

# Use of P22 Challenge Phage to Identify Protein-Nucleic Acid Binding Sites

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## INTRODUCTION

The Challenge phage system is a genetic approach for characterizing DNA-protein interactions. The principle behind the method is to place the expression of a phage reporter gene under the control of a specific DNA-protein interaction (1-3). This approach can be applied to a wide variety of DNA binding proteins because most specific DNA-binding proteins can act as repressors if their binding sites are placed close enough to the promoter (4). The system has also been adapted to study RNA-protein interactions (5,6).

Challenge phage use the P22 *ant* gene as the reporter. The phages are engineered so that expression of *ant* determines the lysis-lysogeny decision of P22 (7). Expression of *ant* is controlled by a specific DNA-interaction which provides a simple, sensitive genetic selection for mutations in the DNA binding site that increase or decrease the strength of the DNA-protein interaction. These features provide general methods for measuring relative *in vivo* affinities of DNA-binding proteins for target sites and for identifying specific base-pairs in DNA that contact the protein. In addition, challenge phages can be constructed where *ant* expression is regulated by the assembly of nucleoprotein complexes containing several different proteins (8,9). This feature provides a method to define cooperative interactions between DNA-binding proteins and to analyze the abilities of mutant proteins to form complexes.

P22 has two regulatory regions that control the decision between lysis and lysogeny (10,11). One region, called *immC*, encodes the c2 repressor which represses transcription from the P<sub>L</sub> and P<sub>R</sub> promoters. The c2 repressor is required both to establish and maintain lysogeny. P22 also has a second regulatory region, called *immI*, which contains the *ant* (antirepressor), *arc* (antirepressor control) and *mnt* (maintenance) genes. Ant binds noncovalently to c2 repressor and inactivates it. Expression of Ant is negatively regulated by the Arc and Mnt proteins.

Challenge phages have three alterations in the *immI* region that affect the lysis-lysogeny decision. They contain a cassette encoding kanamycin resistance (Kan<sup>R</sup>) in the *mnt* gene, an *arc*(Am) mutation that inactivates Arc, and a substitution of a DNA-binding site for *Omnt*. Binding of the cognate protein to the substituted site is the only method of regulating transcription from *Pant* and thus controls the decision between lysis and lysogeny. If no protein is bound to the substituted site, *ant* is expressed and the infected host cell is lysed. If the protein binds to the site, *ant* expression is repressed and the phage forms a Kan<sup>R</sup> lysogen. The efficiency of lysogeny is typically 10<sup>-1</sup> if the host makes a protein that recognizes the substituted site and less than 10<sup>-7</sup> if the site is not recognized. Challenge phage containing *ant::lacZ* or *ant::cam* fusions can also be constructed by genetic recombination. Isolation of the *ant::lacZ* or *ant::cam* lysogens provides a single copy chromosomal fusion as a reporter gene (12).

Protocols for construction and use of challenge phage are detailed below. Basic phage biology, molecular biology, and genetic protocols can be found in Maloy et al. (13).

## METHODS

**Construction of Challenge Phages.** Challenge phage are constructed in two steps. First, the DNA-binding site is cloned into a plasmid that carries the P22 *immI* region. The plasmid pPY190 is a pBR322 derivative that carries a 500 bp *EcoRI-HindIII* fragment from the *immI* region (Figure 1). The *immI* DNA cloned onto pPY190 includes *Pant* and the 5' end of *arc*. The *Omnt* site has been replaced with *SmaI* and

*EcoRI* sites. Blunt-ended DNA fragments such as complementary oligonucleotides or restriction fragments can be cloned into the *SmaI* site. This places the insert 3 bp upstream of the initiation site of *Pant*. The presence of the insert can be easily verified by PCR analysis.

The substitution in pPY190 can be moved onto P22 *mnt::Kan arc(Am)* (P22-1000) by homologous recombination *in vivo*. A host containing the plasmid is infected with the phage and a lysate is prepared. During lytic growth, the phage and the plasmid can undergo recombination between the sequence homology on the plasmid and the phage. Recombinant phage that contain the substituted DNA-binding site in place of *Omnt* can be identified by infecting a MS1582 cells. MS1582 contains a P22 prophage which expresses the *c2* and *Mnt* proteins. *Mnt* represses *Omnt* of incoming parental phages preventing lytic growth. In contrast, phages carrying the substituted DNA-binding site are not repressed by *Mnt* and thus express *Ant*, forming plaques. The frequency of recombination between the phage and plasmid is high. Typically between 10 - 20% of the progeny phage are recombinants. One caveat is that if the *Salmonella* host chromosome encodes the protein that recognizes the DNA-binding site, it should be mutated so that the protein does not repress the challenge phage.

