L'Expérience en Action

OTECI

www.oteci.asso.fr

Expression systems: perspectives for 2025 "between evolution and revolution".

Regis Sodoyer

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adebiotech

28 & 29 novembre 2016

#Proteinov2016

Protéines

Impacts des Procédés

Immunogénicité / Allergénicité

Cité des entreprises de santé et de biotechnologies, BIOCITECH ROMAINVILLE-GRAND PARIS

Novel routes to make / improve expression systems

- Looking for an universal expression platform ?
  - Will never exist !!!
- Monoclonal Antibody production
  - The major driver for the definition and improvement of expression systems
  - CHO is dominating the market
- Genomic Engineering
- Regulatory feedback on:
  - acceptance of use of alternative expression systems
  - Alternatives to antibiotic-based selection
- Microbial expression:
  - New genetic tools and concepts to increase solubility of protein production in *E. coli*

Any alternative to CHO?

- Antibody production, the major driver
- Productivity
  - Reaching the physiological limits
  - Different metabolic pathways can be impacted
- Cost of Culture Media
- Other Mammalian Cell line?
- Unicellular Eukaryote?

Unicellular Eukaryote

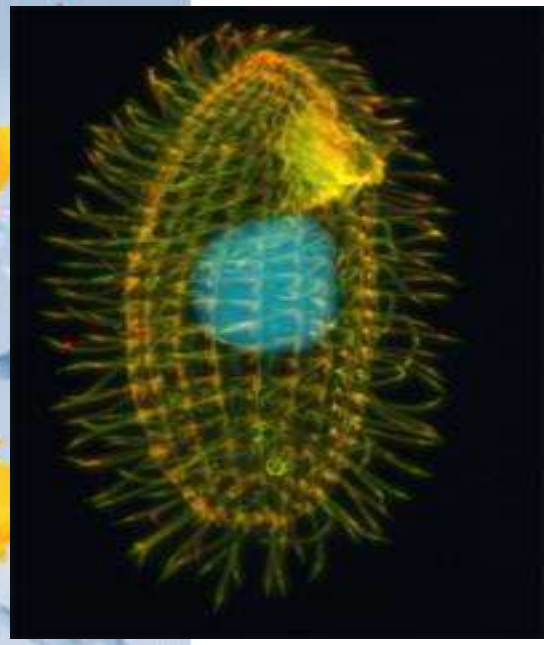
- Yeast & humanized Yeast
  - Enginered glycosylation pathway
- Non conventional hosts
  - *Tetrahymena thermophila*
  - *Leishmania tarentolae*
- Others
  - Filamentous fungi
  - Micro-Algae
- Regulatory point of view

Leishmania tarentolae

Natural Host

Jena Bioscience

Parasite



BMC Biotechnol. 2006 Mar 16;6:19.

Secretion of functional human enzymes by Tetrahymena thermophila.

Weide T, Herrmann L, Bockau U, Niebur N, Aldag I, Laroy W, Contreras R, Tiedtke A, Hartmann MW.

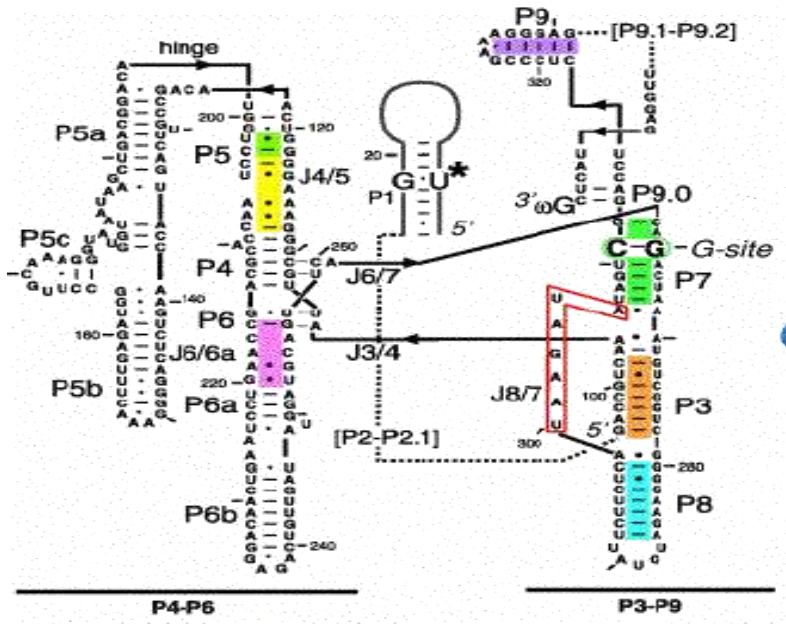
Cilian AG, Johann-Krane-Weg 42, D-48149 Munster, Germany. weide@cilian.de

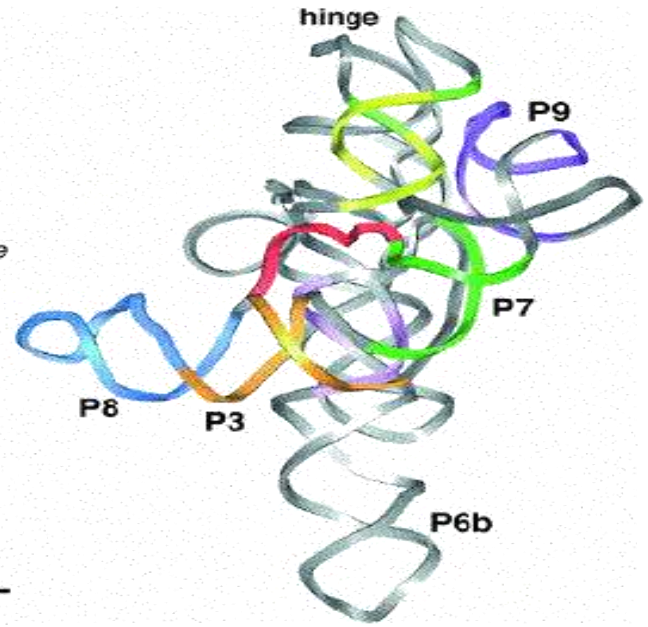
The ciliate has the potential to become an excellent expression system. However, additional optimisation steps including host strain improvement as wells as measures to increase the yield of expression are necessary to be able to provide an alternative to the common *E. coli* and yeast-based systems

The Nobel Prize in Chemistry 1989

The discovery of **catalytic RNA**, also called **ribozyme**, has been of great importance to both research and industry.

