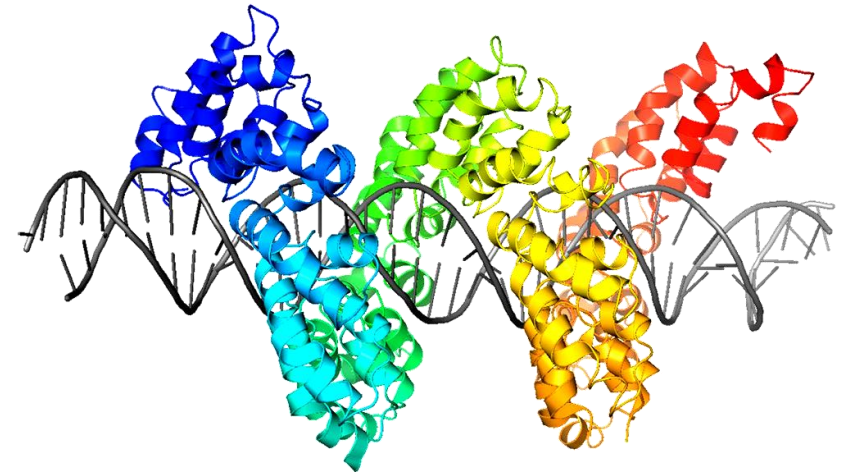


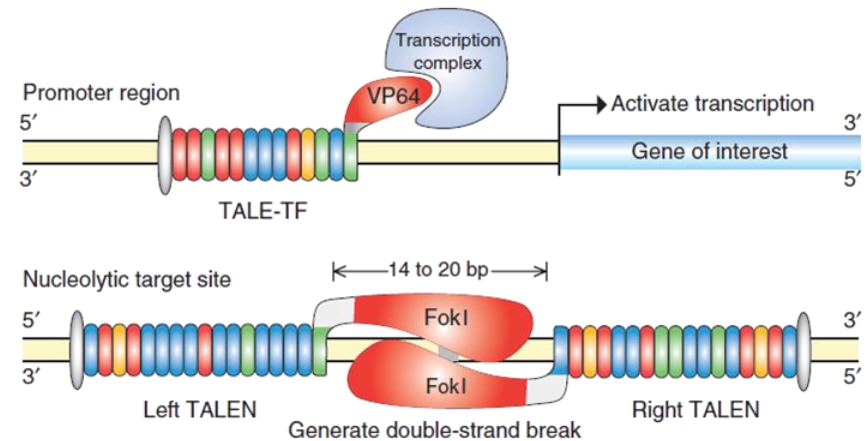
# TAL effectors

- transcription activator-like effectors
- modular DNA binding domains



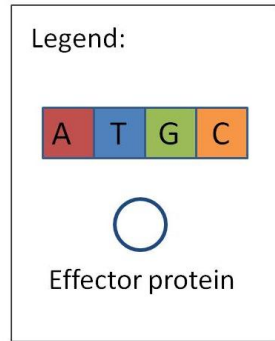
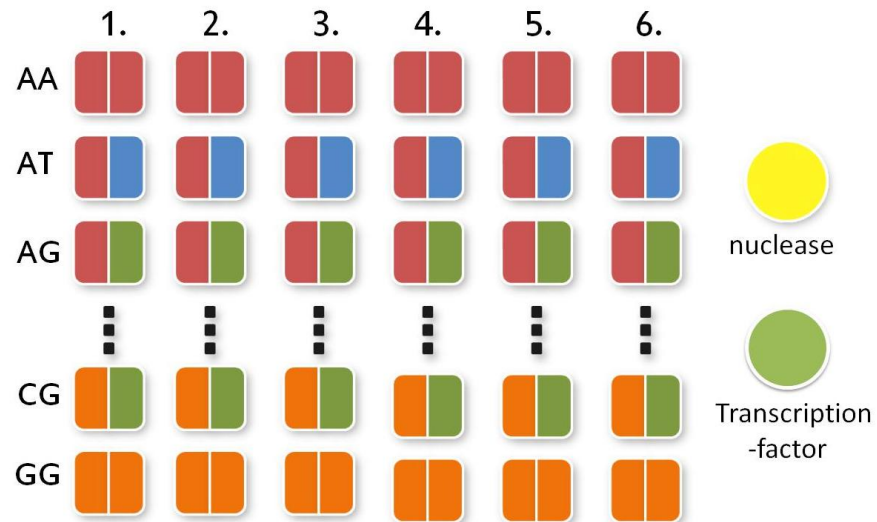
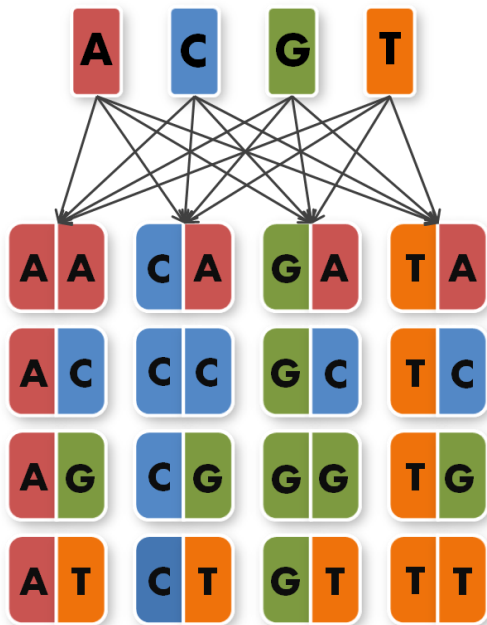
The laboratory of Barry L. Stoddard, Ph.D., Fred Hutchinson Cancer Research Center

- artificial transcription factors
- TAL effector nucleases



Sanjana et. al 2012

# Freiburg 2012 - Toolkit



Target Sequence: T-AATGAGGCATCT-T

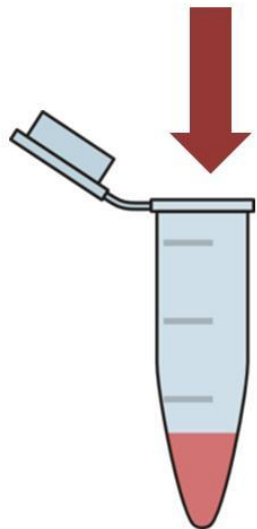


# Freiburg 2012 - Toolkit

Target Sequence: AATGAGGCATCT



1.AA + 2.TG + 3. AG + 4. GC + 5. AT + 6.CT + TF (VP64)



Combine the sequences in one step of Golden Gate Cloning to get the ready-to-use sequence of your entire TAL-TF effector.



The VP64 domain will activate the transcription of the GOI

# Jamboree Results

- Europe:  
53 registered Teams
- Advance to World  
Championship in Boston:  
18 Teams from Europe  
71 Teams in total  
up to 1000 participants



