ECOLOSA

E.coli producing cellulose

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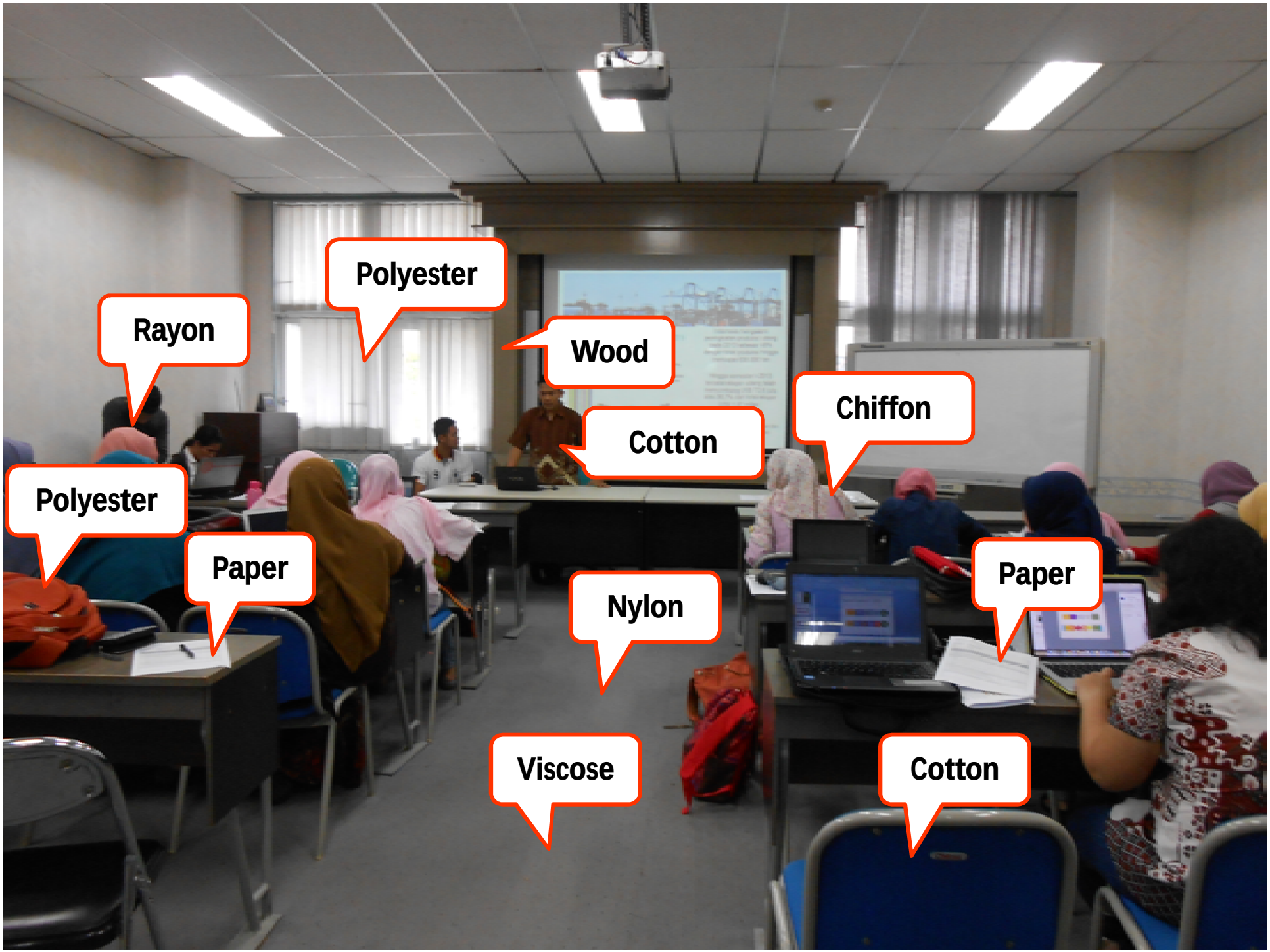
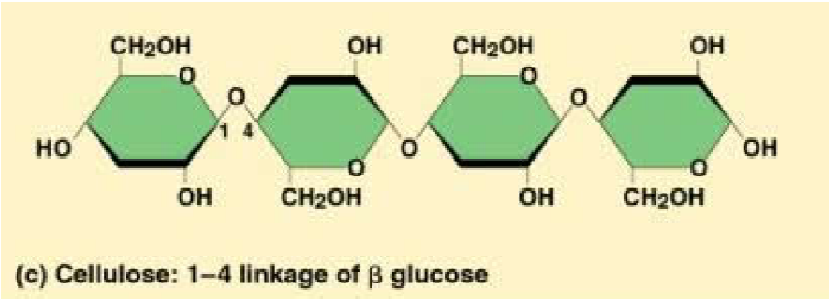
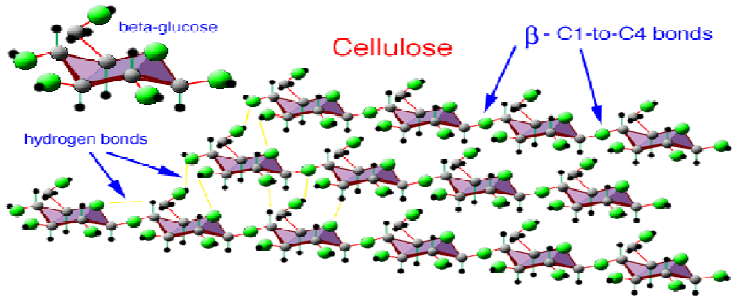