Professor **Sidney Altman**, Yale University, New Haven, Connecticut, USA
Professor **Thomas Cech**, University of Colorado, Boulder, USA





Desired features for unicellular eukaryotic systems

- Eeasy handling known from bacterial expression systems
- Potential of an eukaryotic protein expression / folding / modification system.
- Cultivation in simple cost effective serum-free or full synthetic media
- Short doubling time
- Linear scale-up
-Transformation by electroporation
- Capacity for secretion
- Non pathogenic to mammals (Biosafety level 1)

Prokaryotic systems

- Engineering
  - What is expected?
- Solubility / proper folding
  - Codon usage / speed of translation
- Safety / Regulatory compliance
  - Antibiotic-free selection

TRANSLATIONAL CONTROL AND FOLDING

- Why some recombinant proteins are not properly folded?
  - Intrinsic insolubility
  - Not enough time to be properly folded during translation
- Translation rate
  - In eukaryotes – 2 amino acids per sec.
  - In prokaryotes – 15 amino acids per sec

Codon choice is not random, but is highly selected across a broad range of organisms to optimize protein production


Tab
A Z E R T Y U I O P
Entrée
Verr Maj
Q S D F G H J K L M
Maj
W X C V B N
Maj
Ctrl
Alt
Alt Gr
Ctrl


Underwood

doi:10.1016/j.jmb.2009.12.042 J. Mol. Biol. (2010) 396, 1310–1318

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

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L'Expérience en Fiction

Slowing Bacterial Translation Speed Enhances Eukaryotic Protein Folding Efficiency

Efraín Siller[1], Diane C. DeZwaan[2], John F. Anderson[1], Brian C. Freeman[2] and José M. Barral[1]*

Published in final edited form as:
Cell. 2010 April 16; 141(2): 227–229. doi:10.1016/j.cell.2010.03.033.

How the sequence of a gene can tune its translation

Kurt Fredrick and **Michael Ibba**
Department of Microbiology, Ohio State Biochemistry Program, and Center for RNA Biology, The Ohio State University, Columbus, Ohio 43210, USA

Abstract

Sixty-one codons specify 20 amino acids offering cells many options for encoding a polypeptide sequence. Two new studies (Cannarozzi et al, 2010, Tuller et al., 2010) now foster the idea that patterns of codon usage can control ribosome speed, fine-tuning translation to increase the efficiency of protein synthesis.

SEQUENCE AND MODULATING THE SPEED OF TRANSLATION

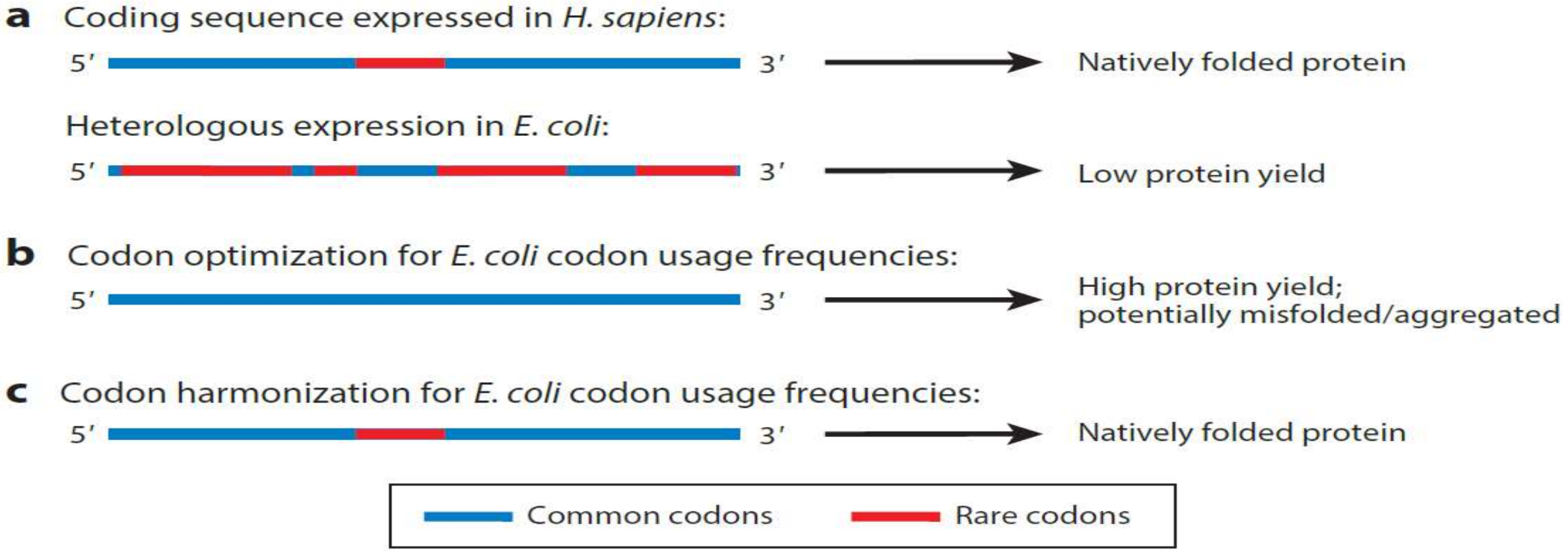
- Reducing the speed
  - Appropriate incorporation of rare or less frequent codons in the sequence
- Speed limit is not enough
  - Speed of translation should be modulated according to structure of the protein
  - The sequence contains accelerating and speed decreasing signals

Review in Advance first posted online on February 26, 2015. (Changes may still occur before final publication online and in print.)

Roles for Synonymous Codon Usage in Protein Biogenesis

Julie L. Chaney[1] and Patricia L. Clark[1,2]

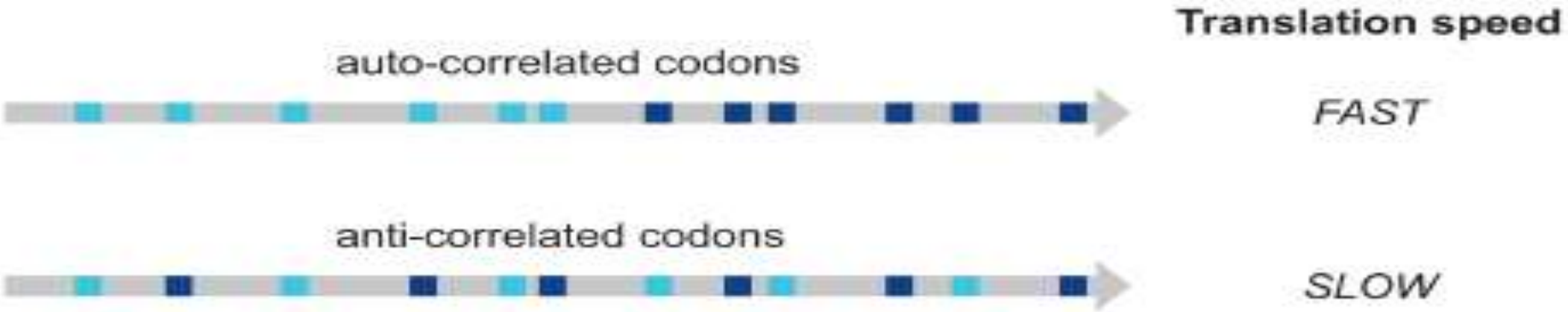
[1]Department of Chemistry & Biochemistry, [2]Department of Chemical & Biomolecular Engineering, University of Notre Dame, Notre Dame, Indiana 46556; email: jchaney@nd.edu, pclark1@nd.edu