# Championship results:

## Sweet Sixteen:

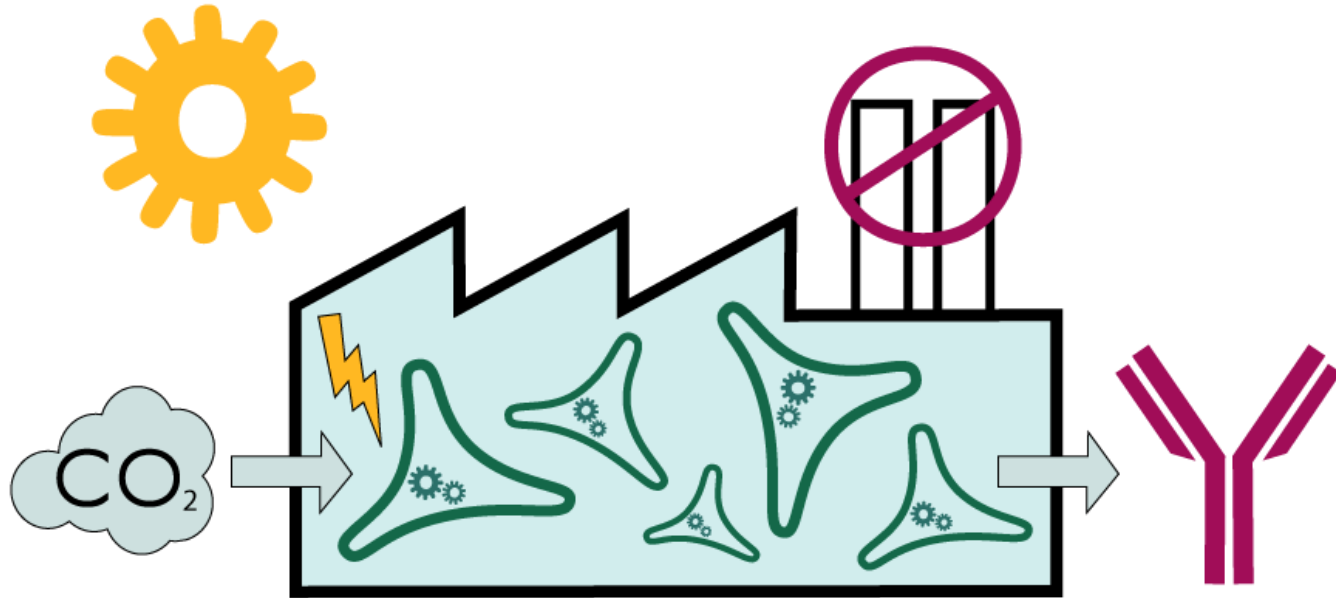
- Bielefeld-Germany
- Calgary
- Carnegie Mellon
- Cornell
- Freiburg
- Groningen
- LMU-Munich
- Paris Bettencourt
- Penn
