Short Technical Reports

- Su and J.M. Trent. 1996. Use of a cDNA microarray to analyze gene expression patterns in human cancer. *Nature Genet.* 14:457-460.
6. Feinberg, A.P. and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.
7. Horstmann, H.J., J.W. Rohen and K. Sames. 1983. Age-related changes in the composition of proteins in the trabecular meshwork of the human eye. *Mech. Ageing Dev.* 21:121-136.
8. Jordan, B.R. 1998. Large-scale expression measurement by hybridization methods: from high density membranes to "DNA chips". *J. Biochem.* 124:251-258.
9. Lennon, G., C. Auffray, M. Polymeropoulos and M.B. Soares. 1996. The I.M.A.G.E. consortium: an integrated molecular analysis of genomes and their expression. *Genomics* 33:151-152.
10. Nguyen, C., D. Rocha, S. Granjeaud, M. Baldit, K. Bernard, P. Naquet and B.R. Jordan. 1995. Differential gene expression in the murine thymus assayed by quantitative hybridization of arrayed cDNA clones. *Genomics* 29:207-216.
11. Patel, P.H. and B.D. Preston. 1994. Marked infidelity of human immunodeficiency virus type 1 reverse transcriptase at RNA and DNA template ends. *Proc. Natl. Acad. Sci. USA* 91:549-553.
12. Pietu, G., O. Alibert, V. Guichard, B. Lamy, F. Bois, E. Leroy, R. Mariage-Sampson, R. Houlgatte, P. Soularue and C. Auffray. 1996. Novel gene transcripts preferentially expressed in human muscles revealed by quantitative hybridization of a high density cDNA array. *Genome Res.* 6:492-503.
13. Schena, M., D. Shalon, R.W. Davis and P.O. Brown. 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270:467-470.
14. Schena, M., D. Shalon, R. Heller, A. Chai, P.O. Brown and R.W. Davis. 1996. Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. *Proc. Nat. Acad. Sci. USA* 93:10614-10619.
15. Stamer, W.D., R.E. Seftor, S.K. Williams, H.A. Samaha and R.W. Snyder. 1995. Isolation and culture of human trabecular meshwork cells by extracellular matrix digestion. *Curr. Eye Res.* 14:611-617.

This work was supported by National Eye Institute Grant Nos. EY11906 and EY01894, the American Health Assistance Foundation and the Research to Prevent Blindness. T.B. is a Jules and Doris Stein Research to Prevent Blindness Professor Awardee. Address correspondence to Dr. Teresa Borrás, Duke University Medical Center, Wadsworth Building, Erwin Road, Box 3802, Durham, NC 27710, USA. Internet: borra001@mc.duke.edu

Received 17 September 1998; accepted 2 February 1999.

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Increasing DNA Transfer Efficiency by Temporary Inactivation of Host Restriction

BioTechniques 26:892-900 (May 1999)

ABSTRACT

E. coli and Salmonella typhimurium are widely used bacterial hosts for genetic manipulation of DNA from prokaryotes and eukaryotes. Introduction of foreign DNA by electroporation or transduction into E. coli and Salmonella is limited by host restriction of incoming DNA by the recipient cells. Here, we describe a simple method that temporarily inactivates host restriction, allowing high-frequency DNA transfer. This technique might be readily applied to a wide range of bacteria to increase DNA transfer between strains and species.

INTRODUCTION

Molecular biology relies on the ability to efficiently introduce foreign DNA into different hosts. Cloned DNA from bacteria, eukaryotes and archaea is routinely moved into bacterial hosts for subsequent manipulations. The efficiency of acquisition of foreign DNA is limited by host restriction, the ability of the recipient strain to distinguish incoming DNA from its own chromosome and, preferentially, digest the invading DNA. *Escherichia coli* and *Salmonella* possess potent type I restriction modification systems (4,10). These systems comprise three proteins. HsdS is the subunit that recognizes the

specific DNA sequences that are to be methylated or restricted. Methylation by HsdM marks the DNA as belonging to the host and prevents it from being digested by the restriction subunit, HsdR. Naive DNA entering a bacterium is not methylated and is digested by HsdR. The restriction activity results in linear double-stranded (ds)DNA, which is rapidly degraded by the RecBCD complex (15). With a very low frequency, HsdM modifies unmethylated DNA before it is restricted by HsdR. The resulting methylated DNA is replicated and recognized as host DNA in future bacterial generations.