Theory

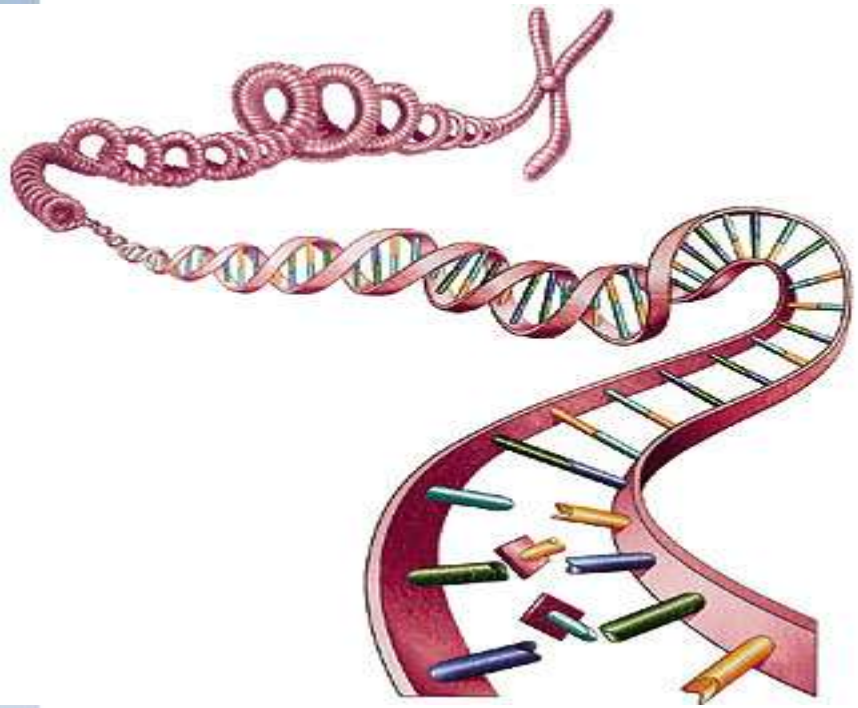
A Role for Codon Order in Translation Dynamics

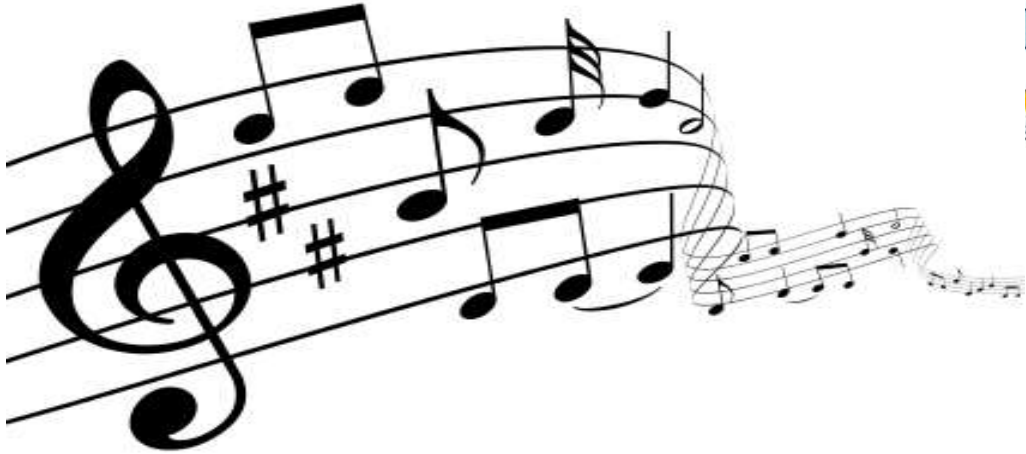
Gina Cannarozzi,[2,3,4] Nicol N. Schraudolph,[2,4] Mahamadou Faty,[1,4] Peter von Rohr,[2] Markus T. Friberg,[2] Alexander C. Roth,[2,3] Pedro Gonnet,[2] Gaston Gonnet,[2,3,*] and Yves Barral[1,*]
[1]Institute of Biochemistry, ETH Zurich, 8093 Zurich, Switzerland
[2]Institute of Computational Science, ETH Zurich, 8092 Zurich, Switzerland
[3]Swiss Institute of Bioinformatics, Quartier Sorge - Batiment Genopode, 1015 Lausanne, Switzerland
[4]These authors contributed equally to this work
*Correspondence: gonnet@inf.ethz.ch (G.G.), yves.barral@bc.biol.ethz.ch (Y.B.)
DOI 10.1016/j.cell.2010.02.036



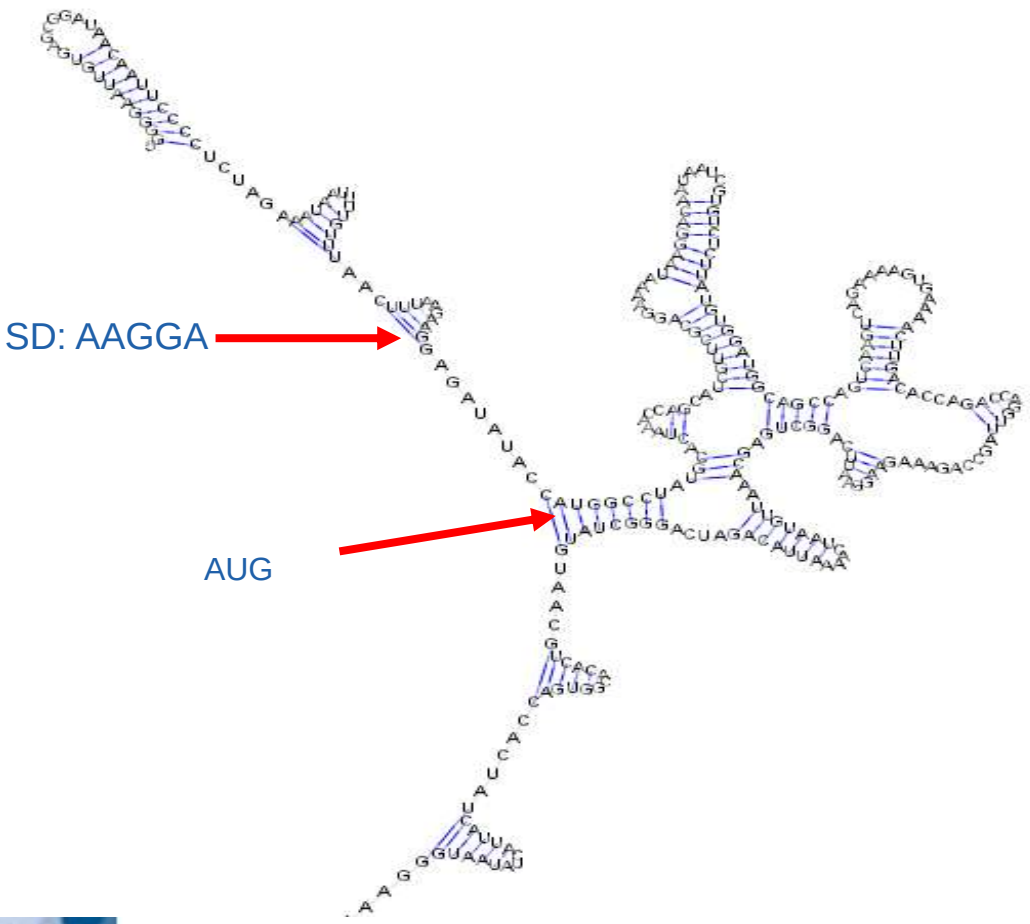
Messenger RNA sequences set the speed limit

The arrangement of synonymous codons along a gene influences translation speed. Shown is a simple example in which two different codons (represented by different shades of blue) encode the same amino acid. When the identical codons are consecutively arranged along the mRNA (auto-correlated), translation is faster than when they are alternatively arranged (anti-correlated).

