniprep kit (Qiagen) or PhasePrep BAC DNA kit (Sigma-Aldrich, Irvine, UK) using instructions provided by the kits’ manufacturer. GeneJET genomic DNA purification kit (Thermo Scientific, Loughborough, UK) was used to isolate genomic DNA from *B. subtilis*. DNA sequencing was performed by Source Bioscience. Oligonucleotide primers for PCR amplification were synthesized by Sigma-Aldrich and the longer DNA fragments by Integrated DNA Technologies (IDT). PCR amplification of DNA fragments was conducted in a 25 μl or 50 μl final reaction volume with Dream Taq master mix kit (Thermo Scientific), Phusion DNA polymerase (Thermo Scientific) or Q5 high-fidelity DNA polymerase (NEB) using instructions recommended by the supplier. Assembly of DNA fragments was performed using Gibson Isothermal Assembly in a 5.2 μl final reaction volume (Gibson et al., 2009; Merryman and Gibson, 2012; Juhas et al., 2014a). Confirmation of the correctly assembled DNA fragments was performed by diagnostic PCR using flanking primers and DNA sequencing.

DNA integration into the *E. coli* chromosome

The chemically competent *E. coli* and the electro-competent *E. coli* were generated using the modified Hanahan et al. (1991) and Miller and Nickoloff (1995) methods respectively. Lambda Red recombinating to integrate DNA into the *E. coli* chromosome was performed using the previously described procedure (Juhas et al., 2014a; Juhas and Ajioka, 2015a,b). Briefly, plasmid pKM208 encoding IPTG-inducible lambda Red recombinase system was electroporated into *E. coli*. The cells were selected on the ampicillin plates and then grown in the liquid LB with ampicillin at 30°C overnight, diluted (1:100 dilution) into fresh LB with ampicillin and grown at 30°C to OD₆₀₀ 0.2. After the addition of IPTG (1 mM) at OD₆₀₀ 0.2, the bacterial culture was grown to OD₆₀₀ 0.5. Cells were washed twice and then resuspended in 10% glyceral and electrooporated with 100 μl of the amplified DNA fragments harbouring sequences homologous to the integration sites in the *E. coli* chromosome. Transformants
with integrated DNA were selected by growth on selective plates at 30°C overnight. pKM208 was cured out by growth at 42°C. Integration of DNA fragments into the E. coli chromosome was verified by diagnostic PCR and sequencing. The kanamycin resistance marker was flipped off from the chromosome using the thermosensitive plasmid pEP-FLP encoding constitutively expressed flippase (FLP) recombinase (St-Pierre et al., 2013).

DNA transfer from E. coli to B. subtilis

DNA transfer from E. coli to B. subtilis was performed using the modified culture mix method (Itaya and Kaneko, 2010; Kaneko and Itaya, 2010) described previously (Juhas et al., 2016). Briefly, E. coli grown in liquid LB medium at 30°C for 24 h was diluted (1:200 dilution) into a fresh 20 ml LB with appropriate antibiotics and grown at 30°C for 5 h. Cells were centrifuged at 5000 r.p.m. for 8 min and then resuspended in a fresh 20 ml LB and grown for 1 h. B. subtilis grown in a liquid LB at 37°C for 17 h was diluted (1:200 dilution) into a 20 ml TFI medium [5 x minimal salts solution, glucose (0.5% w/v), casamino acids (2% w/v), tryptophan (50 μg ml⁻¹), threonine (50 μg ml⁻¹), leucine (50 μg ml⁻¹), arginine (50 μg ml⁻¹)] and cultivated at 37°C for 5 h. E. coli and B. subtilis cells were mixed (1:1 ratio) and grown at 42°C for 6 h. Then, 200 μl of the mixed bacterial culture was transferred on LB plate supplemented with chloramphenicol. The remaining E. coli cells were eliminated by inducing B. subtilis sporulation and heat treatment. First, colonies scraped off the plates were resuspended in 1 ml of 2 x SG medium [Difco nutrient broth (Difco Laboratories, Detroit, MI, USA); 16 mg ml⁻¹], glucose (0.1% w/v), 1 mM FeSO₄ (0.1% v/v), 0.1 M MnCl₂.4H₂O (0.1% v/v), KCl (2 mg ml⁻¹), MgSO₄.7H₂O (0.5 mg ml⁻¹), 1 M Ca(NO₃)₂ (0.1% v/v) and grown on SG plates for 72 h. Then, cells scraped off the 2 x SG plates were washed with water and incubated at 90°C for 10 min. Subsequently, 100 μl was transferred on LB plates supplemented with chloramphenicol and grown at 37°C overnight. The CFUs and standard deviations were calculated from three biological replicates.

