

# Gene Deletion

Status: Failed



## Background

*E. coli* bacteria use the EnvZ/ompR Two-component Signaling system to sense changes in their environment. The EnvZ/OmpR system consists of an EnvZ histidine kinase protein with an external domain that senses osmolarity changes in the environment. At high osmolarity, the internal domain of the EnvZ autophosphorylates. The phosphorylated EnvZ then transfers its phosphate to the response regulator ompR. The phosphorylated ompR activates the promoter Pomc which regulates production of membrane porin protein ompC. (Heyde, Laloi, & Portalier, 2000) (Forst & Roberts, 1994) (See Figure 1)

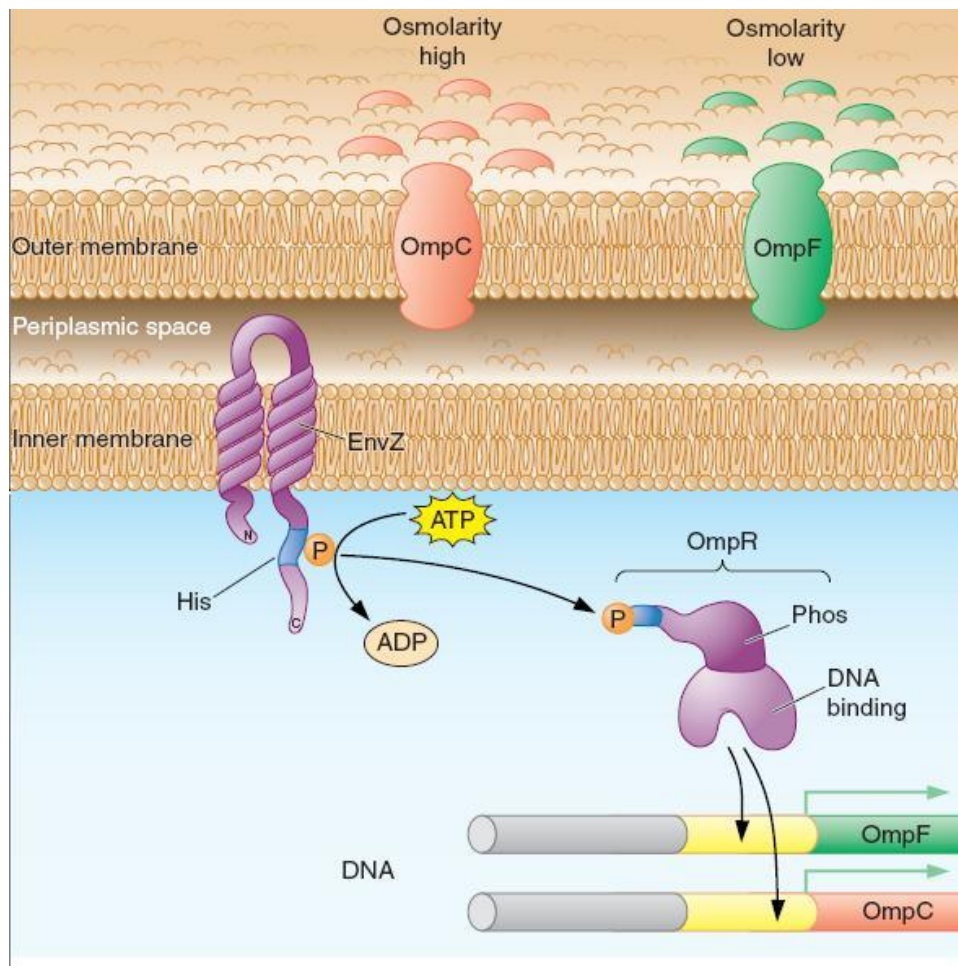


Figure 1: A diagram of the EnvZ/OmpR two-component signaling system (Kenney)

## Method

To be able to utilize the **EnvZ/OmpR two-component-signaling**<sup>1</sup> system for our project, we need to ensure that the natural EnvZ/OmpR system does not interfere, introducing noise to the system, giving a false signal.

