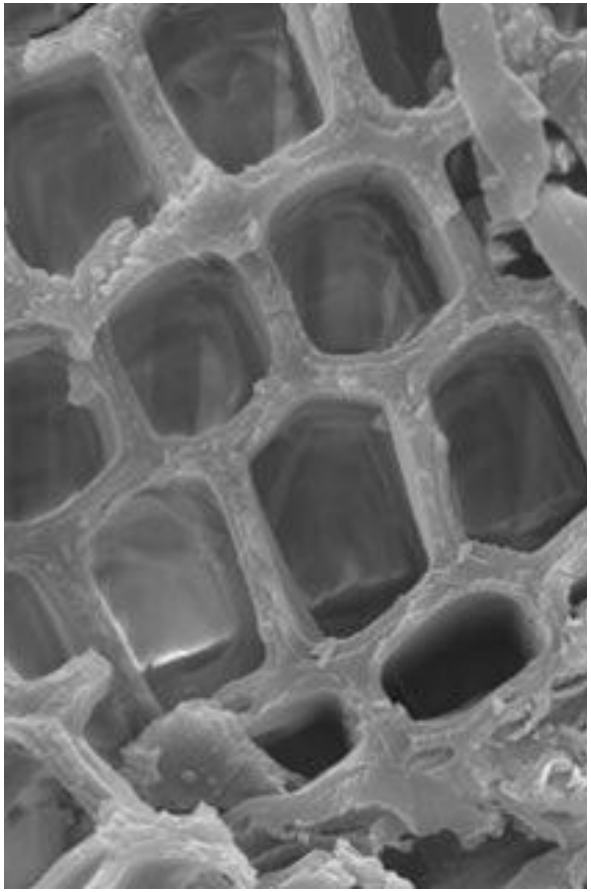
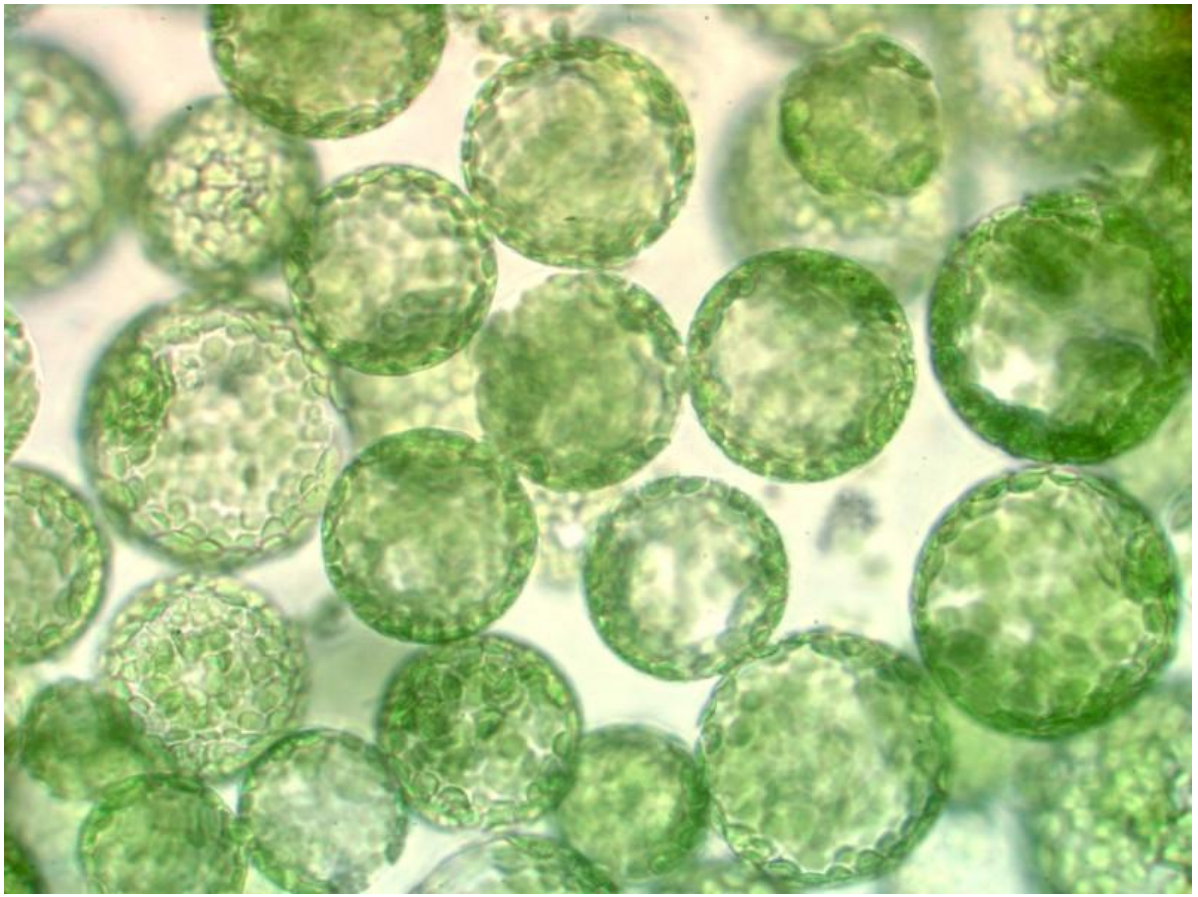
Трансгенные растения

Что нужно, чтобы получить трансгенное растение?