Since the parental phage carries the *arc(Am)* mutation and the pPY190 plasmid contains the wild-type allele, recombinants are either *arc(Am)* or *arc<sup>+</sup>* depending upon which side of the mutation the crossover occurred. Both types of recombinants form plaques on MS1582. The *arc<sup>+</sup>* recombinants form small, turbid plaques because *Arc* can partially repress *Pant*. The *arc(Am)* recombinants form large, clear plaques which can be purified and used in subsequent experiments.

The desired recombinants contain both the DNA binding site and the *arc(Am)* mutation. The *Omnt* substitution in pPY190 contains an *EcoRI* site that is not present in the parental phage, and the *arc(Am)* mutation destroys a *Fnu4H* site (Figure 2). Thus the desired recombinants can be confirmed by PCR amplification of the *immI* region followed by restriction fragment length polymorphism (RFLP) mapping with *EcoRI* and *Fnu4H* (Figure 3).

Essentially any DNA binding site can be cloned into pPY190 and recombined onto P22 to form a challenge phage. It is also possible to clone large fragments that contain multiple DNA-binding sites. For simple DNA-protein interactions the extent of repression of *Pant* by a DNA-binding protein depends upon the proximity of the protein to the transcription start site. For example, a consensus  $\lambda$  *cI* binding site functions well when placed at the +1 or +4 position but does promote repression when placed at position +24 (4). It is also possible to construct phages where DNA looping regulates expression from *Pant*. In the case of the  $\lambda$  *attL* site, repression required an IHF-mediated loop containing an *Int* protein monomer bound simultaneously to both the *Pant* region and a sequence more than 50 bp downstream of *Pant* (14).

#### *Construction of challenge phage by in vivo recombination*

1. Grow the MS1883 containing a pPY190 subclone with the desired DNA-binding site in LB-broth + Amp at 37°C overnight.
2. Mix 10<sup>6</sup>-10<sup>7</sup> plaque forming units (pfu) of P22 *mnt::Kan9 arc(Am)* phage with 50  $\mu$ l of the overnight culture. Leave at room temperature for 15-20 min to allow phage adsorption.
3. Add 2 ml LBEG and grow at 37°C with vigorous aeration for 3 h or until the culture is lysed.

4. Add several drops of chloroform and vortex thoroughly. Allow the chloroform to settle. Pour the supernatant (avoiding the chloroform) into a microfuge tube. Centrifuge 2 min in a microfuge to pellet the cell debris.
5. Transfer the supernatant to a new tube, add a few drops of chloroform, vortex, and allow the chloroform to settle. Store the lysate at 4°C.
6. Dilute the lysate 1/100 by adding 10  $\mu$ l to 1 ml LB-broth. Vortex. Mix 0.2 ml of the diluted lysate with 0.1 ml of a fresh overnight culture of MS1582 (to give a MOI < 1).
7. Leave at room temperature for 15-20 min to allow phage adsorption.
8. Add 3 ml of melted TBSA to a test tube, place in a 50°C heating block, and allow the temperature of the TBSA to equilibrate to 50°C.
9. Add 3 ml TBSA to the cells + phage, mix, and quickly pour onto a LB-agar plate. Allow the top agar to solidify at room temperature then incubate overnight at 37°C.
10. Pick large clear plaques with sterile toothpicks and streak for isolation on a LB-agar plate overlaid with 3 ml TBSA containing 0.1 ml of fresh MS1582. Incubate overnight at 37°C.
11. Pick large, clear, well isolated plaques and streak for isolation on a LB-agar plate overlaid with 3 ml TBSA containing 0.1 ml of fresh MS1883. Incubate overnight at 37°C.
12. Pick and restreak the large, clear plaques. Incubate overnight at 37°C. The larger, clear plaques contain potential recombinant challenge phage.

**Identification of challenge phage recombinants by RFLP mapping.** The desired challenge phage recombinants have a restriction fragment length polymorphism (RFLP) that can be identified by digesting the *imm1* PCR fragment with *EcoR1* or *Fnu4H1*.