### How did we change make sure the natural EnvZ/OmpR system doesn't disrupt our system?

We needed to use a strain of *E. coli* that has an EnvZ deletion ( $\Delta$ EnvZ). The **Keio Collection**<sup>2</sup> (Baba, Ara, Hasegawa, Takai, & Okumura, 2006) contains a strain of *E. coli* with exactly this deletion (strain JW3367-3).

### Great! So we are all set right?

Wrong.

Our mentor, Lior Levy, said that the ompR can be phosphorylated not only by the histidine kinase EnvZ but also by an acetyl phosphate dependent mechanism. (Heyde, Laloi, & Portalier, 2000) This would introduce a low level of noise into the system. Since our detector needs to be precise to be able to detect low concentrations, even a low level of noise would be problematic.

### What did we do about this?

Well, like all good scientists, we read articles until we found an answer. This is what we found:

Phosphate acetyl transferase (pta) and Acetate kinase (ackA) are involved in the following pathway (Heyde, Laloi, & Portalier, 2000):



We read about research done by Heyde, Laloi and Portalier who tested expression of ompC porin protein (under PompC) in *E. coli* strains with ackA deletions and ackA-pta deletions in the presence or absence of EnvZ and found that the  $\Delta$ ackA-pta strains showed no ompC expression. (Heyde, Laloi, & Portalier, 2000)

So we decided we need a strain of *E. coli* with both  $\Delta$ ackA-pta and  $\Delta$ EnvZ mutations. Unfortunately we had some trouble finding a strain of this kind so we decided to make one ourselves.

### How did we do this?

We asked for help!

We got in touch with Lior Zelcbuch and Elad Hertz from Ron Milo's lab at the Weizmann Institute of Science. They know a lot about deleting genes and they suggested we take the *E. coli* strain JW3367-3 ( $\Delta$ EnvZ) from the Keio Collection and use the Lamda Red technique to delete the genes for ackA and pta.

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<sup>1</sup> EnvZ/ompR Two-component Signaling system (Forst & Roberts, 1994): Bacteria sense changes in their environment using two-component signaling systems. The EnvZ/OmpR system consists of an EnvZ histidine kinase protein with an external domain that senses osmolarity changes in the environment. At high osmolarity, the internal domain of the EnvZ autophosphorylates. The phosphorylated EnvZ then transfers its phosphate to the response regulator ompR. The phosphorylated ompR activates the promoter PompC which regulates production of membrane porin protein ompC. (Heyde, Laloi, & Portalier, 2000)

<sup>2</sup> Keio collection: A research group from Keio University in Japan made a set of single-gene deletions of all non-essential genes in the *E. coli* K-12 strain BW25113 (parent strain) using the lamda red technique. (Baba, Ara, Hasegawa, Takai, & Okumura, 2006)

Since the genes for *ackA* and *pta* are adjacent to each other on the *E. coli* chromosome, we decided to delete them in one go.

With Lior Z. and Elad's guidance and help from Edna Kler from the Technion, we attempted to delete the genes.

## How does the lamda red technique work?

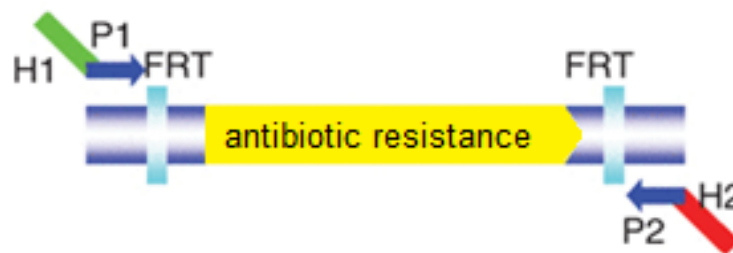
*Diagrams are adapted from (Baba, Ara, Hasegawa, Takai, & Okumura, 2006)*

The lamda Red Recombination technique uses the phage lamda Red recombinase which is synthesized under an inducible promoter on the plasmid pKD46. The technique disrupts chromosomal genes and replaces them with antibiotic resistance flanked by FRT sites. FLP recombinase will then remove the antibiotic resistance leaving a short scar where the FRT sequences are. (Datsenko & Wanner, 2000)

We decided to take the *E. coli* strain JW3367-3 from the Keio collection which has a  $\Delta$ EnvZ knockout mutation that was deleted using this same Lamda-Red Recombinase technique and knock out the *ackA-pta* genes in one go.