In the laboratory, host restriction hinders the introduction of novel DNA, such as phage or plasmids, into bacterial strains and limits the construction of mutants and their analysis. Many of the common laboratory *E. coli* strains used for recombinant DNA experiments contain mutations that prevent restriction (*r*⁻ strains). In contrast, restriction deficient derivatives of most other species of bacteria are not available. Thus, host restriction must be overcome before foreign DNA can be efficiently introduced into these strains.

Plasmids can be moved between strains by conjugation, and it has previously been shown that the efficiency of DNA transfer by conjugation between *Salmonella* and *E. coli* can be increased by a simple heat treatment (9). In this report, we describe the use of heat treatment to temporarily and specifically inactivate the restriction subunit of type I restriction modification complexes in enteric bacteria, thereby allowing high-efficiency introduction of foreign DNA into wild-type *r*⁺ strains. We show that this technique is equally applicable to *E. coli* and *Salmonella* strains and might also be widely used to enhance the efficiency of DNA transfer into other bacterial species.

MATERIALS AND METHODS

Strains and Phage

Table 1 describes all strains and phage used in this study. To isolate phage with altered modification phenotypes, high-titer lysates were streaked on the appropriate host growing in a top

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agar plate. Individual plaques were used to inoculate cultures for high-titer lysates (7).

Bacterial Growth Conditions

Bacteria were grown in LB medium (7). When antibiotics were required, they were added at the following final concentrations: 30 µg/mL ampicillin, 10 µg/mL tetracycline, 45 µg/mL kanamycin and 30 µg/mL chloramphenicol.

Inactivation of Host Restriction

Phage titers and transductions. Immediately before transduction, 0.1 mL of an overnight culture of bacteria was heated to 50°C in an 0.5-mL polymerase chain reaction (PCR) tube in a PTC-100™ Thermal Cycler (MJ Research, Watertown, MA, USA). For phage titers and efficiency of plating (EOP) calculations, the bacteria were mixed with 5 mL of top-agar (7) held at 50°C and immediately poured onto the surface of a room-temperature LB plate. The agar solidified within 3 min. EOP was determined by titering phage lysates as follows: 10-fold dilutions of a high-titer phage lysate were made from 10¹–10⁸ in sterile 0.85% NaCl solution, then 10 µL of each dilution were spotted onto the surface of a top-agar plate. Transductions were performed using the delayed expression technique (7). Briefly, 0.1 mL of bacteria was mixed with enough phage to give a multiplicity of infection (MOI) between 0.1 and 1. After a 20-min incubation at 37°C, 1 mL of LB supplemented with 10 mM EGTA was added, and the samples were incubated for an additional 20 min at 37°C. The samples were concentrated by centrifugation, resuspended in 0.1 mL of LB and spread on selective plates. All plates were incubated overnight at 37°C. All EOPs and transduction frequencies are the average of at least two independent experiments.

Electroporations. Before electroporation, 0.3 mL of a mid-log phase culture was incubated at 50°C as described above. Following heat treatment, the sample was cooled on ice for 2 min, and the cells were made competent by washing three times with ice-cold sterile 10% glycerol. Electropora-

Table 1. Strains, Plasmids and Phage Used in this Study

Strain	Restriction Phenotype ^a	Reference
Salmonellae:		
<i>S. typhimurium</i> LT2	(r ⁺ m ⁺) _{LT} (r ⁺ m ⁺) _{LTII} (r ⁺ m ⁺) _{LTIII}	(16)
<i>S. typhimurium</i> JS107	(r ⁺ m ⁺) _{LT} (r ⁺ m ⁺) _{LTII} (r ⁺ m ⁺) _{LTIII} <i>zlg::Kan</i>	(8)
<i>S. enteritidis</i> LK5	(r ⁺ m ⁺) _{SEN} (r ⁺ m ⁺) _{LT} (r ⁺ m ⁺) _{LTIII}	(6)
E. coli:		
<i>E. coli</i> K-12 MG1655	(r ⁺ m ⁺) _K	(5)
<i>E. coli</i> C	(r ⁻ m ⁻)	(3)
Phage:		
P22 _(St)	P22HT <i>int</i> modified by <i>S. typhimurium</i>	(12)
P22 _(Se)	P22HT <i>int</i> modified by <i>S. enteritidis</i>	(12)
λ <i>vir</i> _(K)	<i>E. coli</i> K modified λ	(13)
λ <i>vir</i> _(C)	unmodified λ	(13)