#### *Preparation of challenge phage lysates*

1. For each potential recombinant challenge phage to be tested, add 0.1 ml of MS1883 to 5 ml LBEG. Prepare an identical culture as a control. Incubate on a shaker at 37°C until the cells reach early exponential phase (about 1-2 h).
2. Using a sterile pasteur pipet, plug an independent, well isolated, large clear plaque from the potential challenge phage and add 1 plaque to each tube of MS1883. To the control culture, add a plaque of the P22-1000 parental phage. Incubate at 37°C with vigorous aeration for about 3 h or until lysed.
3. Add several drops of chloroform, vortex, allow the chloroform to settle, then transfer the supernatant (avoiding the chloroform) to microfuge tubes. Centrifuge for 2 min in a microfuge to pellet the cell debris.
5. Transfer the supernatant from each phage into clean microfuge tubes. Centrifuge for 30 min at 4°C in a microfuge to pellet the phage.
6. Carefully pour off the supernatant. Add 0.1 ml 0.85% NaCl to each phage pellet and vortex to resuspend the phage. Combine the supernatants from a single phage into one tube. Add several drops of chloroform and vortex. Store the concentrated lysate at 4°C.

#### *PCR and RFLP analysis*

1. Phage lysates can be amplified directly without purifying phage DNA. Include the parent P22-1000 and a challenge phage with the *Pant* region of pPY190 recombined onto P22 *arc*(Am) as controls.
2. Combine the following in a 500  $\mu$ l microfuge tube:
  - a. 10  $\mu$ l P22 lysate

- b. 1  $\mu$ l Intra-Arc primer (20 pmol; 5' CCG CTA CCT TGC GTA CCA AAT CC 3') or intra-Ant primer (20 pmol; 5' CAA GGC TGT TTG CTT CTT TTG CAG 3')
  - c. 1  $\mu$ l Anti-*Omnt* primer (20 pmol; 5' GAT CAT CTC TAG CCA TGC 3')
  - d. 37  $\mu$ l Sterile deionized H<sub>2</sub>O
  - e. 10  $\mu$ l 10X PCR buffer (supplied by the manufacturer)
  - f. 10  $\mu$ l Each of 2 mM dATP, dGTP, dTTP, and dCTP
  - g. 1  $\mu$ l Taq DNA polymerase (Perkin-Elmer Corporation, 2.5 u/ $\mu$ l)
3. Mix and amplify in a thermocycler using the following program:

Step	Temperature	Time
1	95°C	90 sec
2	53°C	30 sec
3	74°C	60 sec
4	cycle steps 1 - 3	repeat 30 times
5	74°C	5 min
6	4°C	hold

4. Purify the PCR products by phenol extraction followed by ethanol precipitation or by using a commercially available method such as Qiagen Affinity columns (Qiagen), Wizard Preps (Promega), or Millipore MC filters (Millipore).
5. In separate tubes digest 10  $\mu$ l of the PCR product with *Eco*RI and *Fnu*4H (NEB).
6. Electrophorese the DNA and undigested controls on a 3.0% NuSieve GTG (FMC) or 3:1 (Sigma) agarose gel in 1X TBE at 100 volts until the bromphenol blue is 3/4 the length of the gel. Include a lane with low molecular weight standards.
7. Stain the gel with ethidium bromide. Visualize the gel with UV and photograph the gel. Compare the restriction digests with the expected results for P22-1000 and pPY190 shown in Figure 3.

**Challenge phage DNA-binding assays.** The challenge phage assay depends upon binding of a protein to the cognate DNA-binding site. To vary the levels of a DNA-binding protein for use with challenge phage, it is usually necessary to express the DNA-binding protein under control of a regulated promoter, such as *Plac*, *Ptac*, or *PBAD*. The genetic background of the host strain will depend upon the type of regulated promoter used: for *Plac* and *Ptac* the *lacI<sup>q</sup>* gene should be provided on a plasmid; for *PBAD* the chromosomal copy of the *ara* operon should be deleted.

When a DNA-binding protein is expressed from a regulated promoter, the relative *in vivo* affinity of a DNA-binding protein for the binding site on the challenge phage can be quantitated by measuring the frequency of Kan<sup>R</sup> lysogens in media with different concentrations of inducer (and hence different concentrations of the DNA-binding protein). The relative DNA-binding affinity can be expressed by plotting the log(percent lysogeny) vs the log[inducer].