The full protocol we used can be found at: [http://openwetware.org/wiki/NanoBio: Protocol\\_for\\_gene\\_knockout](http://openwetware.org/wiki/NanoBio: Protocol_for_gene_knockout).

### Step 1: PCR amplification of FRT-flanked Chloramphenicol resistance gene

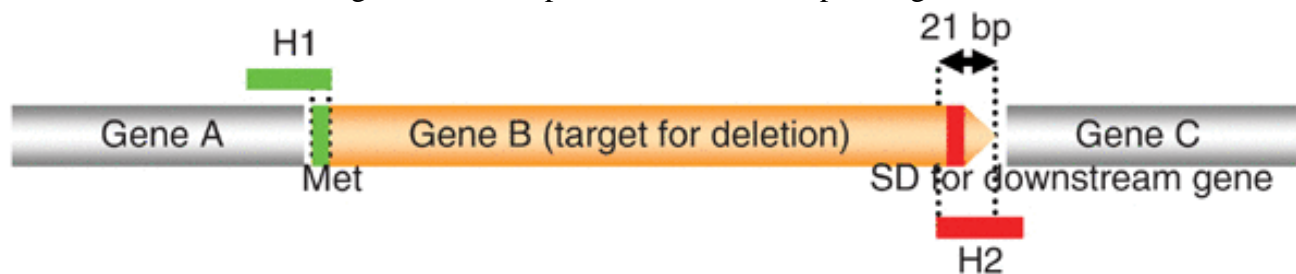


We took the pKD3 plasmid which contains Chloramphenicol resistance flanked by FRT sequences and amplified this segment using primers which were partly homologous to the chloramphenicol resistance gene (P1 and P2), and partly homologous to the area around the *ackA-pta* genes in the *E. coli* genome (H1 and H2).

### Step 2: Transform *E. coli* strain with $\lambda$ -Red Recombinase (on pKD46 plasmid)

The pKD46 plasmid contains the  $\lambda$ -Red Recombinase gene under a promoter induced by L-arabinose. We transformed this plasmid into the *E. coli* JW3367-3 strain.

### Step 3: Transform linear PCR fragment from step 1 into the *E. coli* expressing $\lambda$ -Red Recombinase

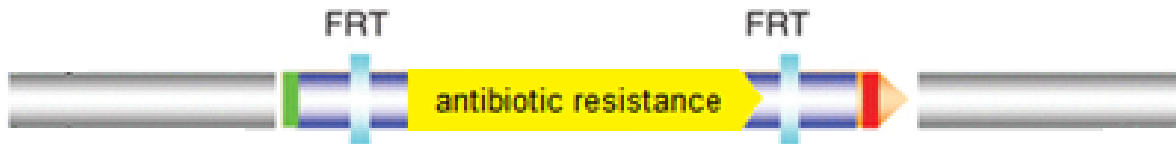


After making the strain from step 2 electrocompetent, we electrotransformed the linear PCR product (Chloramphenicol resistance flanked by FRT sequences) into the bacteria.

We did the following tests:

- A. L-arabinose induced + transformed PCR product (expect positive result)
- B. L-arabinose induced + no PCR product (control)
- C. Non-induced + PCR product (control)
- D. Non-induced + no PCR product (control)

