

# MutL induced endonuclease activity in Cyanobacteria

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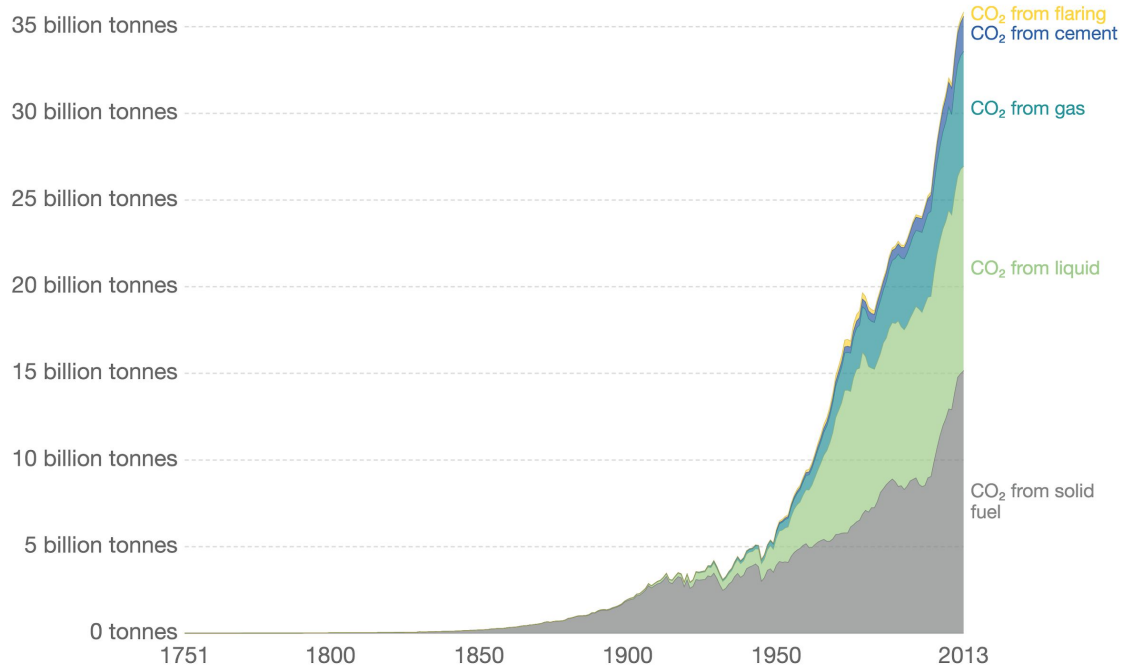
# Energy Crisis

- Fossil fuels consumption
- Toxic emissions
- Climate Change
- Public Health Risk

## CO<sub>2</sub> emissions by source, World

Annual carbon dioxide (CO<sub>2</sub>) emissions from solid fuel (e.g. coal); liquid (e.g. oil); gas (e.g. natural gas); cement production and gas flaring, measured in tonnes per year.

