# The Influence of Non-Steroidal Anti-Inflammatory Drugs on Quasi-Palindrome Driven Template-Switch Mutagenesis in *E. coli*

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## Abstract

- Quasi-palindrome (QP) regions are characterized by almost perfect inverted repeats of DNA bases which can form secondary structures known as hairpins[1]
- Harpins are known to block DNA replication. Once DNA replication is blocked by these DNA structures, the DNA replication fork needs to find a solution to continue to replicate the DNA
- At some frequency, DNA polymerase (responsible for replicating the DNA) can use alternative DNA strands as a template to make more DNA[2,5]
- One alternative method is called “Template-switching” and it results in a mutation that creates a perfect palindrome from a quasi-palindromic sequence. This class of mutation has been associated with multiple human diseases including osteogenesis imperfecta and cancer[3,4]
- FDA approved non-steroidal anti-inflammatory drugs are commonly used to treat many different types of human ailments[6]
- Using a library of 300 FDA-approved drugs, we aim to assess the propensity of QP mutations after exposure to chemicals - known to block or stall DNA polymerase
- Using a QP mutation reporter in *E. coli*, we are screening hundreds of drugs for their ability to promote QP mutations[3,4]
- Elucidating the consequences of these drugs in template-switching QP mutations is essential in providing full understanding of the potential side effects for the current FDA-approved drugs

## Objectives

- Determine what Non-steroidal anti-inflammatory drugs (NSAID) might influence the stalling of Polymerase and thus increase QP mutation rates
- To confirm our model prediction for template switch mutations where DNA polymerase leaves the DNA after stalling to search for an alternative template. NSAID cause DNA polymerase to dissociate from the fork

## Materials & Methods

- We selected four NSAID from our library[7] .

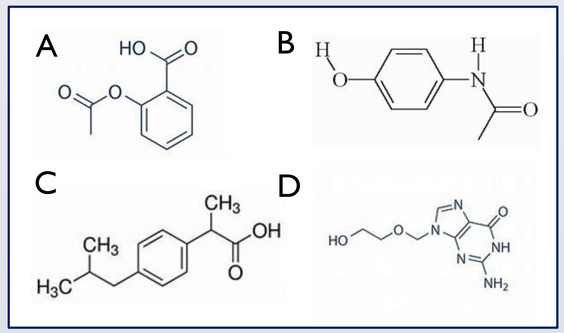


**Figure 1**: Drugs selected. (A) 2-Acetoxybenzoic acid. (B) Acetaminophen. (C) Ibuprofen. (D) Acyclovir

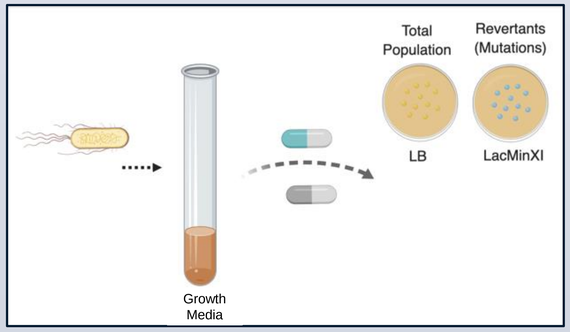


**Figure 2**: Schematic of fluctuation assay

- We will assess mutation rates by performing Disk diffusion and fluctuation assays [3,4]

## Expected Results

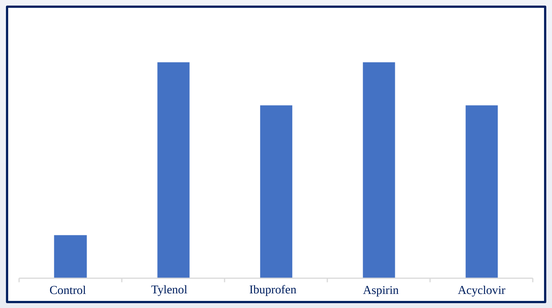


**Figure 3:** NSAID promote template-switch mutations. Rates of mutations increased for all tested drugs

- We hypothesize that mutation rates will increase after treatment of all the tested drugs and expect their mutation rates to be similar among the different drugs
- It is known that Ibuprofen and Acetaminophen act strongly in bacterial cells, therefore their rates might be different than the other drugs possibly due to viability

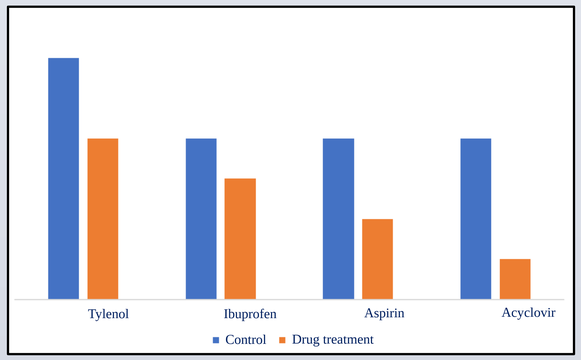


**Figure 4:** NSAID reduces template-switch mutation rates in *rec*A strains.
Rates of mutations increased for all tested drugs

- Publications have shown that the rate of template-witch mutations is independent to RecA activity after treatment of specific drugs. Given that our selected drugs interact in different mechanisms, it is possible to affect mutation rates in a different manner

## Discussion

- We predict that NSAID will cause an increase in QP mutations since they can promote the sliding clamp to dissociate at a QP region, and therefore allowing more chances for a template-switch mutation (TSM) to occur
- Results will indicate if the addition of different drugs promoted TSM. A higher increase on mutation rates will confirm our model and predictions
- We also would like to confirm the effect of these drugs in *rec*A strains. This protein is involved in the bacterial SOS response. Previous publications indicate a higher rate of mutation in these mutants. We would like to test if NSAID affect the rate of mutations dependent of the SOS response pathway

## References

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4 Laranjo, L.T.; Klaric, J.A.; Lovett, S.T. Stimulation of Replication Template-Switching by DNA-Protein Crosslinks. 2018, 2018110046 (doi: 10.20944/preprints201811.0046.v1)

5 “DNA Polymerase III, Beta Sliding Clamp” interpro7-Client, www.ebi.ac.uk/interpro/entry/InterPro/IPR001001/.

6 Yin, Zhou, et al. “DNA Replication Is the Target for the Antibacterial Effects of Nonsteroidal Anti-Inflammatory Drugs.” Chemistry & Biology, Cell Press, 13 Mar. 2014,

7 Please refer to #5932-Library of FDA-Approved Compounds "A Library of 159 'FDA-Approved' compounds – a subset of the Tocriscreen Plus Compound Collection“ for a complete Certificate of Analysis

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