^aAll strains are prototrophic.

tions were performed immediately in 1.5-mm cuvettes. The time constant was approximately 4 kV using a Gene Pulser® Electroporator (Bio-Rad, Hercules, CA, USA). Bacteria were then diluted in 1 mL room-temperature LB and incubated at 37°C for 1 h to allow expression of the antibiotic-resistance gene encoded by the plasmid. Following centrifugation, samples were resuspended in 0.1 mL of LB and spread on selective plates. All electroporation frequencies are the average of at least two independent experiments.

Viable counts. The viability of each culture was assayed before and after each heat treatment. Viable counts were measured by diluting bacteria in sterile 0.85% NaCl and spotting 10 µL of each dilution onto an LB plate. The spot was allowed to dry at room temperature, and the plate was incubated overnight at 37°C. All viable counts are the average of at least two independent experiments.

RESULTS AND DISCUSSION

Effect of Heat Treatment on Viability

A previous report indicated that mild heat pretreatment might increase the conjugation frequency between *E. coli* and *S. typhimurium* (14–16). However, there was no indication whether this treatment would increase the fre-

quency of interspecies transduction or electroporation, or whether it could be extended to other enteric bacteria in addition to *S. typhimurium*. Therefore, we examined the effect of temperature on overcoming restriction barriers to phage infection or electroporation of plasmids in enteric bacteria.

To examine the effect of heat treatment on host restriction, we first needed to determine the viability of the recipients at different temperatures. Figure 1 shows the survival of *S. enteritidis* and *S. typhimurium* after heating to 50° and 60°C. At 60°C, the majority of the *Salmonella* were killed within the first 5 min, and viability was completely lost by 30 min. In contrast, at 50°C, the viability decreased much slower, such that after 1 h at 50°C, the loss was less than 2 logs. The decrease in viability did not appear to be related to the growth phase of the culture. Because brief heat treatment at 60°C is lethal to *Salmonella*, the effect of higher temperatures on host restriction was not assessed. In contrast, heating to 40°C for up to 1 h had no effect on either the viability or host restriction by *Salmonella* (data not shown).

Effect of Heat Treatment on Host Restriction

Transfer of DNA between *Salmonella* species (intraspecies transfer). Previously, it has been shown that *S.*

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Table 2. Effect of Restriction Modification Systems on EOP of P22

Recipient	EOP of Donor Phage	
	P22 _(St)	P22 _(Se)
<i>S. typhimurium</i>	1.0	0.4
<i>S. enteritidis</i>	0.0003	1.0

enteritidis and *S. typhimurium* share some restriction systems (4). The three host restriction systems of *S. typhimurium* are the LT, LTII and LTIII (also called the LT, SA and SB systems, respectively) (2,4). *S. enteritidis* contains the LT and LTIII systems (but not the LTII system) and also contains a unique restriction system, the SEN system. The efficiencies with which *Salmonella* restricted phage infections were not equal (Table 2). The SEN system restricted P22_(St) (SEN-) approximately 4 to 5 logs, but the LTII restriction system restricted P22_(Se) (LTII-) approximately one log (4).

The titer of either P22_(St) or P22_(Se) on *S. typhimurium* was not affected by heating (Figure 2A). In contrast, heating *S. enteritidis* to 50°C inactivated the potent SEN restriction system (Figure 2B). The inactivation of the SEN restriction system was maximal after approximately 20 min. From 20 min to 1 h, there was no significant loss of viability (Figure 1) and only minor changes in restriction inactivation. During the first 20 min that the recipient was heated, the phage titer decreased in parallel with the loss of bacterial viability (Figure 1). Because the phage were not heated, and the decrease in titer was observed for both P22 and λ vir, this decrease in titer is likely to be the result of phage adsorption to bacteria that have been killed by heating, thereby preventing a productive infection.

