BioBricks assigment

Sensor for botulinum neurotoxin B

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What is botulinum neurotoxin B?

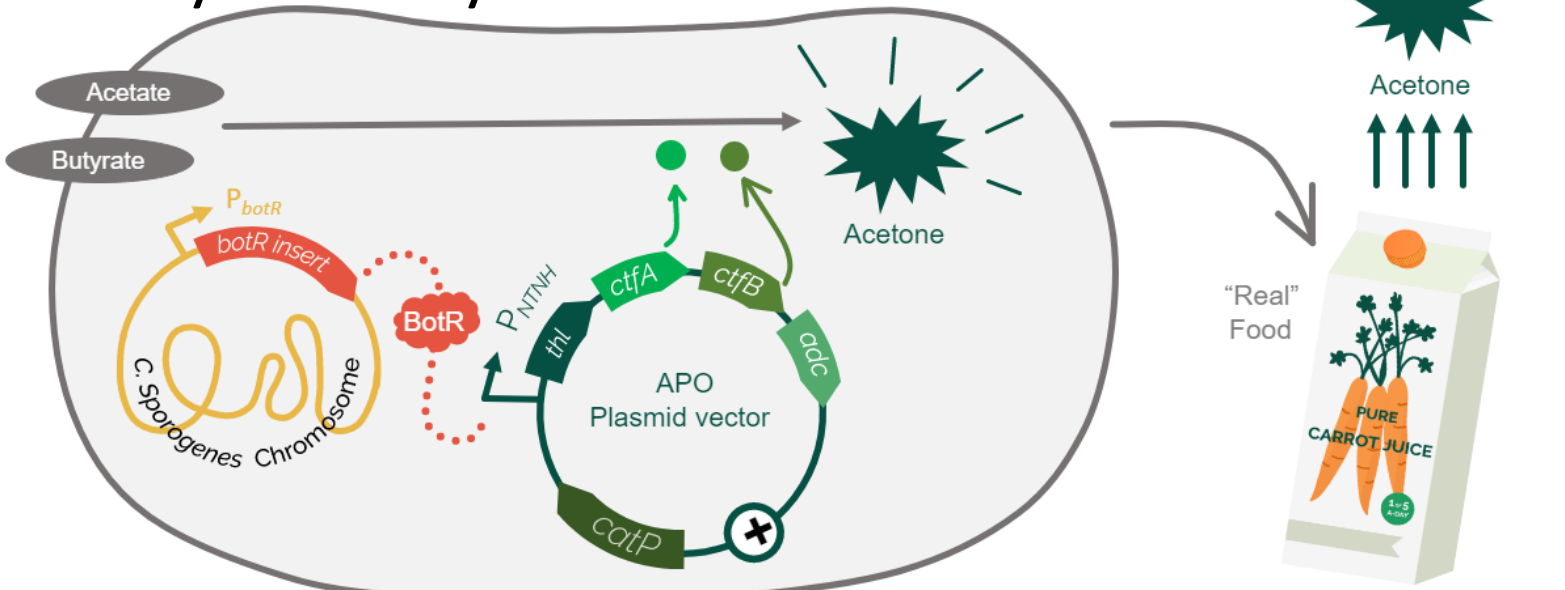
- Botulinum neurotoxins (BoNTs) are a family of bacterial toxins with 7 serotypes: BoNT/A-G
- One of the most potent toxins known
  - LD_{50} 1-5 ng/kg
- Produced by bacteria in the *Clostridium* genus
  - Gram-positive, spore-forming, anaerobic
- Major cause of food and wound-borne poisonings
- BoNTs also used for therapeutic and healing purposes
  - Botulinum neurotoxin B (BoNT/B): FDA approved