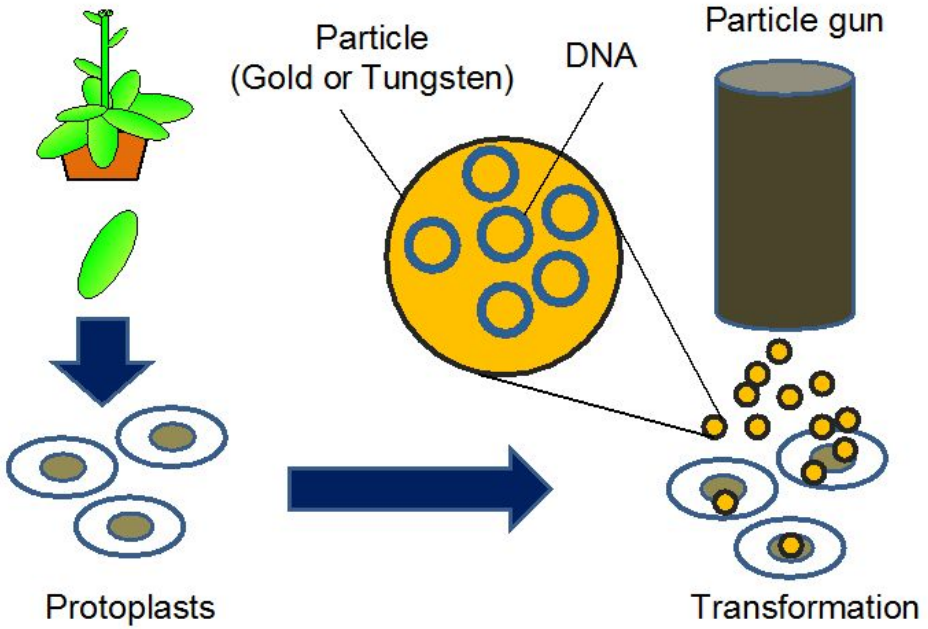
Чужеродная ДНК должна проникнуть в клетку

