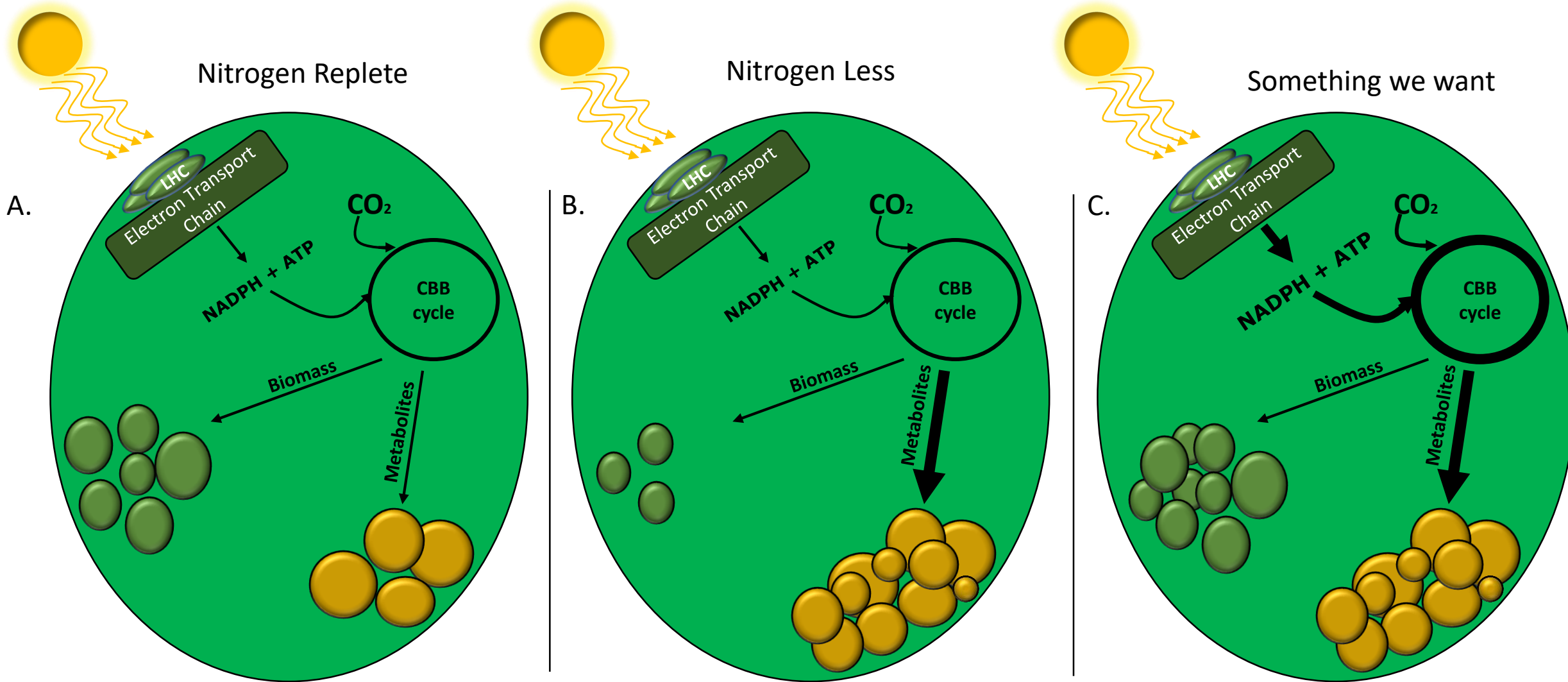


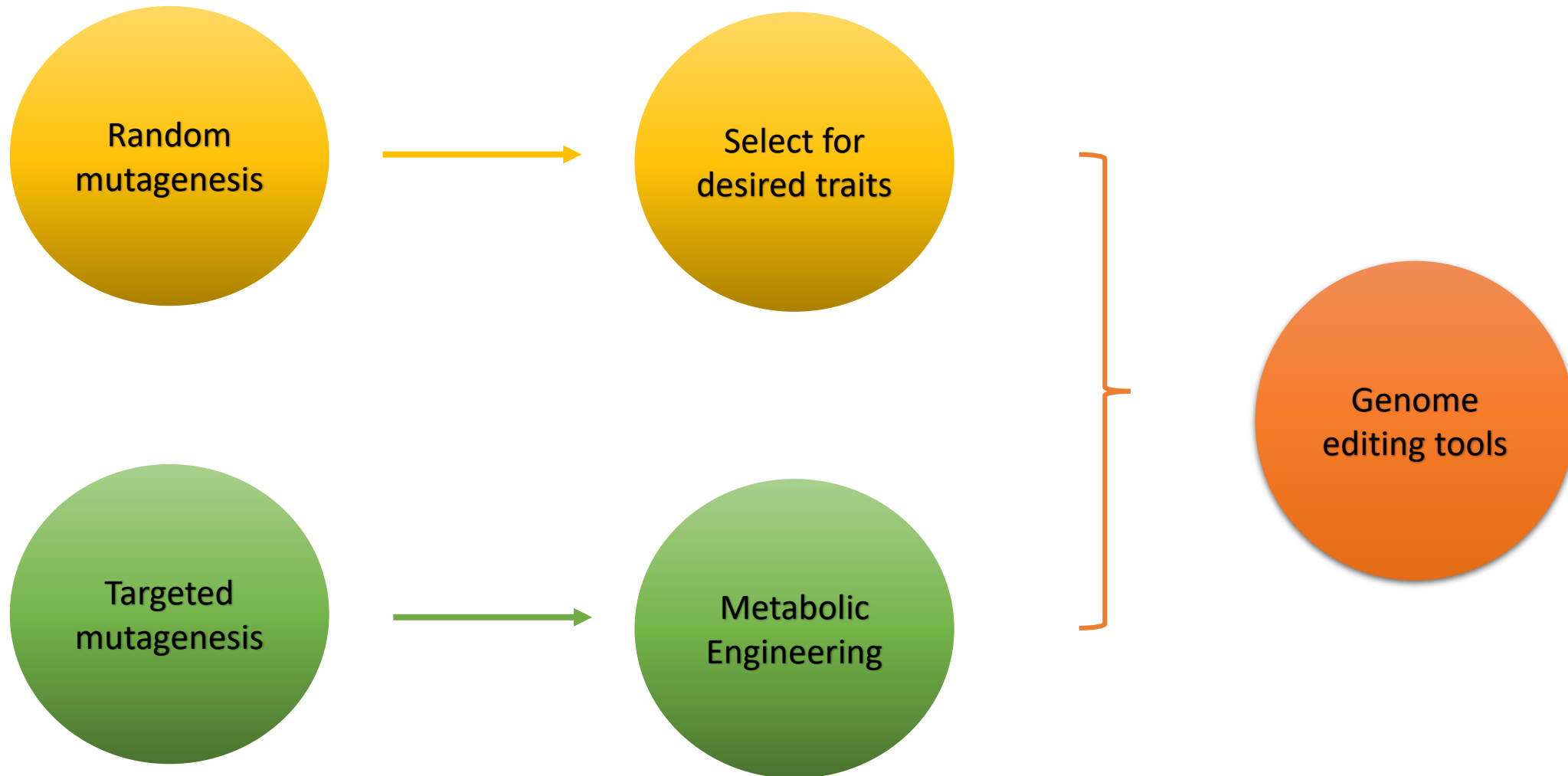
# A CRISPy tale on microalgal genome editing *ft. Nannochloropsis oceanica*