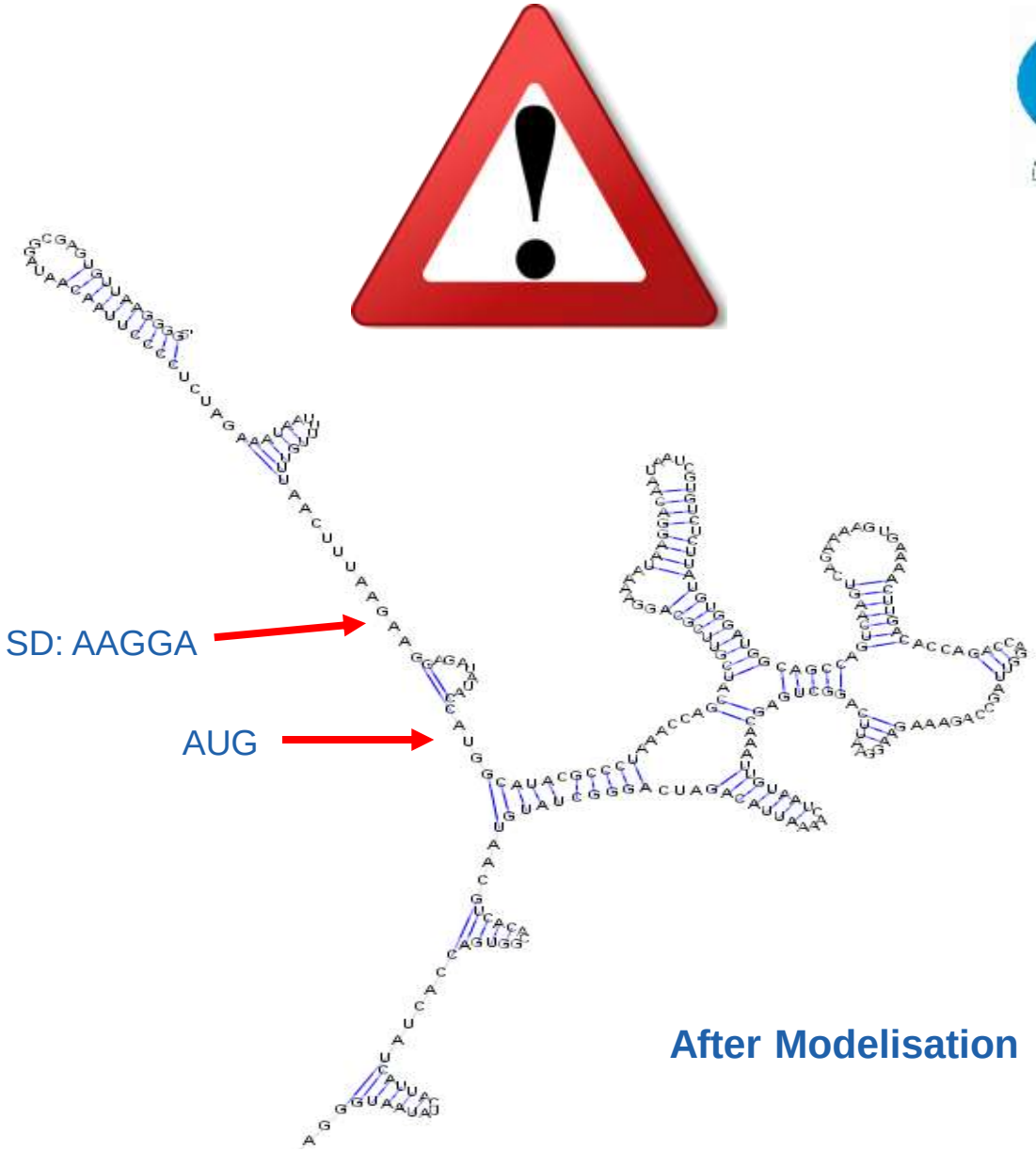




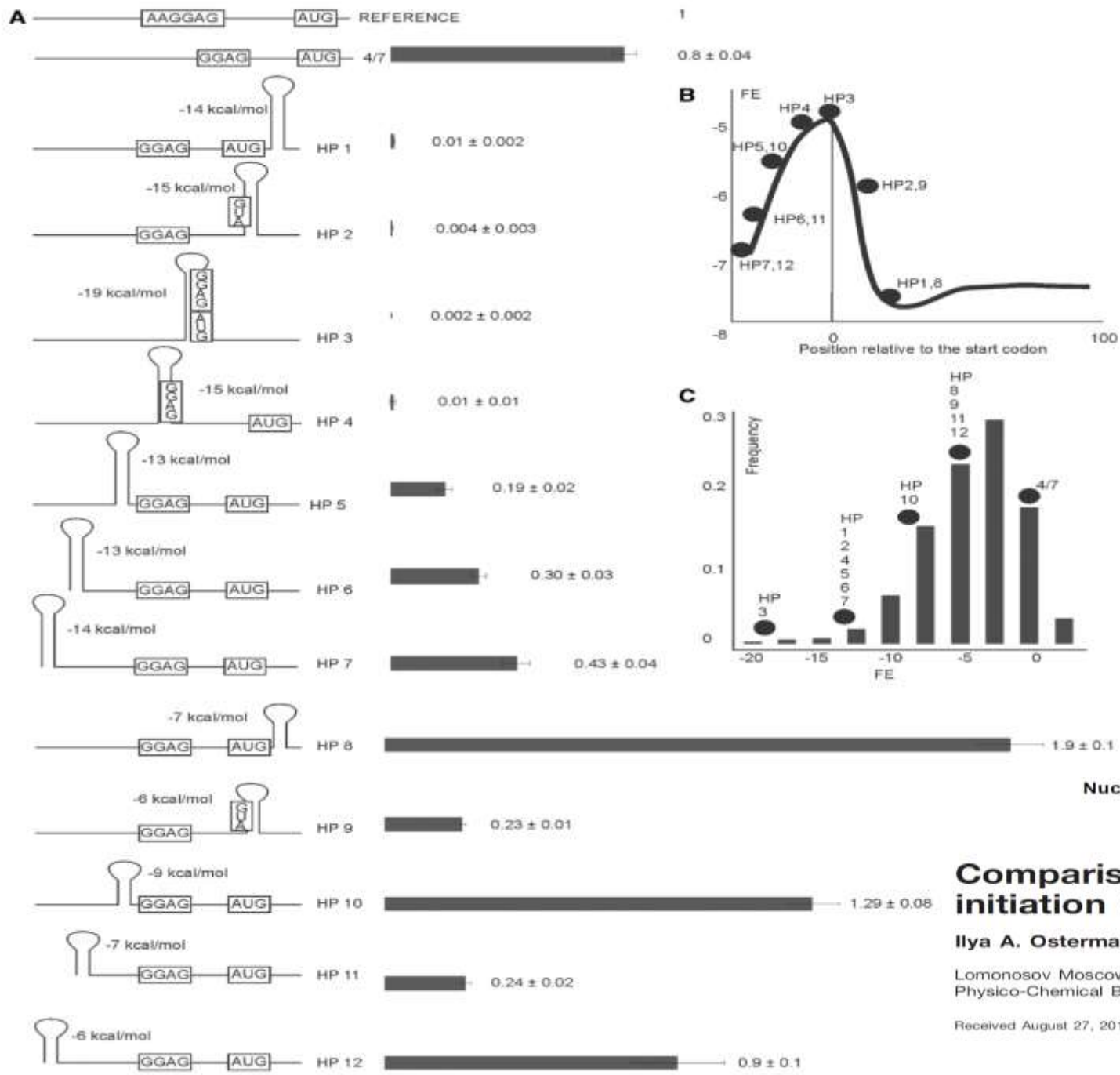
LISTEN TO THE MUSICAL RHYTHM OF THE SEQUENCE