«Избавление» от клеточной стенки

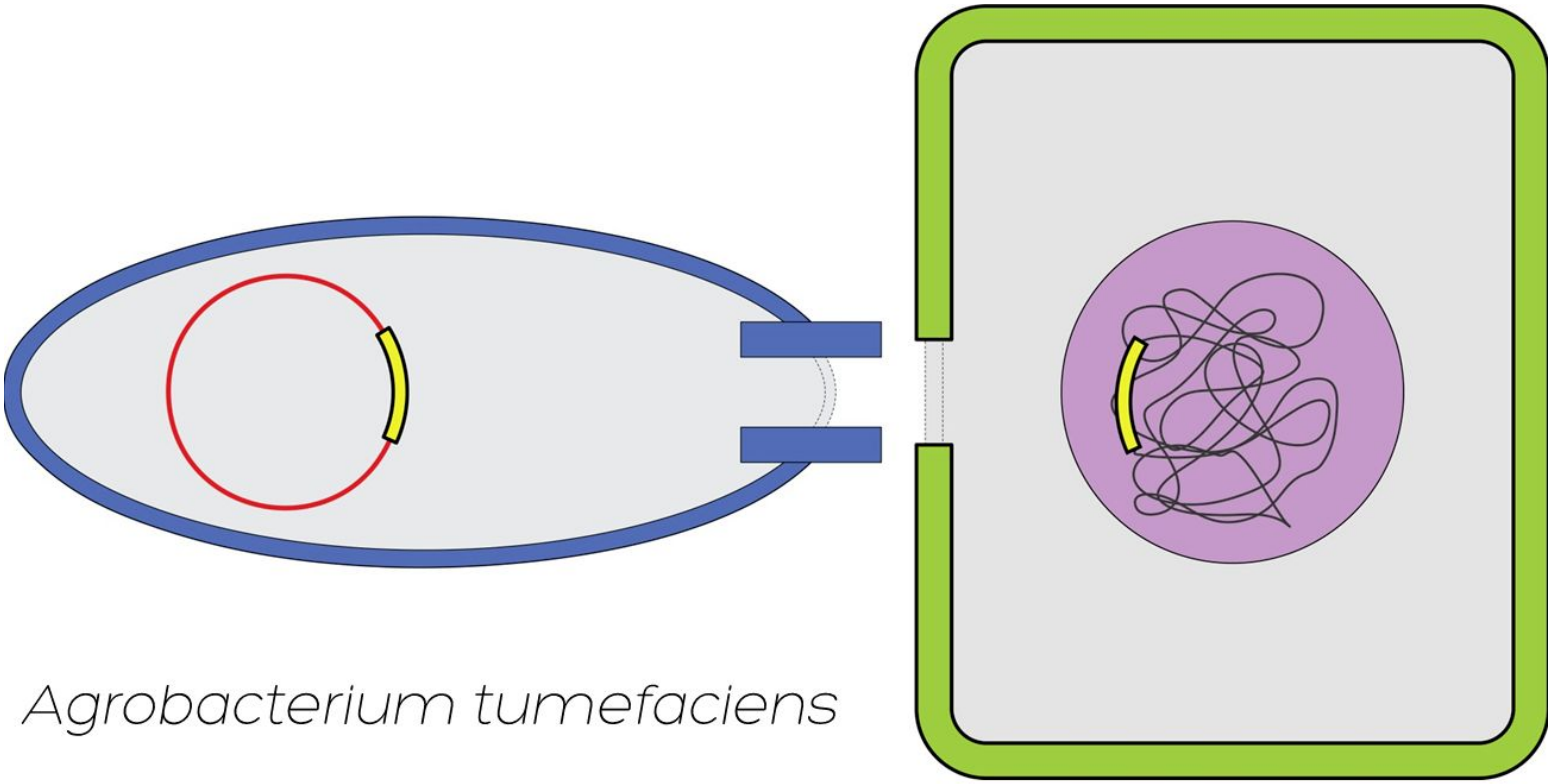




Бомбардировка клеток



Использование потенциала фитопатогенов



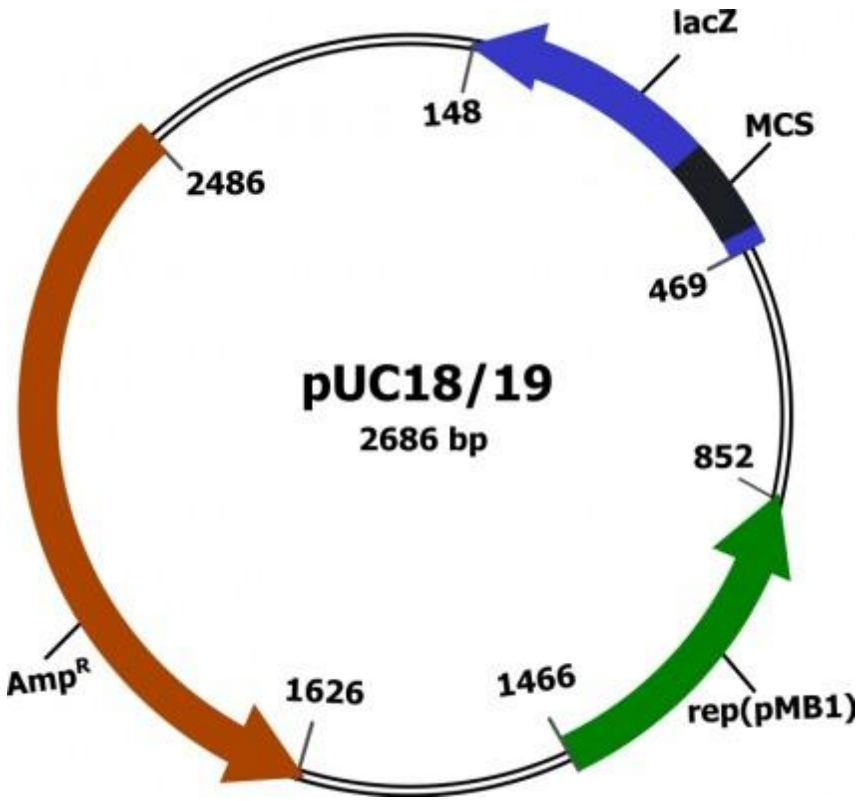
Agrobacterium tumefaciens

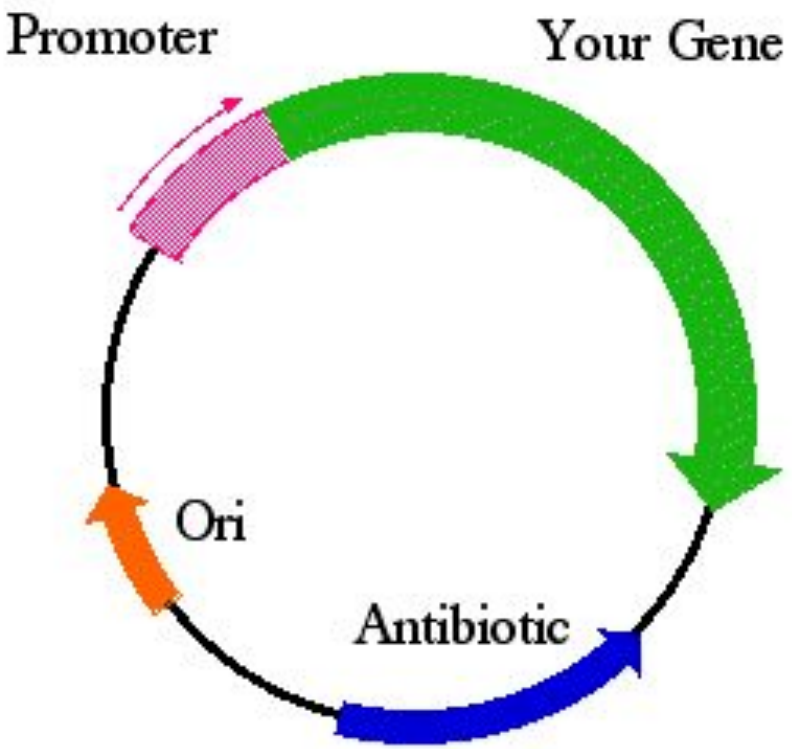
Нужно чтобы целевой участок ДНК:

Встроился в геном

Экспрессировался (чтобы генетическая информация считывалась)

