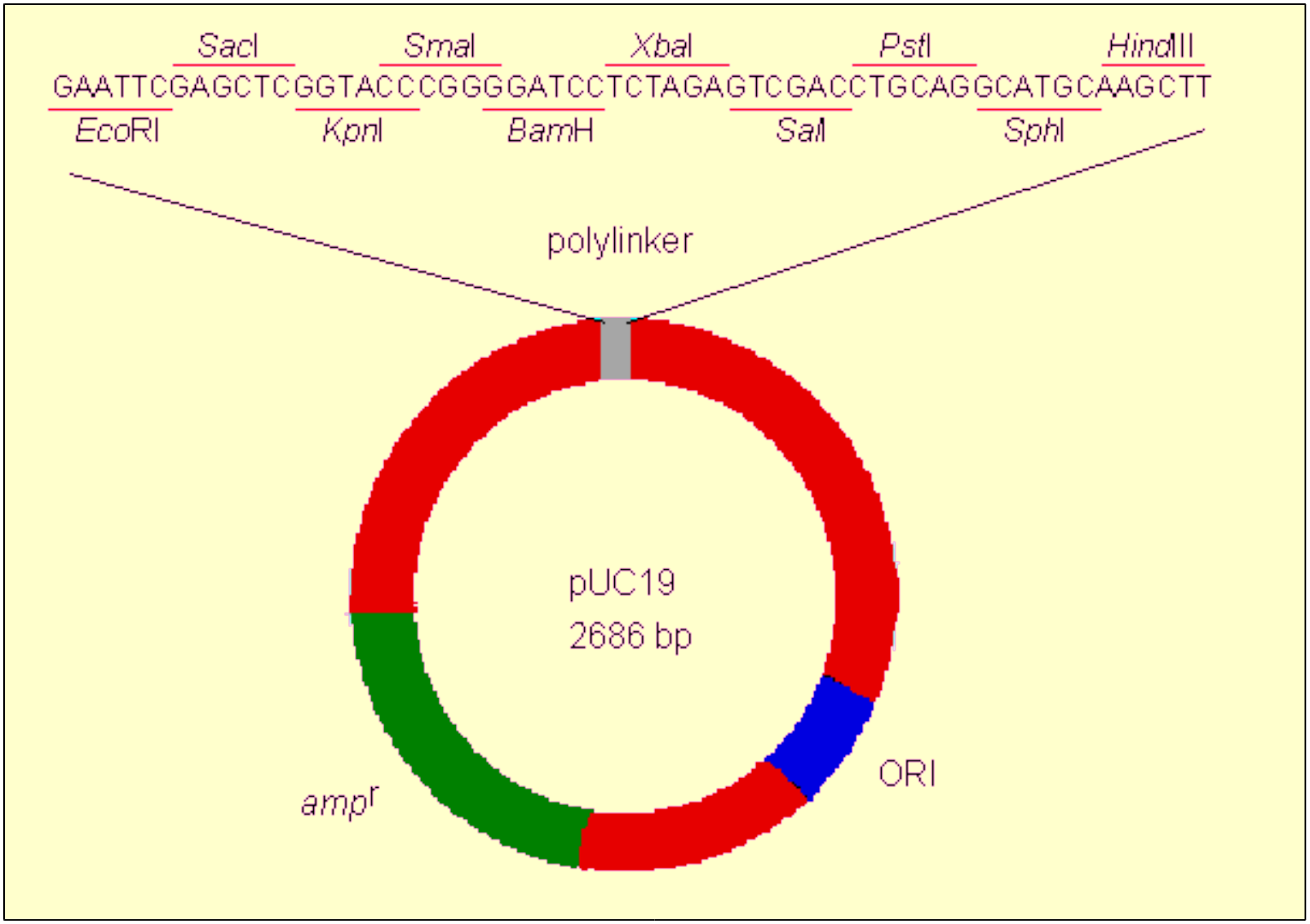
I'm an athletic mature, independent, 33 yrs. old, with a youthful appearance of 23. I'm a single white male and a working professional in the research industry. I'm 5'10" tall, 175 lbs, with light brown hair and hazel eyes. I'm a non-drinker and non-smoker. I've never been married and have no dependants. Currently seeking a female companion who enjoys the outdoors and understands DNA replication. If interested in finding out more, call Box 1044.

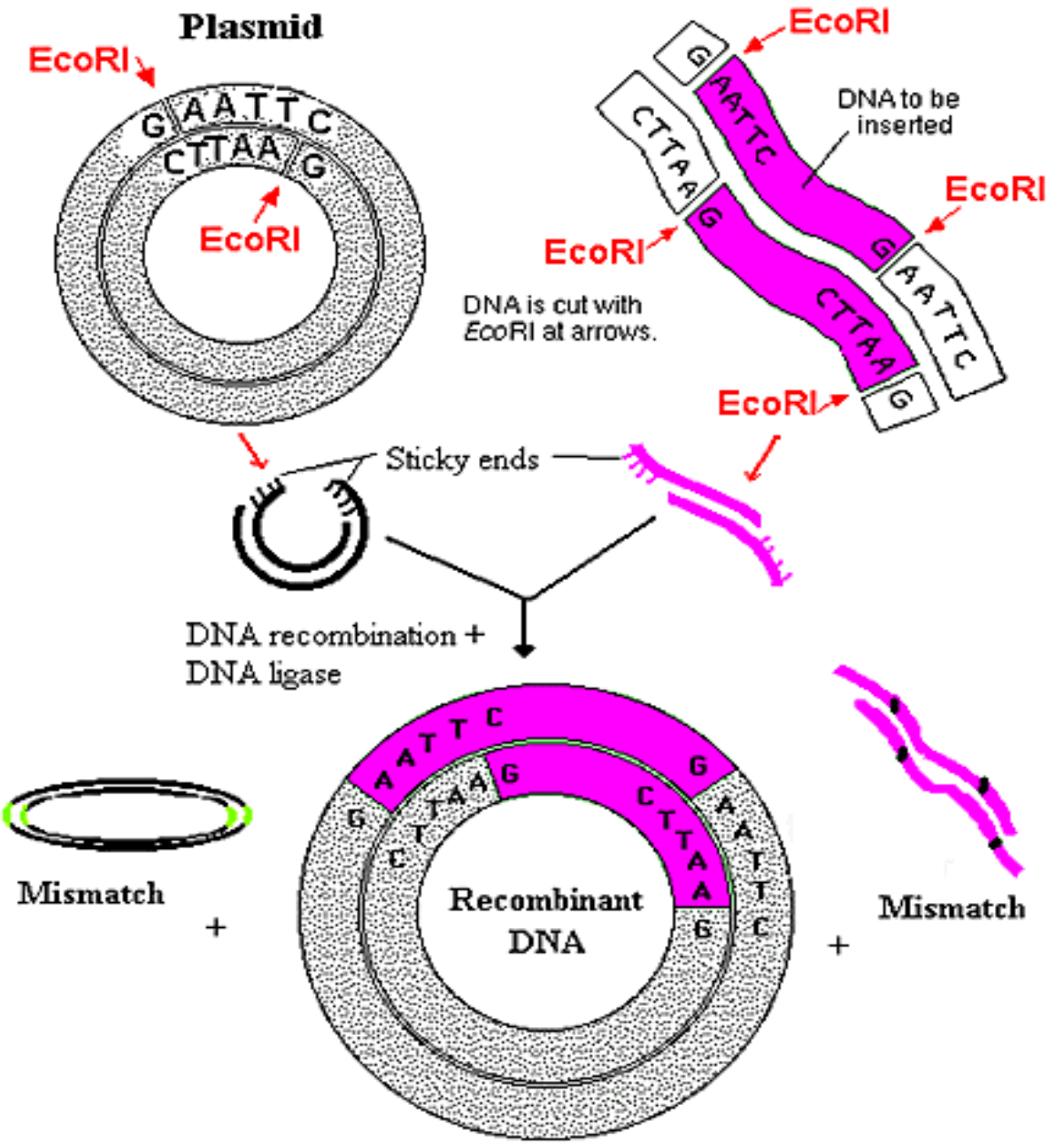
Vybrané experimentální postupy pro manipulaci s DNA a proteiny

segmentace enzymy a rekombinace DNA
transformace organizmů cizí DNA
klonování DNA
cDNA knihovny
elektroforéza DNA
hybridizace se značenými sondami
PCR (Polymerase Chain Reaction)
určování sekvence DNA