The proctols below are based upon the regulated expression of a DNA-binding protein from the *Ptac* promoter under the control of the *lacI<sup>q</sup>* gene product. In this case, expression of the DNA-binding protein is induced by the addition of isopropyl- $\beta$ -D-galactopyranoside (IPTG). Typically 0.1-1 mM IPTG results in maximal induction from *Ptac*. However, the optimal concentration of IPTG for each specific DNA-

binding protein should be determined empirically by testing 10-fold dilutions of IPTG to ascertain the concentration that promotes maximal lysogeny of the challenge phage. The cell viability should be measured at each concentration of inducer because overexpression of DNA-binding proteins often inhibits cell growth. All of the dilutions can be done in microtiter plates. Using a multichannel pipettor 48 samples can be spotted onto each agar plate for phenotypic selection or screening.

#### *Challenge phage assays*

1. Titer the challenge phage on MS1883.
2. Subculture 50  $\mu$ l of MS1868 cells with the P<sub>tac</sub>-DNA-binding protein expression plasmid into 2 ml LB-broth containing + an appropriate antibiotic to maintain selection for the expression plasmid. Incubate on a shaker at 37°C for about 1-2 h until the cells reach early exponential phase.
3. Subculture 0.5 ml of the early exponential phase culture into 1.5 ml of LB-broth + antibiotic, and LB-broth + antibiotic + IPTG. Incubate on a shaker at 37°C for about 1-2 h until the cells reach early exponential phase.
4. Prepare serial 10-fold dilutions of both cultures to 10<sup>-5</sup> in LB-broth. To determine the viable cell count, remove 5  $\mu$ l from the 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> dilutions and spot onto LB-agar plates. Add 100  $\mu$ l LB-broth to each spot and spread. Incubate the plates overnight at 37°C.
5. Calculate the volume of each phage required to give multiplicity of infection (MOI) of 10-50 pfu/cell. For example, if the cultures are at about 10<sup>8</sup> cells per ml, for an MOI of 25: Add the calculated volume of each challenge phage lysate to 0.1 ml of the undiluted cells. Include a P22-1000 phage control.
6. Mix the cells and phage by gently pipeting the solution up and down then leave at room temperature for 1 h to allow phage adsorption and phenotypic expression.
7. Prepare sequential 10-fold dilutions of each infected cell culture to 10<sup>-4</sup> in LB-broth.
8. Remove 10  $\mu$ l from the 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, and 10<sup>-4</sup> dilutions of infected cells grown without IPTG and spot onto a LB-agar-Kan + antibiotic plate.
9. Remove 10  $\mu$ l from the 10<sup>-1</sup>, 10<sup>-2</sup>, and 10<sup>-3</sup> dilutions of the cells grown with IPTG and spot onto a LB-agar-Kan + antibiotic + IPTG plate.
10. Allow the spots to dry at room temperature then incubate 1-2 days at 37°C.
11. Count the number of colonies on each plate. Calculate the number of Kan<sup>R</sup> lysogens per viable cell. If the DNA-binding protein is properly expressed, challenge phage will form Kan<sup>R</sup> lysogens at a much higher frequency in cells grown with inducer than in cells grown without inducer, and the control phage will not form Kan<sup>R</sup> lysogens at a significant frequency in either condition.

**Selection for DNA-binding site mutations.** Challenge phage form lysogens on a host that expresses a DNA-binding protein which binds to the substituted *O<sub>mnt</sub>* site. It is possible to select for "operator constitutive" (O<sup>c</sup>) mutations in the DNA-binding site by isolating challenge phage mutants that are not repressed by the DNA-binding protein and thus form plaques on a lawn of cells that express the DNA-binding protein. This provides a very strong selection for mutations that affect the DNA-protein interaction in vivo. Mutations in a DNA-binding site are usually rare, but the resulting rare plaques can be easily selected from a population of >10<sup>8</sup> infecting phage.

DNA-binding site mutants may result from spontaneous mutagenesis. However, spontaneous mutants are often due to deletion mutations which are less valuable for defining DNA-protein interactions than point

mutations. The frequency of point mutations may be enhanced by mutagenizing the phage. It is useful to use several mutagens with different specificities to obtain a wide spectrum of mutations (13). Two methods of mutagenesis that are particularly useful are UV mutagenesis and hydroxylamine mutagenesis. Phage can be directly treated with both of these mutagens *in vitro*.