Our World  
in Data

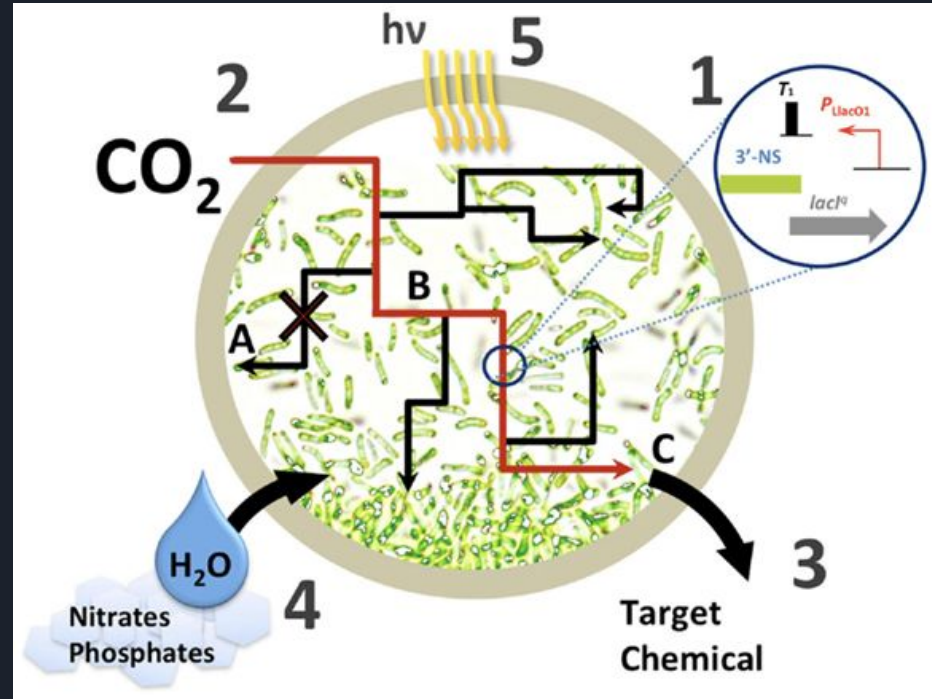


Source: CDIAC

OurWorldInData.org/co2-and-other-greenhouse-gas-emissions/ • CC BY-SA

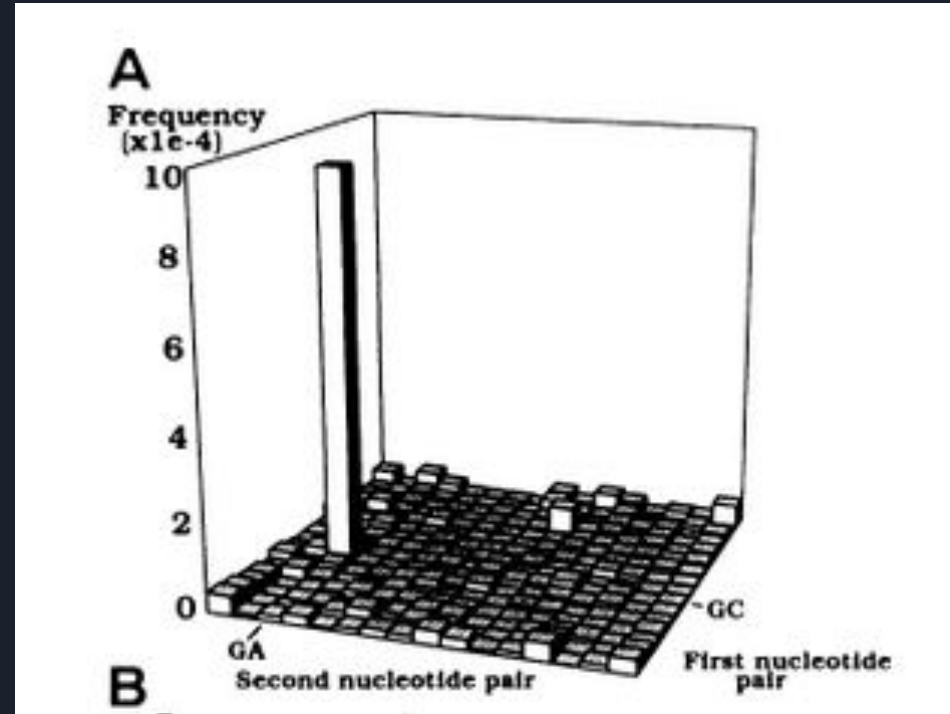
# Cyanobacteria as a platform for biofuel production

- Photosynthetic
- Ubiquitous and robust
- Microbial factories
- Limitation = Genetic Stability

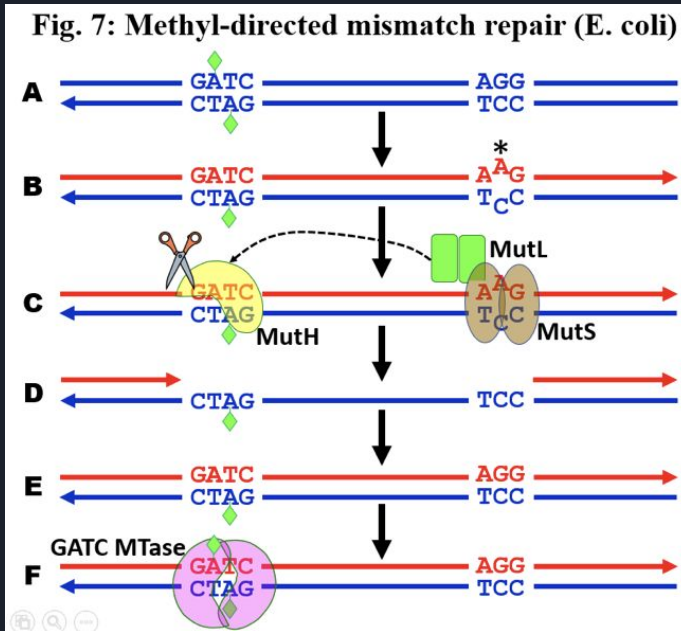


# The role of repetitive sequences...

- HIP1- octameric palindrome (5'-GCGATCGC-3')
- Why so frequent/conserved?
- Functional Role?
- Proposed ideas.....