Mihris Naduthodi

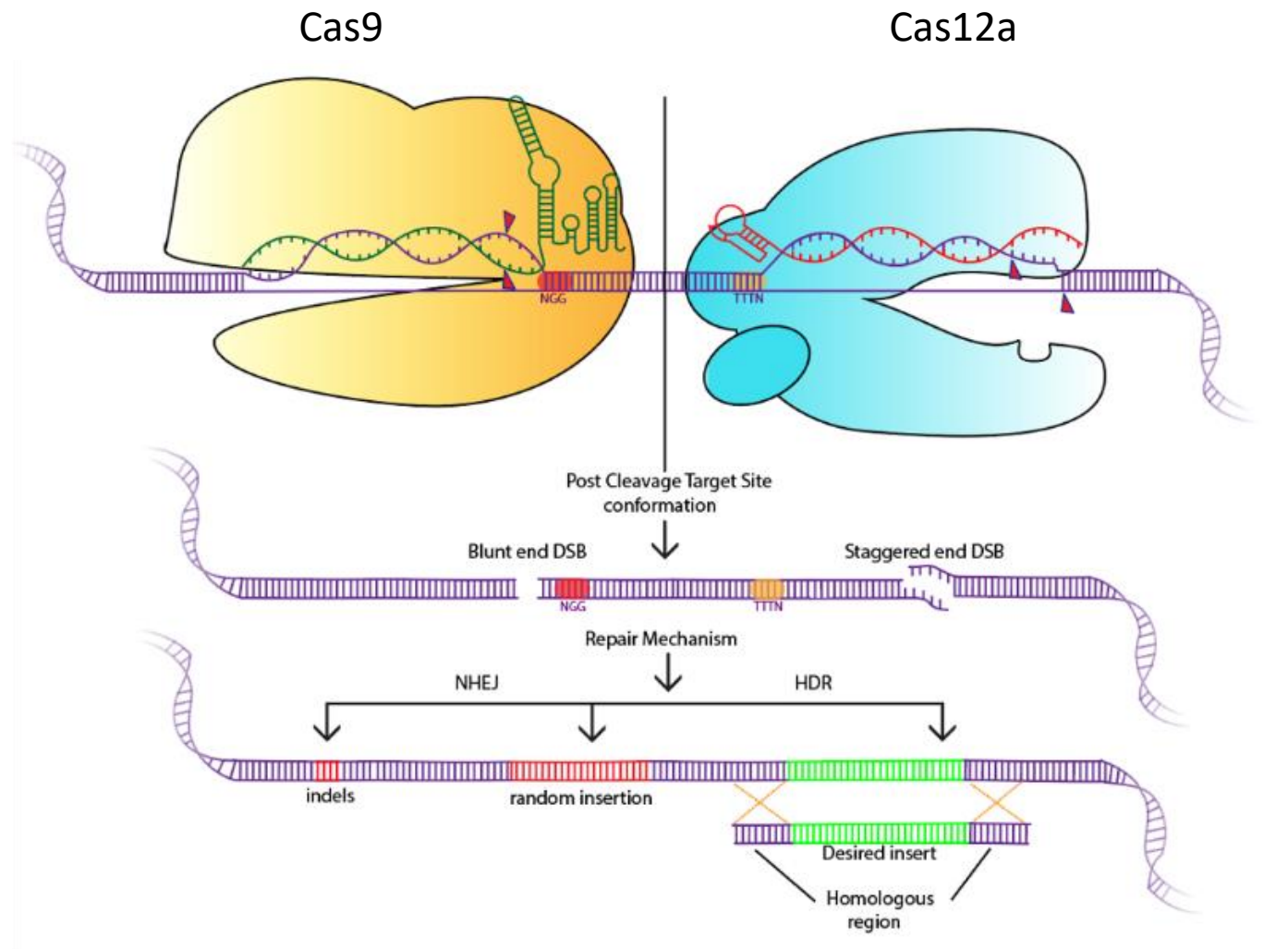
# *Nannochloropsis* spp – Strain improvement



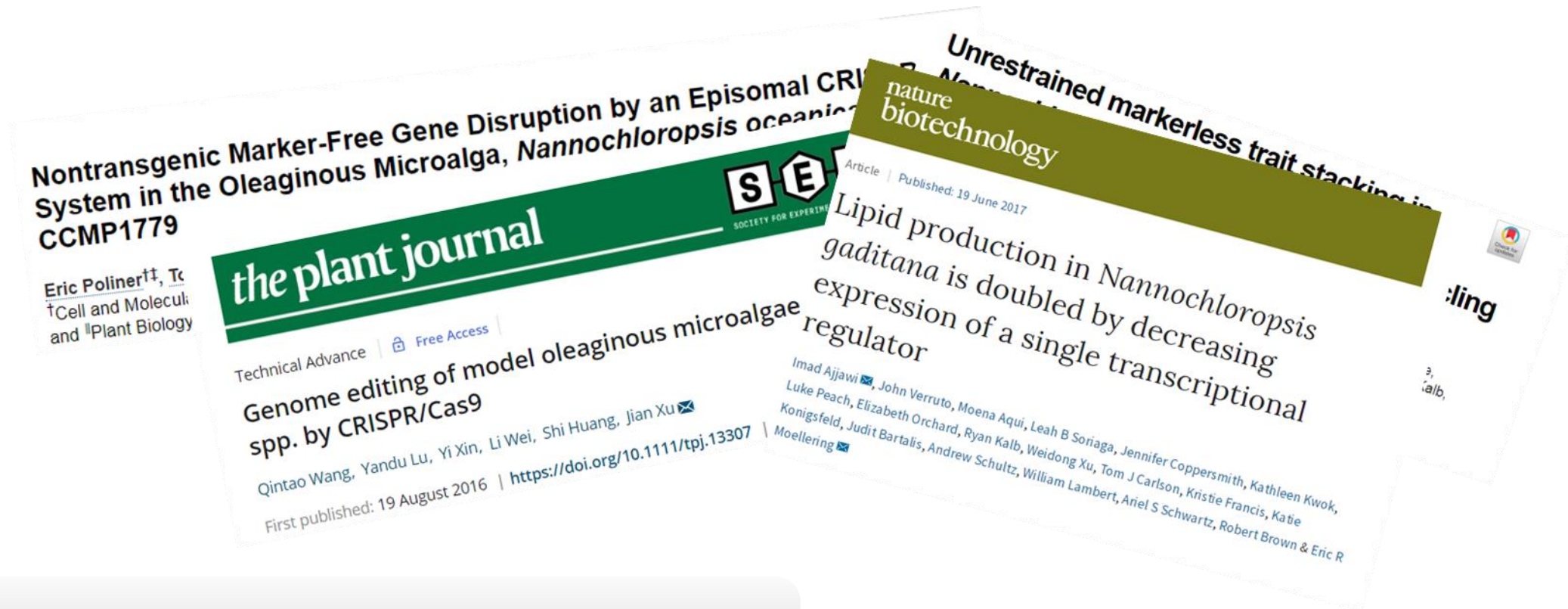
# *Nannochloropsis* spp – Strain improvement



