SynBio projects at RIKEN (and India)

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1. creating a non-natural DNA

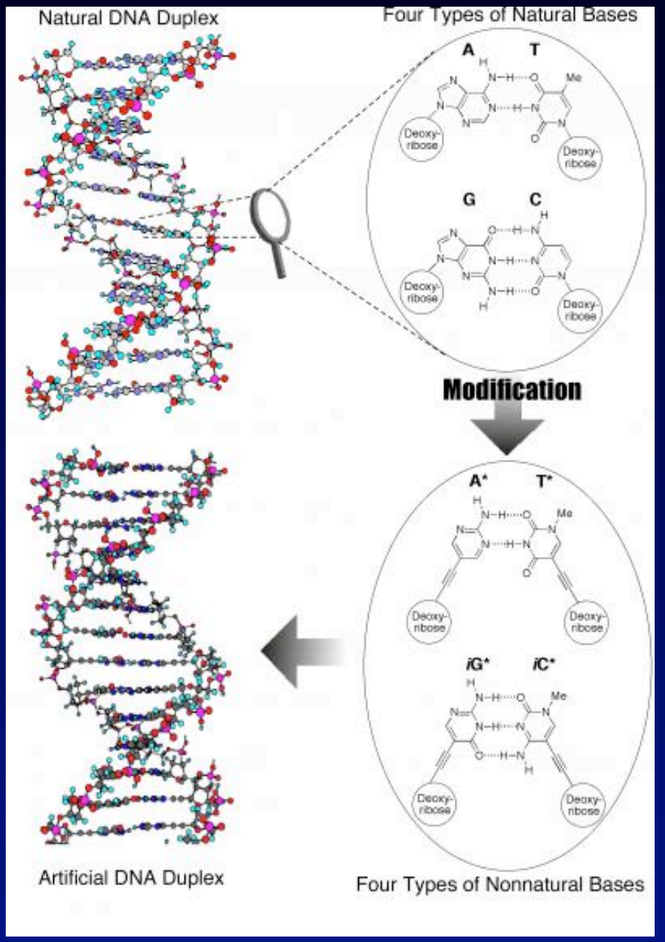
A*, T*, iG* and iC*
(Nonnatural C-Nucleosides)

moving beyond ATGC....

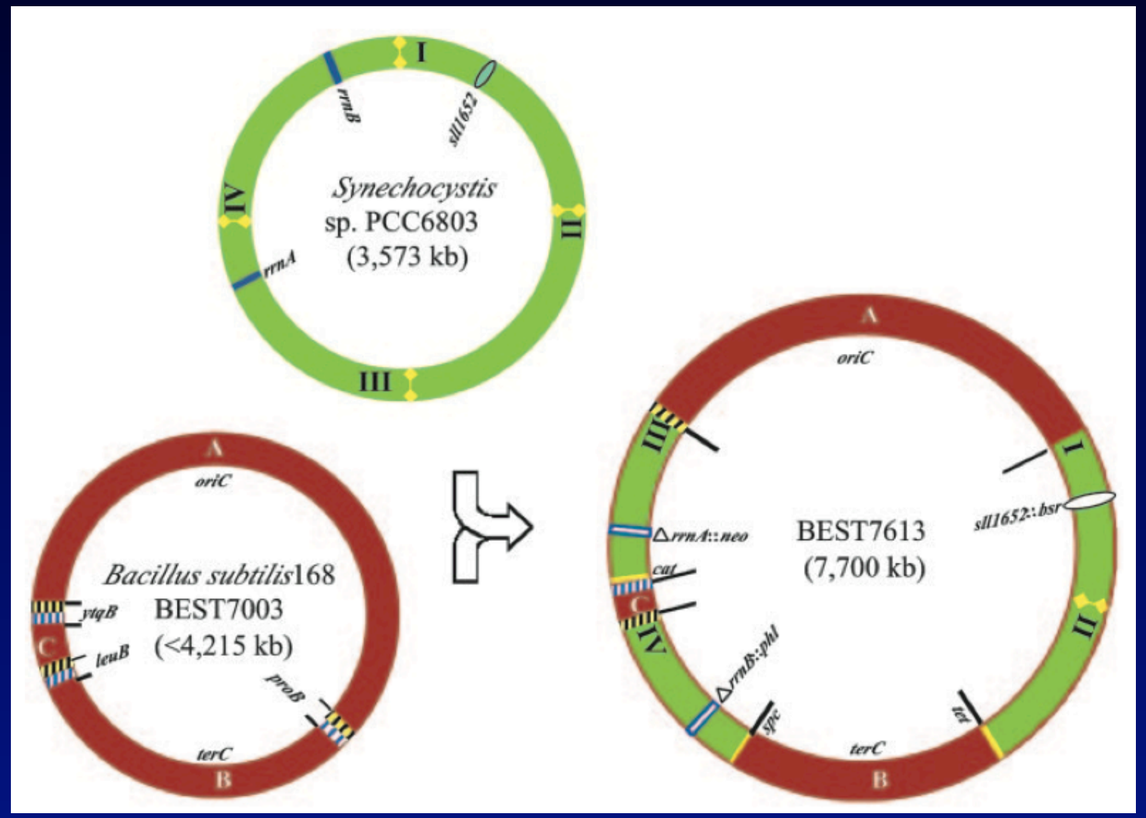
Key people:

Masahiko Inouye (University of Toyama, Japan)

Ichiro Hirao (RIKEN, Japan)



2. Making new species by mixing genomes



Cyanobacteria

B.subtilis

Cyanobacillus

M.Itaya et al , PNAS 2006

3. Bioplastics (microbe & plant based)

starting soon... @ RIKEN

Dr. Minami Matsui

4. Cholesterol eating bacteria

design cells that ingest cholesterol to decrease blood cholesterol levels

Takuya Ueda

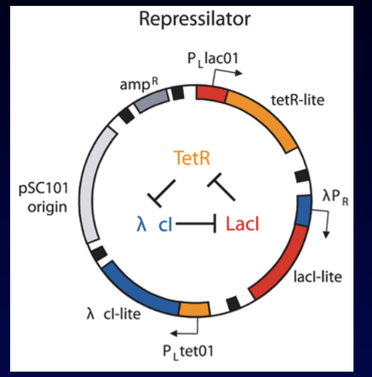
Yoshihiro Shimizu

Tokyo University

India

5. synchronous oscillators

MAPK pathway and repressilator-based



combinatorial network synthesis

Prof. V. Kulkarni
IIT Bombay

6. Building complex devices (oscillators)

i GEM 2009: Best presentation

i GEM 2008: Best presentation

i GEM 2007: Best Modeling / Sim

Mukund Thattai

National Centre for
Biological Sciences, Bangalore

Dhar Lab

Centre for Systems and Synthetic Biology
www.cssb.in

1. Genome Designer (bioCAD platform)

ver 1. summer 2010

BioBricks Support
Genome sequence editor
Pathway editing
Multi format support
Design creation
Design optimization
Database interfacing
Interaction prediction
muti-level design integration