Чужеродные гены находятся в составе векторных плазмид

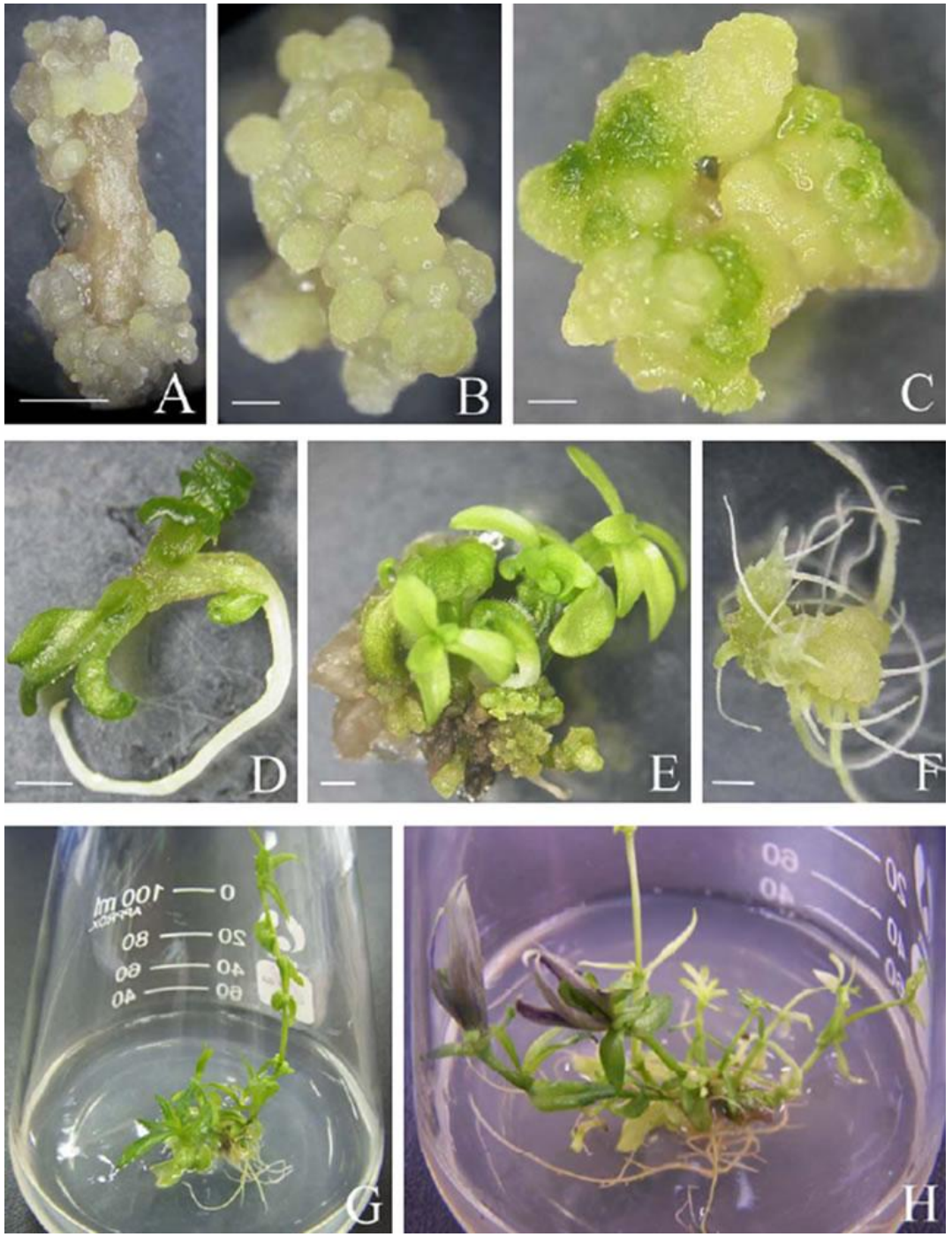




Введение векторных конструкций в клетки растений

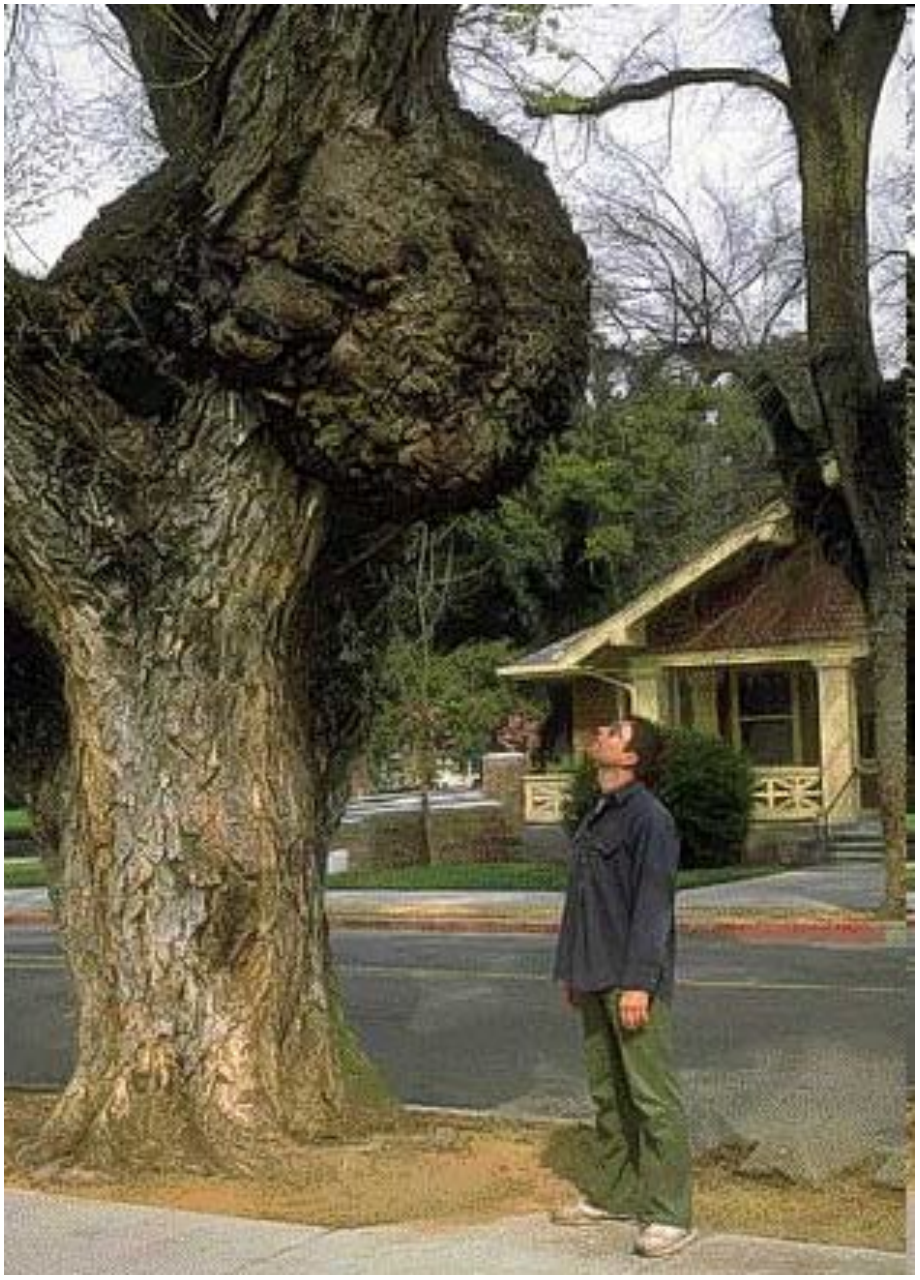
Станет ли после этого растение трансгенным?