Polyester
Rayon
Wood
Chiffon
Cotton
Polyester
Paper
Nylon
Paper
Viscose
Cotton

Cellulose

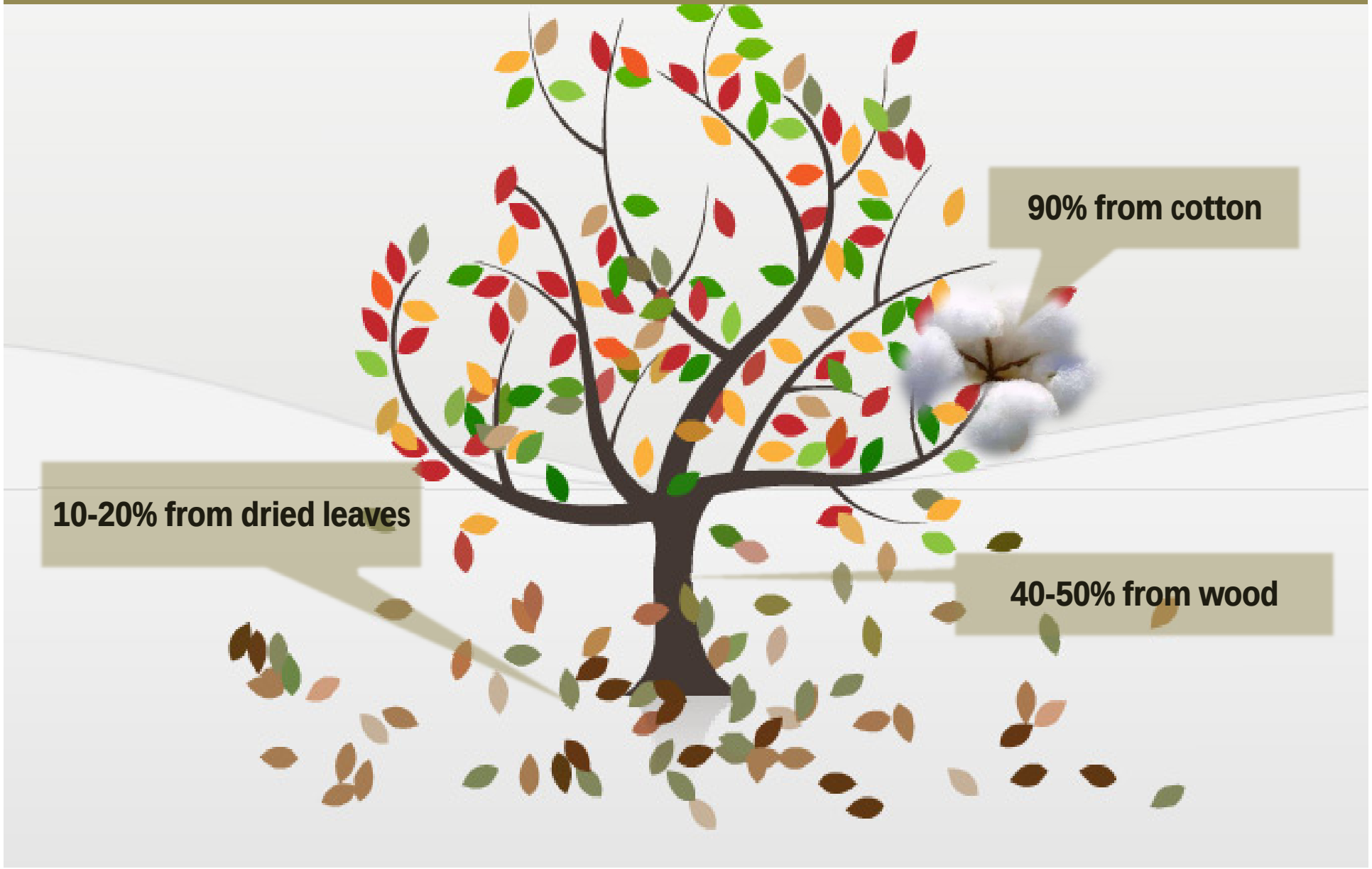
- A water-insoluble polysaccharide, is the most abundant macromolecule on earth and is mostly produced by vascular plants (Brown, 2004)
- Consisting of the monosaccharide glucose in (1→4) β-glycosidic bonds
- Is the most abundant renewable carbon resource on earth
- Is an indispensable raw material for the wood, paper, and textile industries





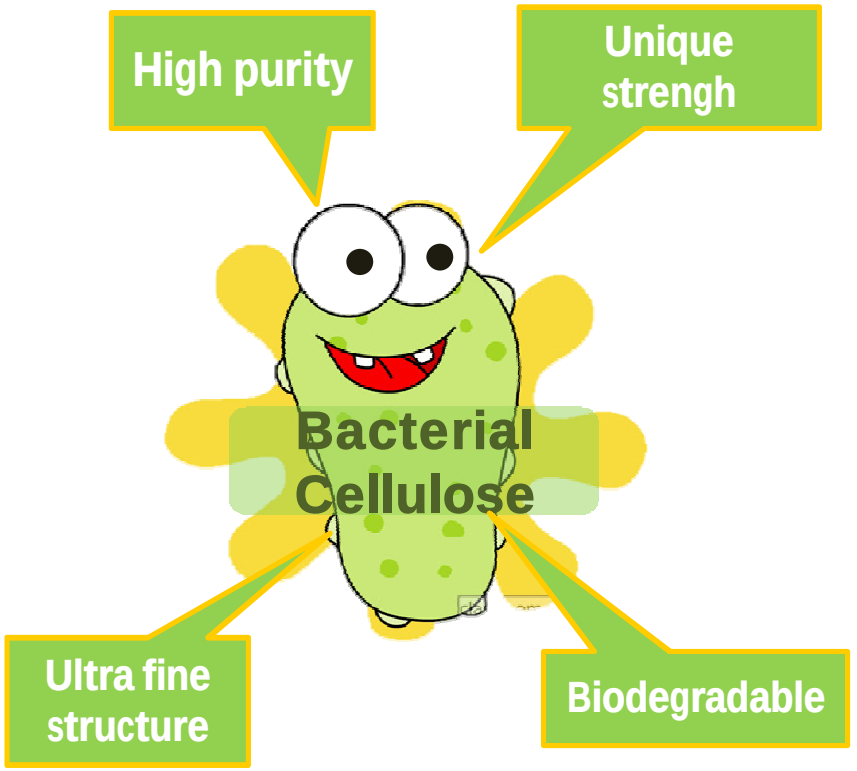
Picture 1. Cellulose Structure (http://www.quarkology.com/12-chemistry/92-production-materials/92B-biological-polymers.html

Plant Cellulose



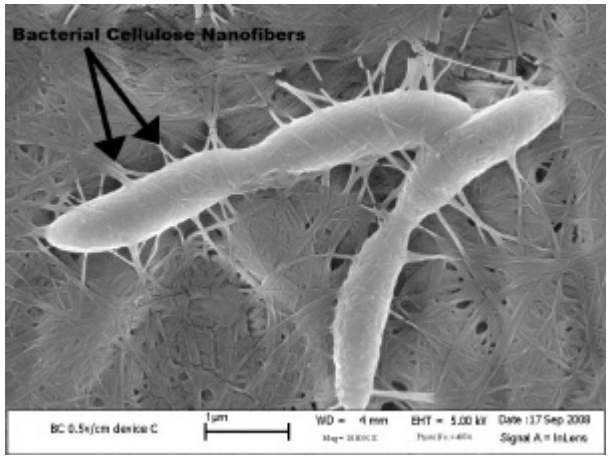
Bacterial Cellulose (BC)

- Microorganisms also produce cellulose which possesses considerably different properties and, therefore, has applications other than those of plant cellulose.
- Genera: *Gluconacetobacter, Rhizobium, Agrobacterium, Rhodobacter* and *Sarcina* have been reported (Brown 2004; Morgan et al. 2013)