Using this approach, it is possible to quickly isolate a large number of O<sup>c</sup> mutations in the substituted DNA-binding site. The resulting mutations define critical residues of the DNA-binding site necessary for recognition by a DNA-binding protein. Many DNA-binding sites are palindromes bound by identical subunits of dimeric regulatory proteins: each monomer recognizes one of the half-sites. For palindromic DNA-binding sites, it may be necessary to construct symmetric double mutants to determine their effect on DNA binding. Such symmetric, double mutants can be constructed by cloning the appropriate double stranded oligonucleotide into the *SmaI* site of pPY190 to yield the corresponding challenge phage.

In the following procedure, the challenge phage are mutagenized with UV light. A *S. typhimurium* strain carrying a plasmid which expresses the cognate DNA-binding protein and a second compatible plasmid carrying the *mucA*<sup>+</sup>*B*<sup>+</sup> genes is infected with the irradiated phage. The *mucAB* gene products increase the frequency of error prone repair of UV induced DNA damage. Clear plaque mutations that inactivate the binding site will be rare (typically about 10<sup>-6</sup> to 10<sup>-7</sup>) and the background will have numerous faint turbid plaques. Therefore it is necessary to plaque purify the clear plaque mutants several times.

#### *Isolation of mutations in a DNA-binding site*

1. Grow an overnight culture of the MST2781 cells with the P<sub>tac</sub>-DNA-binding protein expression plasmid in 2 ml LB-broth + Tet + antibiotic at 37°C.
2. Subculture 0.1 ml of the overnight culture into 2 ml LB-broth + Tet + antibiotic. Incubate with vigorous aeration at 37°C for 2-3 h or until mid-exponential phase.
3. Dilute 0.5 ml of the mid-exponential phase culture into 1.5 ml LB-broth + Tet + antibiotic + IPTG. Incubate the cells for 1-2 h with vigorous aeration at 37°C.
4. Dilute the challenge phage to approximately 10<sup>9</sup> pfu/ml in 0.85% NaCl (final volume needed is 1 ml). Save 0.5 ml of the diluted phage as a control.
5. Place 0.5 ml of the diluted phage in a small sterile petrie dish, remove the lid, and UV irradiate to 12,000 μJ/cm<sup>2</sup>.
6. Prepare serial 10<sup>-1</sup> and 10<sup>-2</sup> dilutions of the UV irradiated phage and the control phage in 0.85% NaCl.
7. Mix 200 μl of the IPTG induced culture with 100 μl of each phage dilution.
8. Leave at room temperature for about 20 min to allow phage adsorption.
9. To each mixture of cells + phage, add 3 ml TBSA top agar that has been melted then cooled to 50°C. Mix gently then quickly pour onto an LB-agar plate and swirl the plate to cover the surface evenly. Allow the top agar to solidify for about 15 min at room temperature then incubate overnight at 37°C.
10. Examine the plates for clear plaques.

#### *Purify clear plaque mutants*

1. Subculture 0.1 ml of the overnight culture of TH564 cells with the P<sub>tac</sub>-DNA-binding protein expression plasmid into 2 ml LB-broth + antibiotic. Incubate with vigorous aeration at 37°C about 2-3 h until the cells reach mid-exponential phase.

2. Dilute 0.5 ml of the mid-exponential phase culture into 1.5 ml LB-broth + antibiotic + 1 mM IPTG. Incubate the cells for 1-2 h with vigorous aeration at 37°C.
3. Add 0.1 ml of the culture induced in LB-broth+ antibiotic + 1 mM IPTG to 3 ml TBSA that has been melted then cooled to 50°C. Mix gently then quickly pour onto an LB-broth+ 1 mM IPTG plate and swirl the plate to cover the surface evenly. Allow the top agar to solidify at room temperature.
4. Pick large clear plaques from challenge phage with an O<sup>c</sup> mutation in the DNA-binding site and streak on the cell lawn. Also streak out the unmutagenized challenge phage and P22-1000 as controls. Incubate the plates at 37°C overnight.
5. The next day, add 0.1 ml of a fresh, overnight culture of MS1883 to 3 ml TBSA that has been melted and cooled to 50°C. Mix gently then quickly pour onto an LB-broth plate and swirl the plate to cover the surface evenly. Allow the top agar to solidify at room temperature.
6. Pick large clear plaques from step 4 and restreak on the MS1883 lawn. Incubate the plates at 37°C overnight.
7. Remove a well isolated large clear plaque from each streak with a sterile Pasteur pipet.
8. Add the agar plug to 2 ml LBEG. Vortex. Add 0.1 ml of an overnight culture of MS1883. Incubate with vigorous aeration at 37°C for about 3 h or until the culture lyses.
9. Centrifuge for 1 min in a microfuge to pellet the cells and debris.
10. Pour the supernatant into a clean tube. Add several drops of chloroform and vortex.
11. Titer the phage on MS1883. Assign allele numbers to the mutants and save pickates of the mutant phage lysates at 4°C. The DNA sequence of the mutations can be determined by PCR using the anti-*Omnt* and intra-Arc primers or by subcloning the *EcoRI* fragment which carries the *Omnt* substitution and *mnt::Kan* onto a plasmid and sequencing the plasmid DNA.