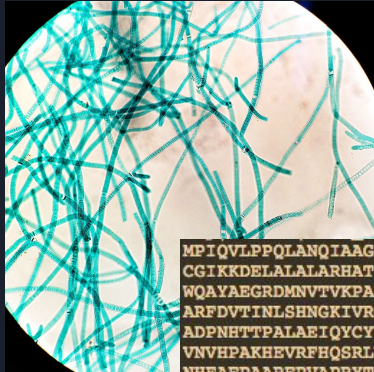
# MMR in E.Coli vs Cyanobacteria



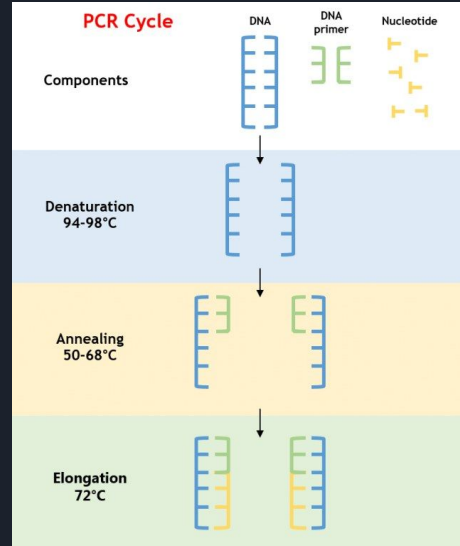
- MutH does not exist in Cyanobacteria
- Role of MutL and MutS remains unknown

# How to measure MutL endonuclease activity

- MutL gene plasmid isolated from Cyanobacterial strain and replicated using PCR

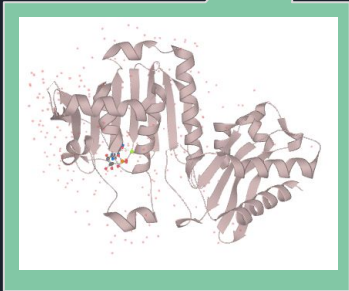
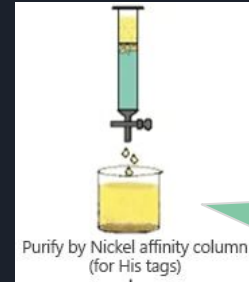
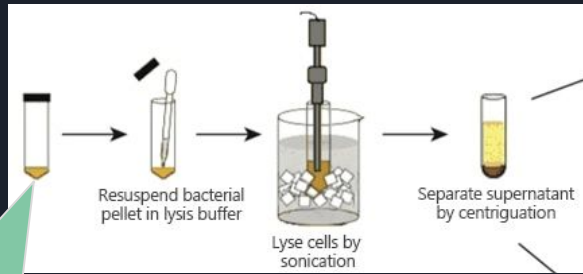


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MPIQVLPPQLANQIAAGEVW  
CGIKKDELALALARHATSKI  
WQAYAEGRDMNVTVKPAHF  
ARFDVTINLSHGKIVRQYF  
ADPNETTPALAEIQCYVNG  
VNVHPAKHEVRFHQSRVHI  
NHFAEPAAREPVAPRYTPAF  
PEPQEPALAANSQSFRVLI  
AOPLLIPLRLKVSAAEKSAL
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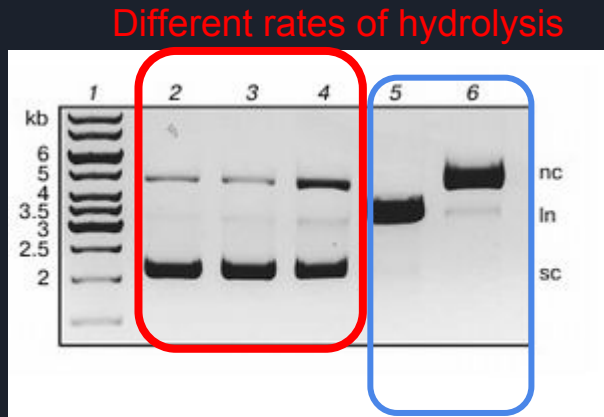
# How to measure MutL endonuclease activity