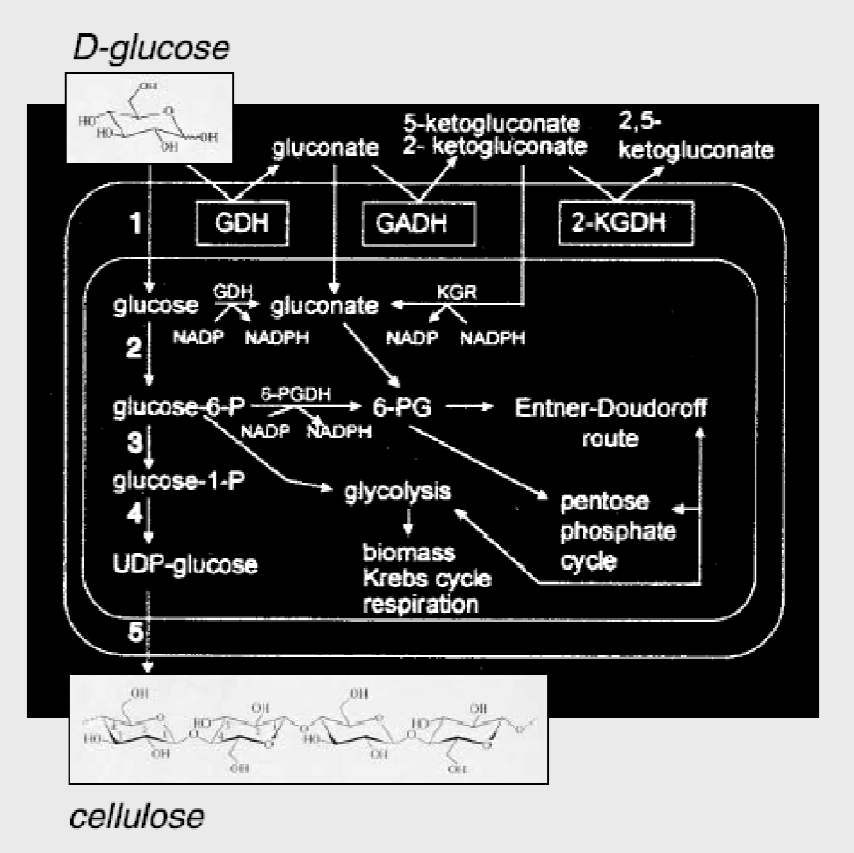
Acetobacter xylinum

- A model system to study the mechanism of cellulose biosynthesis
- Gram-negative, strictly aerobic bacterium
- Produce pure cellulose as an extracellular product: requires no intensive processing to remove unwanted impurities and contaminants such as lignin, pectin and hemicellulose.
- BC, with several remarkable physical properties, can be grown to any desired shape and structure to meet the needs of different applications.
- BC has been commercialized as diet foods, filtration membranes, paper additives, and wound dressings
- Low production rate, slow growth due to the inhibition of acetic acid produced by *A. xylinum*.



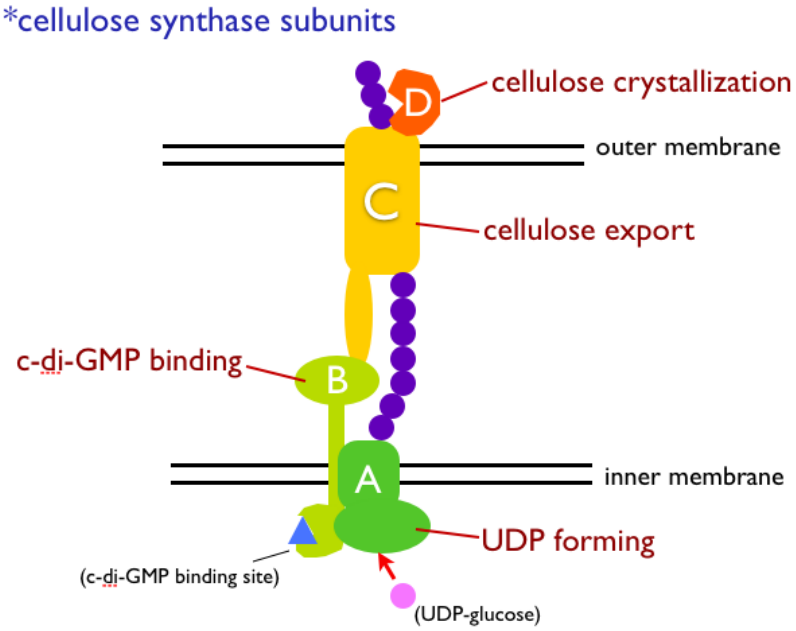
Picture 2. *A. xylinum* (http://meddic.jp/Acetobacter_xylinum)

Cellulose Biosynthesis



Picture 3. Pathway of Carbon metabolism in *G. xylinus* (Klemm *et al.*, 2001)

The key factor of this process is cellulose synthase: UDP-glucose → cellulose (BcsA, BcsB, BcsC, BcsD form a complex of membrane)

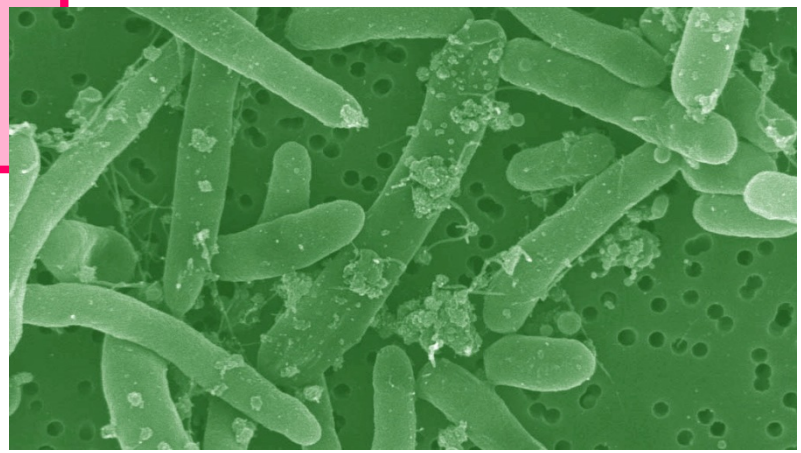


Picture 4. Cellulose Synthase subunit

Synthetic *Escherichia coli*

Why *E. coli?*

Bacterial model that its protein expression systems has been well characterized, grows quickly (faster than *A. xylinum*) and compatible with BioBricks.