Bacterial viability and growth assays

To quantify the viability and growth of the bacterial strains, several assays were performed.

In the first assay, the absorbance of the bacterial cultures was measured using 96-well microplates (clear, Sterilin Sero-Well, Loughborough, UK). Overnight, bacterial cultures were normalized to OD₆₀₀ of 0.05 and 200 μl of the normalized cultures was transferred into the microplate wells. Microplates were incubated in the microplate reader (Fluostar Omega, BMG Labtech, Aylesbury, UK) at 30°C for 3 h and at 42°C for another 21 h or at 30°C for 24 h. Absorbance was quantified using the automatically repeated protocol (double orbital shaking at 500 r.p.m., cycle time 60 min, absorbance filter 600 nm). The average absorbance and the standard deviations were calculated from three experiments.

In the second assay, the overnight bacterial cultures adjusted to optical density OD₆₀₀ of 0.05 were incubated at 30°C for 3 h and then at 42°C for another 6 h on a rotary shaker at 200 r.p.m. Bacterial cultures were serially diluted up to 10⁻⁶ in a fresh LB media (pre-heated to 42°C) 6 h after the induction of cell lysis; 100 μl of the diluted bacterial cultures was spread on solid LB media plates pre-heated to 42°C and incubated overnight at 42°C. The average numbers of CFUs and standard errors were calculated from three independent biological replicates.

In the third assay, the viability of the E. coli cells was analysed using the Live/Dead BacLight™ bacterial viability kit (Lifetechnologies, Paislay, UK), according to supplier’s instructions. First, bacterial cells were incubated on a rotary shaker at 200 r.p.m. and 30°C for 6 h and then at 42°C for another 6 h. Bacterial cultures were washed with 0.85% NaCl and adjusted to 1 x 10⁶ bacteria per 1 ml of 0.85% NaCl. Bacteria were then mixed with the red-fluorescent nucleic acid stain propidium iodide and the green-fluorescent nucleic acid stain SYTO9 (3 μl of stain per 1 ml of medium containing bacteria) and examined by fluorescent microscopy (Nikon Microphot-SA, Kingston Upon Thames, Surrey, UK). The numbers of the dead bacteria (propidium iodide-stained) and the live bacteria (SYTO-9 stained) were calculated 6 h after the induction of the cell lysis. The means and standard errors were calculated from three biological replicates.

Chemical lysis of E. coli

The control chemical lysis of E. coli cells was performed by the modified method (Song et al., 1999), described earlier. Briefly, bacterial cultures were serially diluted up to 10⁻⁶ in LB media and 500 μl thereof was centrifuged at 8000 r.p.m. for 5 min. The pellets were resuspended in 500 μl of the 1:1 of phenol and chloroform solution (v/v). Then, the samples were mixed vigorously by vortexing for 10 s, incubated with shaking at room temperature for 30 min and centrifuged at 13 000 r.p.m. for 5 min. The pellets were resuspended in 100 μl of a fresh LB media, spread on solid LB media plates and incubated overnight at 37°C. The mean CFU values and standard errors were calculated from three replicates.

Sequence analyses

DNA sequences were analysed using tBLASTX, BLASTN (Altschul et al., 1990) and position-specific iterated BLAST
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Conflict of interest

There is no conflict of interests related to this manuscript.

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**Supporting information**

Additional Supporting Information may be found online in the supporting information tab for this article:

**Fig. S1.** Genetic circuits *pT7*L123, Repr-ts-1 and *pLT7*pol. Figure shows schematic view of the genetic circuits *pT7*L123, Repr-ts-1 and *pLT7*pol, which were integrated into the *E. coli* chromosome to generate the T7 RNA polymerase-driven inducible cell lysis system.

**Table S1.** Primers used in this study.

**Data S1.** Plasmids pFRT*pT7*L123 and pFRT*pLT7*. Sequence of plasmids pFRT*pT7*L123 and pFRT*pLT7* harbouring genetic circuits *pT7*L123 and *pLT7*pol (highlighted green) respectively.