Designing engineering systems

Plan
computer

Parts

Model
computer

Process

Optimization
computer

Designing biological systems

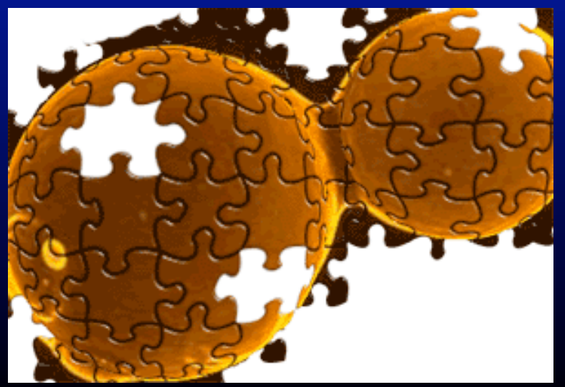
GEN ME DESIGNER

Parts

GEN ME DESIGNER

Process

GEN ME DESIGNER



top level abstraction

Input: feature

computational engine

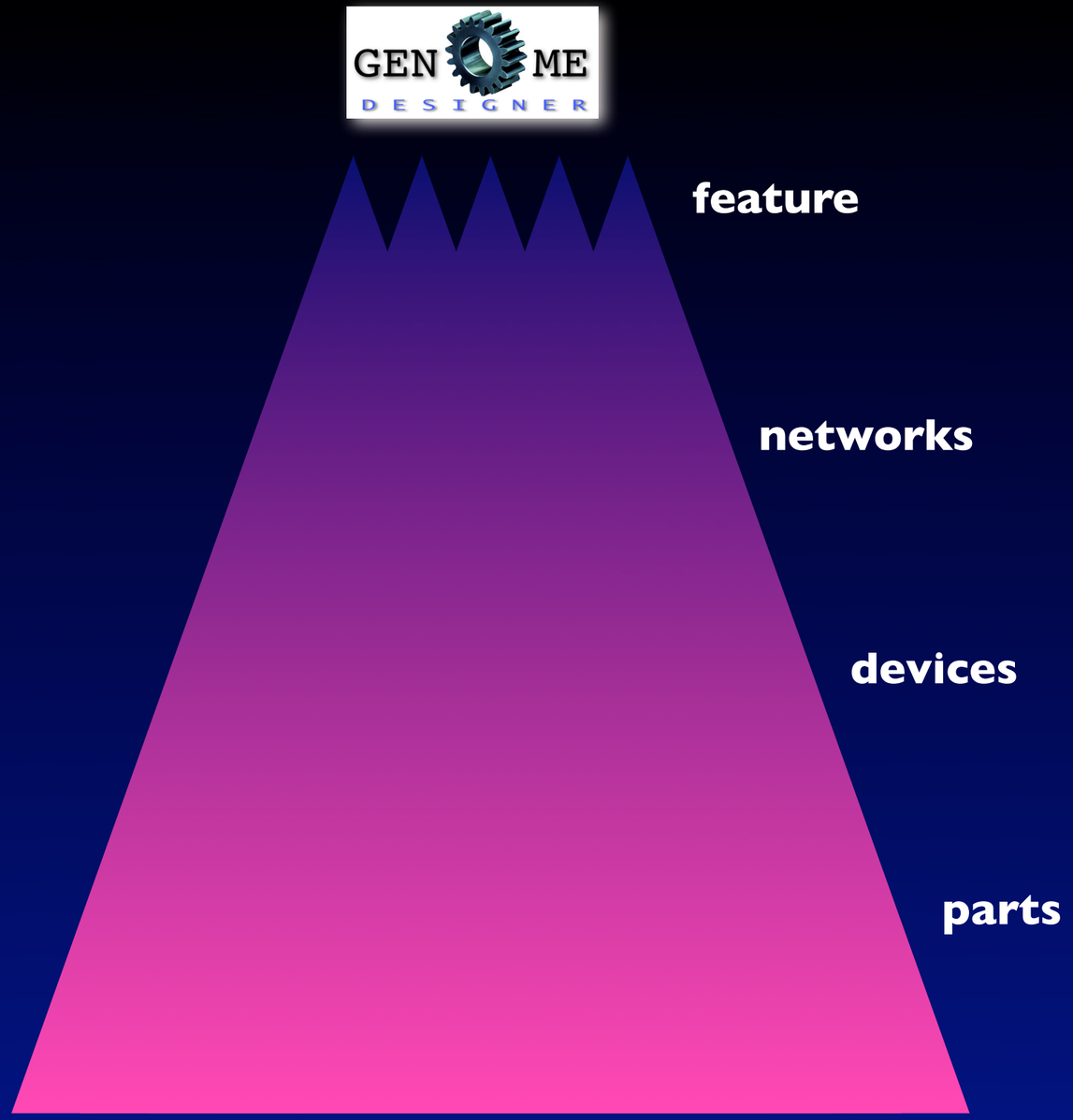
view, edit, compile

show alternate designs

add constraints

final design

print DNA sequence



2. Making genes out of 'junk DNA'

We are reasonably good in making ...

junk out of genes

point mutations
knock downs
knock outs

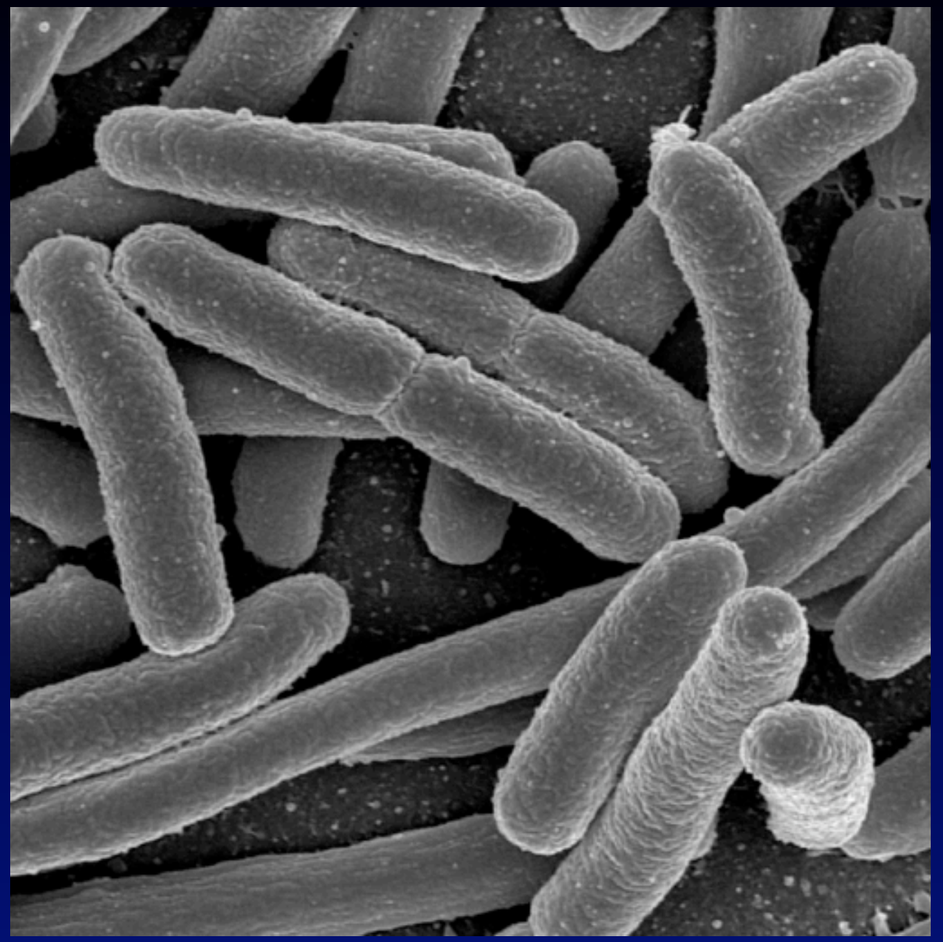
Can we make genes out of “junk DNA”?