**Bacteria and phage.** The *Salmonella typhimurium* host strains for growing P22 challenge phage are shown in Table 1. Although *S. typhimurium* can cause gastrointestinal infections in humans, the *S. typhimurium* LT2 strains used for challenge phage assays are not very virulent in humans (15,16). However, *S. typhimurium* can cause infections in immunocompromised hosts and many of the strains carry drug-resistance plasmids or transposons that could be transferred to other enteric bacteria. Therefore, good microbiological technique should be followed when working with *Salmonella* (see the CDC recommendations for Biosafety in Microbiological and Biomedical Laboratories [<http://siri.org/library/cdc.html>]).

A typical P22 lysate contains about 10<sup>11</sup> phage per ml and is stable for many years when stored over chloroform at 4°C. Lytic growth is favored when the host cells are growing rapidly and the multiplicity of infection (MOI) is low. Lysogeny is favored when cells are growing slowly and the MOI is high. When the MOI of P22 is greater than 10, more than 95% of the infected cells form lysogens (17). P22 diffuses rapidly throughout a soft agar overlay on a petri dish upon extended storage. This can result in contamination of one plaque with phage from a nearby plaque. Therefore, to ensure the genetic homogeneity of phage lysates, recombinant phage should be purified by streaking for isolated, single plaques several times before growing large-scale lysates.

## REAGENTS

Antibiotics: When needed, add antibiotics to LB medium at the following concentrations: 50 µg/ml kanamycin sulfate (Kan), 20 µg/ml tetracycline hydrochloride (Tet), 50 µg/ml sodium ampicillin (Amp), 50 µg/ml spectinomycin dihydrochloride.

E medium (13,18): 5x E medium contains 1 g MgSO<sub>4</sub>•7H<sub>2</sub>O, 10 g Citric acid•H<sub>2</sub>O, 50 g K<sub>2</sub>HPO<sub>4</sub>, and 17.5 g NaH<sub>2</sub>NH<sub>4</sub>PO<sub>4</sub>•2H<sub>2</sub>O in 1000 ml deionized H<sub>2</sub>O.

LBEG (13): 1x LB-broth containing 1x E medium and 0.2% glucose

TBSA (13): 10 g tryptone, 5 g NaCl, and 7 g agar in 1000 ml deionized H<sub>2</sub>O.

Luria-Bertani broth (LB-broth): LB-broth contains 10 g tryptone, 5 g yeast extract, and 5 g NaCl in 1000 ml deionized H<sub>2</sub>O. LB-agar contains 15 g agar per liter LB-broth.

TE: 10 mM TrisHCl, 1 mM EDTA, pH 8

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Table 1. Some examples of DNA-protein and RNA-protein interactions that have characterized using Challenge phage<sup>a</sup>.

<u>DNA-protein interaction</u>	<u>Source</u>	<u>Reference</u>
λ repressor	Phage λ	(19)
Cro repressor	Phage λ	(19)
Integrase	Phage λ	(20,21)
Fis-Xis-Int	Phage λ	(9)
<i>attL</i> complex	Phage λ	(8,14)
EcoRI	RY13 plasmid	(22)
TetA repressor	<i>Tn10</i>	(7)
TrpR repressor	<i>E. coli</i>	(1,23)
LacI repressor	<i>E. coli</i>	(7)
GalR repressor	<i>E. coli</i>	(7)
Integration host factor	<i>E. coli</i>	(24-27)
Hin recombinase	<i>S. typhimurium</i>	(12)
PutA repressor	<i>S. typhimurium</i>	(P. Ostrovsky and S. Maloy, unpublished)
Nac activator	<i>Klebsiella aerogenes</i>	(28)
OxyR/MtrA	<i>Mycobacterium</i>	(T. Zahrt and V. Deretic, unpublished)
<i>nifH</i> promoter	<i>Rhizobium meliloti</i>	(30)
ToxR	<i>Vibrio cholerae</i>	(29)
FLP recombinase	<i>S. cerevisiae</i>	(31)
<u>RNA-protein interaction</u>	<u>Source</u>	<u>Reference</u>
Coat proteins	Phage R17 and MS2	(6,32)

<sup>a</sup>Numerous other DNA-protein interactions have been studied as well. The critical requirement for any specific DNA-protein interaction is that the protein is expressed and active in *Salmonella*.