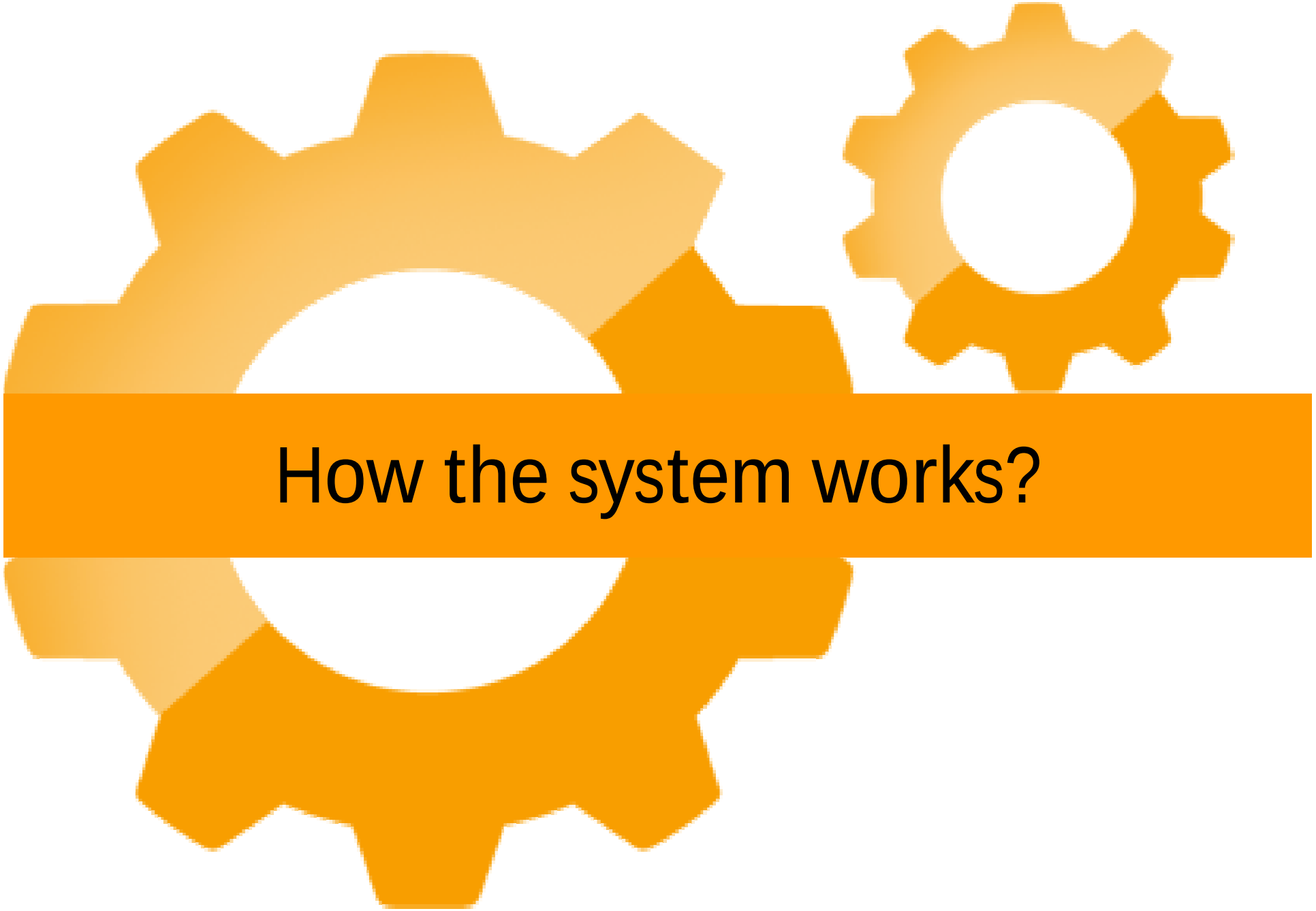


Picture 5. *E.coli* (Keasling, 2013)

Aim of Project

Using bacteria to produce greater number of cellulose cheaply, efficiently

Bacterial cellulose can saves tree


How the system works?

Chassis

Escherichia coli DH5α

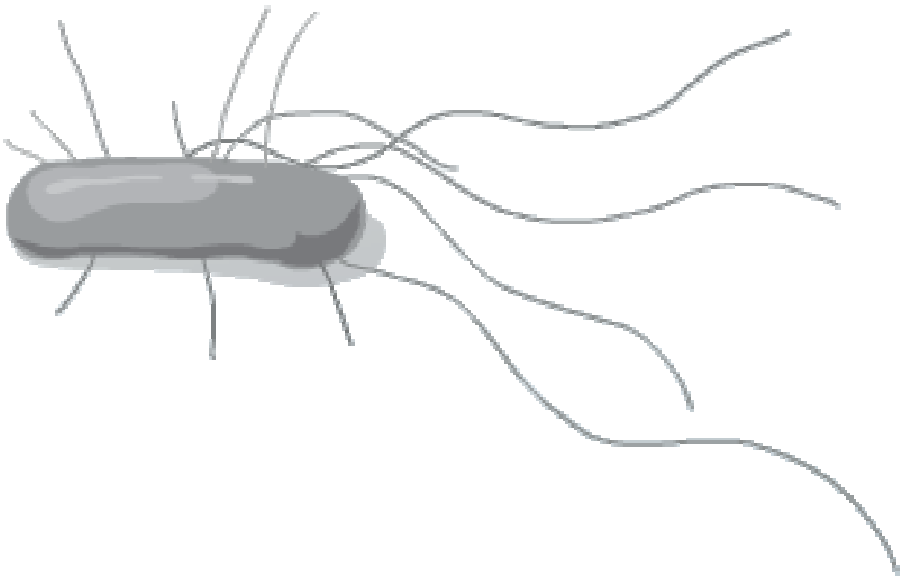
Escherichia coli BL21

Cloning

Expression

E. coli bacterium

3 x 0.6 µm

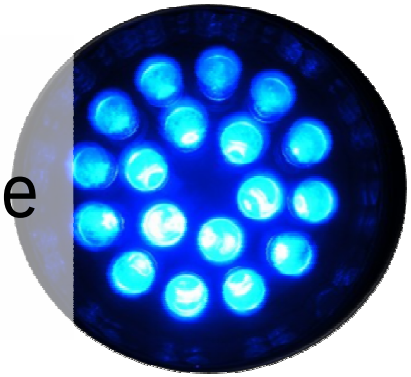


Module

Module I

Blue Sensor

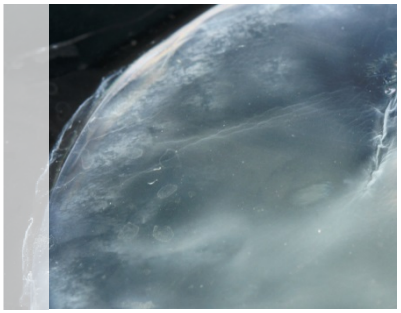
It use to activate Cellulose Biosynthesis