Q 1: What kind sequences should we choose ?

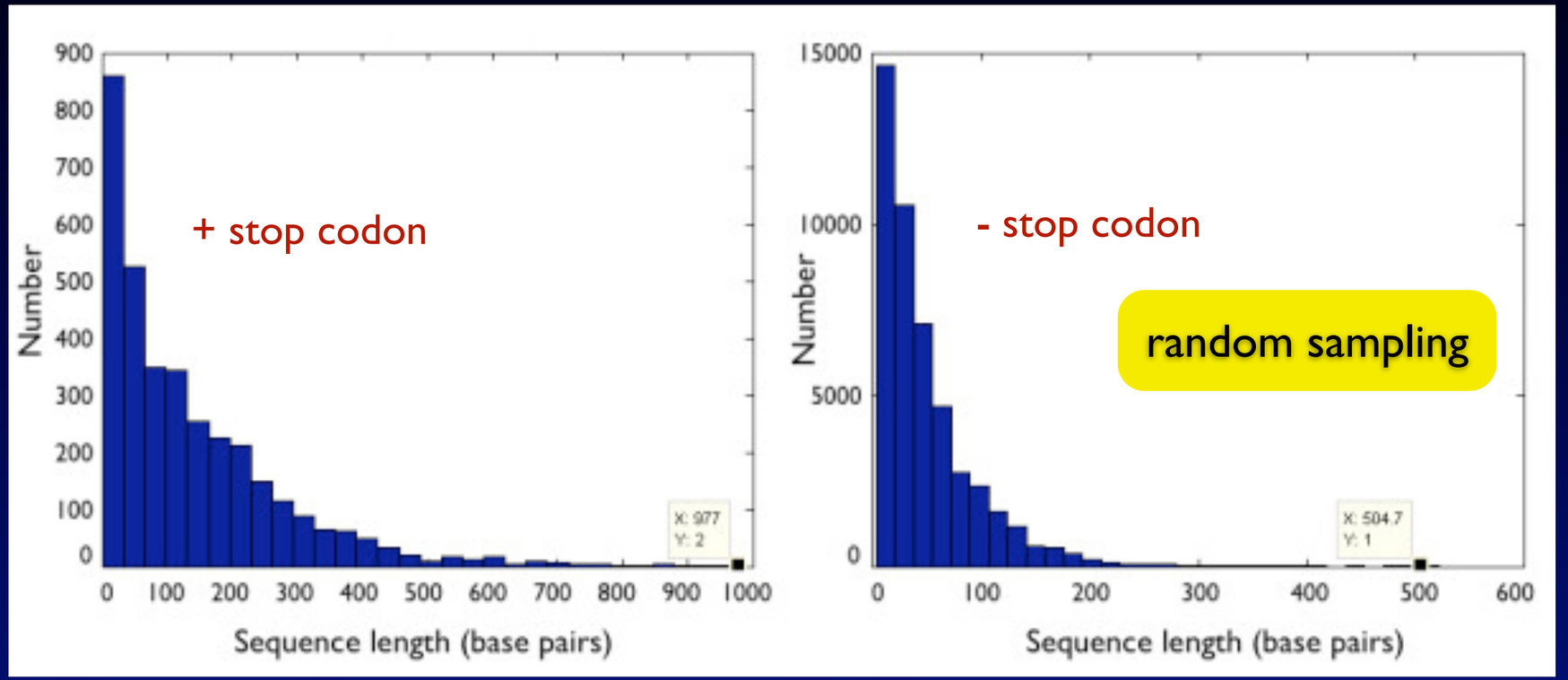
intergenic regions with
no evidence of transcription