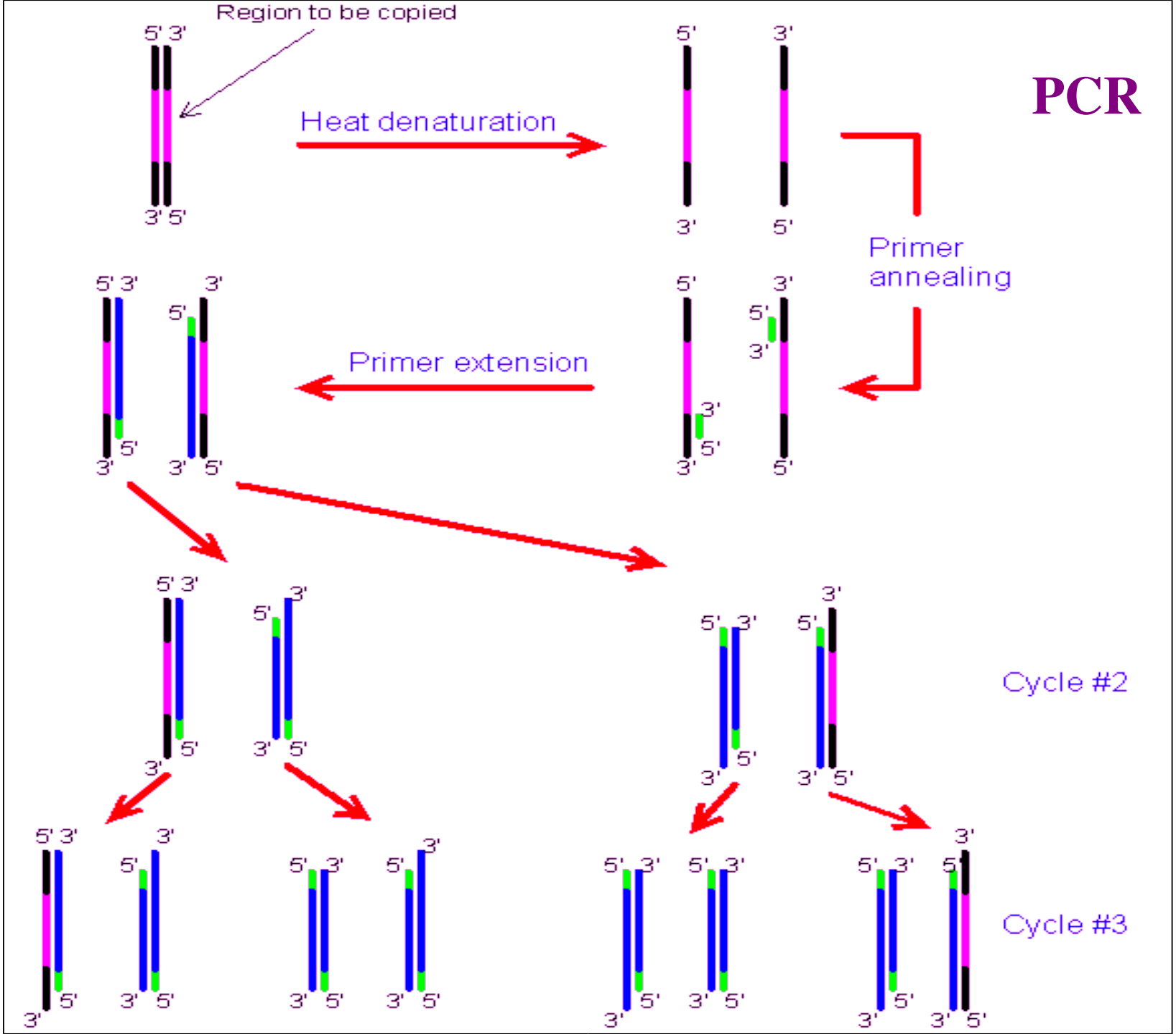
produkce rekombinantních proteinů
určování struktury proteinů (X-ray, NMR)

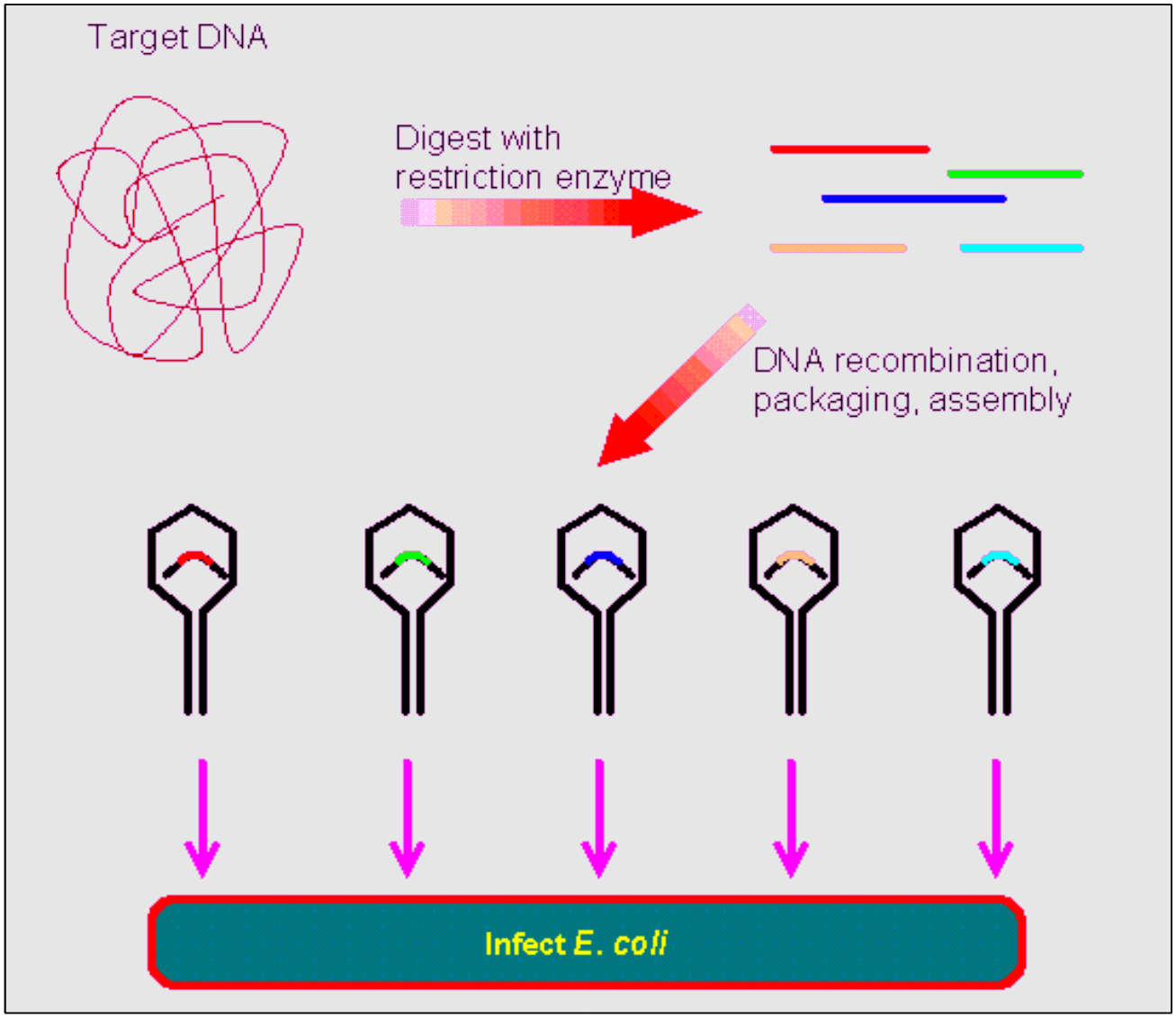
Enzyme	Source	Recognition site	Average cleaved size (kb)
AluI	*Arthrobacter luteus*	AG↓CT TC↑GA	0.3
BamHI	*Bacillus amyloliquefaciens H*	G↓GATC C C CTAG↑G	7.0
EcoRI	*Escherichia coli R factor*	G↓AATT C C TTAA↑G	3.1
HaeIII	*Hemophilus aegyptus*	GG↓CC CC↑GG	0.6
HindIII	*Hemophilus influenzae Rd*	A↓AGCT T T TCGA↑A	3.1
NotI	*Norcadia otitidis-caviarum*	GC↓GGCC GC CG CCGG↑CG	< 9700
PstI	*Providencia stuartii*	C TGCA↓G G↑ACGT C	7.0
TaqI	*Thermus aquaticus*	T↓CG A A GC↑T	1.4