- SJTU-BioX-Shanghai
- Slovenia
- Stanford-Brown
- TU Munich
- UNITN-Trento
- University London
- Wageningen





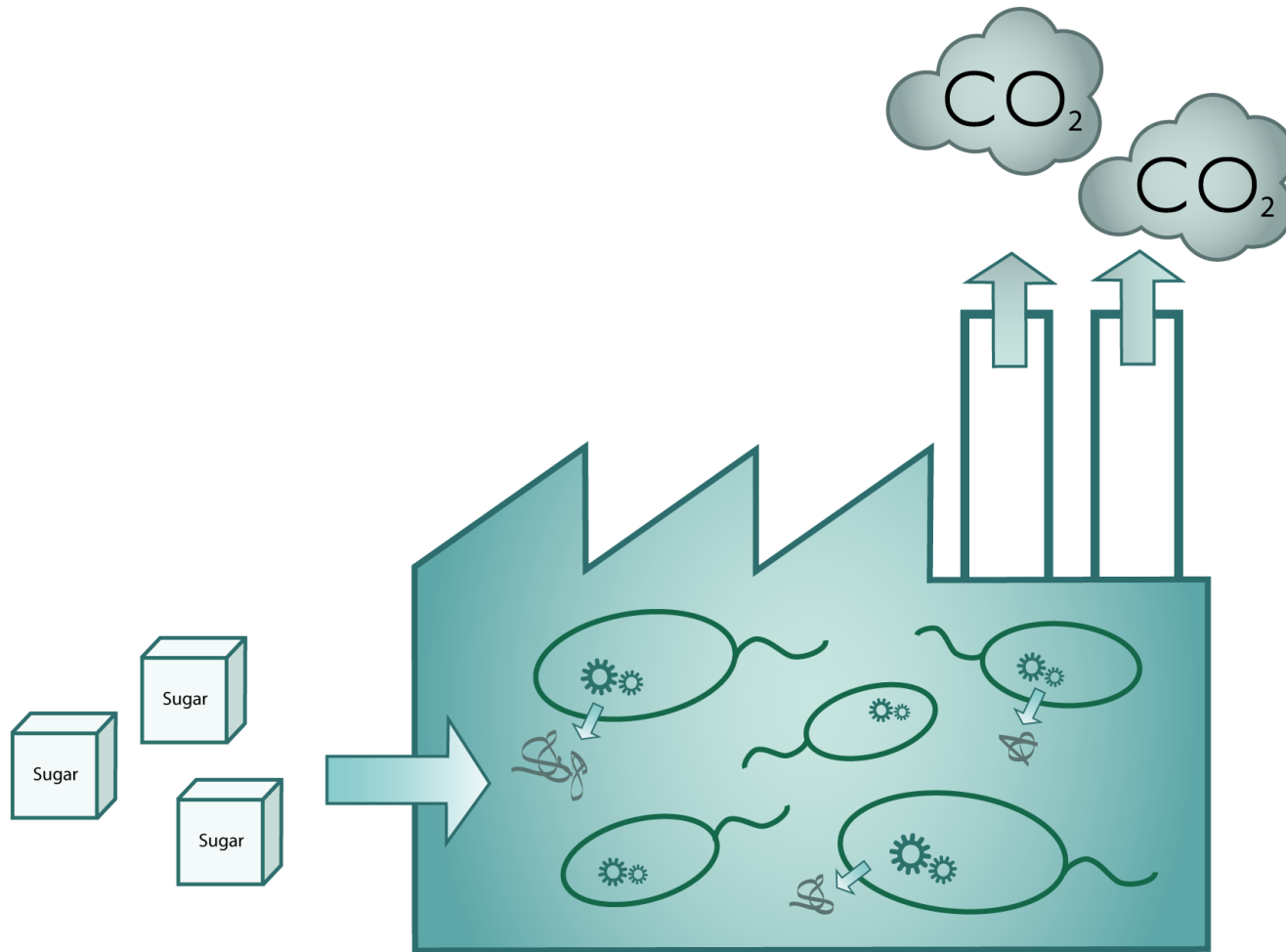
# Missions for synthetic biology: iGEM Marburg 2013