Organism: E.coli

~ 2500 Intergenic regions



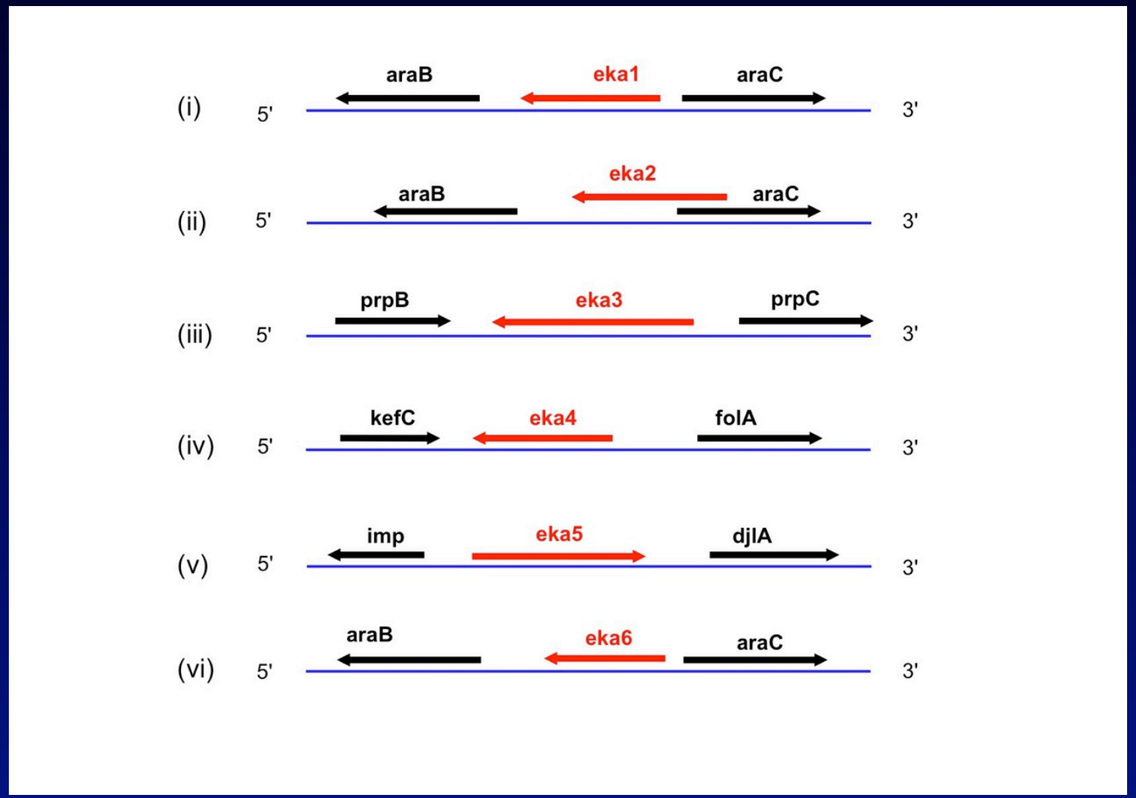
E.coli: Intergenic regions



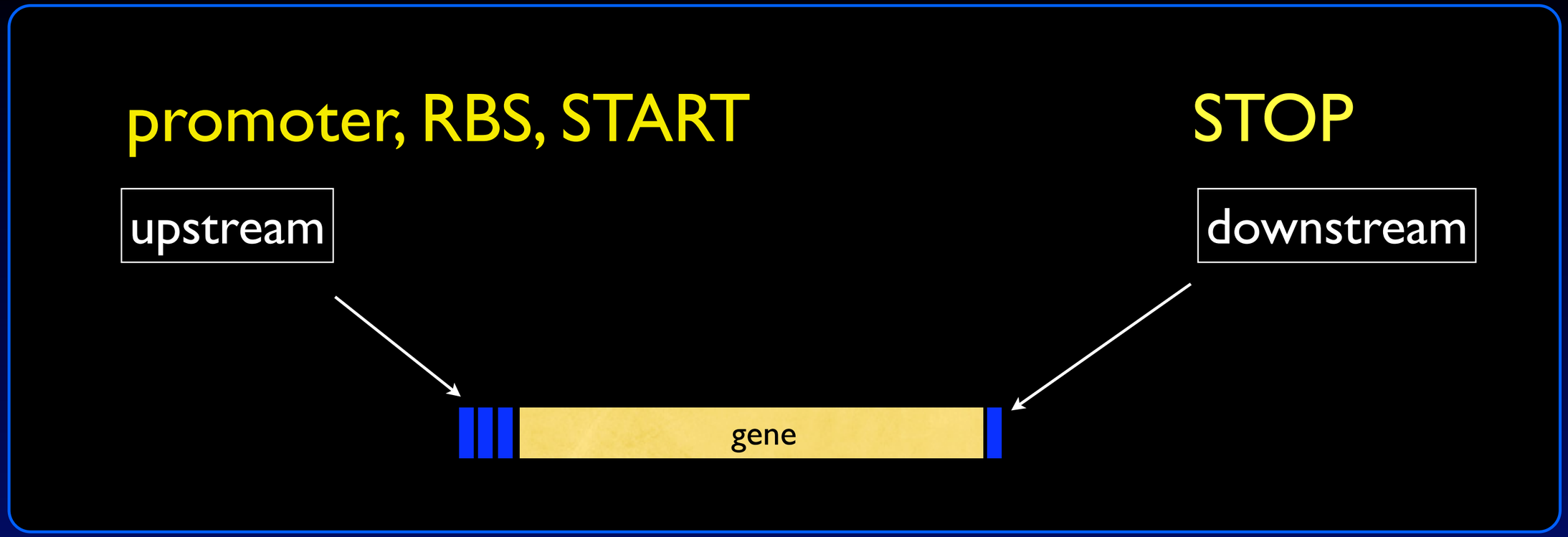
range: 100 - 400 bases

(SEQUENCE SIMILARITY SEARCH AGAINST NCBI'S NON-REDUNDANT PROTEIN DATABASE)

The predicted full length protein sequences **did not** resemble any known protein