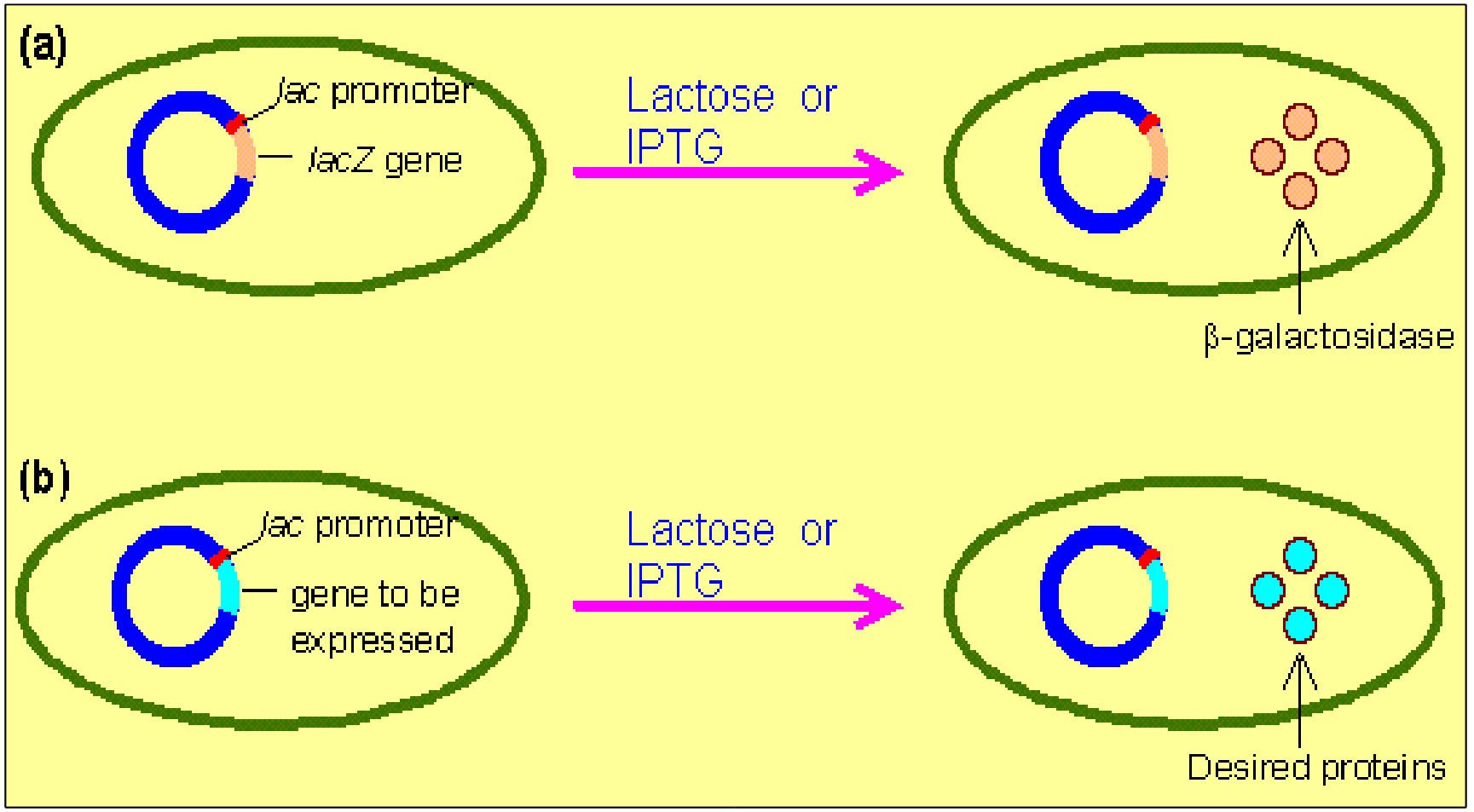

SacI
SmaI
XbaI
PstI
HindIII
GAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTT
EcoRI
KpnI
BamH
SalI
SphI
polylinker
pUC19
2686 bp
ampr
ORI



Inserting a DNA Sample into a Plasmid


PCR
Region to be copied
Heat denaturation
Primer annealing
Primer extension
Cycle #2
Cycle #3
5'
3'


Target DNA
Digest with
restriction enzyme
DNA recombination,
packaging, assembly
Infect E. coli


(a)
lac promoter
lacZ gene
Lactose or
IPTG
β-galactosidase
(b)
lac promoter
gene to be
expressed
Lactose or
IPTG
Desired proteins

Sekvence DNA

- Poradi nukleotidu ve smeru 5’ k 3’ ulozeno pro budouci generace jako dlouhy retezec symbolu A, C, G a T
- Dobra sprava pro budoucnost bioinformatiku: zatim je osekvenovano jenom asi 150 organizmu, data budou pribyvat
- Polymorfizmus: Rozdil mezi jedinci Homo sapiens sapiens 1/1000 bazi