Consumption of improperly processed foods

- The consumption of improperly processed food can result in foodborne botulism
  - Rare but potentially fatal disease if not diagnosed rapidly and treated with antitoxin
- C. botulinum growth and toxin formation occurs in anaerobic conditions in combination to certain storage temperature and preservative parameters
  - Lightly preserved foods and inadequately processed, home-canned or home-bottled foods
  - C. botulinum does not grow in acidic conditions → toxin not formed if pH < 4.6
  - Low storage temperature and salt contents also used to prevent botulism
- Spores of *C. botulinum* are heat-resistant, but boiling destroys the toxin produced by the bacteria growing from the spores under anaerobic conditions
  - Internal temperature > 85 °C for 5 minutes or longer
  - Ready-to-eat foods in low oxygen-packaging often involved in cases of foodborne botulism
- Food with suspected contaminations must be handled specifically and sent to be analysed for botulism immediately in order to prevent poisonings

NoTox

- iGem project from 2019
- Method for monitoring *C. botulinum* toxin production in food
- Alternative to mouse lethality bioassay
- Using *C. Sporogenes* to produce acetone as a reporter



Biosensor for detecting BoNT/B in foods

- Inspired by the NoTox project
- *Clostridium sporogenes*, harmless relative of *C. botulinum* used as expression host
- Native botulinum toxin expression mechanism used to control expression of an easily detectable reporter (mimicking toxin expression)
  - Integration of the toxin expression machinery into chromosome of C. sporogenes
  - The reporter gene placed downstream of the toxin expression promoter into a shuttle plasmid -> plasmid transformed into expression host
  - Transcription should only occur when the reporter and the toxin-expression machinery are both present in the same cell
- β-glucuronidase (GusA) used as reporter instead of acetone
  - An enzyme that can convert specific colorless/non-fluorescent substrates into stable colored/fluorescent products -> detected with GUS assay

GusA detection

Utilization of 4-methyl-umbelliferyl-β-d-glucuronide (4-MUG) as a substrate to measure GUS activity

Measuring the level of fluorescence with a plate reader at the Optical Density of 600 nm (OD_{600})

The suitable time intervals for the measurements are 0,4,8,24 and 48 hours

Comparison of BioBricks

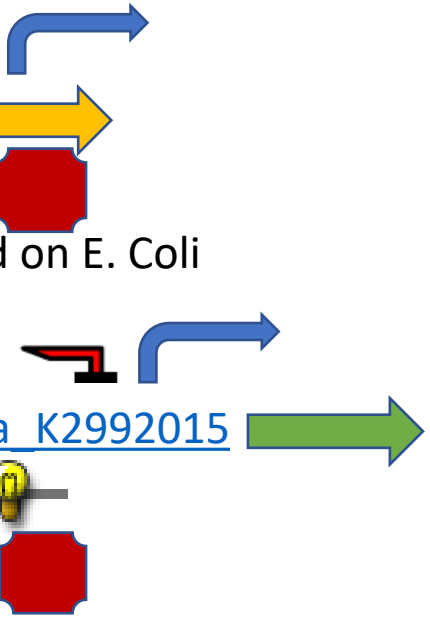
Our Biosensor (GusA reporter system)

C. Sporogenes reporter strain

- Promoter ***PbotR*** BBa_K2992012
- Coding ***botR*** BBa_K2992002
- Terminator Tfdx BBa_K2284012

Reporter-expression plasmid based on E. Coli (pMLT82151)

- Promoter **P*ntnH*** BBa_K2992001
- UTR containing RBS for ntnH BBa_K2992015
- Reporter ***gusA*** BBa_K330002
- Terminator Tfdx BBa_K2284012



NoTox Biosensor (Acetone reporter system)