# CRISPR-Cas nuclease based genome editing



# CRISPR-Cas in *Nannochloropsis* spp.



Similar strategy for generating mutants

Plasmid based Cas9 expression → Indels at target site

Our Aim: Use Ribonucleoproteins to generate precise mutants instead of indels

# RNPs for introducing specific mutation via HDR

Possibility of low off target effects

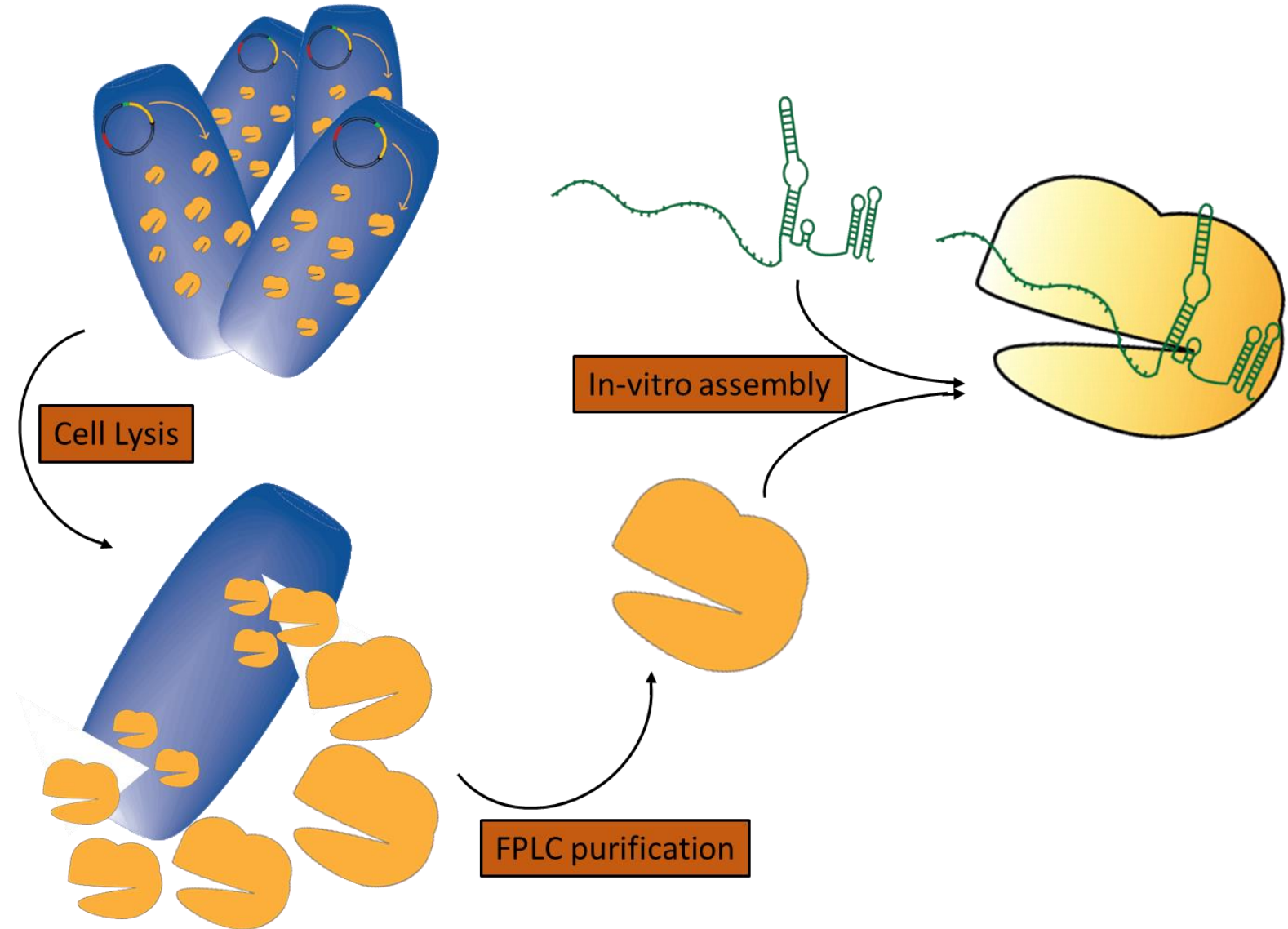
Dispensability of regulatory systems and gene sequence