Sekvence DNA

- Sekvenace se dela ve dvou fazich
  - Za pouziti DNA polymerazy a smesi normalnich a modifikovanych deoxyribonukleotidu se syntetizuji vlakna DNA, templatem je sekvenovana molekula DNA
  - Produkty polymerizace se v automatickych strojich chromatograficky deli podle velikosti a vypovidaji o sekvenci
- Jedna reakce poda informaci o 500-1000 bazich

DNA Polymerase reads the template strand and synthesizes a new second strand to match:

IF 5% of the T nucleotides are actually dideoxy T, then each strand will terminate when it gets a ddT on its growing end:

5' - TACGCGGTAACGGTATGTTCGACCGTTTAGCTACCGAT•

5' - TACGCGGTAACGGTATGTTCGACCGTTTAGCT•

5' - TACGCGGTAACGGTATGTTCGACCGTTT•

5' - TACGCGGTAACGGTATGTTCGACCGTT•

5' - TACGCGGTAACGGTATGTTCGACCGT•

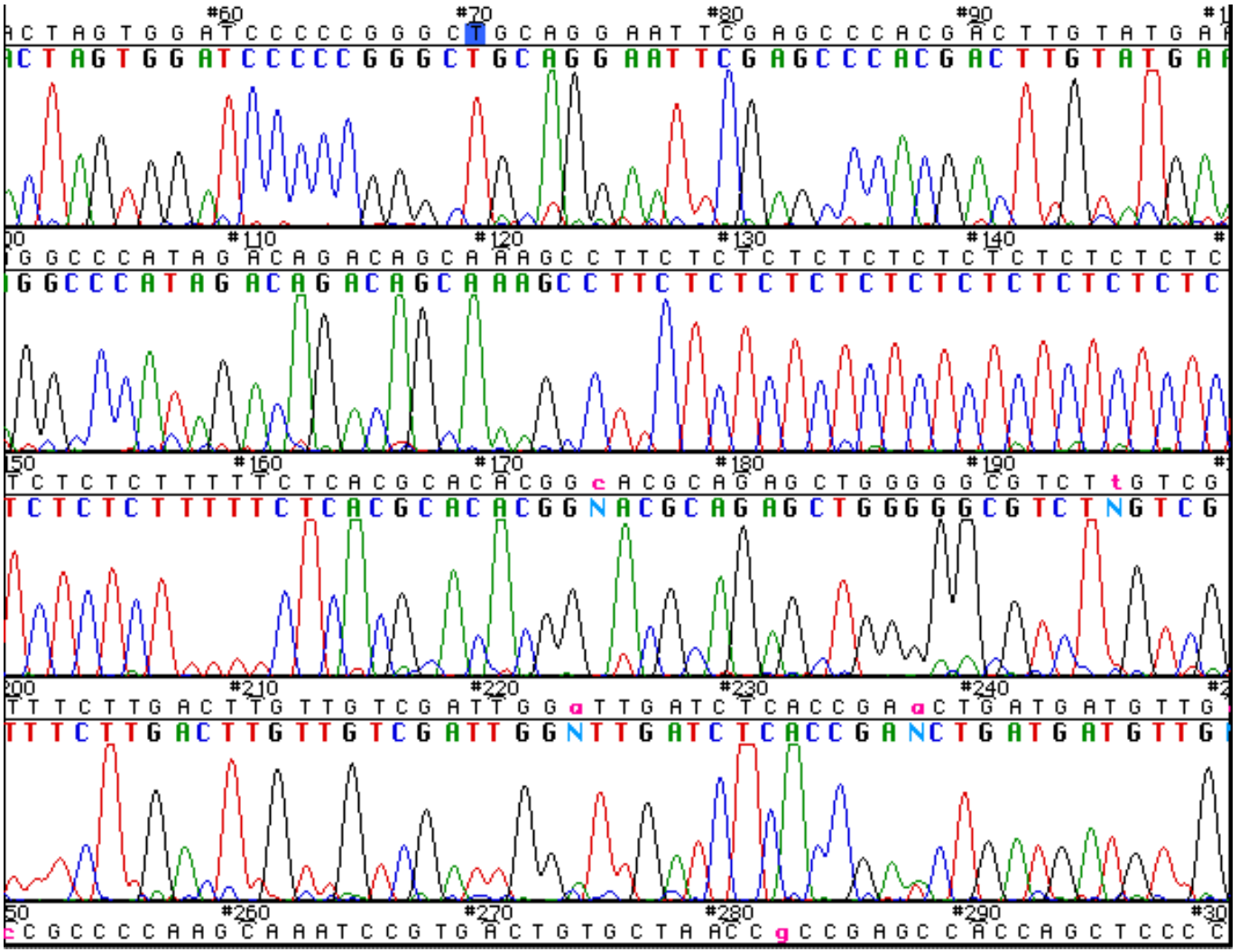
5' - TACGCGGTAACGGTATGTT•

5' - TACGCGGTAACGGTATGT•

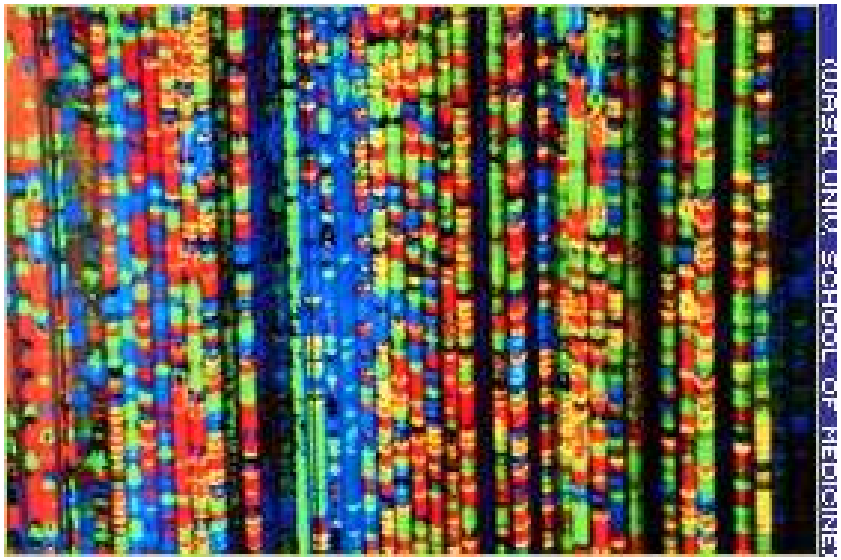