Heat treatment only affected the restriction system of *S. enteritidis* and did not affect DNA entry into the cell or recombination into the chromosome. The frequency with which a kanamycin-resistance marker was transferred between *S. typhimurium* and *S. enteritidis* was restored to the same level as for transfer between *S. typhimurium* and *S. typhimurium* following heat treatment

Table 3. Effect of Heat Pretreatment on Transduction Efficiency

Recipient	Colonies Recovered	
	No Treatment	50°C Pretreatment
<i>S. enteritidis</i>	0	133
<i>S. typhimurium</i>	168	142

The kan^R marker from JS107 (*S. typhimurium* modified) was transduced into *S. typhimurium* and *S. enteritidis*.

Table 4. Effect of Heating *E. coli* on the EOP of λ vir

<i>E. coli</i> Strain	λ vir _(K) EOP		λ vir _(C) EOP	
	Not Heated	Heated	Not Heated	Heated
K	1	1	0.01	1
C	0.7	0.4	1	1

of the recipient strain (Table 3). These results demonstrate two differences in host restriction in *S. enteritidis* and *S. typhimurium*: (i) The LTII restriction system is present in *S. typhimurium* but not in *S. enteritidis*. This is a relatively weak restriction system that reduces unmodified phage titers by approximately one log and is not affected by temperature (Figure 2A). (ii) The SEN restriction system is present in *S. enteritidis* but not in *S. typhimurium*. This is a more efficient system, which reduces unmodified phage titers by 4 to 5 logs, and, unlike the LTII system, is inactivated by tempera-

Figure 1. Survival of *S. typhimurium* and *S. enteritidis* at 50° and 60°C. Viability of *S. typhimurium* (open symbols) and *S. enteritidis* (filled symbols) were assessed after incubation at 50° (triangles) or 60°C (squares) for up to 1 h.

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ture treatment (Figure 2B).

DNA transfer between *E. coli* strains (intraspecies transfer). Two *E. coli* strains have different restriction and modification phenotypes. *E. coli* K strains have a functional restriction system, but *E. coli* C strains are phenotypically $r^{-}m^{-}$ (1). To analyze host restriction in *E. coli*, we assayed the titer of differentially modified λ vir lysates. When *E. coli* K was infected with λ vir grown in *E. coli* C, host restriction decreased the EOP by 2 logs. However, as described for *Salmonella*, pretreatment of *E. coli* K for 30 min at 50°C was sufficient to inactivate the restriction system (Table 4).

Transfer of DNA between *E. coli* and *Salmonella* (interspecies transfer). The method of choice for introducing DNA into many bacterial species is electroporation. Therefore, we used interspecies DNA transfer to assay the effect of heat treatment on *Salmonella*

host restriction following electroporation. After heat treatment, the cell viability decreased by approximately 2 logs, similar to that shown in Figure 1. However, the additional treatments involved in electroporation (both making

competent cells and the electric shock) had little effect on cell viability. DNA of a pBR322 plasmid derivative was modified in the *E. coli* K strain and then electroporated into the *Salmonella* strains. Without treatment, host restriction

Figure 2. Effect of heating *S. typhimurium* and *S. enteritidis* on P22_(Si) and P22_(Se) titers. The EOP of P22_(Si) (closed symbols) and P22_(Se) (open symbols) on (A) *S. typhimurium* and (B) *S. enteritidis* following heat treatment at 50°C for up to 1 h.

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Table 5. Effect of Heat Pretreatment on Electroporation Efficiency

Recipient	Colonies Recovered	
	No Treatment	50°C Pretreatment
<i>S. typhimurium</i>	9	1800
<i>S. enteritidis</i>	0	2600
<i>E. coli</i> K modified plasmid DNA was transformed into <i>Salmonella</i> .		

severely reduced the electroporation efficiency of both *S. enteritidis* and *S. typhimurium*; however, after heat treatment at 50°C for 30 min, host restriction was effectively inactivated (Table 5).

CONCLUSIONS

The results indicate that a simple heat treatment of *E. coli* or *Salmonella* at 50°C results in inactivation of major restriction barriers, allowing the trans-

fer of DNA between different strains and species of these enteric bacteria. The stable inheritance of the foreign DNA suggests that the restriction system is either preferentially inactivated or that the modification system is reactivated before the restriction system.