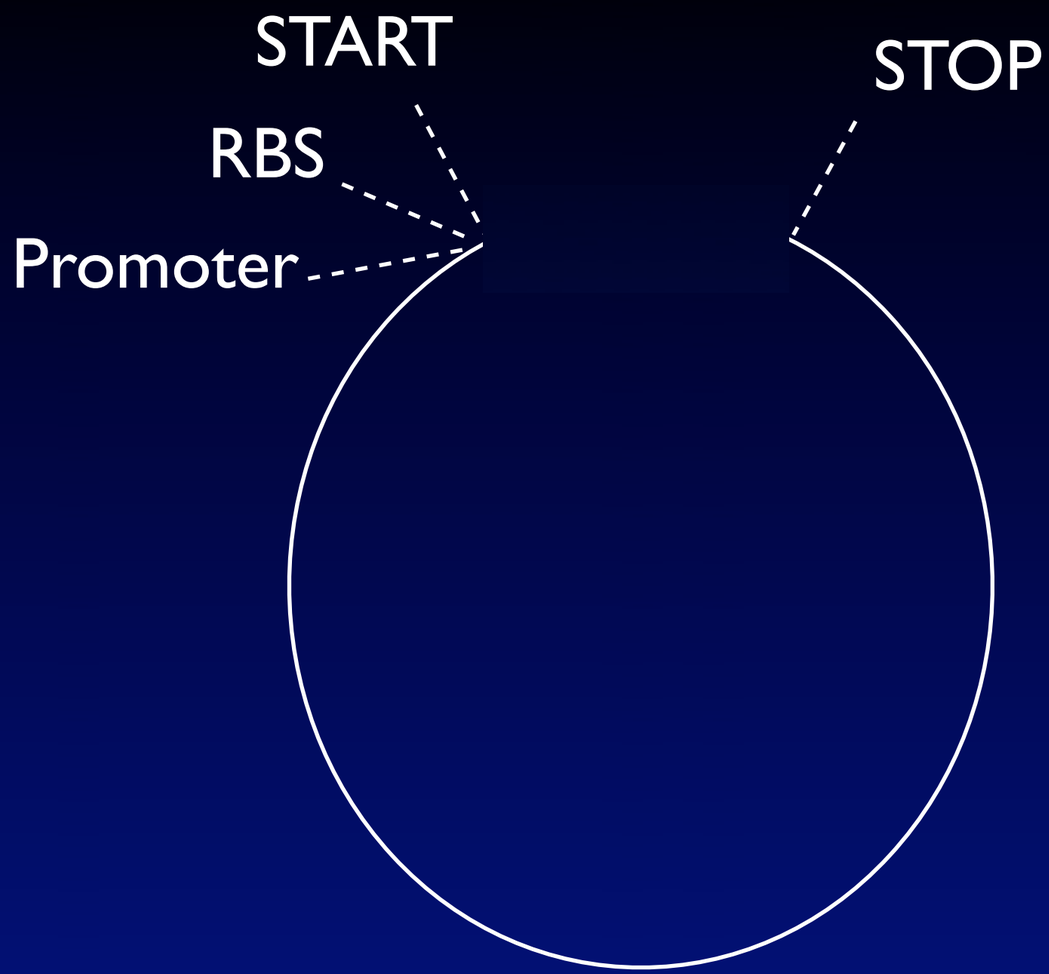


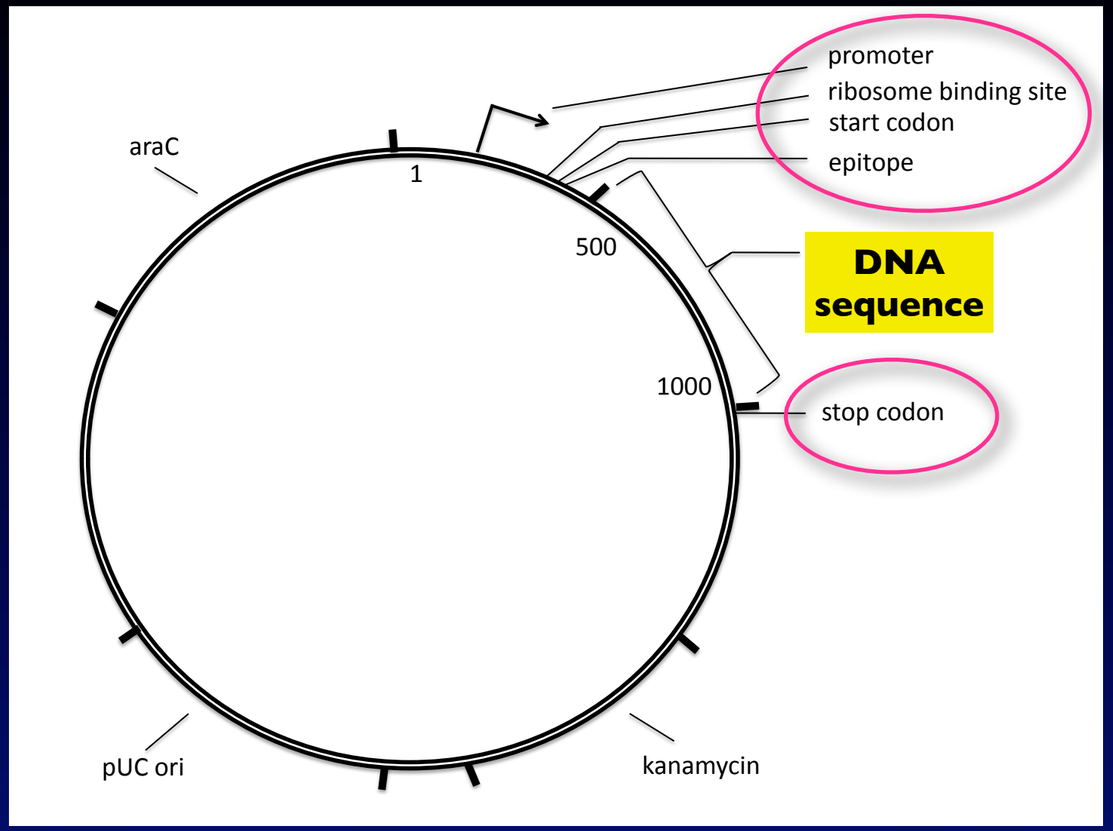
Q 2 : How to make a gene out of a non-gene sequence ?



where ... ?