5' - TACGCGGTAACGGTAT•

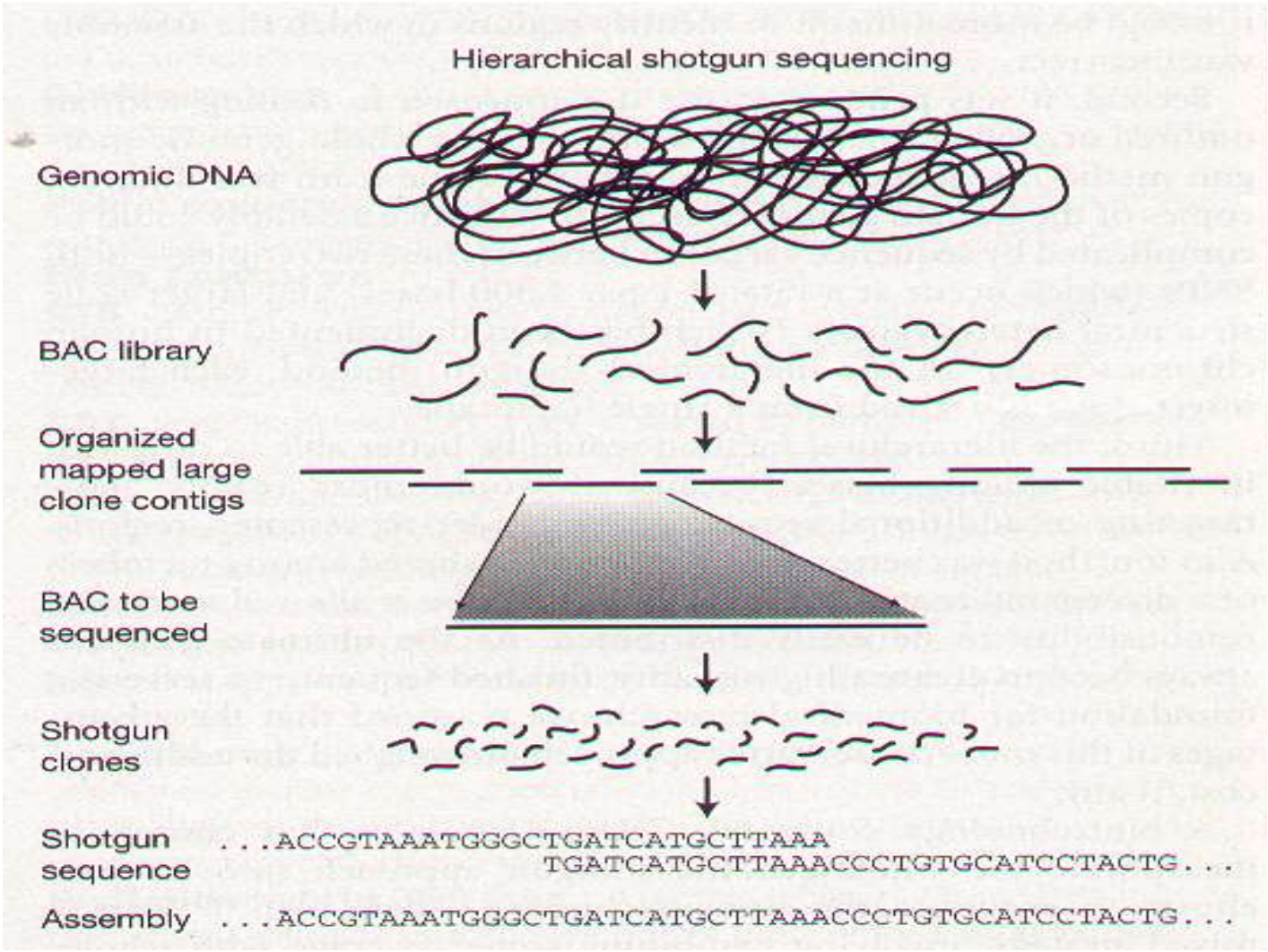
5' - TACGCGGTAACGGT•

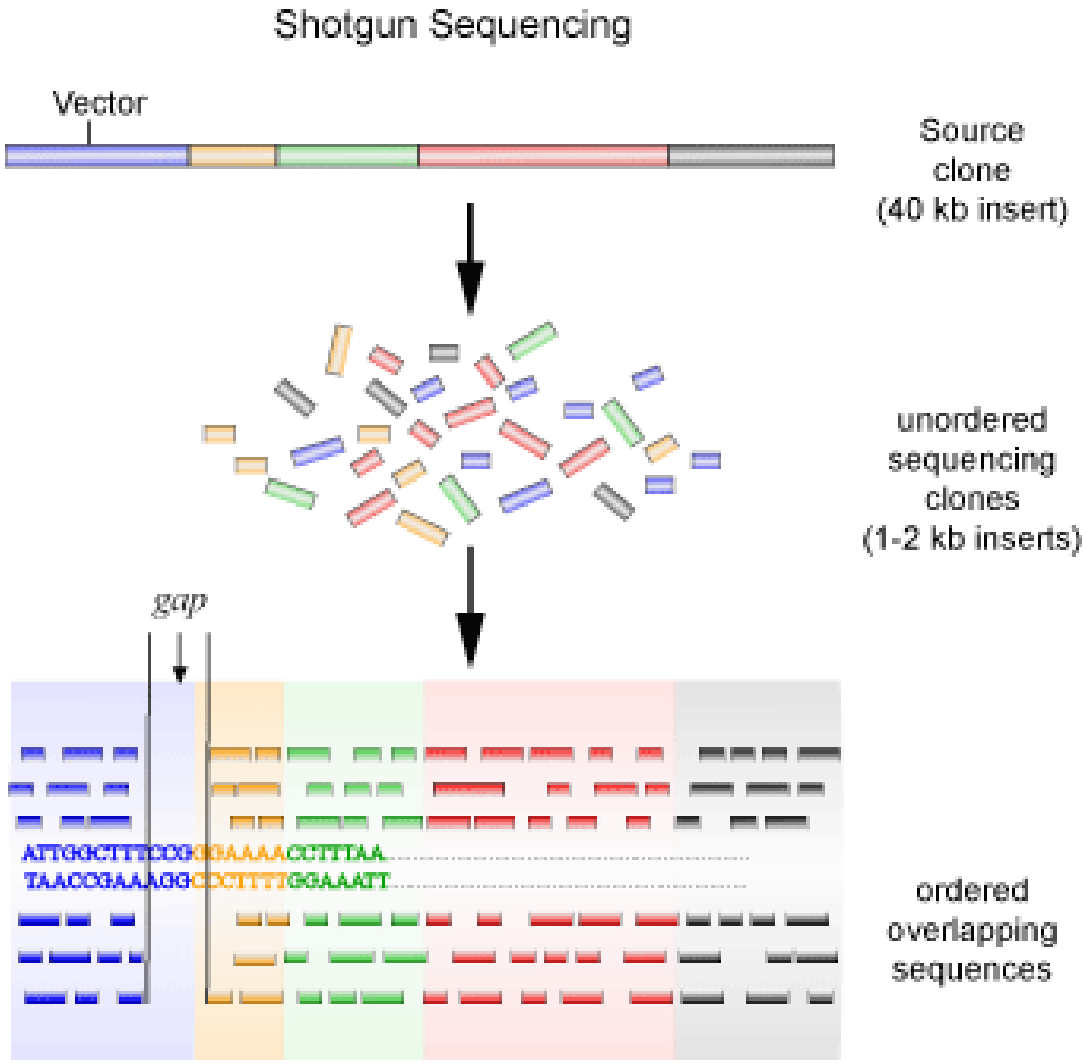
5' - TACGCGGT•


#60
#70
#80
#90
ACTAGTGGATCCCCCGGGCTGCAGGAATTCGAGCCCACGACTTGTATGAA
#110
#120
#130
#140
TGGCCCATAGACAGACAGCAAAGCCTTCTCTCTCTCTCTCTCTCTCTCTC
#160
#170
#180
#190
TCTCTCTTTTTCTCACGCACACGGcACGCAGAGCTGGGGGCGTCTtGTCG
TCTCTCTTTTTCTCACGCACACGGNACGCAGAGCTGGGGGCGTCTNGTCG
#210
#220
#230
#240
TTCTTGACTTGTTGTCGATTGGaTTGATCTCACCGAaCTGATGATGTTG
TTCTTGACTTGTTGTCGATTGGNTTGATCTCACCGANCTGATGATGTTG
#260
#270
#280
#290
CGCCCCAAGCAAATCCGTGACTGTGCTAACCgCCGAGCCACCAGCTCCCC

Sekvence DNA




Hierarchical shotgun sequencing
Genomic DNA
BAC library
Organized mapped large clone contigs
BAC to be sequenced
Shotgun clones
Shotgun sequence
...ACCGTAAATGGGCTGATCATGCTTAAA
TGATCATGCTTAAACCCTGTGCATCCTACTG...
Assembly
...ACCGTAAATGGGCTGATCATGCTTAAACCCTGTGCATCCTACTG...