Possibility of using the mutants as non-GMO

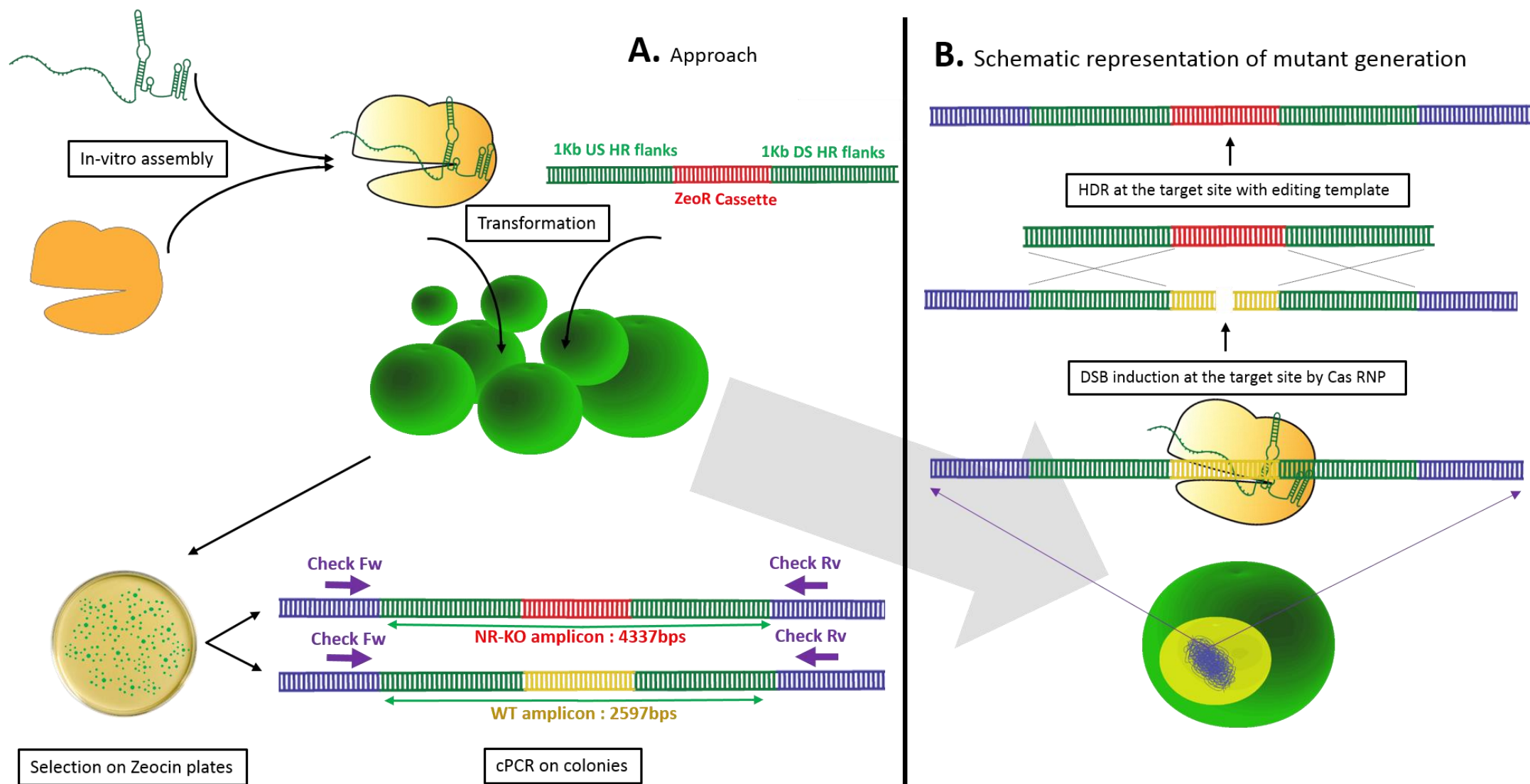
HDR for precise generation of mutants in place of generating indels