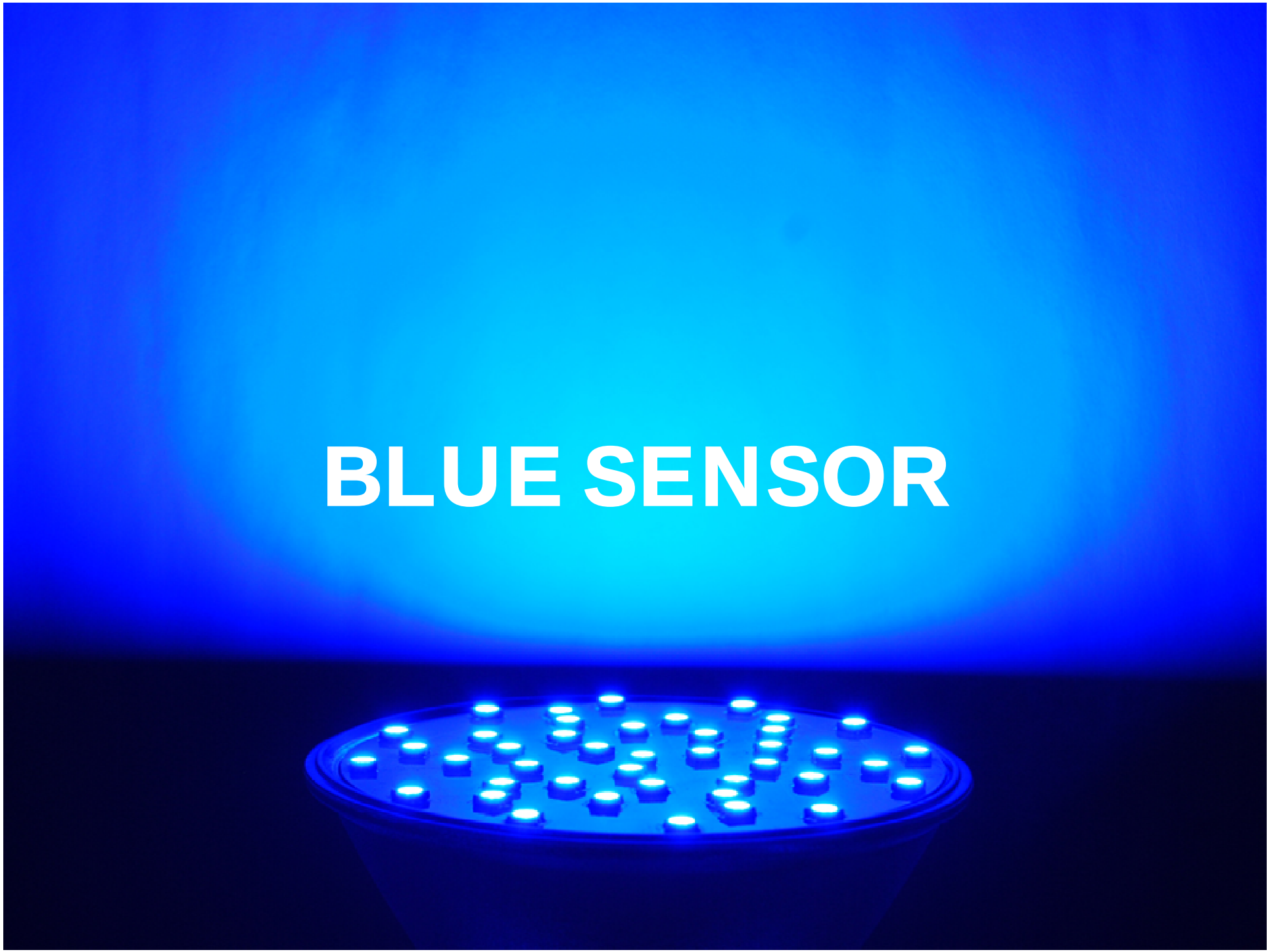


Module II

Cellulose Biosynthesis

It use to production Bacterial Cellulose




BLUE SENSOR

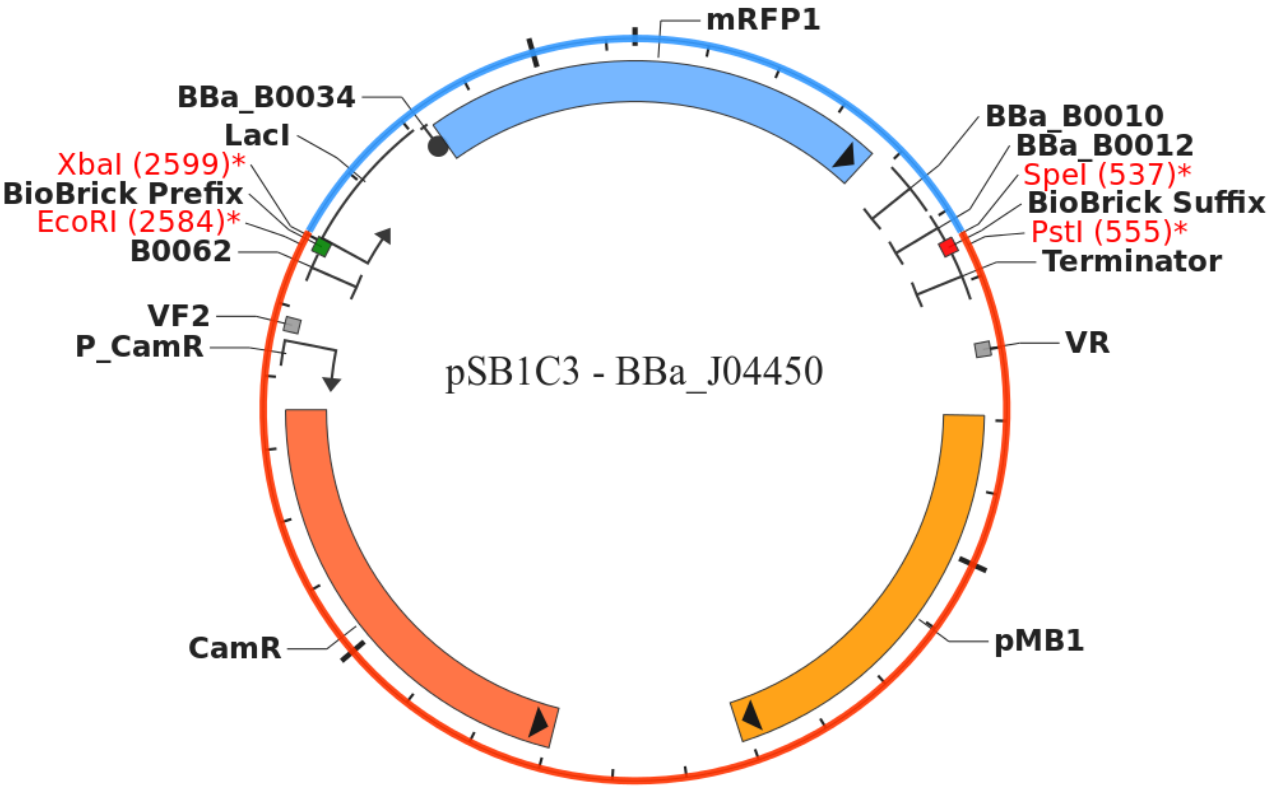
Module I : Blue Sensor

Backbone pSB1C3

2070 bp (default)