Shotgun Sequencing
Vector
Source clone (40 kb insert)
unordered sequencing clones (1-2 kb inserts)
gap
ordered overlapping sequences

Sekvence DNA

1975	bakteriofag MS2	
1977	bakteriofag PhiX174	5,375 bp
1982	bakteriofag Lambda	
1984	HIV-1	
1990	virus HCMV	230 Kbp
1995	H. influenzae	1,83 Mbp
1996	E. coli	4,60 Mbp
	S. cerevisiae	12,00 Mbp
1998	C. elegans	96 Mbp
2000	D. melanogaster	120 Mbp
	A. thaliana	130 Mbp
2001	H. sapiens	3 000 Mbp
	M. musculus	3 000 Mbp
2004-5	R. norvegicus, A. melifera, G. gallus, C. familiaris, B. taurus	

RESEARCH ARTICLE

Environmental Genome Shotgun Sequencing of the Sargasso Sea

J. Craig Venter,[1]* Karin Remington,[1] John F. Heidelberg,[3] Aaron L. Halpern,[2] Doug Rusch,[2] Jonathan A. Eisen,[3] Dongying Wu,[3] Ian Paulsen,[3] Karen E. Nelson,[3] William Nelson,[3] Derrick E. Fouts,[3] Samuel Levy,[2] Anthony H. Knap,[6] Michael W. Lomas,[6] Ken Nealson,[5] Owen White,[3] Jeremy Peterson,[3] Jeff Hoffman,[1] Rachel Parsons,[6] Holly Baden-Tillson,[1] Cynthia Pfannkoch,[1] Yu-Hui Rogers,[4] Hamilton O. Smith[1]

We have applied "whole-genome shotgun sequencing" to microbial populations collected en masse on tangential flow and impact filters from seawater samples collected from the Sargasso Sea near Bermuda. A total of 1.045 billion base pairs of nonredundant sequence was generated, annotated, and analyzed to elucidate the gene content, diversity, and relative abundance of the organisms within these environmental samples. These data are estimated to derive from at least 1800 genomic species based on sequence relatedness, including 148 previously unknown bacterial phylotypes. We have identified over 1.2 million previously unknown genes represented in these samples, including more than 782 new rhodopsin-like photoreceptors. Variation in species present and stoichiometry suggests substantial oceanic microbial diversity.

Microorganisms are responsible for most of the biogeochemical cycles that shape the environment of Earth and its oceans. Yet, these organisms are the least well understood on Earth, as the ability to study and understand the metabolic potential of microorganisms has been hampered by the inability to generate pure cultures. Recent studies have begun to explore environ-

characterization. To help ensure a tractable pilot study, we sampled in the Sargasso Sea, a nutrient-limited, open ocean environment. Further, we concentrated on the genetic material captured on filters sized to isolate primarily microbial inhabitants of the environment, leaving detailed analysis of dissolved DNA and viral particles on one end of the size spectrum and eukaryotic inhabitants on

chlorococcus, that numerically dominate the photosynthetic biomass in the Sargasso Sea.

Surface water samples (170 to 200 liters) were collected aboard the RV Weatherbird II from three sites off the coast of Bermuda in February 2003. Additional samples were collected aboard the SV Sorcerer II from "Hydrostation S" in May 2003. Sample site locations are indicated on Fig. 1 and described in table S1; sampling protocols were fine-tuned from one expedition to the next (*5*). Genomic DNA was extracted from filters of 0.1 to 3.0 μm, and genomic libraries with insert sizes ranging from 2 to 6 kb were made as described (*5*). The prepared plasmid clones were sequenced from both ends to provide paired-end reads at the J. Craig Venter Science Foundation Joint Technology Center on ABI 3730XL DNA sequencers (Applied Biosystems, Foster City, CA). Whole-genome random shotgun sequencing of the Weatherbird II samples (table S1, samples 1 to 4) produced 1.66 million reads averaging 818 bp in length, for a total of approximately 1.36 Gbp of microbial DNA sequence. An additional 325,561 sequences were generated from the Sorcerer II samples (table S1, samples 5 to 7), yielding approximately 265 Mbp of DNA sequence.

Environmental genome shotgun assembly. Whole-genome shotgun sequencing projects have traditionally been applied to identify the genome sequence(s) from one particular organism, whereas the approach taken here is intended to capture representative sequence from many diverse organisms simultaneously. Variation in genome size and relative abundance determines the depth of coverage of any