Step 4: Selection of antibiotic-resistant transformant

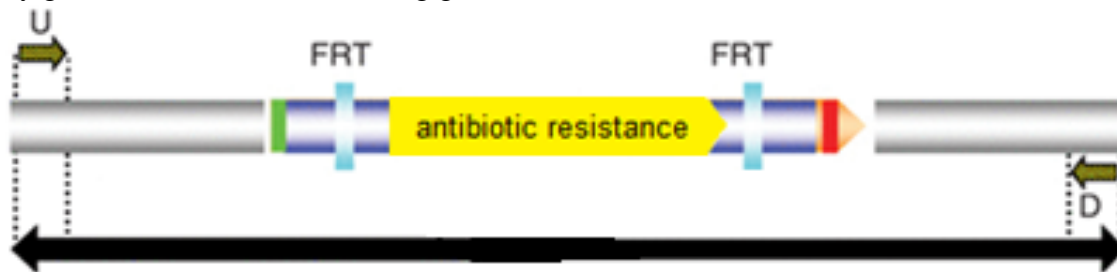


We plated the 4 tested bacteria on LB+agar with chloramphenicol. If we would have seen colonies on the test plates, this is what it would mean:

- A. Four possibilities:
  - 1. **Successful knockout**
  - 2. PCR product was not clean and the pKD3 plasmid was transformed, giving the colonies CM resistance.
  - 3. PCR product was clean but was not integrated into the genome and the bacteria are expressing the chloramphenicol resistance from the linear fragment.
  - 4. contamination
- B. Contamination
- C. Three possibilities:
  - 1. PCR product was not clean and the pKD3 plasmid was transformed, giving the colonies CM resistance.
  - 2. PCR product was clean but was not integrated into the genome and the bacteria are expressing the chloramphenicol resistance from the linear fragment.
  - 3. contamination
- D. contamination

Only plate A showed colonies so we assumed the knockout worked. But we needed to check.

Step 5: Verify genomic DNA deletions using genomic PCR.



Using primers for upstream and downstream of the knockout we expected that if the deletion succeeded, we would see amplification of a fragment 1743bp long. If the deletion failed, we expected amplification of a fragment 4070bp long.

We did colony PCR to see if the colonies actually contained the deletion. We saw a lot of bands so the colony PCR couldn't tell us anything. We tried doing a mini-prep of the colonies to see if any plasmids were present. Most of the colonies showed positive for plasmids. We think that the PCR product wasn't clean and that some pKD3 ended up in them and that's why they grew on Chloramphenicol plates. We also tried purifying the

genomic DNA of the colonies and doing PCR to see if the deletion occurred but that showed that none had the deletion.

We tried several times, once we even went all the way to the Weizmann Institute in Rehovot where Lior Z., Elad and Sagit Yahav helped us. But to no avail! We just couldn't manage to knock out the genes!

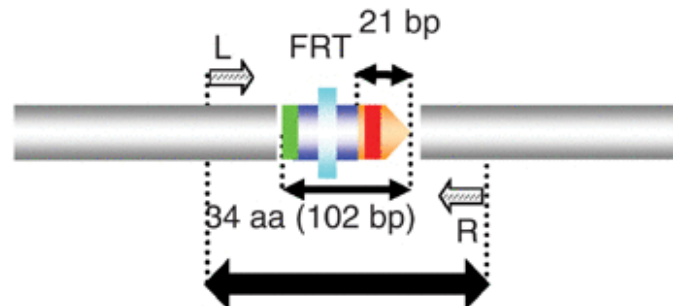
#### Step 5: Eliminate antibiotic resistance gene with FLP recombinase



However, if we had succeeded, the next step would be removal of the chloramphenicol resistance (and the Kanamycin resistance which was already in the JW3367-3 *E. coli* strain from the Keio Collection).

FLP recombinase on the pCP20 plasmid recognizes the FRT sequences and removes the antibiotic resistance between them, leaving a short 102bp scar.

#### Step 6: Genomic PCR amplify and verify by sequencing



To verify that the antibiotic resistance was removed, we would have done several PCR amplifications using upstream and downstream primers, as well as primers inside the resistance gene.

## References

- Baba, T., Ara, T., Hasegawa, M., Takai, Y., & Okumura, Y. (2006). Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Molecular Systems Biology*, 1-11.
- Datsenko, K. A., & Wanner, B. L. (2000). One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proceedings of the National Academy of Sciences of the United States of America*, 97(12), 6640-6645.
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- Kenney, L. (n.d.). *Welcome to the Kenney Lab*. Retrieved from University of Illinois at Chicago: <http://www.uic.edu/labs/kenney1/>