outside the genome

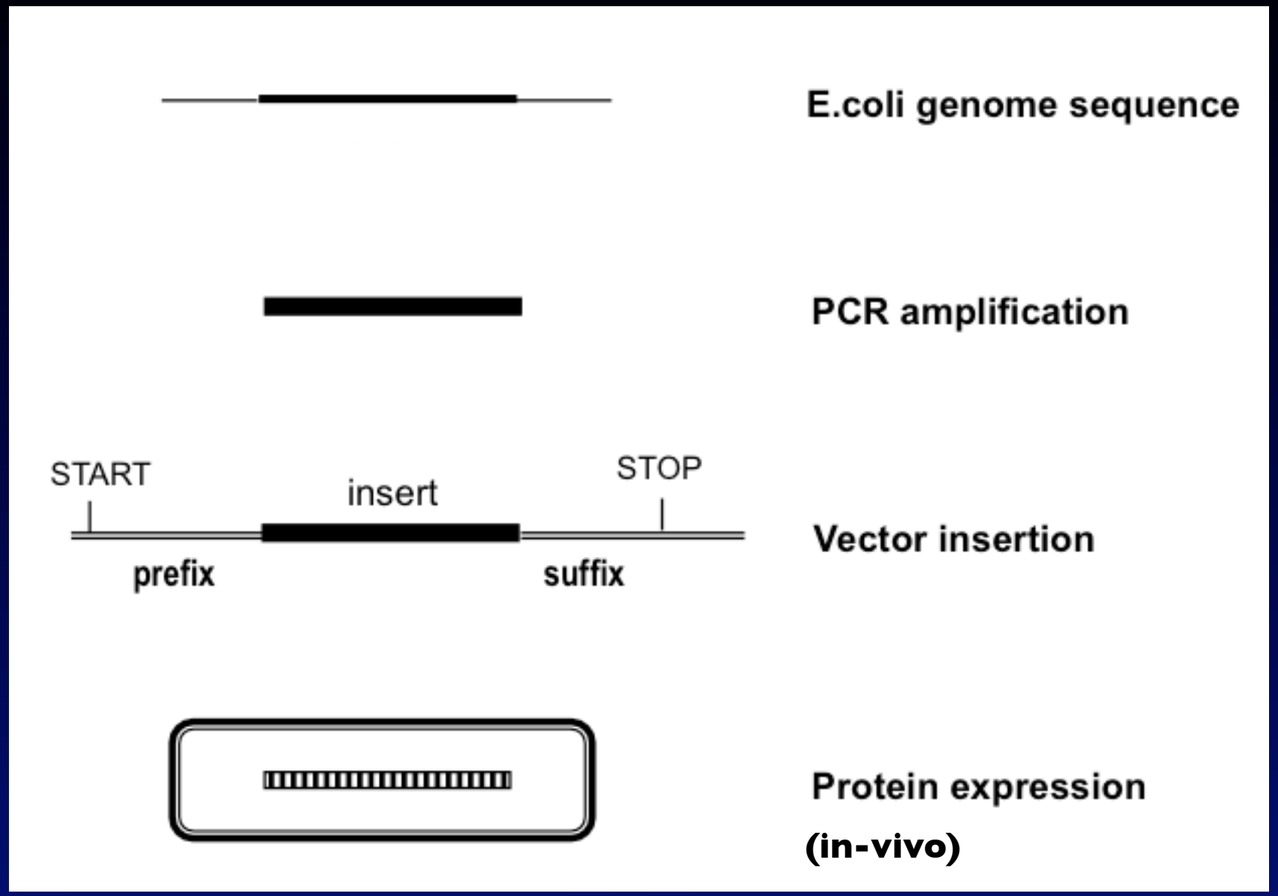

START
RBS
Promoter
STOP

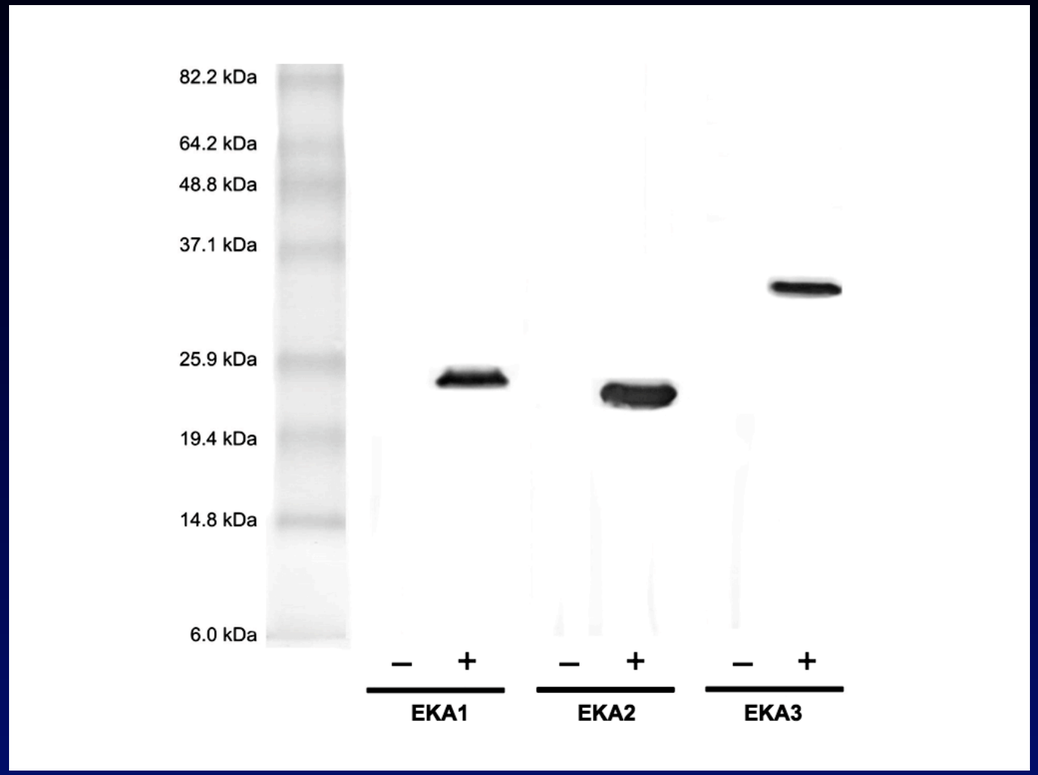


pBAD TOPO expression vector

Invitrogen

provides the infrastructure for artificial expression of a DNA sequence

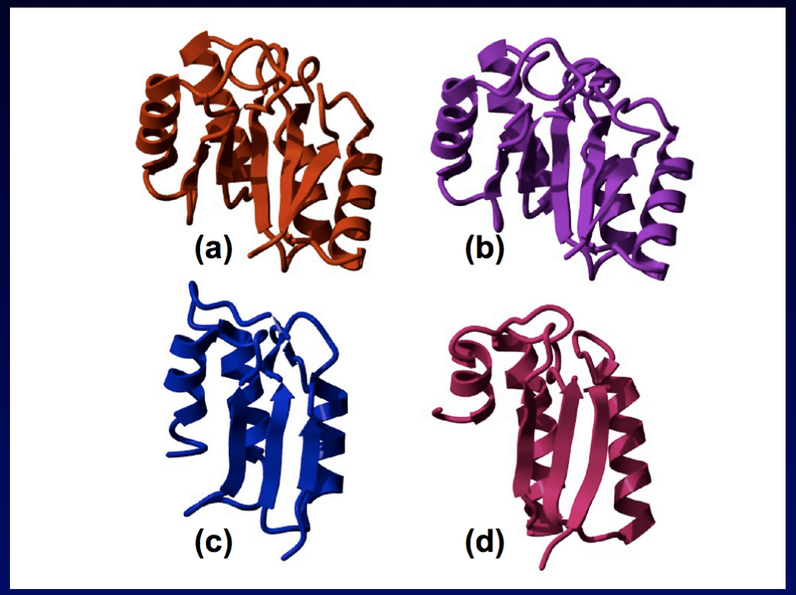

E.coli genome sequence
PCR amplification
START
insert
STOP
prefix
suffix
Vector insertion
Protein expression
(in-vivo)



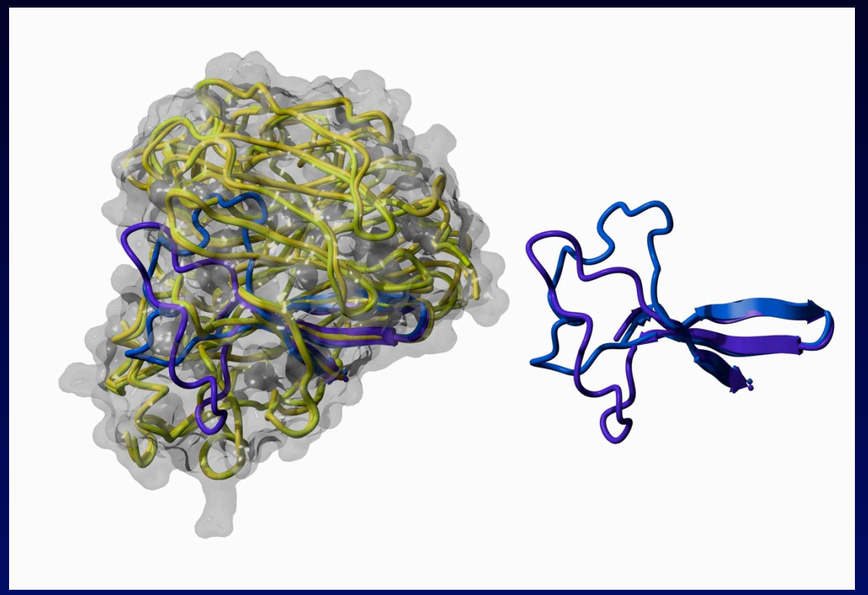
Western Blot
(using His tag)

Q 3: Do these peptides / proteins fold into a certain structure ?

Predictions compatible with forming a tertiary structure



EKA3 protein
(301 aa, 35 kDa)



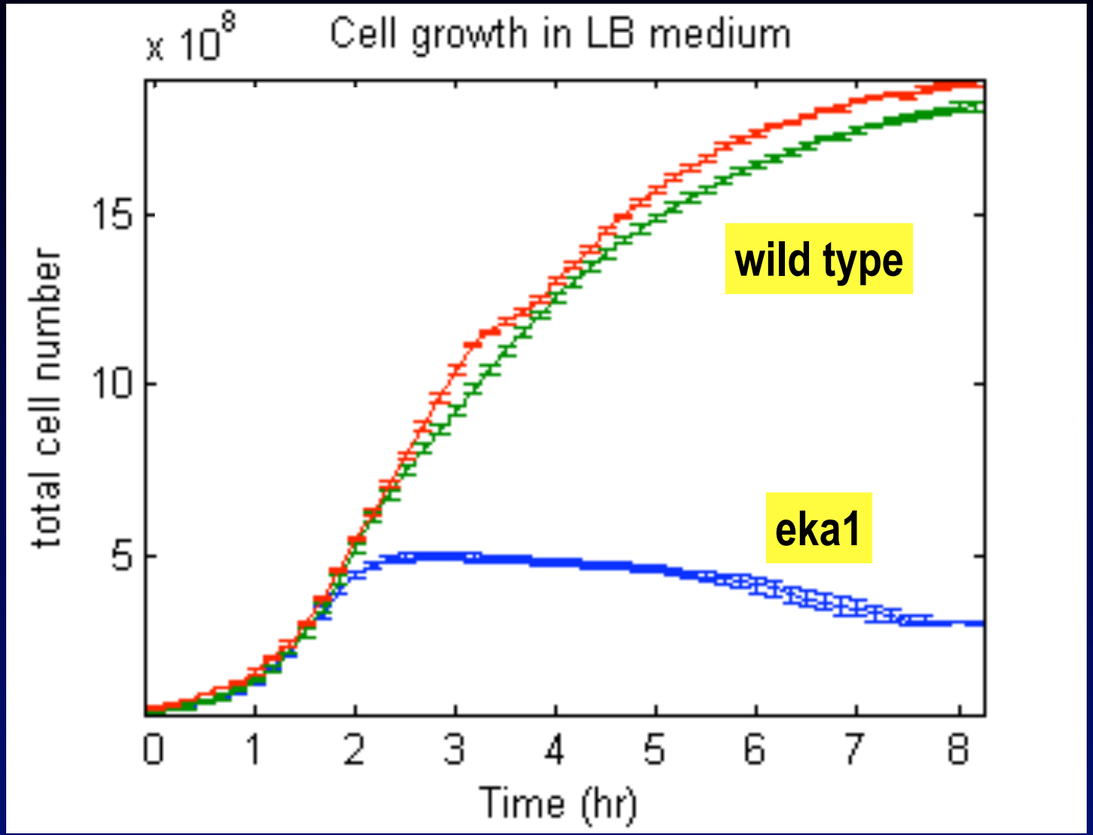
EKA5 protein
(204 aa, 22.2 kDa)