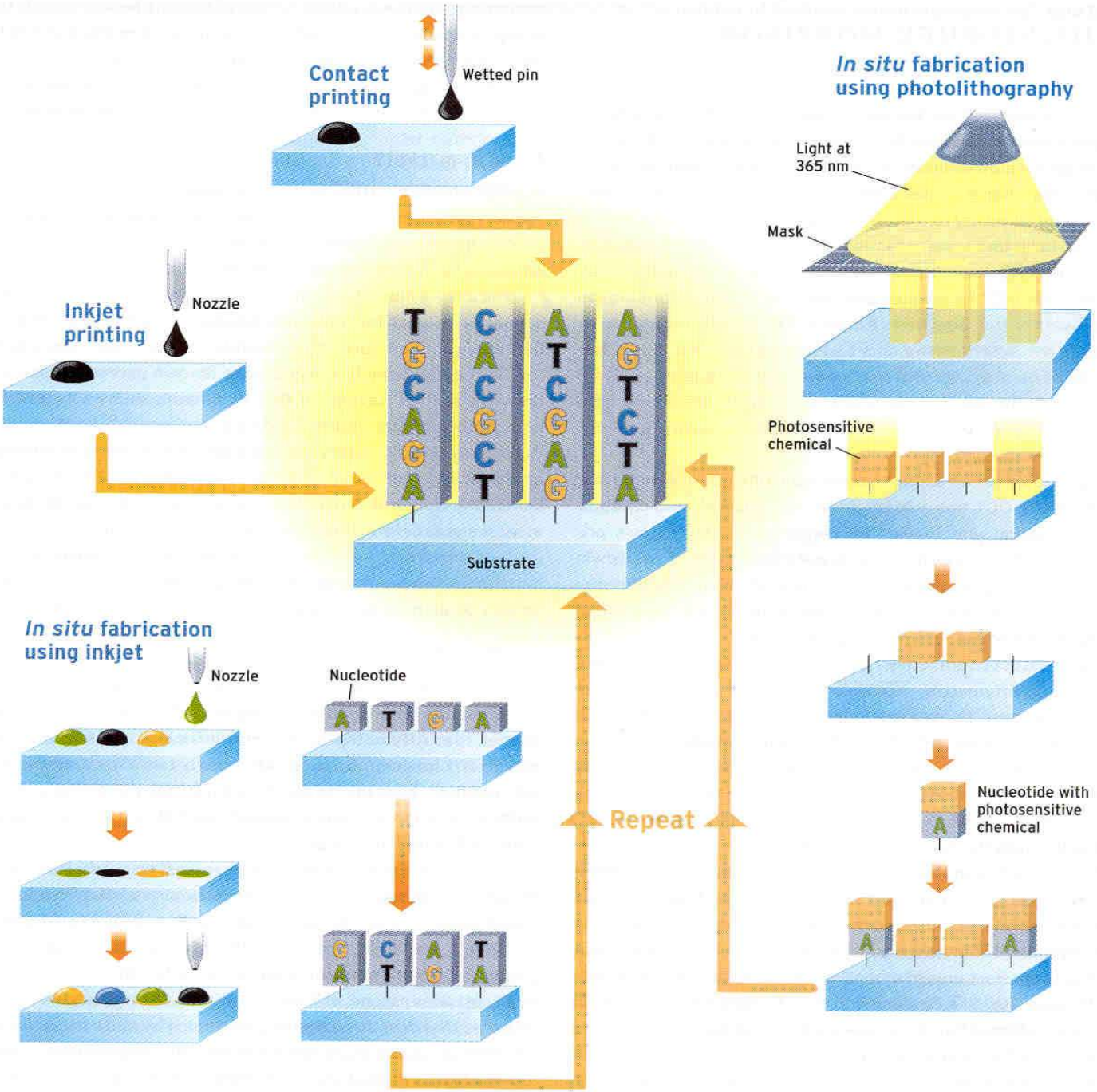
- **DNA Microarray**
- **DNA Chip**

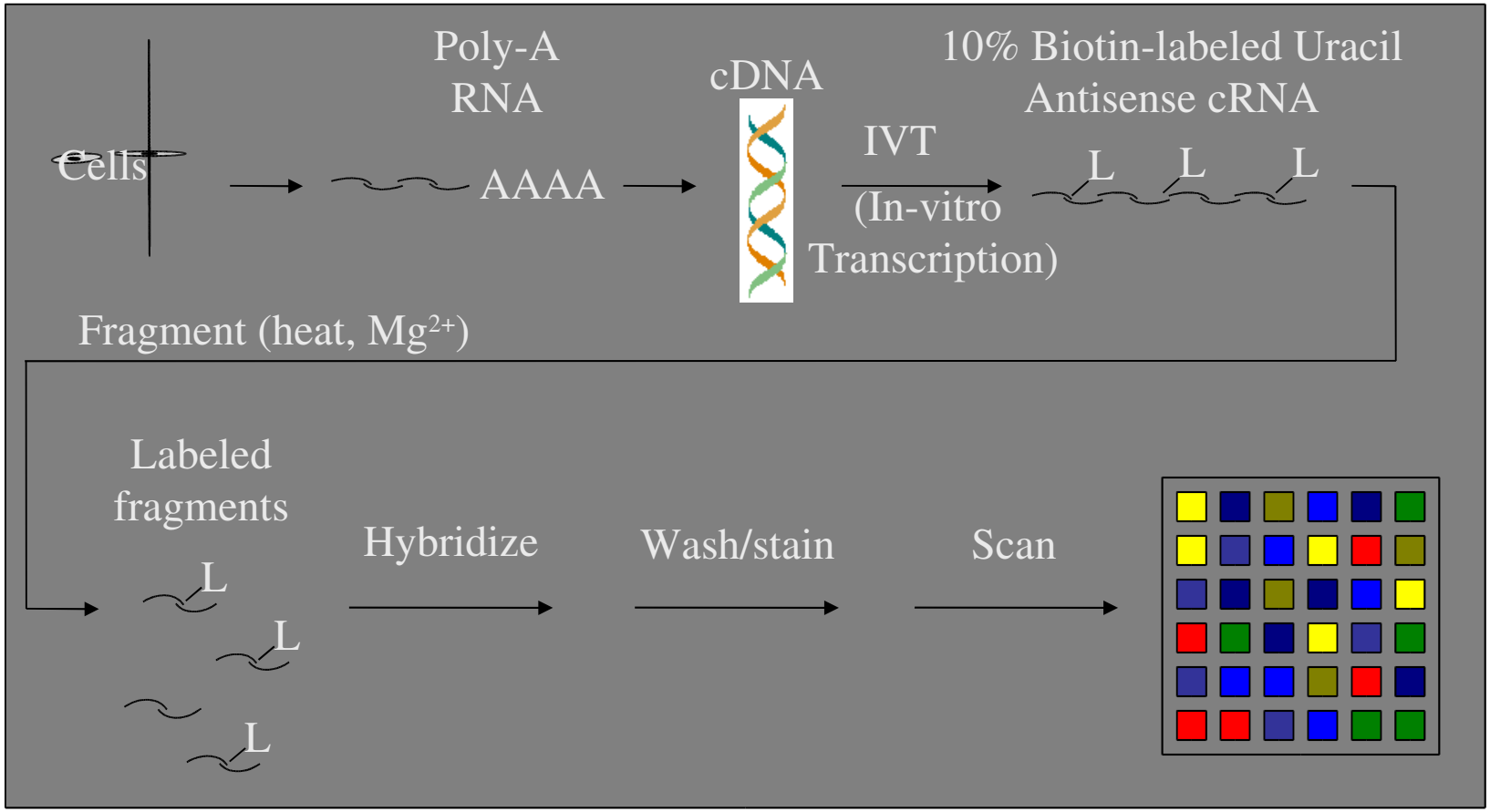
Kazde pole (sonda) nese tisice oligonukleotidu

Desticka o rozmerech 1.28 cm x 1.28+ cm, sklo, silikon

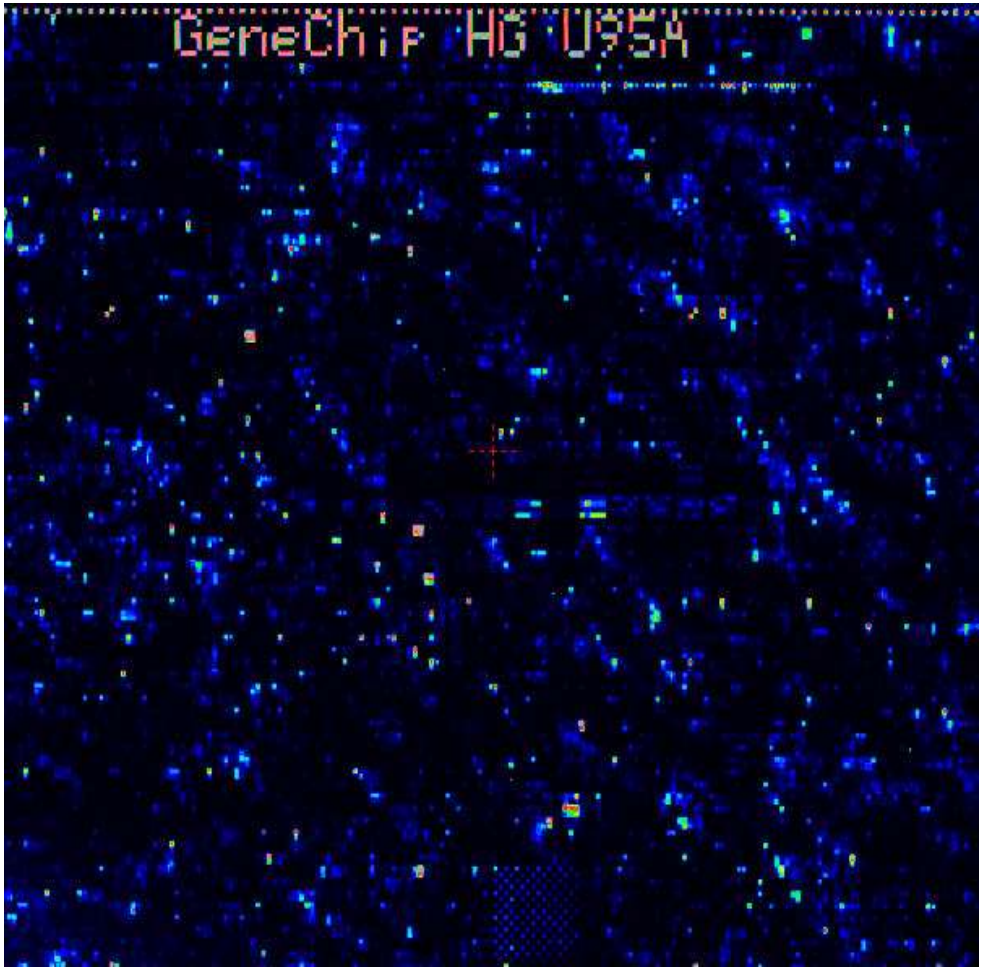
Desticky s nanasenymi oligonukleotidy maji pole asi 100 mikrometru, radove 10000 oligonukleotidovych sond o 60 bazi