# RNPs for introducing specific mutation via HDR

Cas Protein  
Purification



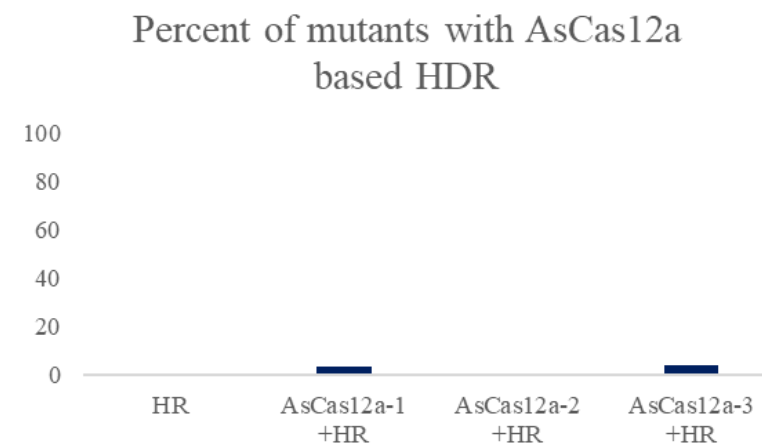
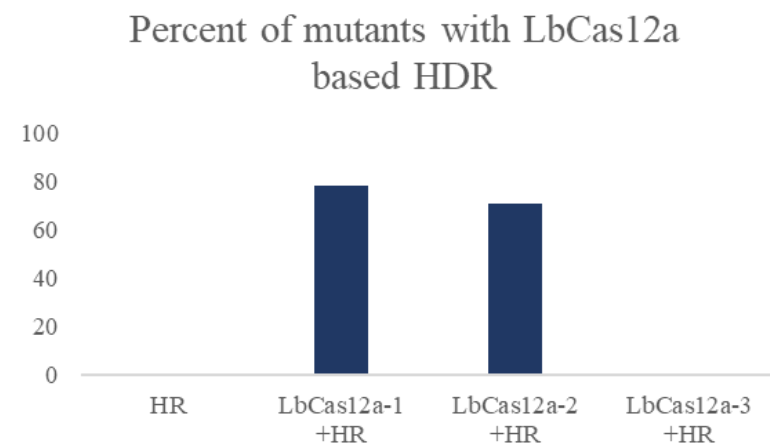
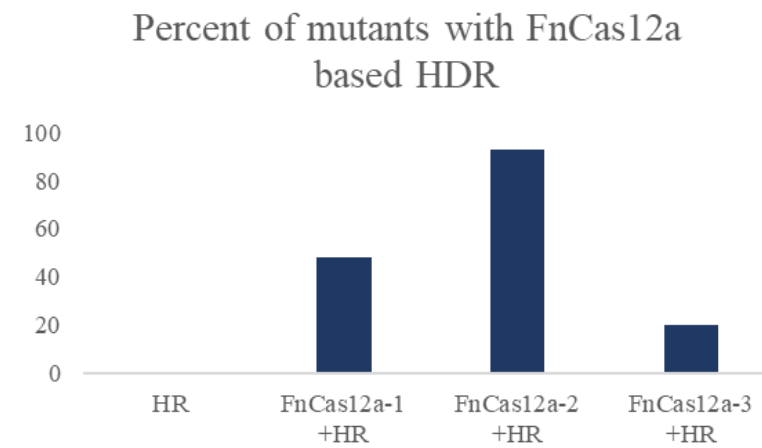
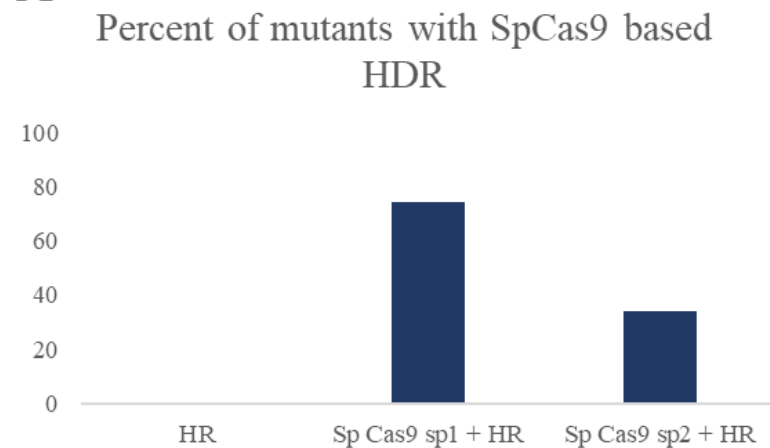
# RNPs for introducing specific mutation via HDR