3D Jury
(mGenThreader)

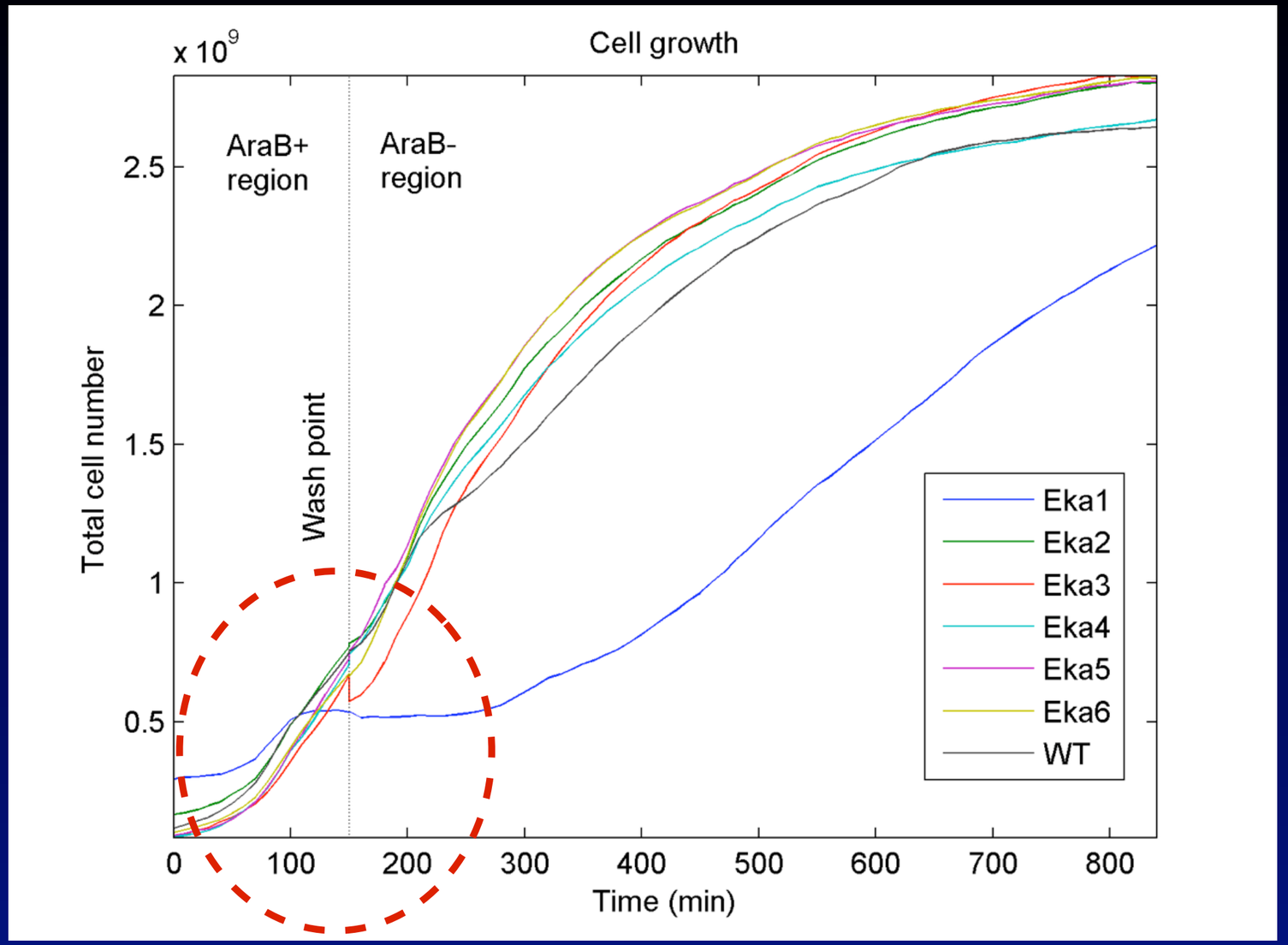
weak similarity to the beta propeller fold of viral neuroaminidase

Q4: What is the phenotype of the transformed cells ?

shape, cell growth


Cell growth in LB medium
x 10^8
total cell number
15
10
5
0
1
2
3
4
5
6
7
8
Time (hr)
wild type
eka1

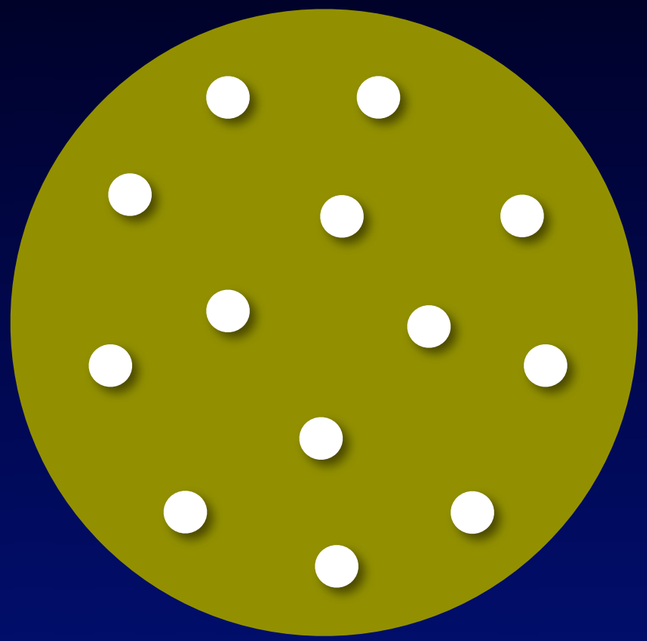
Q 5: Is cell growth inhibition rescued by switching off eka 1 expression ?