Original Sequence



After Modelisation



Nucleic Acids Research Advance Access published October 23, 2012
Nucleic Acids Research, 2012, 1–13
doi:10.1093/nar/gks989

Comparison of mRNA features affecting translation initiation and reinitiation

Ilya A. Osterman, Sergey A. Evfratov, Petr V. Sergiev* and Olga A. Dontsova

Lomonosov Moscow State University, Department of Chemistry and A.N. Belozersky Institute of Physico-Chemical Biology, Moscow 119992, Russia

Received August 27, 2012; Revised September 22, 2012; Accepted September 27, 2012

Why do we have to consider alternatives to antibiotic-based selection?

- The Increasing regulatory requirements to which biotherapeutics are subjected will have a great impact on industrial protein production.
  - There may be “zero tolerance” towards antibiotic-based selection in production systems.
- Besides the antibiotic itself, the antibiotic resistance gene is an important consideration.
  - The complete absence of antibiotic-resistance gene being the only way to ensure that there is no propagation in the environment or transfer of resistance to pathogenic strains.
  - Complete absence is required for DNA immunisation or Gene therapy vectors
- Regulatory status for antibiotic-free selection
  - Preferred < Highly Recommended < Mandatory

The European Agency for the Evaluation of Medicinal Products
Evaluation of Medicines for Human Use

London, 24 April 2001
CPMP/BWP/3088/99

COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

Plasmid DNA products (eg. Plasmid DNA)
"The use of all specific elements or region of DNA should be justified. Special attention should be given to the nature of the selection marker. The use of certain selection markers, such as resistance to antibiotics, which may adversely impact on other clinical therapies in the target population, should be avoided."

Guidance for Industry

Content and Format of Chemistry, Manufacturing and Controls Information and Establishment Description Information for a Vaccine or Related Product

be toxins, carcinogens, teratogens, or allergens. Antibiotics and other components (e.g., growth factors, antibodies) used in the culture but neither required nor specifically intended to be in the final vaccine product should be removed before use. Procedures to

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research (CBER)
January 1999

Guidance for Industry

Guidance for Human Somatic Cell Therapy and Gene Therapy

"Concerning environmental impact and the use of drug resistance traits, consult the NIH Guidelines for Research Involving Recombinant DNA Molecules, Section III-A-1-a (59 FR 34496, amended 61 FR 59732). Non-antibiotic selection systems can also be used."

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
March 1998

Characterization of the Metabolic Burden on *Escherichia coli* DH1 Cells Imposed by the Presence of a Plasmid Containing a Gene Therapy Sequence

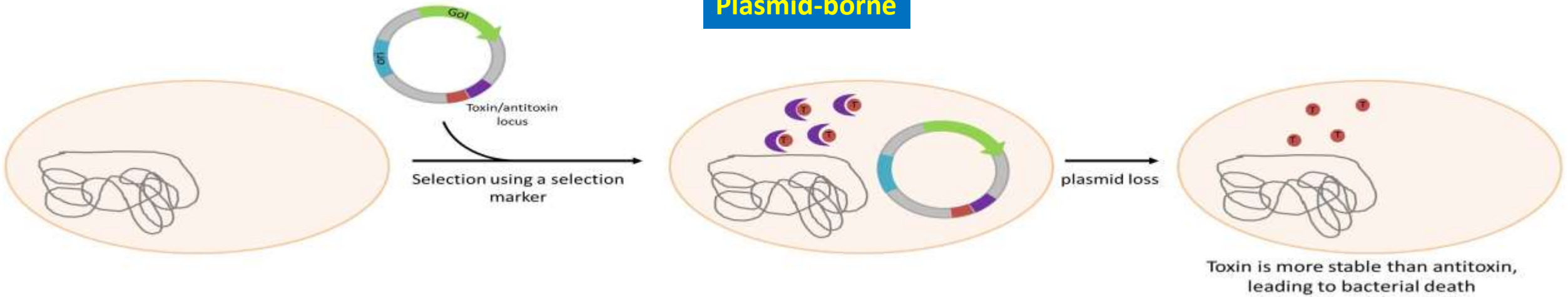
A. Rozkov,[1]* C.A. Avignone-Rossa,[1] P.F. Ertl,[4] P. Jones,[2] R.D. O'Kennedy,[3] J.J. Smith,[2] J.W. Dale,[1] M.E. Bushell[1]

Alternatives to antibiotic-based selection

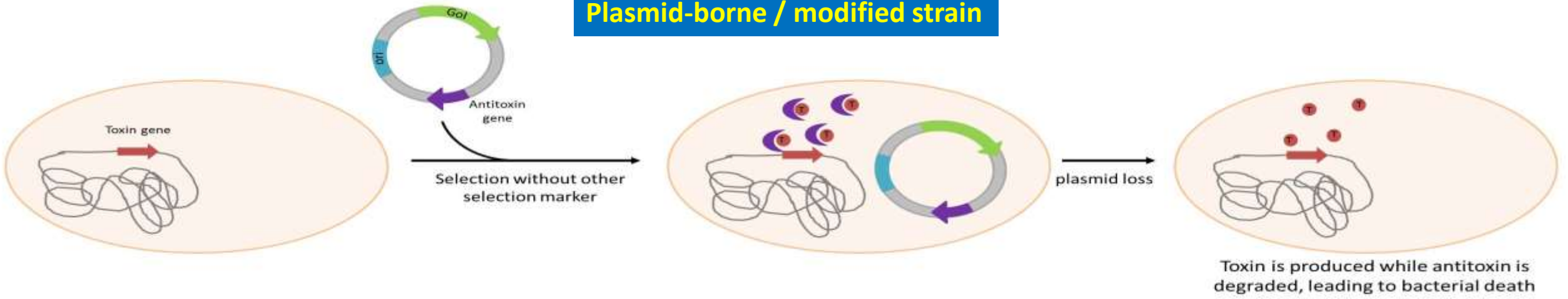
- Chromosomic insertion
- Complementation of essential genes
- RNA-based Systems
- Post-segregational killing
- Minicircles

Post-segregational killing

Plasmid-borne



Plasmid-borne / modified strain



Mode of action

Separate-component-stabilization system for protein and DNA production without the use of antibiotics (Szpirer/Milinkovitch)