Регенерация целых растений из отдельных трансформированных клеток

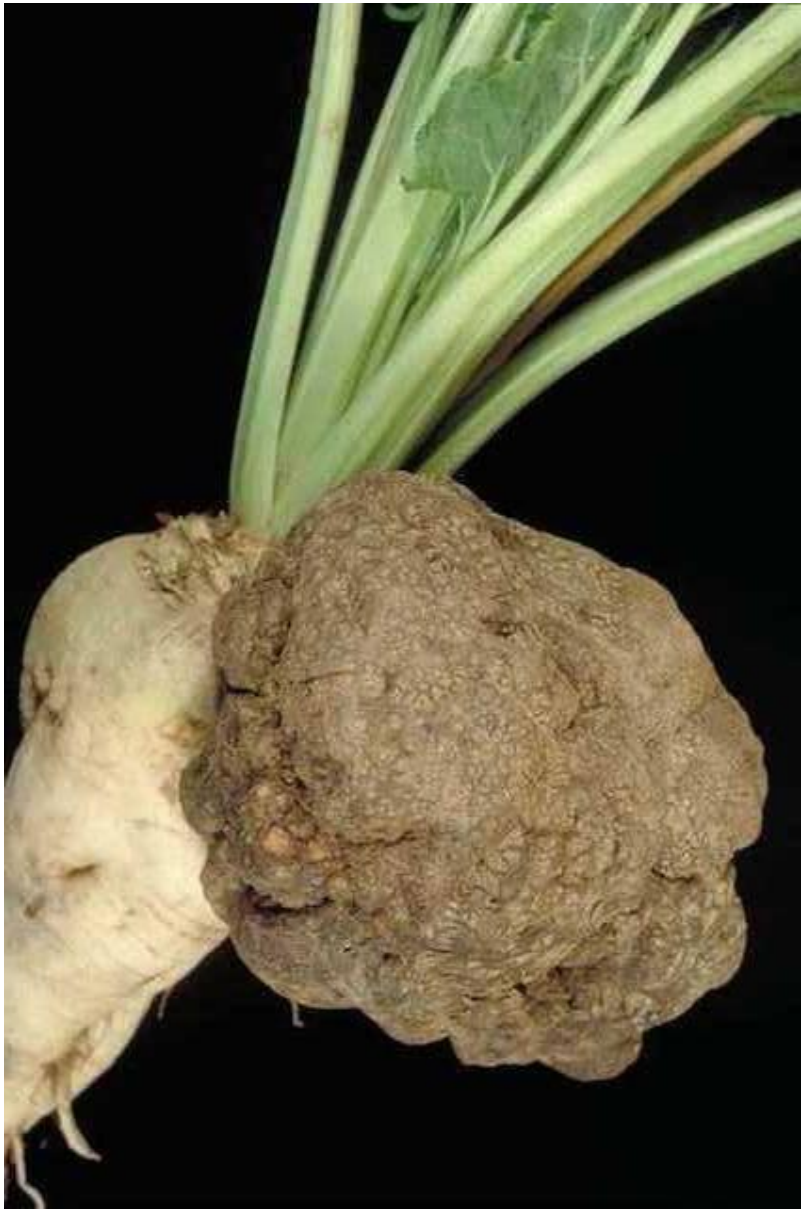


Основной подход для создания трансгенных растений

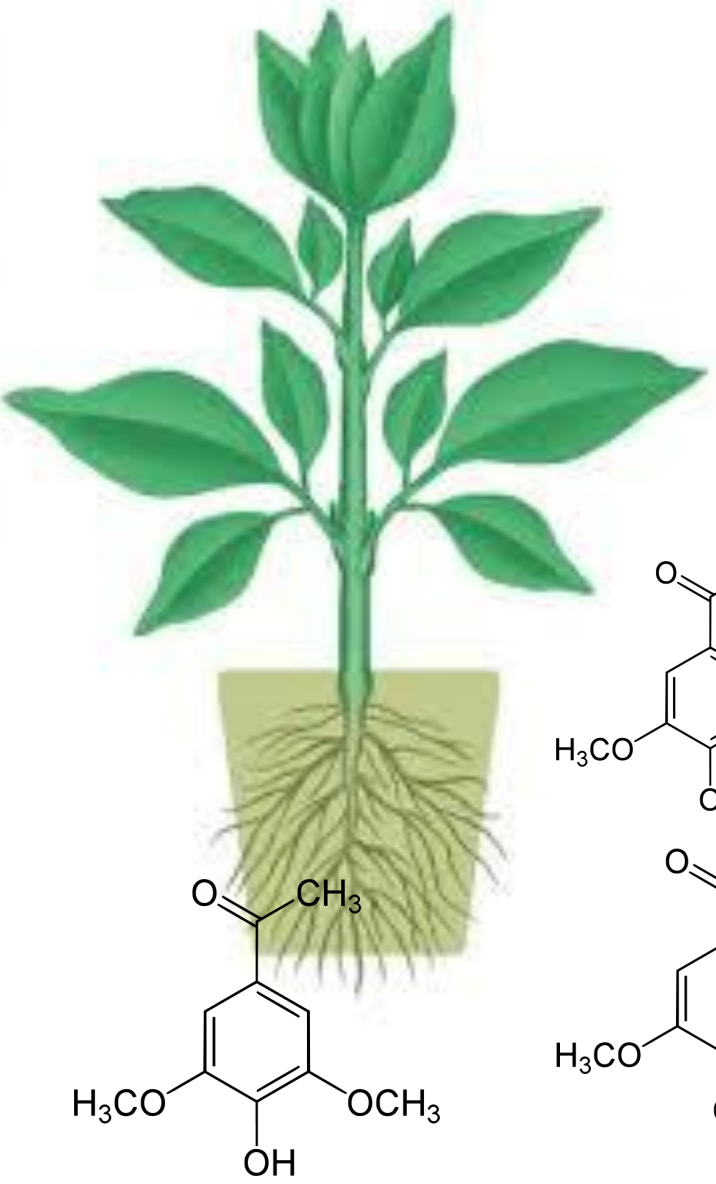