DNA uptake is often detrimental to the recipient. For example, lytic phage can infect a bacterium, reproduce, lyse the cell and subsequently infect other bacteria in the surrounding environment. However, during times of environmental stress, temporary inactivation of host restriction might be beneficial, facilitating horizontal transfer of genes from other bacteria. Such horizontal transfer is proposed to be a driving force in evolution. For example, pathogenesis determinants such as toxins or invasins are frequently transferred as phage, and inheritance of antibiotic resistance is often mediated by transferrable elements such as plasmids or conjugative transposons (11,14).

REFERENCES

1. Arber, W. and D. Dussoix. 1962. Host specificity of DNA produced by *Escherichia coli*. I. Host controlled modification of bacteriophage λ . *J. Mol. Biol.* 5:18-36.
2. Barcus, V.A. and N.E. Murray. 1995. Barriers to recombination: restriction. In S. Baumberg, J.P.W. Young, J.R. Saunders and E.M.H. Wellington (Eds.), *Population Genetics of Bacteria*. Cambridge University Press, Cambridge.
3. Bertani, G. and J.J. Weigle. 1953. Host controlled variation in bacterial viruses. *J. Bacteriol.* 65:113-121.
4. Bullas, L.R., C. Colson and B. Neufeld. 1980. Deoxyribonucleic acid restriction and modification systems in *Salmonella*: chromosomally located systems of different serotypes. *J. Bacteriol.* 141:275-292.
5. Guyer, M.S., R.R. Reed, J.A. Steitz and K.B. Low. 1981. Identification of a sex-factor-affinity site in *E. coli* as gamma delta. Cold Spring Harb. Symp. Quant. Biol.

45:135-140.

6. Keller, L.H., C.E. Benson, K. Krotec and R.J. Eckroade. 1995. *Salmonella enteritidis* colonization of the reproductive tract and forming and freshly laid eggs of chickens. *Infect. Immun.* 63:2443-2449.
7. Maloy, S.R., V.J. Stewart and R.K. Taylor. 1996. Genetic Analysis of Pathogenic Bacteria. CSH Laboratory Press, Cold Spring Harbor, NY.
8. Mann, B.A. and J.M. Schlauch. 1997. Transduction of low-copy number plasmids by bacteriophage P22. *Genetics* 146:447-456.
9. Mojica, A.T., and R.B. Middleton. 1971. Fertility of *Salmonella typhimurium* crosses with *Escherichia coli*. *J. Bacteriol.* 108:1161-1167.
10. Raleigh, E.A. and J.E. Brooks. 1998. Restriction modification systems: where they are and what they do. In G.M. Weinstock (Ed.), *Bacterial Genomes. Physical Structure and Analysis*. Chapman Hall, New York.
11. Salyers, A.A. and N.B. Shoemaker. 1996. Resistance gene transfer in anaerobes: new insights, new problems. *Clin. Infect. Dis.* 23:S36-S43.
12. Schmiegner, H. 1972. Phage P22-mutants with increased or decreased transduction abilities. *Mol. Gen. Genet.* 119:75-88.
13. Silhavy, T.J., M.L. Berman and L.W. Enquist. 1984. Experiments with Gene Fusions. CSH Laboratory Press, Cold Spring Harbor, NY.
14. Waldor, M.K. 1998. Bacteriophage biology and bacterial virulence. *Trends Microbiol.* 6:295-297.
15. Zahrt, T.C. and S. Maloy. 1997. Barriers to recombination between closely related bacteria: *mutS* and *recBCD* inhibit recombination between *Salmonella typhimurium* and *Salmonella typhi*. *Proc. Natl. Acad. Sci. USA* 94:9786-9791.
16. Zinder, N.D. and J. Lederberg. 1952. Genetic exchange in *Salmonella*. *J. Bacteriol.* 64:679-699.

We thank Lise Raleigh (New England Biolabs) for helpful suggestions about inactivation of the restriction system and John Cronan, Jr. (University of Illinois at Urbana-Champaign) for the *E. coli* strains. This work was supported by National Institutes of Health Grant No. PHS GM34715. Address correspondence to Dr. Robert A. Edwards, B103 C&LSL, Department of Microbiology, University of Illinois at Urbana-Champaign, 601 S. Goodwin Ave, Urbana, IL 61801, USA. Internet: edwards2@uiuc.edu

Received 23 November 1998; accepted 27 January 1999.

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