## PHAECTOR<sup>Y</sup>

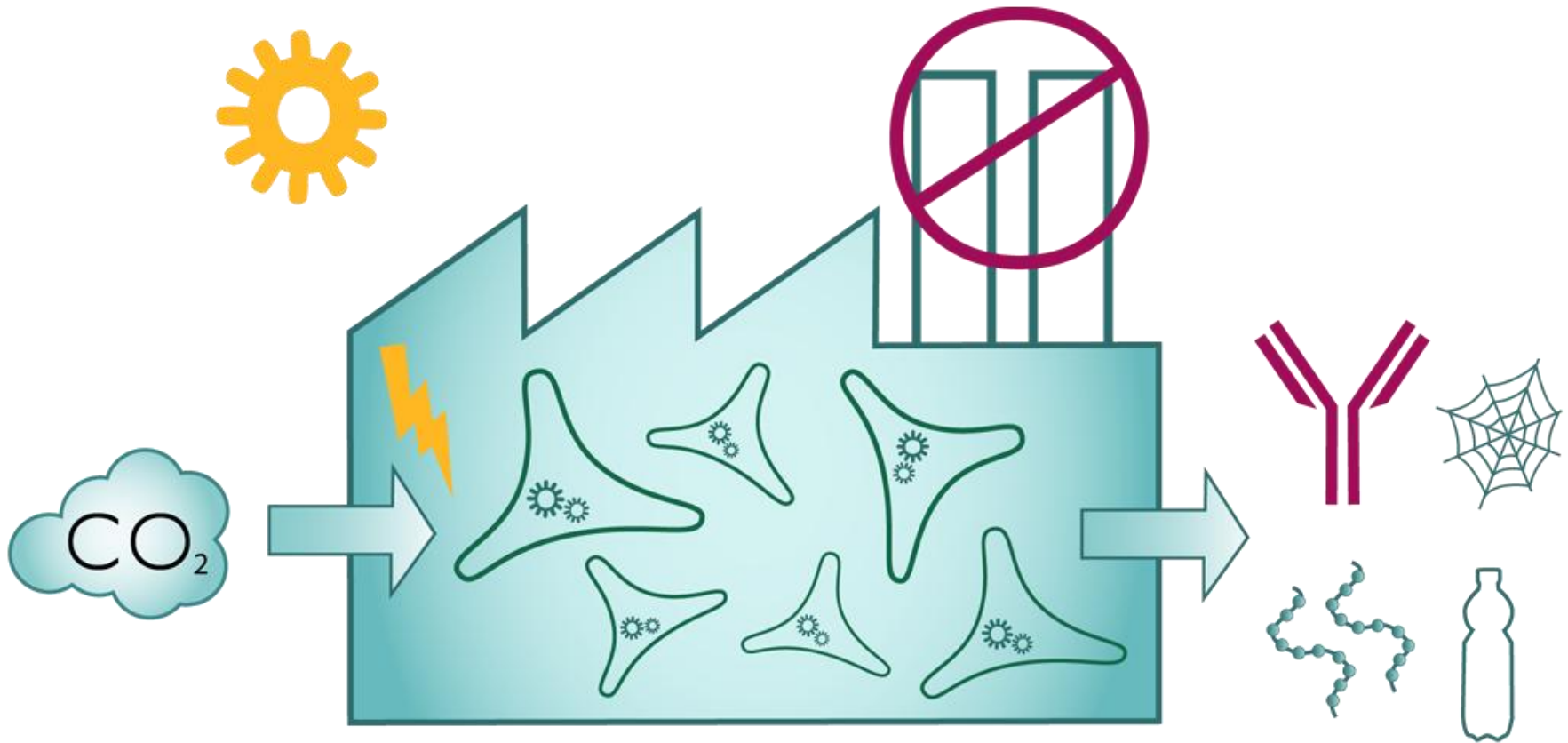


**A green cell factory**

# Microbial cell factory

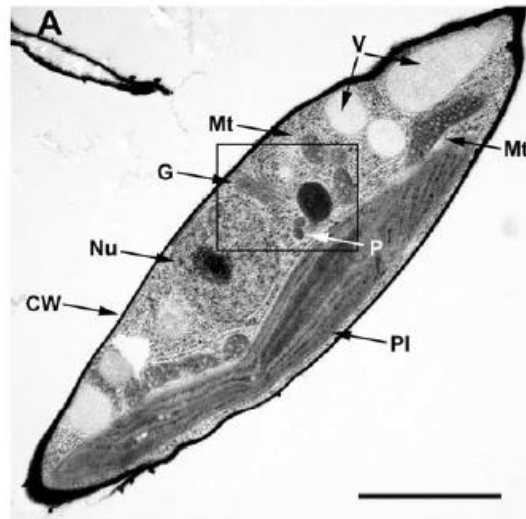
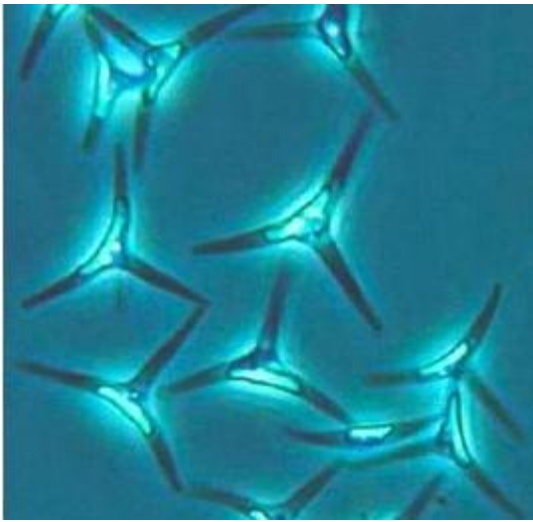