C. Sporogenes reporter strain

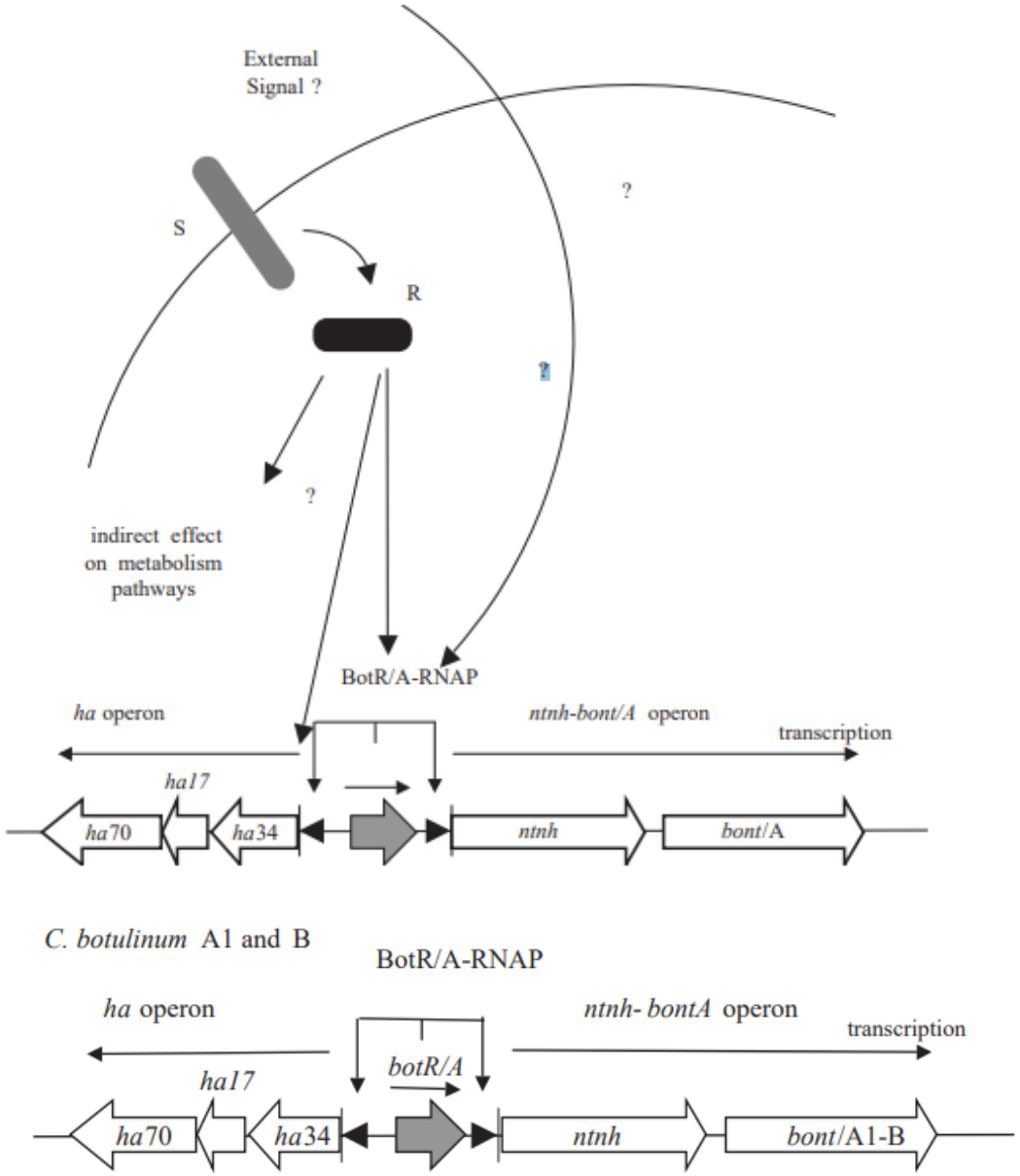
- Promoter ***PbotR*** BBa_K2992012
- Coding ***botR*** BBa_K2992002
- Terminator Tfdx BBa_K2284012
- Terminator Tfad BBa_K2992013

Reporter-expression plasmid based on E. Coli (pMLT82151)

- Promoter **P*ntnH*** BBa_K2992001
- Coding thl BBa_K2992008
- Coding ctfA BBa_K2992003
- Coding ctfB BBa_K2992005
- RBS adc BBa_K2992033