Корончатые галлы

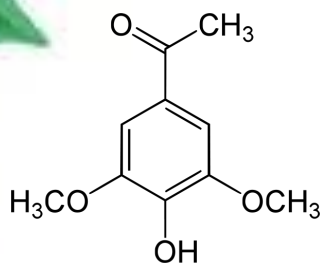


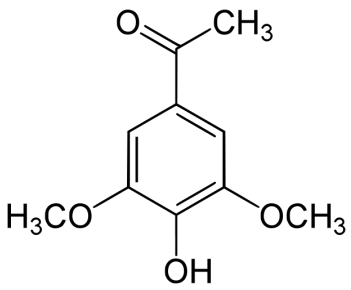
Корончатые галлы

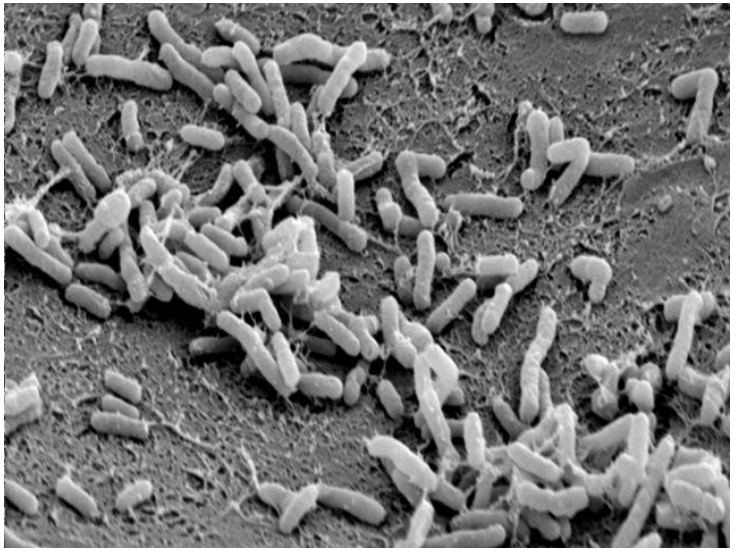