# Algal cell factory





# *Phaeodactylum tricornutum*



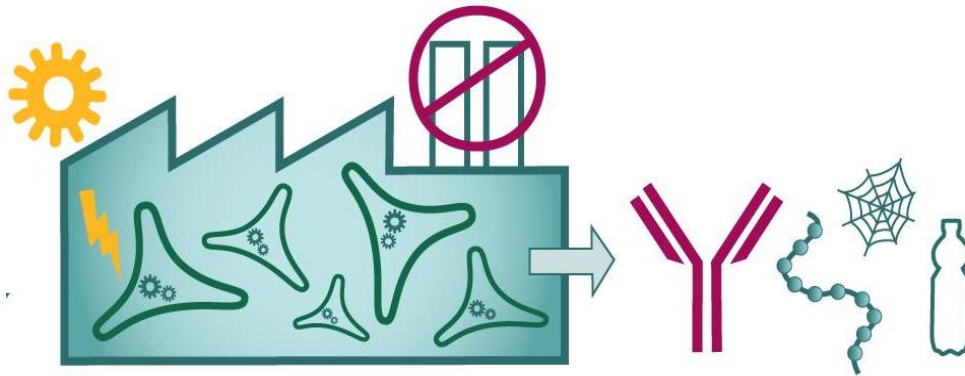
- pennate diatom (heterokont)
- complex plastids
- Genome sequenced (27,4 Mbp)

Microscopic images of *Phaeodactylum tricornutum*

# *Phaeodactylum tricornutum* as a bioreactor

Production of:

- biofuel
- bioplastic
- spider silk
- proteins  
(e.g. therapeutic antibodies)



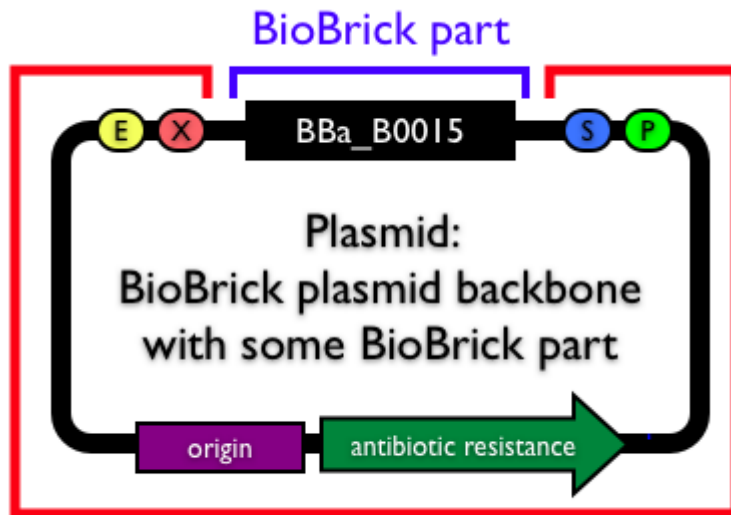
# Practical realization

- Aims:
  - Establishing BioBricks in *P. tricornutum*
  - Quantitative characterization of standardized promoters (e.g. light induction)
  - Targeting of proteins to cell compartments
  - Production of Hepatitis B antibodies



# Assembly standard 23

- Enables combination of two BioBricks without frameshift



BioBrick plasmid backbone





```

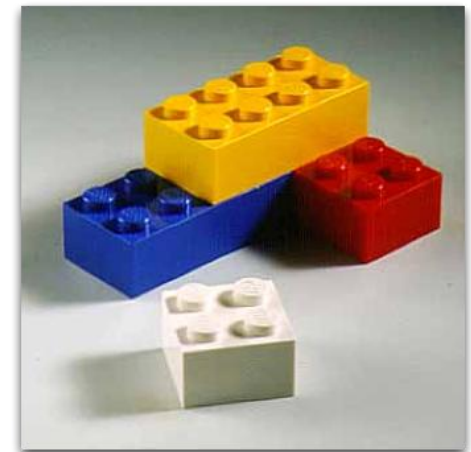
5' -GAATTC GCGGCCGC T TCTAGA part ACTAGT A GCGGCCGC CTGCAG- 3'
3' -CTTAAG CGCCGGCG A ACATCT part TGATCA T CGCCGGC GACGTC- 5'
   EcoRI   NotI   XbaI   SpeI   NotI   PstI
  