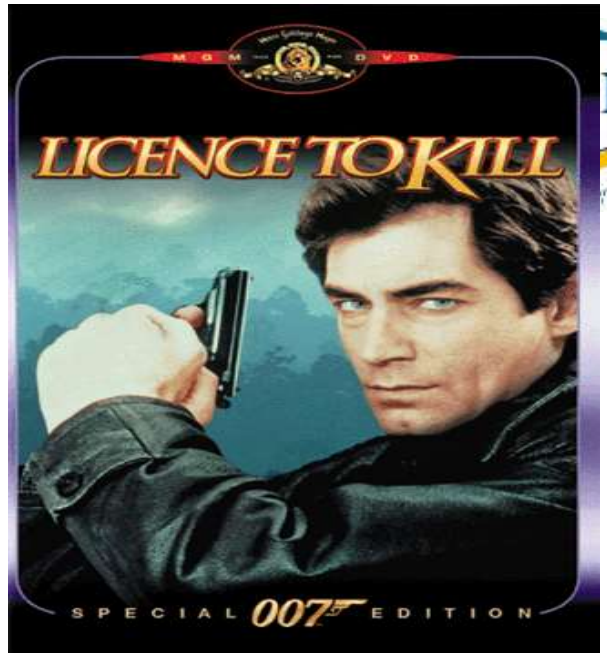
BioTechniques® May 2005

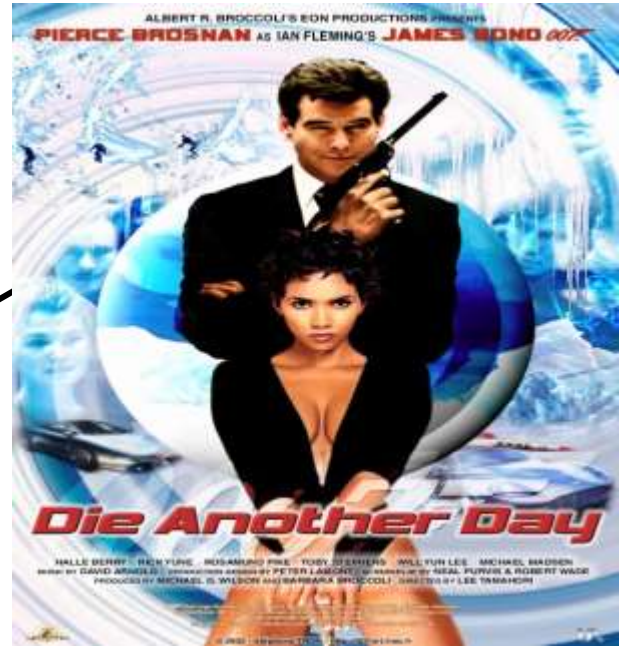
Volume 38, Number 5: pp *775-781*

- Commercial system from Delphigenetics (Belgium)
- System already used in Gateway cloning system (Invitrogen)

- Gene *ccdB* (the poison) ,
  - Insertion into the bacterial genome.
  - Encodes a stable protein (100aa), binding gyrase (essential for cell division) inducing cell death
- Gene *ccdA* (the antidote)
  - Plasmid-borne
  - Under control of a weak promoter, encodes an instable protein (90aa)

Post-segregational killing





Gene *ccdB*

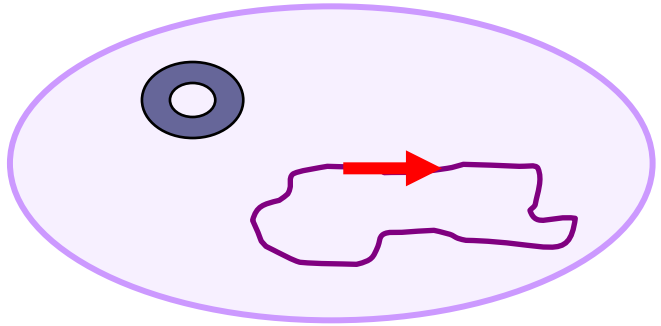
Gene *ccdA*

Gene of interest

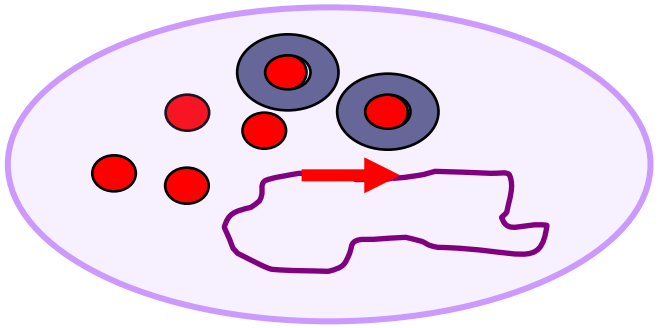
Gyrase (target of the poison)

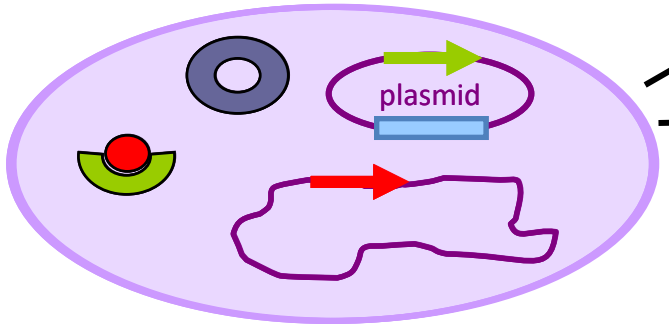
ccdB gene product (Poison)

ccdA gene product(Antidote)



Tranformation





Unexpected increase in yield

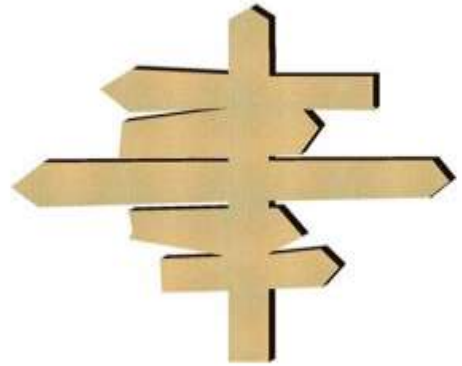
OD=1
To mimick "High Stress" Conditions

OD=25
Standard Fermentation Conditions

	Induction at early stage of growth		Induction at advanced stage of growth	
	System based on kana resistance	Antibiotic free system	System based on kana resistance	Antibiotic free system
Cell Dry Weight (g/L)	28	23	22	24
Plasmid retention (%)	5	100	90	98
Product yield (mg/L)	36	350	280	603
Specific productivity (mg product/ g CDW)	1	15	13	25

Antibiotic-free selection in E. coli: new considerations for optimal design and improved production
Isabelle Peubez, Nicolas Chaudet,Charlotte Mignon, Géraldine Hild, Stéphanie Husson, Virginie Courtois, Karelle De Luca, Régis Sodoyer
Microb Cell Fact. 2010 Sep 7;9:65