Agrobacterium tumifaciens

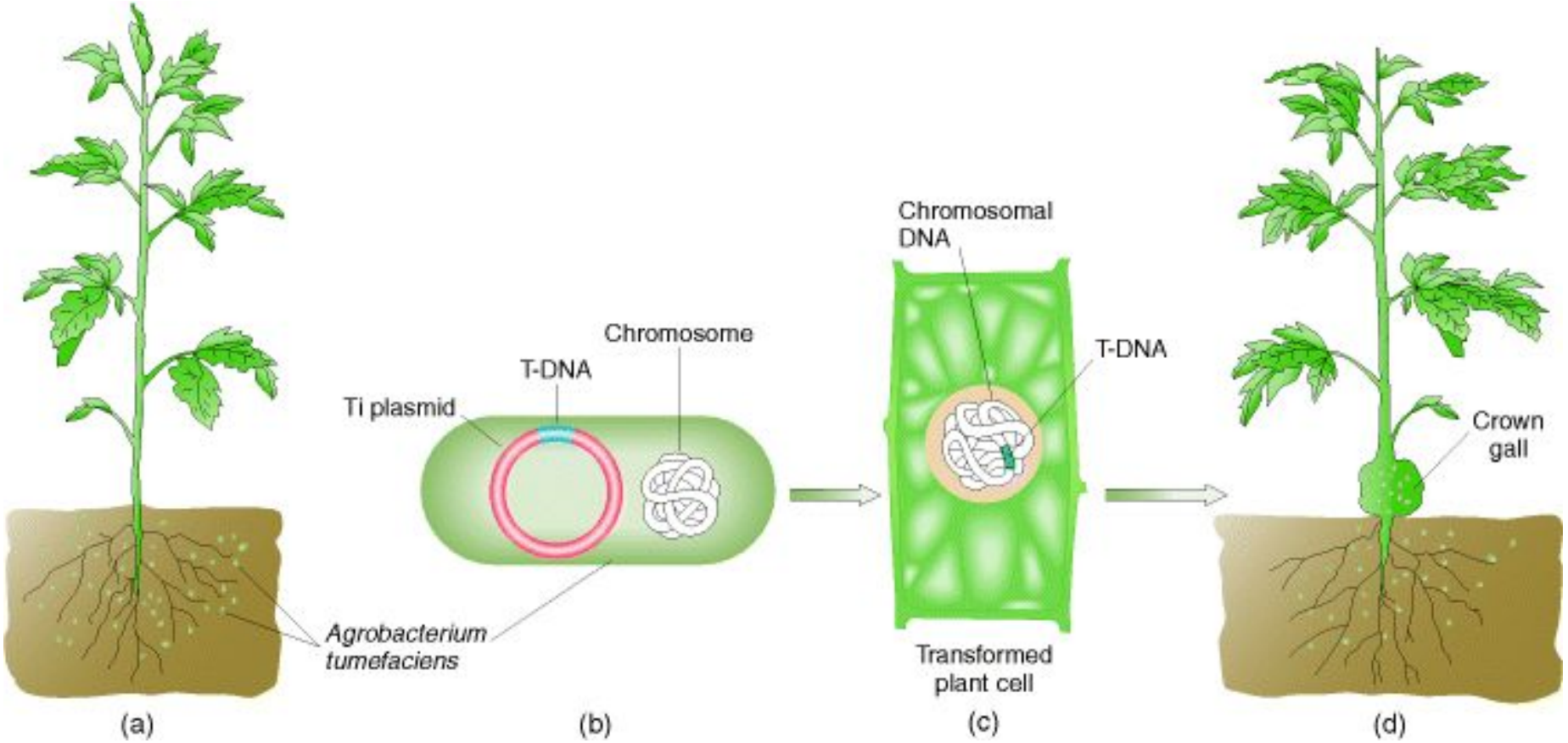




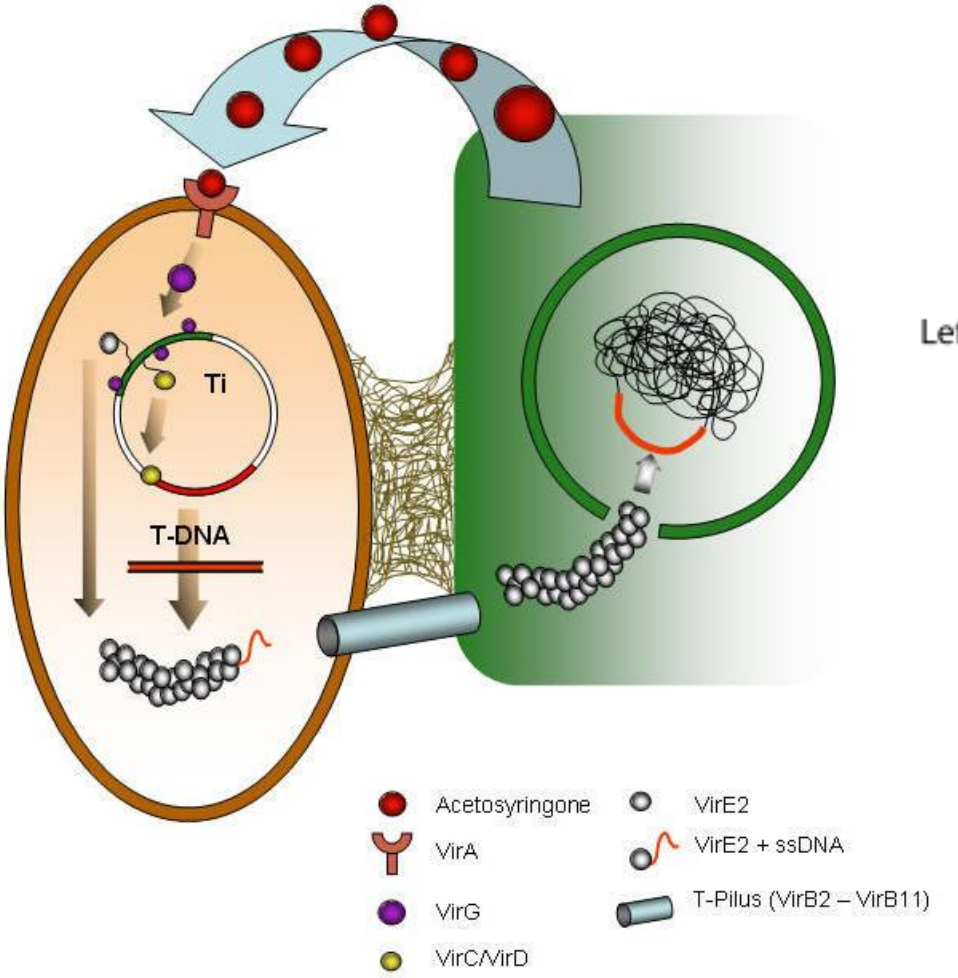


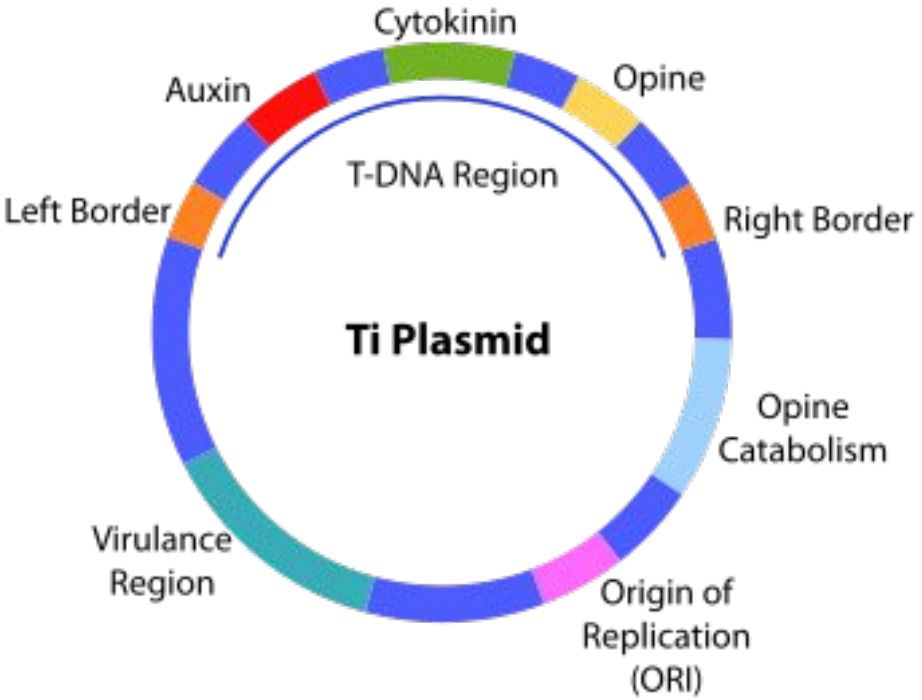


Agrobacterium tumifaciens