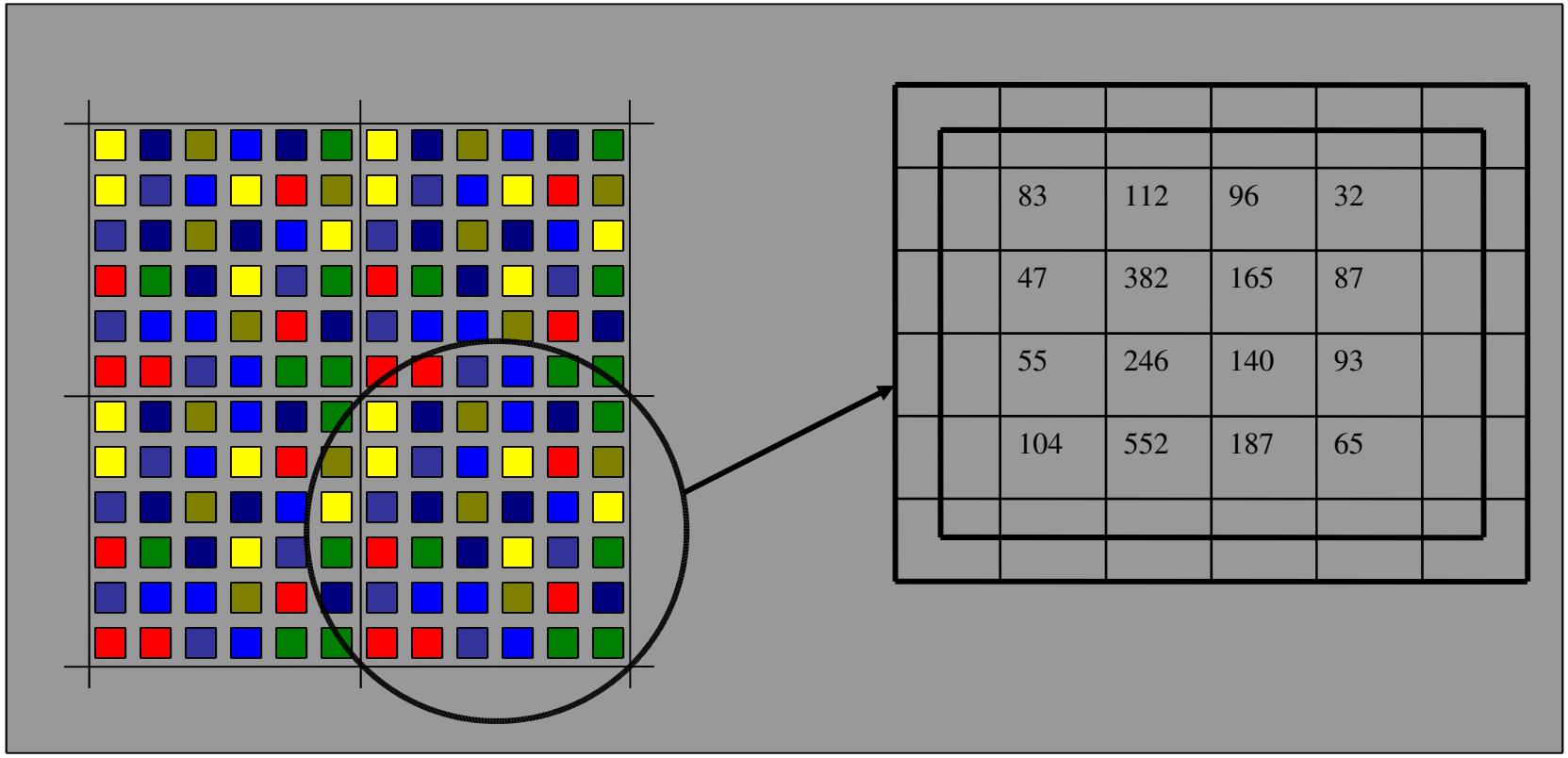
Desticky s oligonukleotidy syntetizovanymi na miste (DNA cipy Affymetrix) maji pole 25 mikrometru, radove 100000 sond 25 bazi


Contact printing
Wetted pin
Inkjet printing
Nozzle
TGCAGA
CACGCT
ATCGAG
AGTCTA
Substrate
In situ fabrication using photolithography
Light at 365 nm
Mask
Photosensitive chemical
Nucleotide with photosensitive chemical
Repeat
In situ fabrication using inkjet
Nozzle
Nucleotide


Cells
Poly-A
RNA
AAAA
cDNA
IVT
(In-vitro
Transcription)
10% Biotin-labeled Uracil
Antisense cRNA
L
L
L
Fragment (heat, Mg2+)
Labeled
fragments
L
L
L
Hybridize
Wash/stain
Scan

GENEARRAY™ SCANNER




83 112 96 32
47 382 165 87
55 246 140 93
104 552 187 65


SHE SAID HER GENOME HAS MORE COMBINATORY COMPLEXITY THAN MINE!

S jakou frekvencí se bude v dostatečně dlouhé náhodné sekvenci DNA vyskytovat sekvence AAGT?

S jakou frekvencí se bude v dostatečně dlouhé molekule DNA vyskytovat sekvence AAGT, pokud víme, že obsah C + G je 2/3 (cca 66%)?

S jakou frekvencí se bude v dostatečně velkém genomu vyskytovat sekvence AAGT, pokud víme, že obsah C+G je 2/3 (cca 66%)?

central dogma
genome
DNA
nucleotide
3’ / 5’
hybridization
replication
DNA polymerase
vector
plasmid
sequence alignment
PCR
DNA sequence
contig

gene
ORF
gene structure
promoter
intron
exon
prokaryote
ATG
GC content
eukaryote
TATA
enhancer
silencer

RNA
transcription
mRNA
gene expression
microarray
probe
EST
translation
codon
protein
proteome
mass spectrometry
signal transduction