# RNPs for introducing specific mutation via HDR


A



# RNPs for introducing specific mutation via HDR

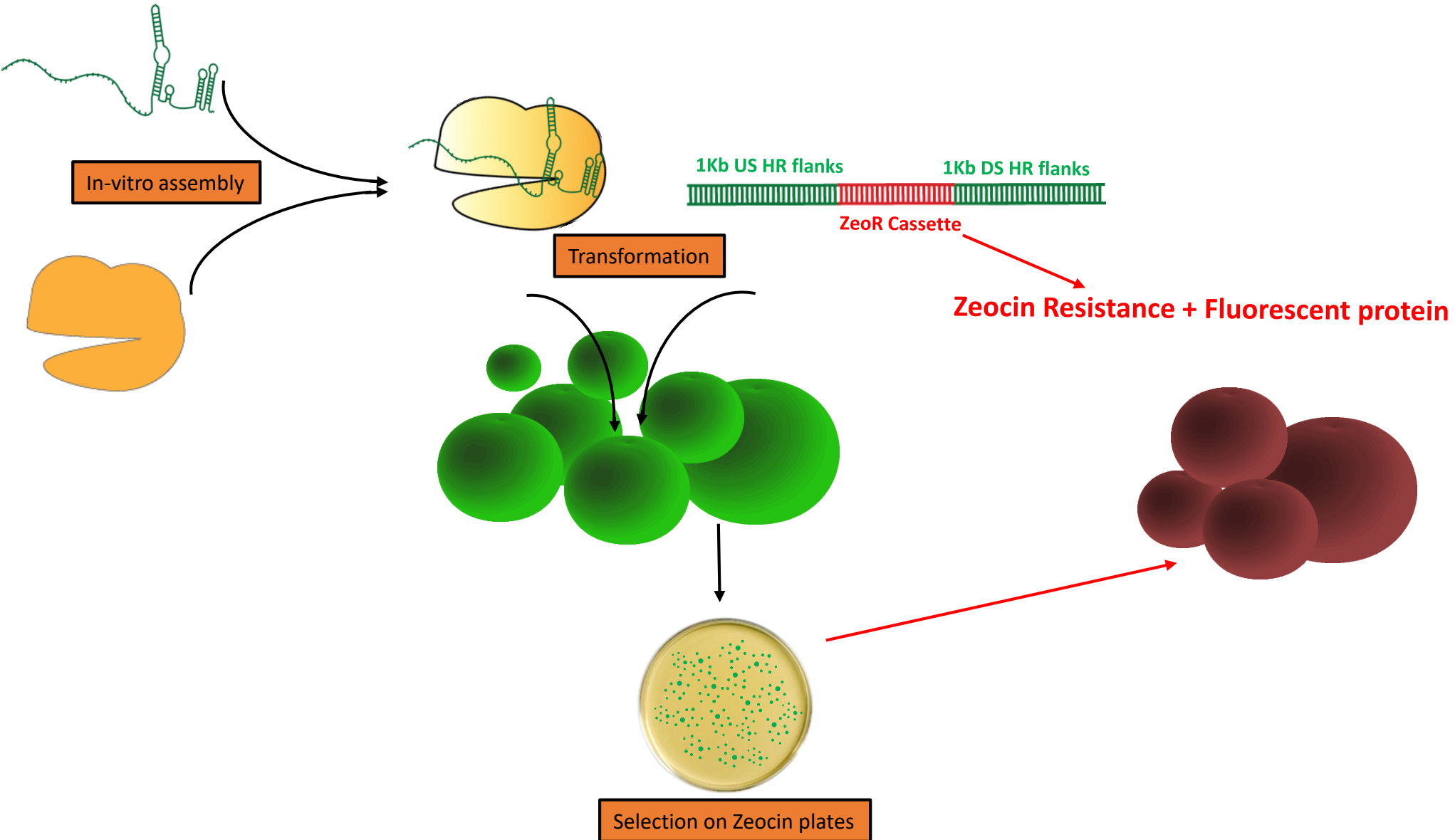
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## **CRISPR–Cas ribonucleoprotein mediated homology-directed repair for efficient targeted genome editing in microalgae *Nannochloropsis oceanica* IMET1**