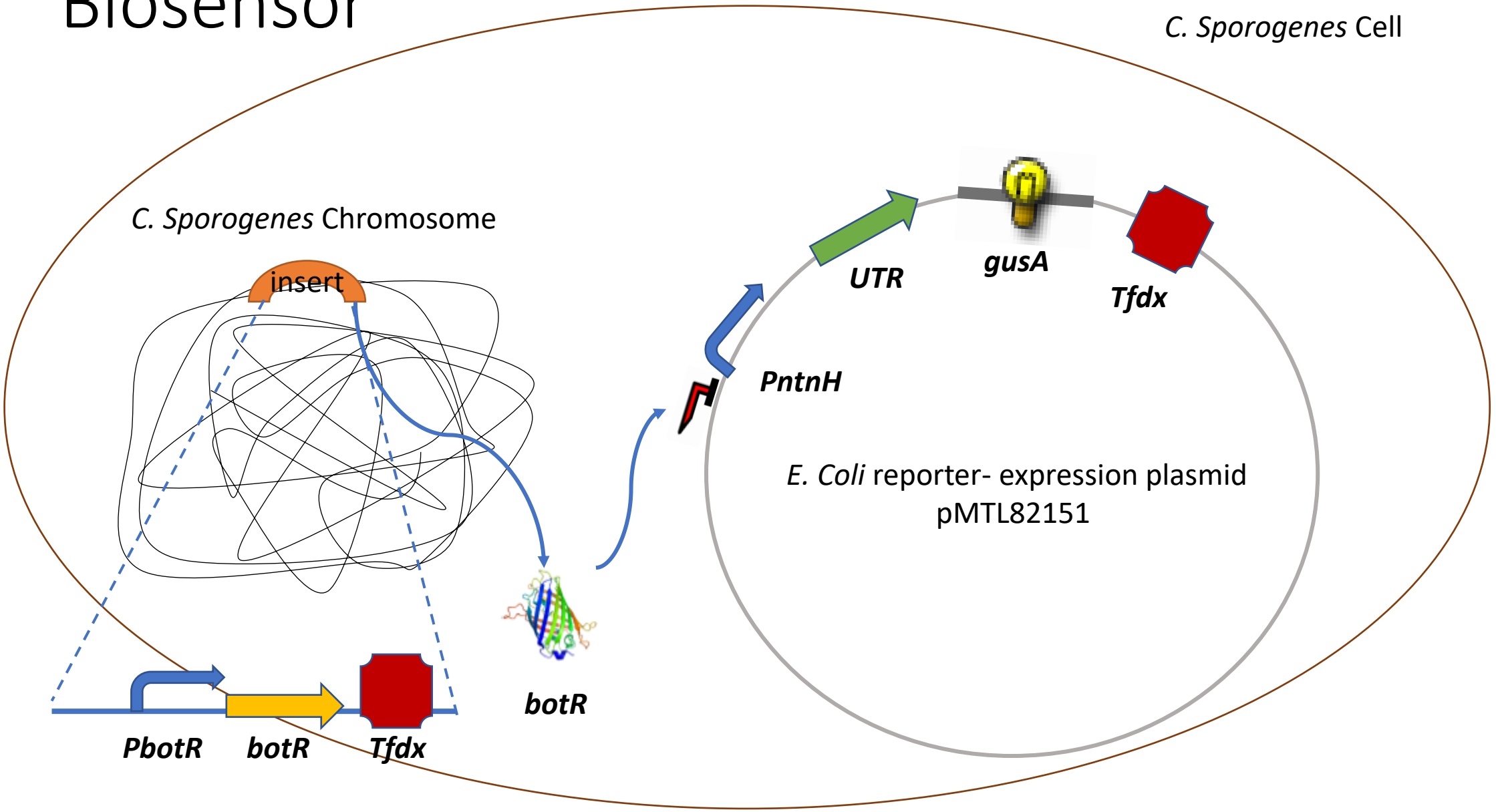
botR Promoter and botR Protein Regulation Mechanism

- botR must bind with the RNA polymerase core enzyme (RNAP) in order to activate botR promoter
- botR-RNAP system directs the transcription from its target gene
- Environmental and nutritional factors (i.e. pH, temperature, availability of CO_2) might effect the toxinogenesis