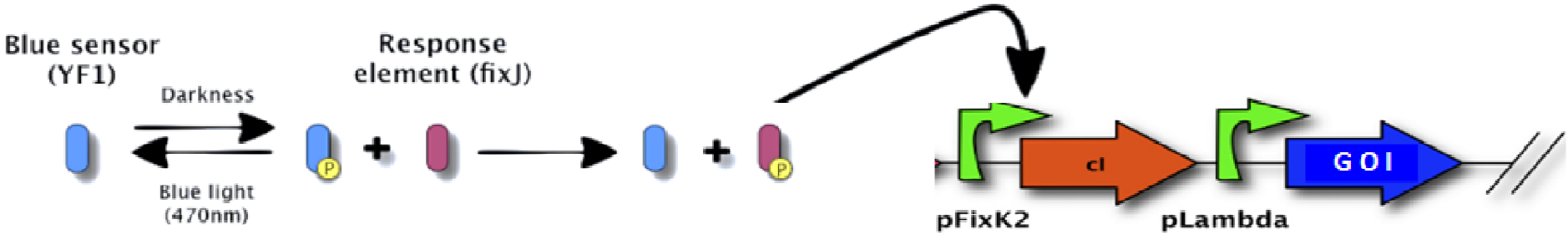
High copy number

Chloramphenicol resistance



Module I : Blue Sensor

Scheme

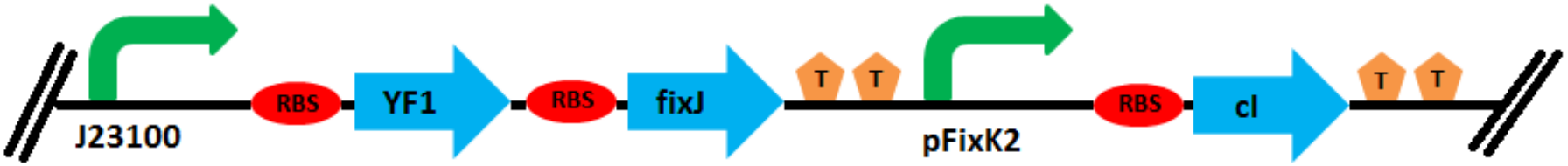


Blue sensor (YF1) in the dark condition will fosforilated, then it can activate response element (fixJ). Response element (fixJ) in active state can induce FixK2 promoter to express cI gene. In the next step, cI Protein act as a repressor for Lambda promoter that can control the expression of gene of interest.

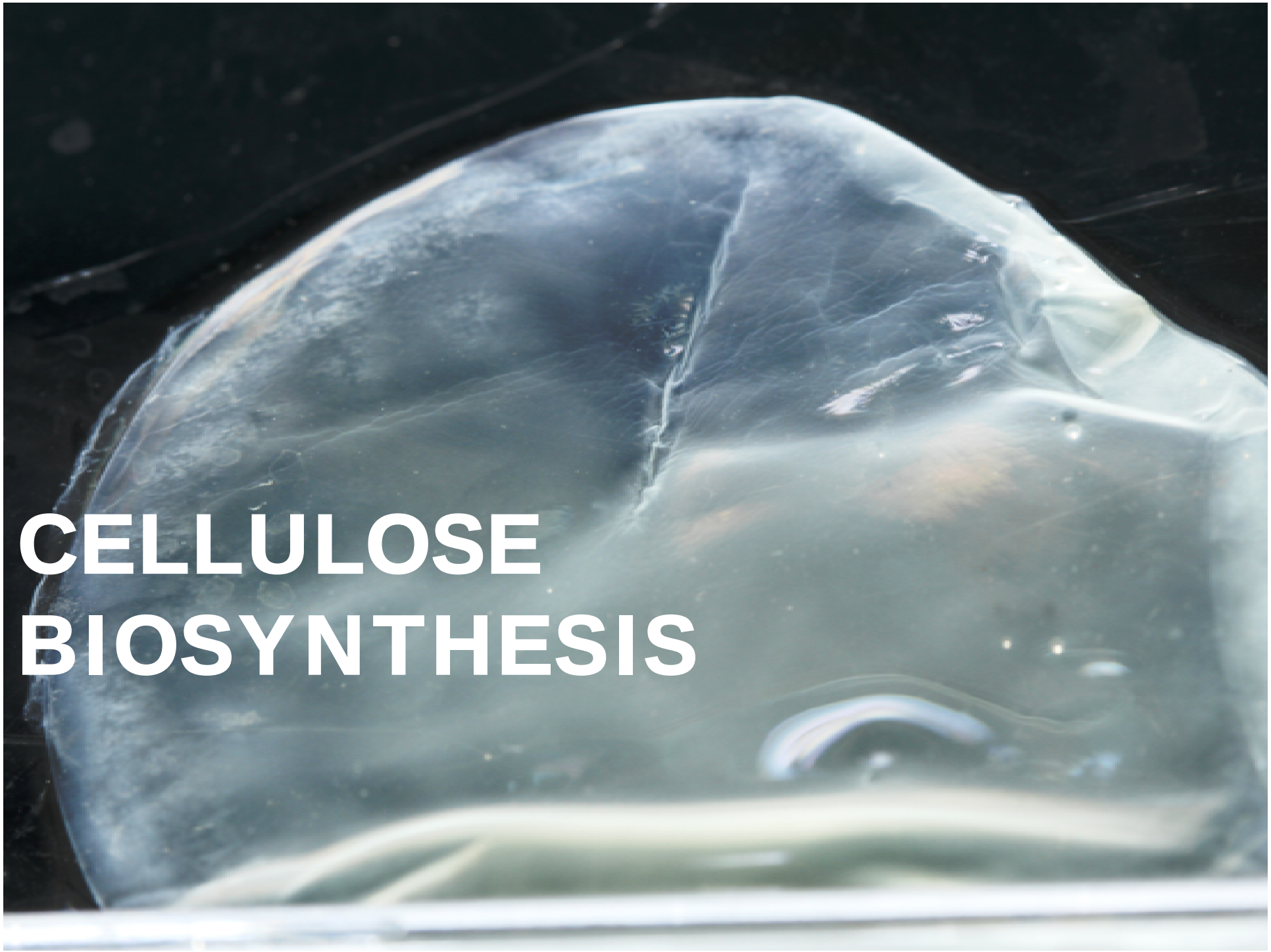
Reverse?