Table 2. Host strains for Challenge phage experiments.

Strain	Species <sup>a</sup>	Genotype	Source
MS1363	<i>St</i>	<i>leuA414</i> (Am) Fels <sup>-</sup> <i>supE40</i>	M. Susskind <sup>b</sup>
MS1868	<i>St</i>	<i>leuA414</i> (Am) Fels <sup>-</sup> <i>hsdSB</i> (r <sup>-</sup> m <sup>+</sup> )	M. Susskind
MS1882	<i>St</i>	<i>leuA414</i> (Am) Fels <sup>-</sup> <i>hsdSB</i> (r <sup>-</sup> m <sup>+</sup> ) <i>endA</i>	M. Susskind
MS1883	<i>St</i>	<i>leuA414</i> (Am) Fels <sup>-</sup> <i>hsdSB</i> (r <sup>-</sup> m <sup>+</sup> ) <i>supE40</i>	M. Susskind
MS1582	<i>St</i>	<i>leuA414</i> (Am) Fels <sup>-</sup> <i>supE40</i> <i>ataP</i> :: [P22 <i>sieA44</i> 16(Am) <i>H1455</i> Tpfr49]	(7)
MST2781	<i>St</i>	<i>leuA414</i> (Am) Fels <sup>-</sup> <i>hsdSB</i> (r <sup>-</sup> m <sup>+</sup> ) <i>supE40</i> <i>ataP</i> :: [P22 <i>sieA44</i> Δ( <i>mnt-a1</i> Tn1) Amp <sup>S</sup> 9 <sup>-</sup> Tpfr184]/ pGW1700 ( <i>mucA</i> <sup>+</sup> <i>B</i> <sup>+</sup> Tet <sup>r</sup> )	(13)
MST2786	<i>St</i>	<i>leuA414</i> (Am) Fels <sup>-</sup> <i>hsdSB</i> (r <sup>-</sup> m <sup>+</sup> ) / pGW1700 ( <i>mucA</i> <sup>+</sup> <i>B</i> <sup>+</sup> Tet <sup>r</sup> )	(13)
TH564	<i>St</i>	<i>leuA414</i> (Am) Fels <sup>-</sup> <i>supE40</i> <i>ataP</i> :: [P22 <i>sieA44</i> Δ( <i>mnt-a1</i> Tn1) Amp <sup>S</sup> 9 <sup>-</sup> Tpfr184]	(12)
EM425	<i>Ec</i>	F <sup>-</sup> <i>supE44</i> <i>recA1</i> <i>gyr-96</i> <i>thi-1</i> <i>endA</i> <i>hsdR17</i> (r <sup>-</sup> m <sup>+</sup> ) λ <sup>-</sup> / pPY190	(13)

<sup>a</sup>*St* indicates *Salmonella typhimurium* T2 derivatives and *Ec* indicated *Escherichia coli* K-12 derivatives. A kit with these strains can be obtained from the authors.