```

```

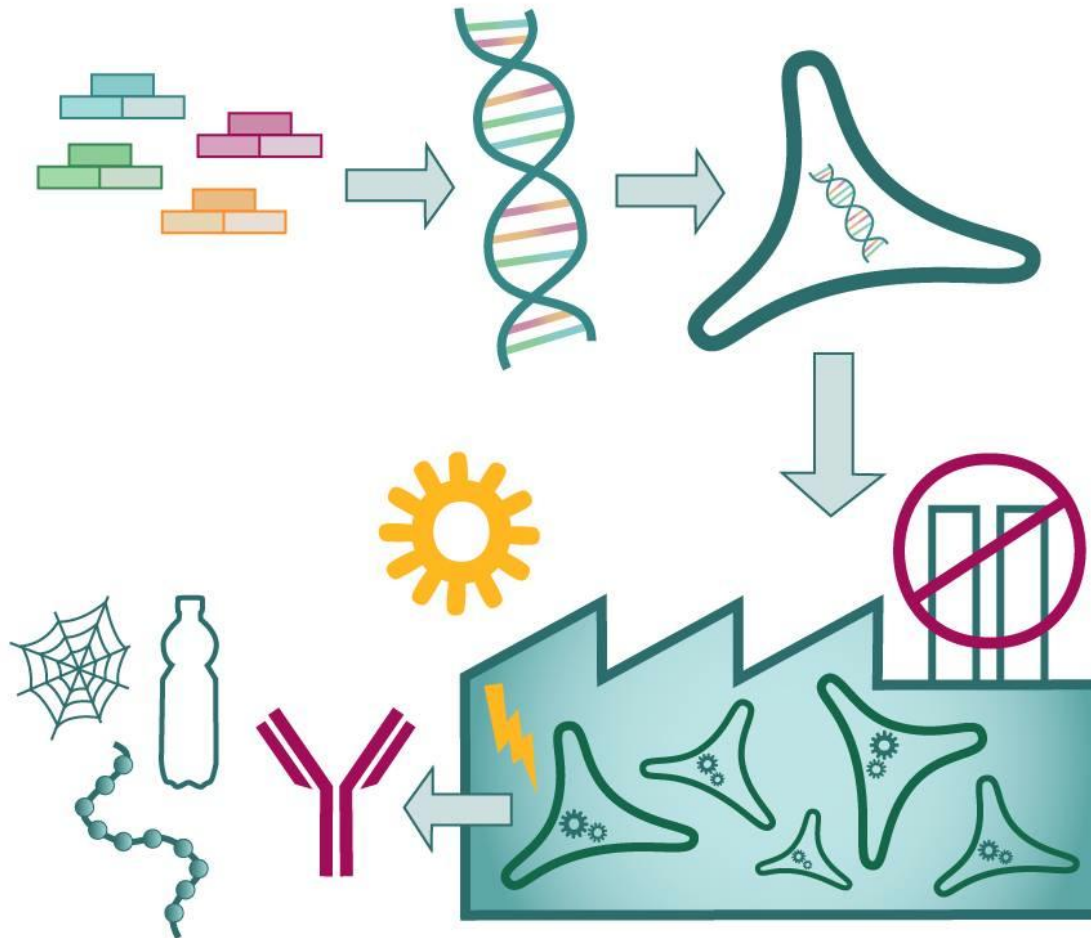
5' -TCTAGA Part_1 ACTAGA Part_2 ACTAGT- 3'
3' -AGATCT Part_1 TGATCT Part_2 TGATCA- 5'
      XbaI      Mixed Site      SpeI
  
```

# Assembled BioBricks

- light- and nitrate-induced promotor   
as well as the corresponding terminator 
- heavy and light chain of the Hepatitis B antibody
- Zeocin and Kanamycin resistance 
- signal peptides (endoplasmic reticulum and stroma)
- eGFP 



# Phaectory





# Human Practice

...to inform and connect



# Human Practice



- composed of the young generation of genetic engineers who represent the synthetic biology community
- Through iGEM:
  - To inform the society about synthetic biology, to wake up the interest in young scientists and to build a functional, effective and communicative network locally and globally