Module I : Blue Sensor

Parts/Composite



Bba_J23100	: Promoter pJ23100 → Constitutive Promoter
Bba_K592016	: RBS (B0034) gen *YF1* (K592004) RBS (B0034) gen *FixJ* (K592005)
BBa_B0024	: Double terminator (B0024)
BBa_K592006	: Promoter pK592006 → Inducible Promoter
BBa_B0034	: RBS (B0034)
BBa_C0051	: gen *cI* (C0051)
BBa_B0024	: Double terminator (B0024).


CELLULOSE
BIOSYNTHESIS

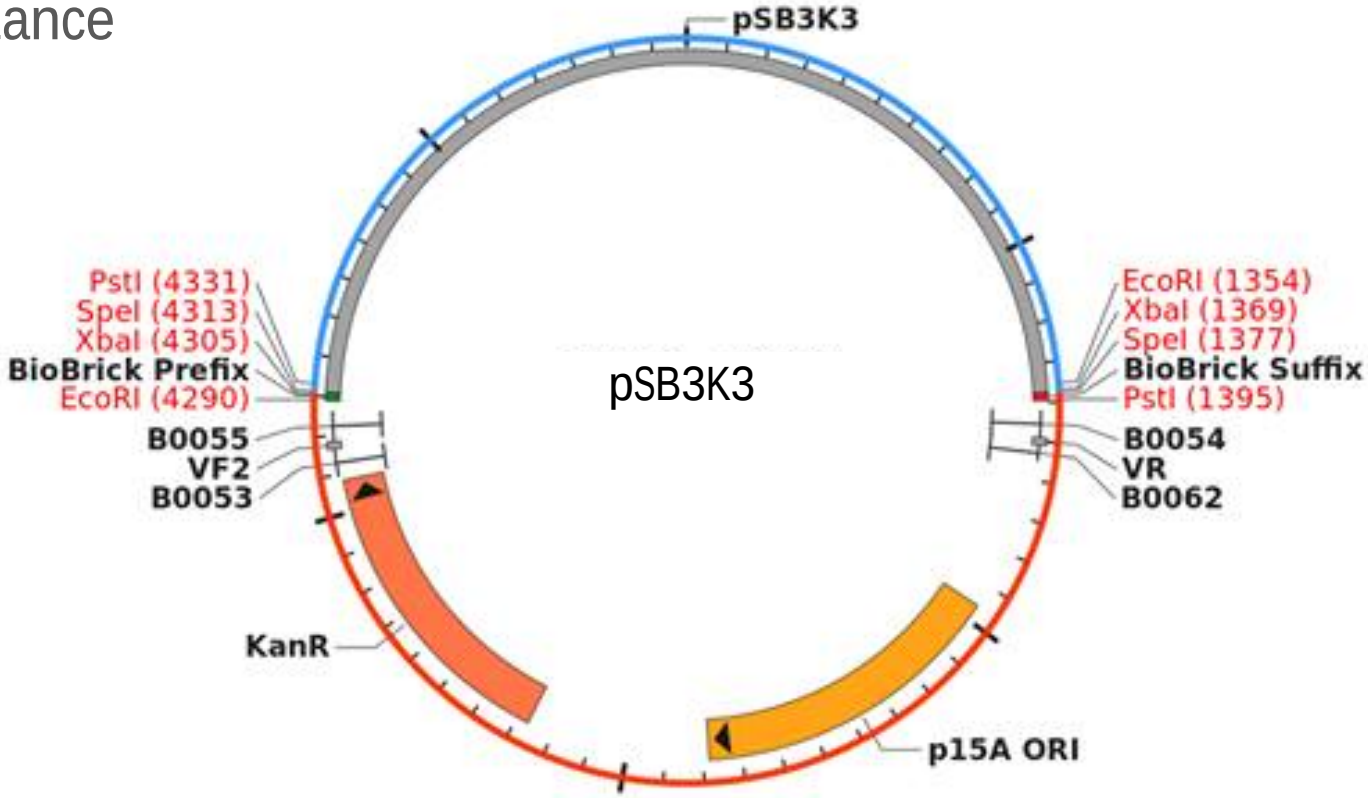
Module II : Cellulose Biosynthesis

Backbone pSB3K3

2750 bp (default)