System developed	Mode of action	Protein expressed	Comments/ potential drawbacks	Ref article
Plasmid-free system	Chromosome based expression system	GFP Human superoxide dismutase(SOD)	Modified *E coli* strain, required	Striedner et al., 2010
FabI-triclosan	Endogenous essential gene	None	Chemical Biocid utilisation	Goh & Good, 2008
***E. coli* strain ΔQAPRTase gene**	Complementation	EGFP	Modified *E coli* strain, required	Dong et al., 2010
Pro BA	Complementation	Fab fragment	Modified *E coli* strain, required Presence of antibiotic	Fiedler & Skerra, 2001
***E coli* strain ΔglyA**	Glycine auxotrophy	RhuA	Modified *E coli* strain, required. Comparable to the conventional system	Vidal et al., 2008
RNA/RNA interference	RNA/RNA interaction	EGFP	Modified *E coli* strain, required	Pfaffenzeller et al., 2006
RNA out	RNA/RNA interaction	EGFP HA vaccine candidate		Luke et al., 2009
pCOr	Complementation of amber mutation tRNA suppressor	Luciferase	Modified *E coli* strain, and minimum medium required	Soubrier et al., 1999
ccdA/ccdB	Toxin/antitoxin	AlpA/rEPA vaccine candidates	Modified *E coli* strain required	Peubez et al., 2010
Kid/Kis	Toxin/antitoxin	EGFP	Presence of antibiotic	Nehlsen et al., 2010



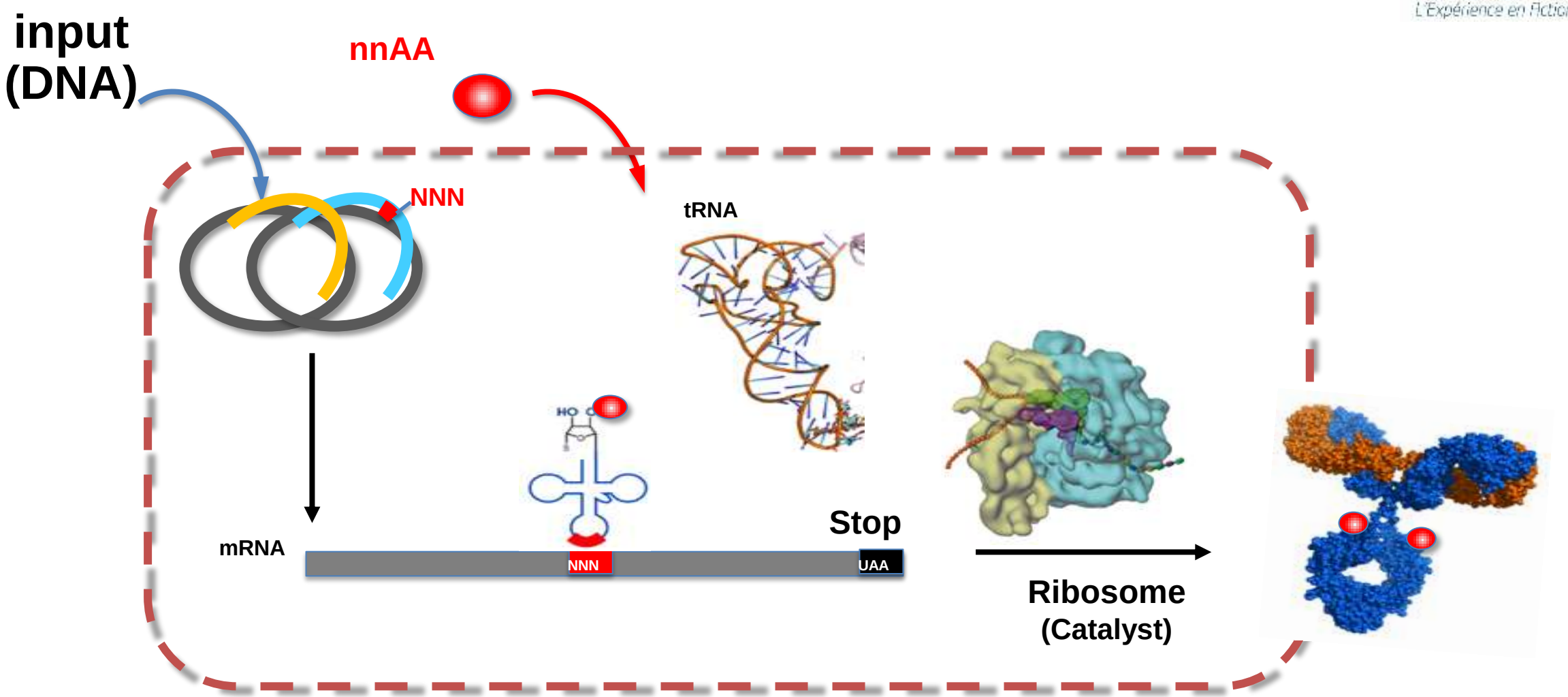
Where to go ?

What Else ?

- Plant-based
- Transgenic Animals
- Cell-free translation
- Synthetic Biology applied to protein production