## Debate on Synthetic Biology



**Wrong beliefs vs. fundamental facts**  
**Tue. 4th of June**

**The Marburg iGEM team proudly presents:**



**DNA preparation *to go*. At the Hessentag in Kassel.  
Sun. 23th of June**

# Human Practice

## Synthetic Biology in the School



*"What's the opposite of 'Eureka!'?"*

**Wake up the interest on green sprouts**  
***To Do: August***



## Abschlusskongress: Bilanz & Ausblick Initiative Biotechnologie 2020+



**Network of the 11 iGEM teams in Germany  
Thu. 27th of June**



# Human Practice

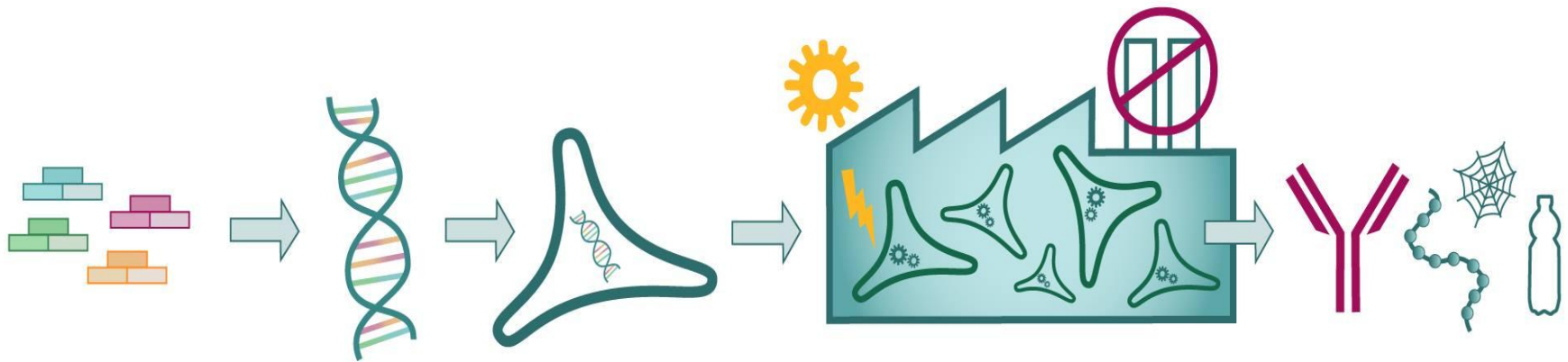
## Cooperation between iGEM teams

- A simultaneous presentation of iGEM and synthetic biology in many cities in Germany
- Cooperation with iGEM teams of Darmstadt and Frankfurt



# Summary

## Marburg PHAECTOR<sup>Y</sup>



1. Synthesis of PhaeoBioBrix

2. Vector Design

3. Transfection

4. CO<sub>2</sub>-neutral, low cost production of POIs

# Problems of Applications of our Phaectory?

## Potential problems:

- Spider silk production

Extraction and the process into fibers

- Bioplastic production

Large scale production open or in containment?

- Therapeutic antibodies

Sterility, FDA-approval

# Large scale production: Optimization and Biosafety?

- Now:
  - *P. tricornutum* BSL1
  - No auxotrophie neither homologous recombination
  - Gene-Silencing for essential enzymes
    - Tetracyclin repressor
  - Low probability of survival outside the test tube

# Meaning of iGEM?

- Why iGEM?
- Why young students?

**Thank you for your  
attention!**





# References

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- Hempel *et al.*: **Algae as protein factories: Expression of a human antibody and the respective antigen in the diatom *Phaeodactylum tricornutum*.** PLoS ONE, Volume 6, Issue 12, December 2011
- Borowitzka and Volcani: **The polymorphic diatom *Phaeodactylum tricornutum*: Ultrastructure of its morphotypes.** Journal of Phycology 14:10-21
- Gonzalez *et al.*: **A Single Peroxisomal Targeting Signal Mediates Matrix Protein Import in Diatoms.** PLoS ONE 6(9): e25316.doi:10.1371/journal.pone.0025316.
  
- <http://bioinformatics.psb.ugent.be/genomes/view/Phaeodactylum-tricornutum>
- [http://parts.igem.org/Assembly\\_standard\\_23](http://parts.igem.org/Assembly_standard_23)
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- <http://www.biotechnologie2020plus.de>
- <http://www.malvorlagen1001.de/malvorlagen/schule/>