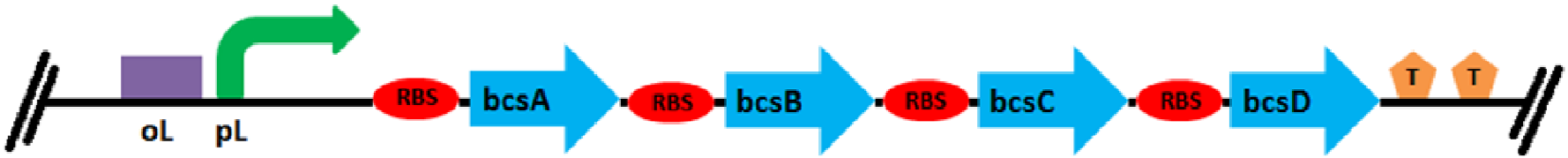
Low to Medium copy number

Kanamycin resistance



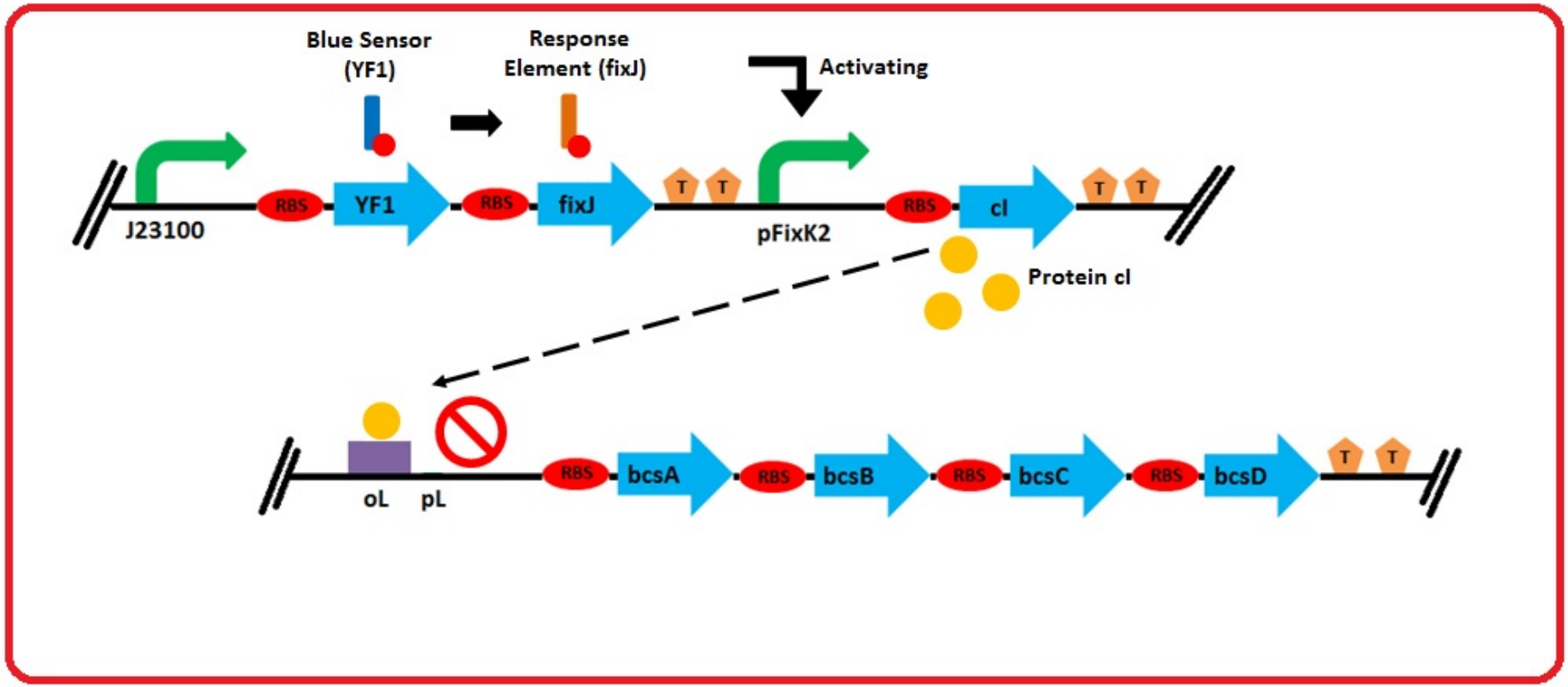
Module II : Cellulose Biosynthesis

Parts/Composite

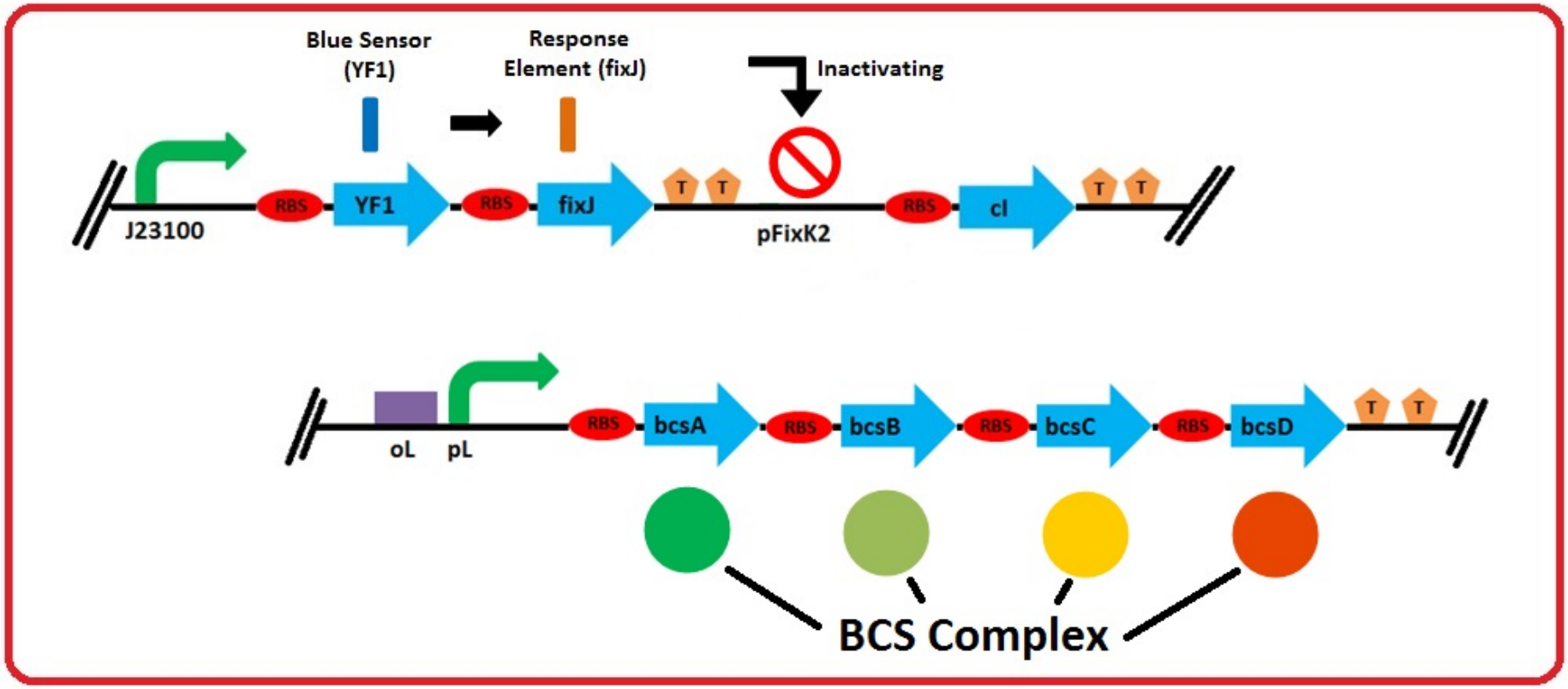


Bba_R0051	: Promoter pJ23100 → Inducible Promoter
BBa_B0034	: RBS (B0034)
Bba_BBa_K861100	: gen *bcsA* (K861100)
BBa_B0034	: RBS (B0034)
Bba_BBa_K861110	: gen *bcsB* (K861110)
BBa_B0034	: RBS (B0034)
Bba_BBa_K861100	: gen *bcsC* (K861130)
BBa_B0034	: RBS (B0034)
Synthetic Gene	: gen *bcsD*
BBa_B0024	: Double terminator (B0024).

Darkness


Blue Sensor
(YF1)
Response
Element (fixJ)
Activating
J23100
RBS
YF1
RBS
fixJ
T
T
pFixK2
RBS
cI
T
T
Protein cI
oL
pL
RBS
bcsA
RBS
bcsB
RBS
bcsC
RBS
bcsD
T
T

Blue Light


Blue Sensor
(YF1)
Response
Element (fixJ)
Inactivating
J23100
RBS
YF1
RBS
fixJ
T
T
pFixK2
RBS
cI
T
T
oL
pL
RBS
bcsA
RBS
bcsB
RBS
bcsC
RBS
bcsD
T
T
BCS Complex

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Thank you