Cell growth
x 10^9
AraB+ region
AraB- region
Wash point
Total cell number
Time (min)
0
100
200
300
400
500
600
700
800
0.5
1
1.5
2
2.5
Eka1
Eka2
Eka3
Eka4
Eka5
Eka6
WT

Q 6 : What are the molecular reasons for the EKA1 phenotype ?

ongoing : microarray, immunoprecipitation, structure studies

EKA 7: phenotype : slow cell growth

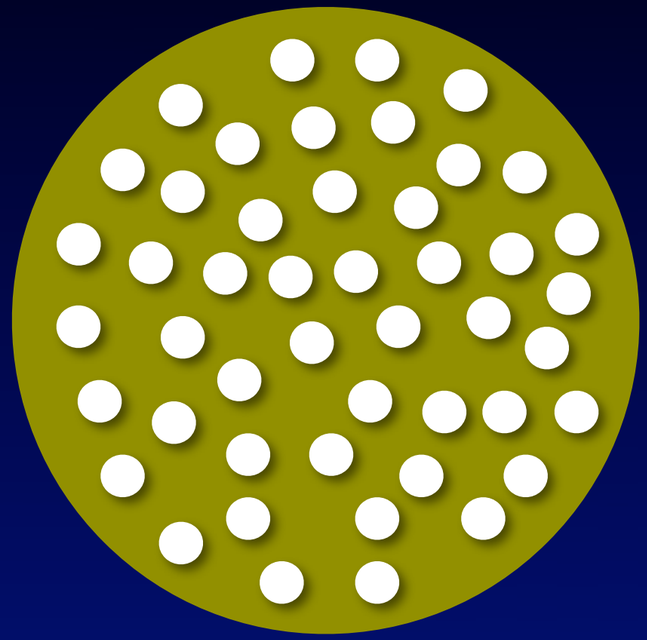


Wild type

(t=n)

EKA 7 transformed

(t=3n)



Biochemical assay

eka 7 protein shows DNA binding property

144 aa residue, 17 kDa

Functional Genomics

over produce

Knock out
Down regulation

(organisms do not need
these proteins by default)

Make your own genes

47% of E.coli intergenic regions show a strong evidence of protein secondary structure

3. BIOPARTS Knowledgebase

Lists functional parts extractable from not-coding regions of genome

The Knowledgbase of proteins extractable from intergenic regions

open access

ver 1: summer 2010

Organism	Part	address	length	GC	protein kDa	predicted p-p	RNA structure prediction	Protein structure prediction	micro-array	Pathway prediction	Pheno-type
E.coli	eka1	... to ...									
	eka2	... to ...									
	eka3	... to ...									
Fly	eka4	... to ...									
	eka5	... to ...									
	eka6	... to ...									

Did Nature sample all possibilities ?

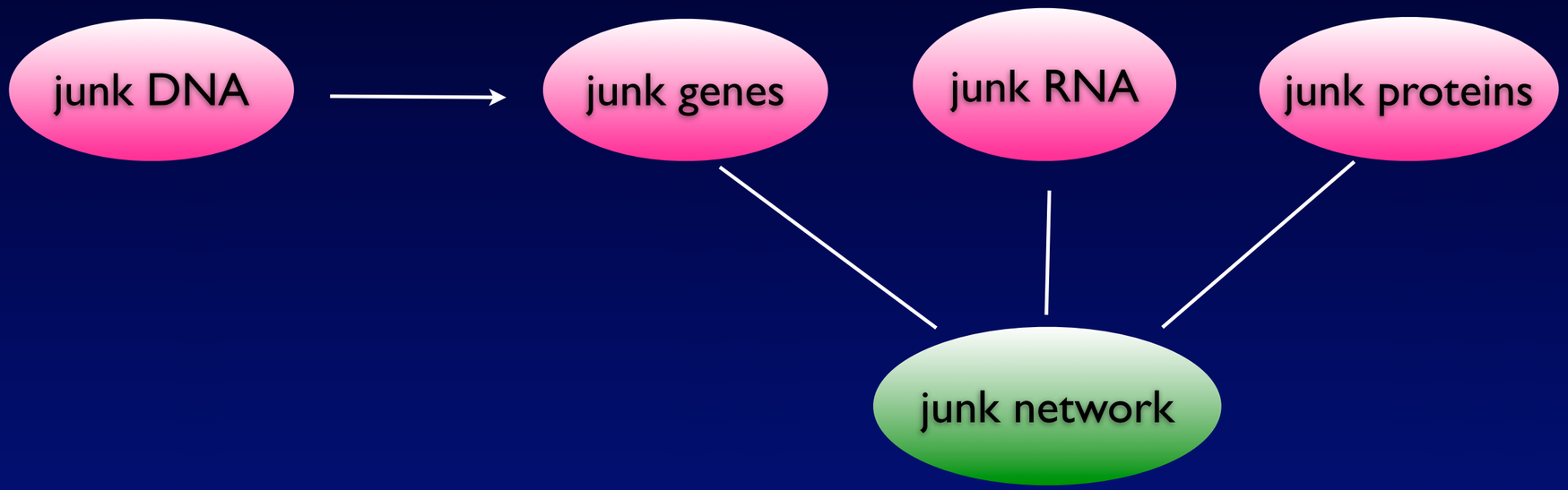
The Big Picture

Re-annotate genomes based on extractable structures

evolutionary biology — applications

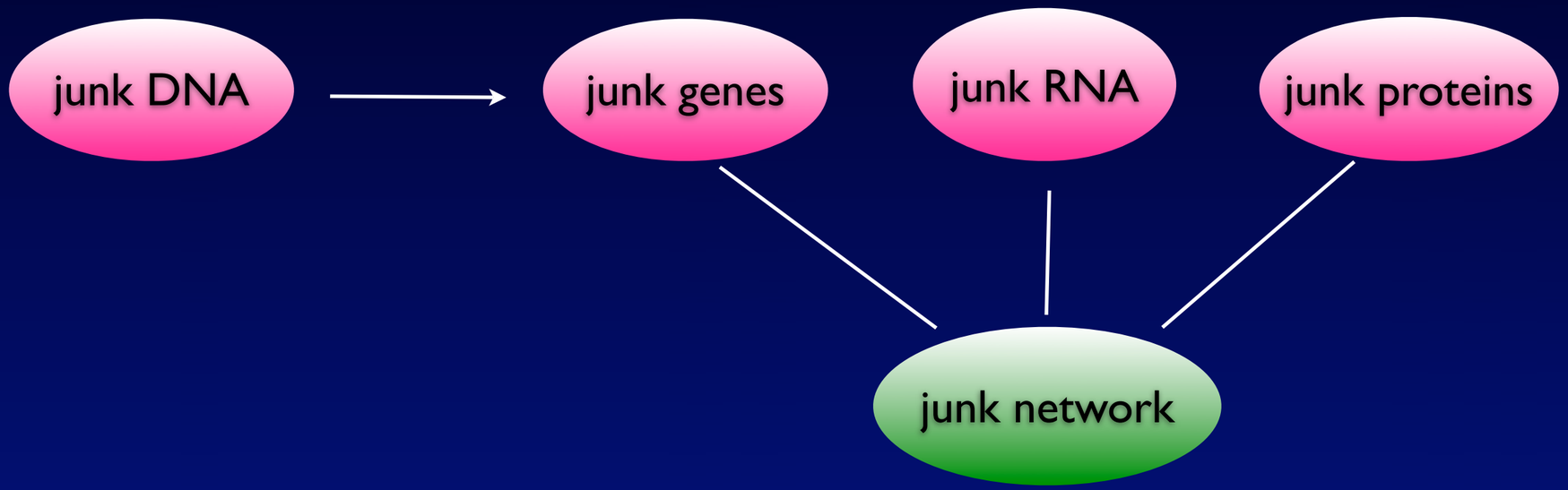
The emergence of combinatorial genomics ?

a new way of doing biology...



Junkomics

a new way of doing biology...



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