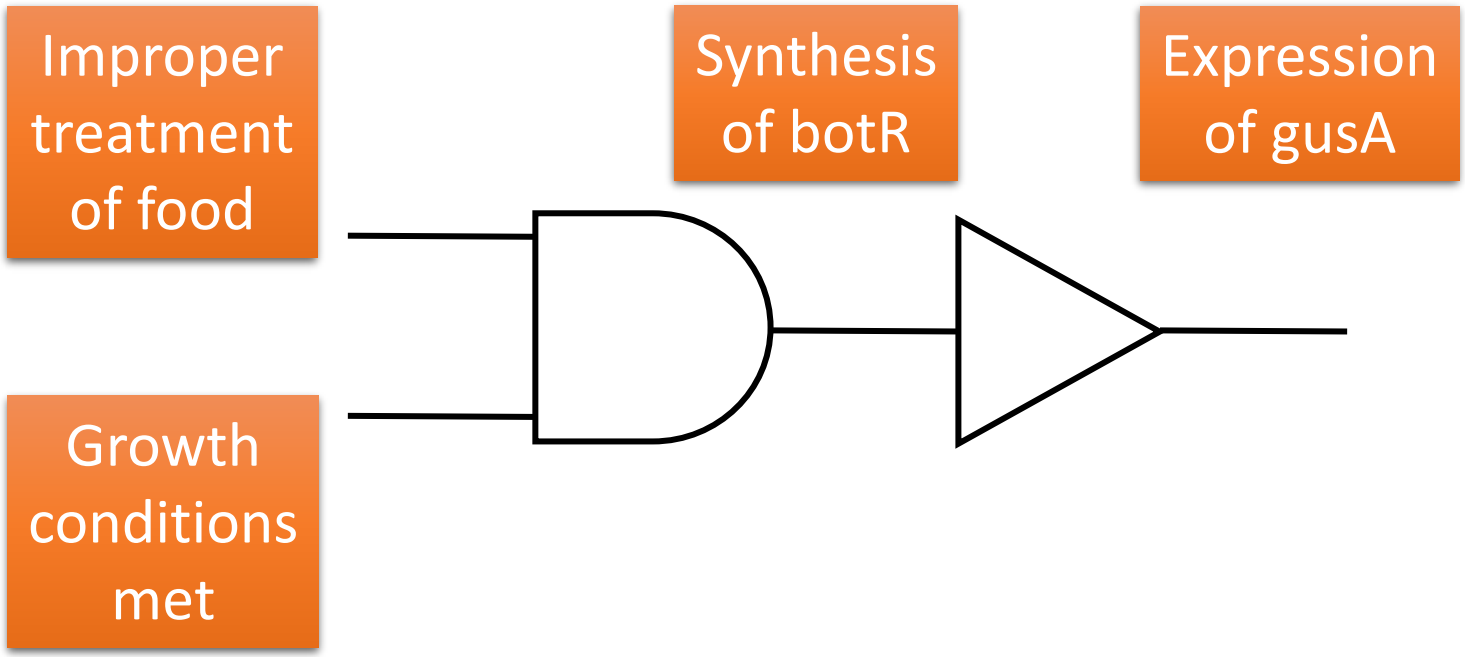


Raffestin, S., Marvaud, J.C., Cerrato, R., Dupuy, B. and Popoff, M.R. (2004). Organization and regulation of the neurotoxin genes in Clostridium botulinum and Clostridium tetani, Anaerobe, 10(2), pp. 93-100. doi: https://doi-org.libproxy.aalto.fi/10.1016/j.anaerobe.2004.01.001.

Biosensor


C. Sporogenes Cell
C. Sporogenes Chromosome
insert
PbotR
botR
Tfdx
botR
PntnH
UTR
gusA
Tfdx
E. Coli reporter- expression plasmid
pMTL82151

Logic gates and truth tables



Improper treatment of food	Growth conditions met	Expression of gusA
0	0	0
0	1	0
1	0	0
1	1	1

Conclusions

- Advantages
  - More ethical and cheaper than mouse lethality bioassay
  - Fluorescence is easily detected
- Limitations
  - Lack of experimental data
  - CRISPR / Cas9
- Further development
  - Integration of reporter
  - Use of a non-toxic strain *C. Botulinum* as a host instead

References:

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