Purification of MutL plasmid to yield homogenous MutL protein

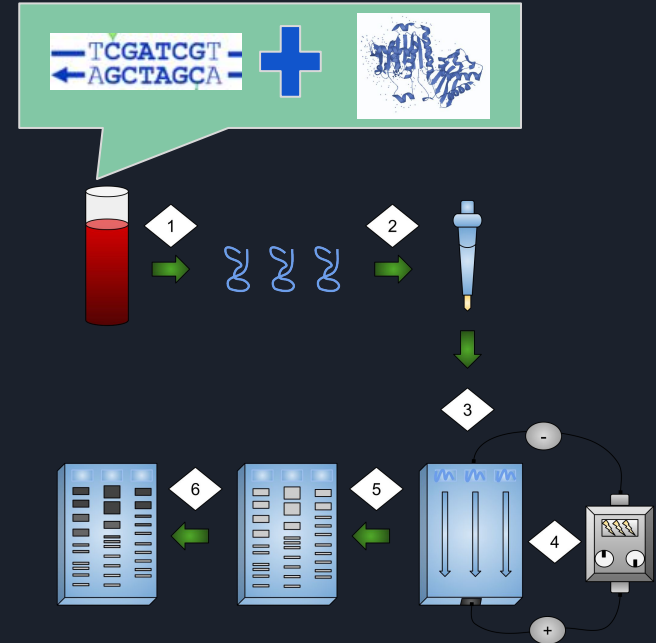


# How to measure MutL endonuclease activity

- A similar process would be done with potential mismatch and hemimethylated GMeC/CG sites (PCR + Purification)
- Use electrophoresis to measure the amount of single stranded breaks



Control by other restriction enzymes





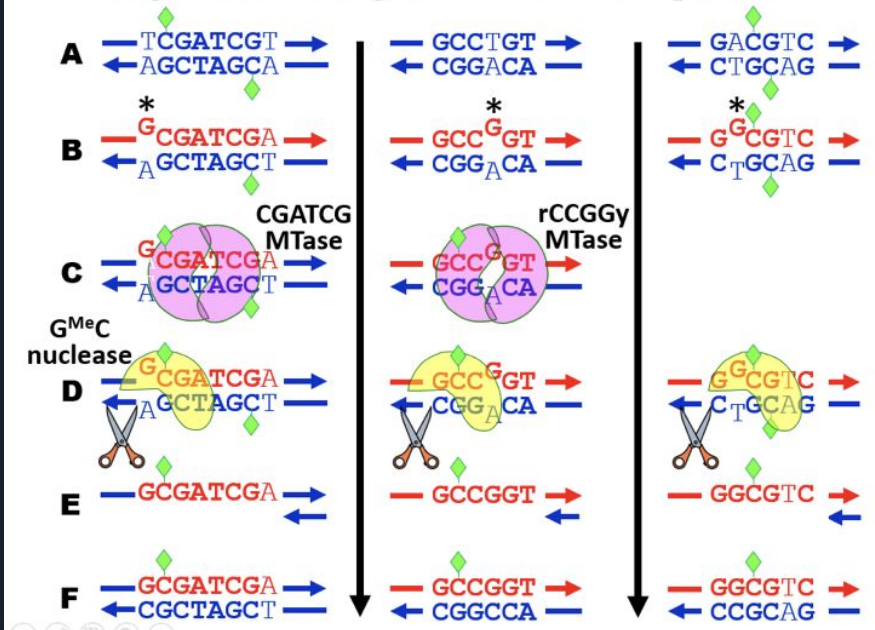


# Predicted Results & Limitations

- MutL endonuclease activity will be present in Cyanobacteria
- Hydrolysis may not be convincing without the use of cations to increase enzymatic activity

# Postulated G<sup>Me</sup>C-dependent mismatch repair system

**Fig. 8: Model for generation of HIP sequences**



- Theory for gain and loss of HIP1
- Association with MTases
- Theory could be supported by endonuclease MutL activity at the hemimethylated G<sup>Me</sup>C/CG sites



# References

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