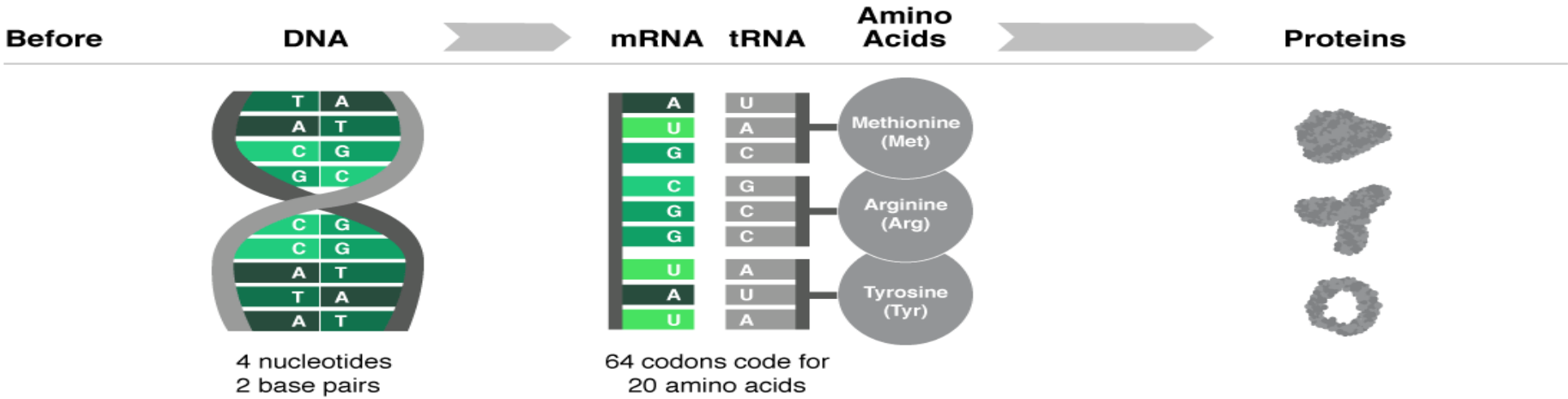
In vitro veritas

Incorporation of Non-Natural Amino Acids



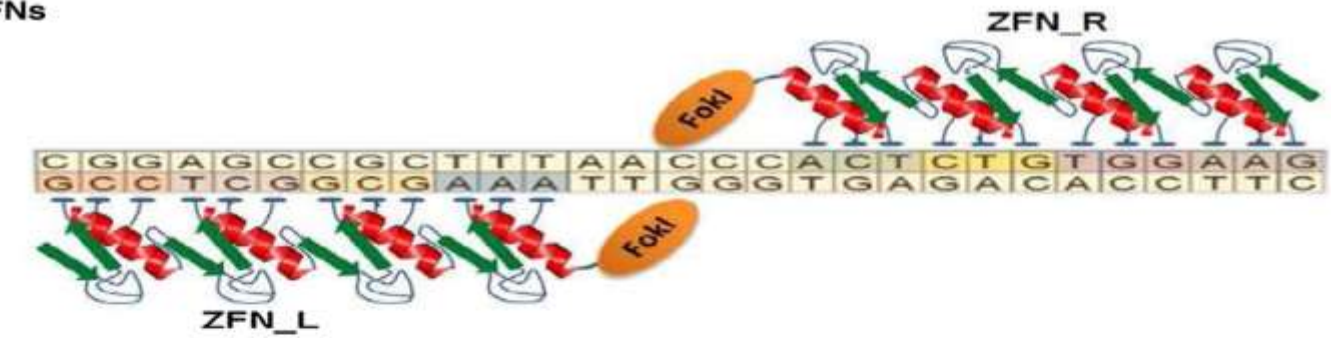
Expanded Genetic Alphabet - In Action

By adding a synthetic base pair—nicknamed X and Y—to DNA, the number of possible amino acids a cell can use to construct proteins increases from 20 to 172. This opens new possibilities to add multiple novel amino acids to create novel and diverse proteins for improved enzymes, drugs, diagnostics, and vaccines.

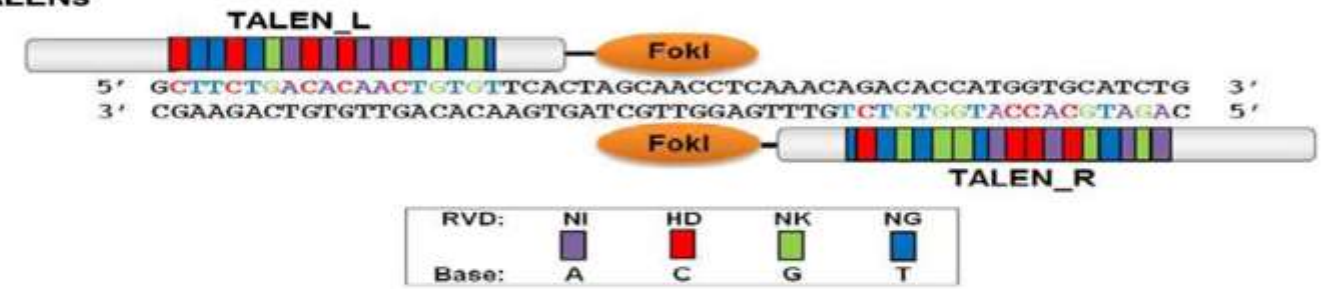


MEGANUCLEASES

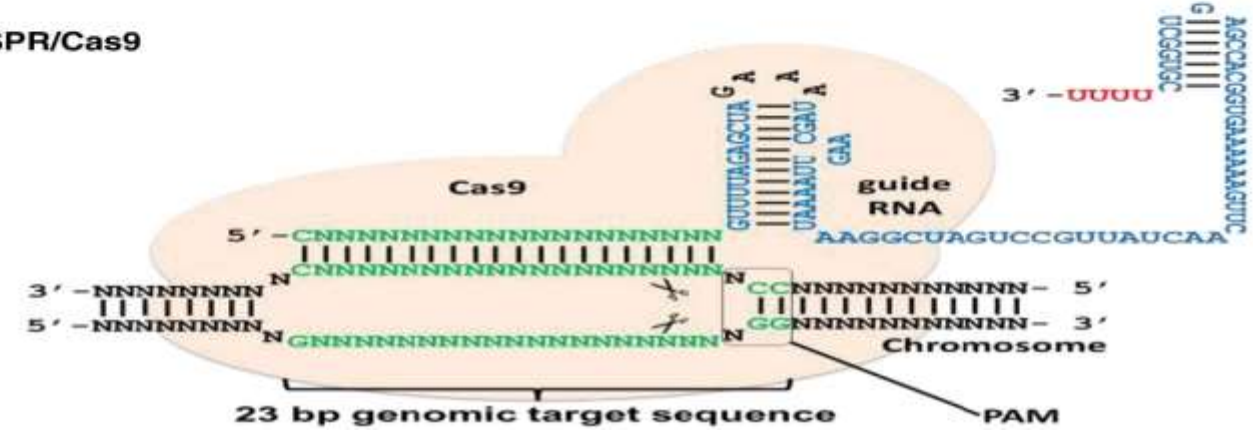
(a) ZFNs



(b) TALENs



(c) CRISPR/Cas9

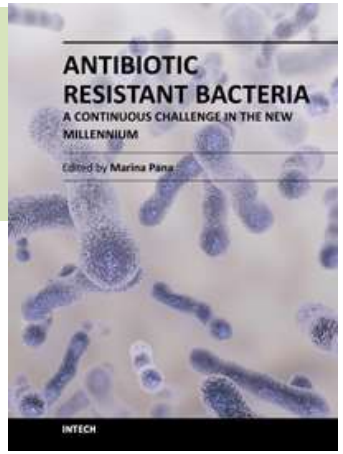


METABOLIC ENGINEERING
NO LIMIT….

Useful readings

Antibiotic-free selection in E. coli: new considerations for optimal design and improved production
Isabelle Peubez, Nicolas Chaudet,Charlotte Mignon, Géraldine Hild, Stéphanie Husson, Virginie Courtois, Karelle De Luca, Régis Sodoyer
Microb Cell Fact. 2010 Sep 7;9:65

Antibiotic Resistance (ISBN 979-953-307-855-6)
Antibiotic-Free Selection for Bio-Production: Moving Towards a New Gold Standard
Regis Sodoyer, Virginie Courtois, Isabelle Peubez and Charlotte Mignon



ANTIBIOTIC-FREE SELECTION IN BIOTHERAPEUTICS: « NOW AND FOREVER »
Charlotte Mignon, Régis Sodoyer & Bettina Werle
Special Issue "Alternatives to Antibiotics: Current Strategies and Future Prospects"
Pathogens. 2015 Apr 3;4(2):157-81

Characterization and immunogenicity in mice of recombinant influenza haemagglutinins produced in Leishmania tarentolae.
Pion C, Courtois V, Husson S, Bernard MC, Nicolai MC, Talaga P, Trannoy E, Moste C, Sodoyer R, Legastelois I.
Vaccine. 2014 Sep 29;32(43):5570-6

"Non-conventional expression systems for the production of vaccine proteins and biotherapeutic molecules".
LEGASTELOIS I, BUFFIN S, PEUBEZ I, MIGNON C, SODOYER R and WERLE B.(2016)
Human vaccines and Immunotherapeutics (in press).

THANK YOUR FOR YOUR ATTENTION