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**FIGURES**

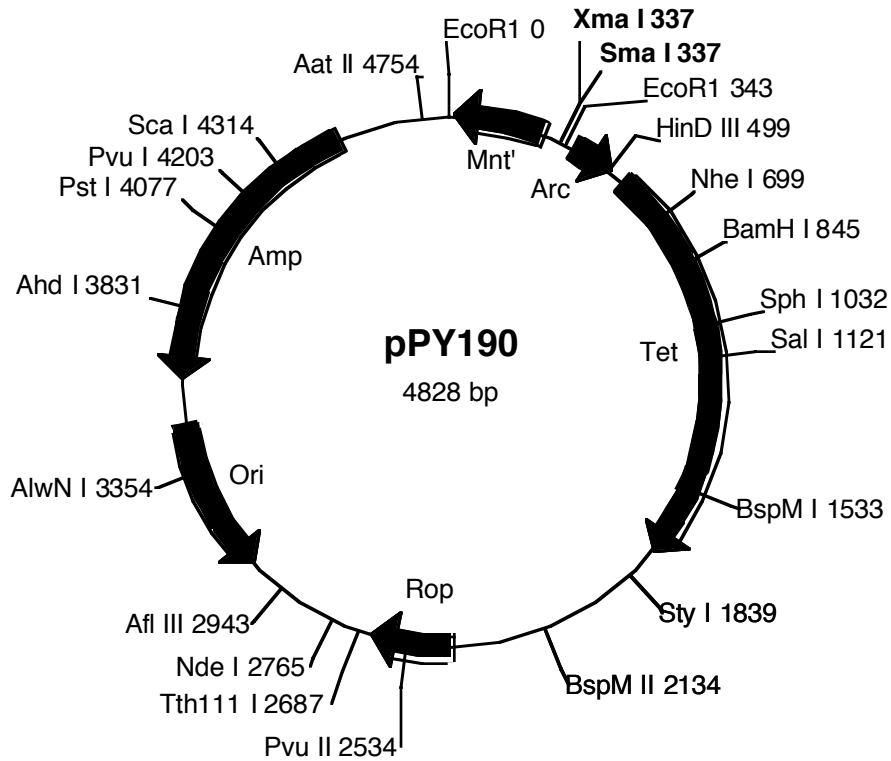
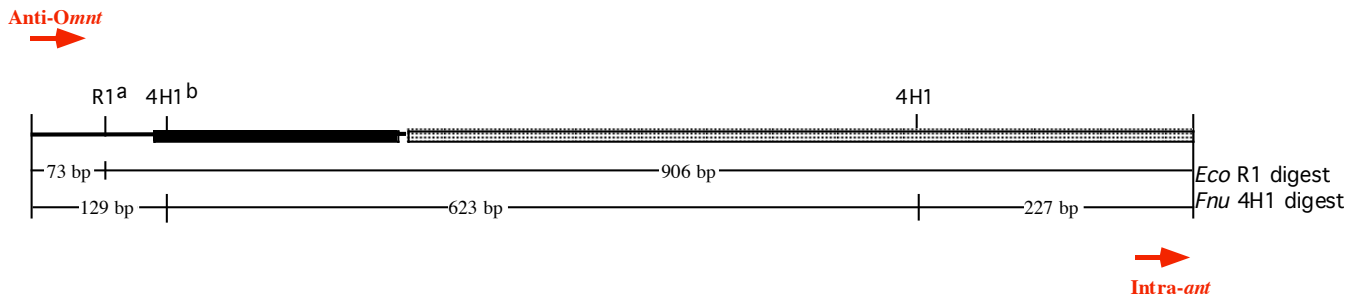


Fig. 1. Plasmid pPY190. pPY190 is a 4.8 Kb plasmid with the 5' end of the *mnt* gene and the entire *arc*<sup>+</sup> gene from the P22 *immI* region cloned into the *EcoRI-HindIII* sites of pBR322. The *Omnt* was replaced with a *SmaI/XmaI* substitution constructed by site directed mutagenesis. Cloning at *SmaI* places the insert at -3 and cloning at *XmaI* places the insert at -1 relative to the transcription start site of *ant*.



**(A) Restriction map of anti-Omnt/intra-ant PCR product:**



**(B) Restriction map of anti-Omnt/intra-arc PCR product:**

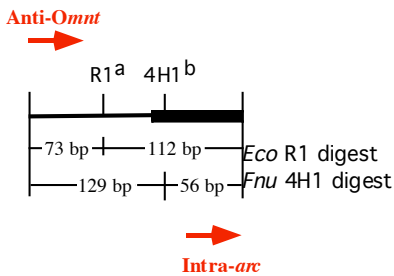


Fig. 3. Restriction maps of PCR products from the *immI* region in pPY190. The primers used are indicated in red. The *arc* gene is indicated by the black box and the *ant* gene is indicated by the gray box. The *EcoR*I site labeled R1<sup>a</sup> and the *Fnu*4H1 site labeled 4H1<sup>b</sup> are present in pPY190 but not in P22-1000. The presence of a cloned fragment in the *Sma*I or *Xmn*I site will increase the size of the 73 bp *EcoR*I and 129 bp *Fnu*4H1 fragments. (A) PCR with the anti-Omnt and Intra-ant primers yields a 979 bp fragment. (B) PCR with the anti-Omnt and intra-arc primers yields a 185 bp fragment.