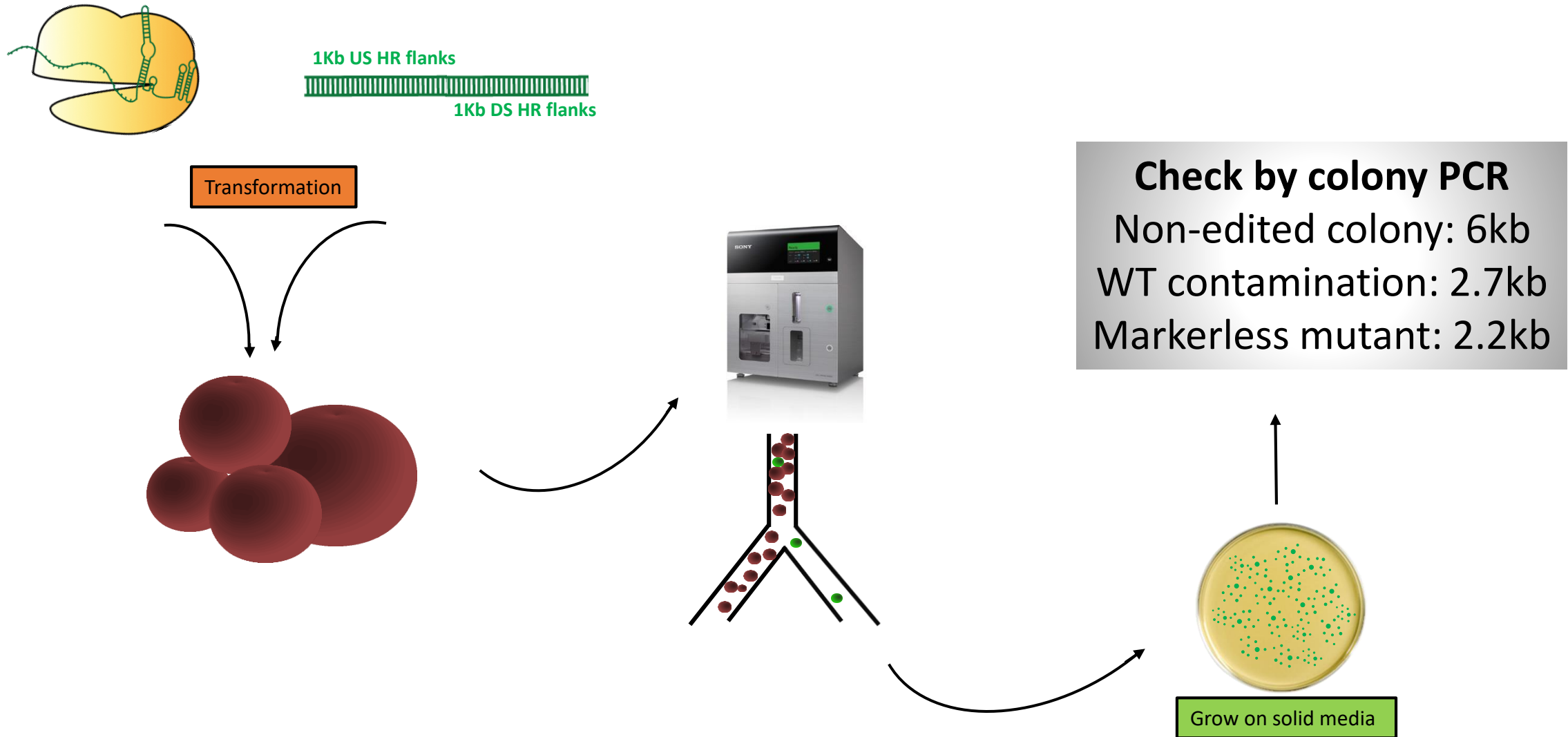
[Mihris Ibnu Saleem Naduthodi](#), [Prarthana Mohanraju](#), [Christian Südfeld](#), [Sarah D'Adamo](#), [Maria J. Barbosa](#)  
& [John van der Oost](#) 

[Biotechnology for Biofuels](#) **12**, Article number: 66 (2019) | [Cite this article](#)

# How can we generate markerless mutants with RNP and HDR?



# How can we generate markerless mutants with RNP and HDR?



# Summary

- Developed RNP based genome editing strategy in *N. oceanica*
- HDR based strategy was shown for the first time in *N. oceanica* for generation of precise mutants
- This strategy was combined with high throughput selection by FACS to obtain markerless mutants