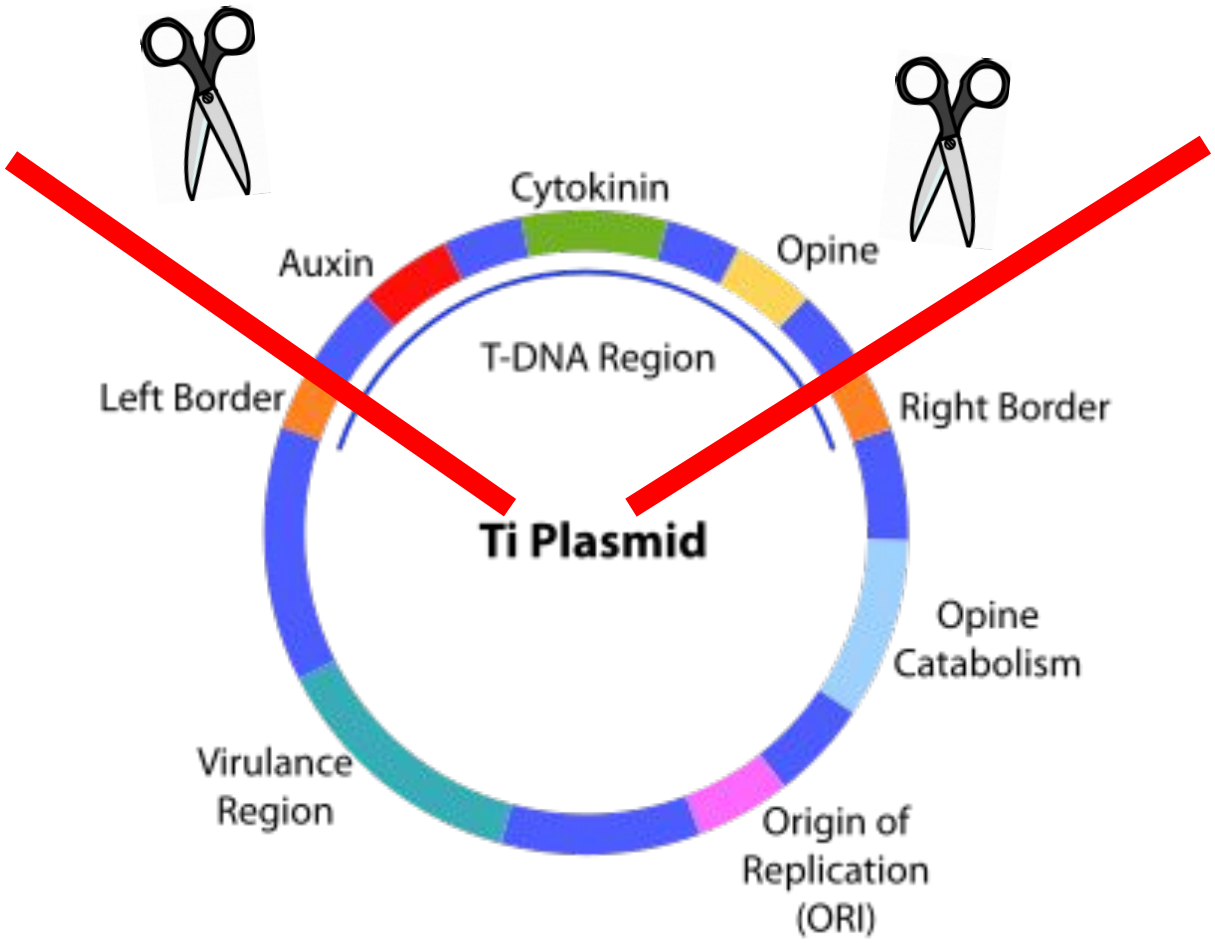


Интеграция тДНК в геном растения

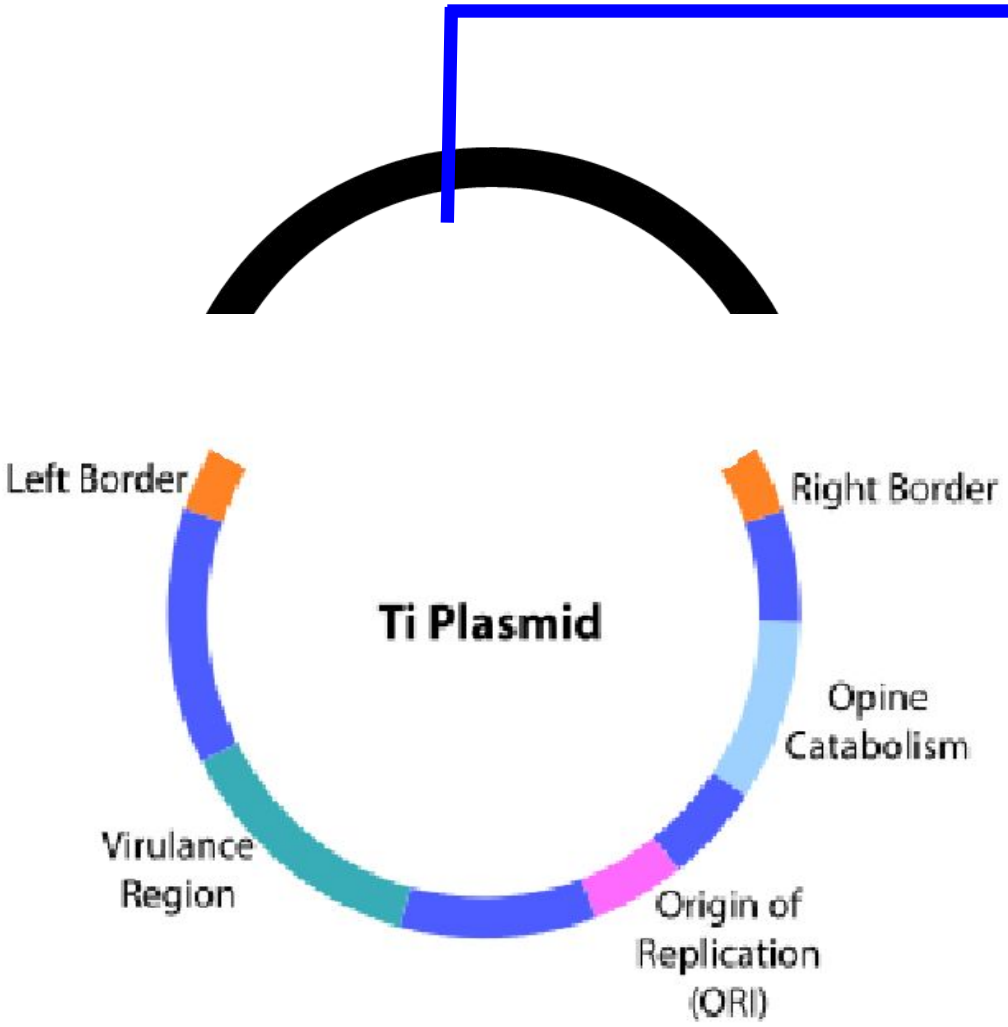




Модификация Ti – плазмиды для создания трансгенных растений



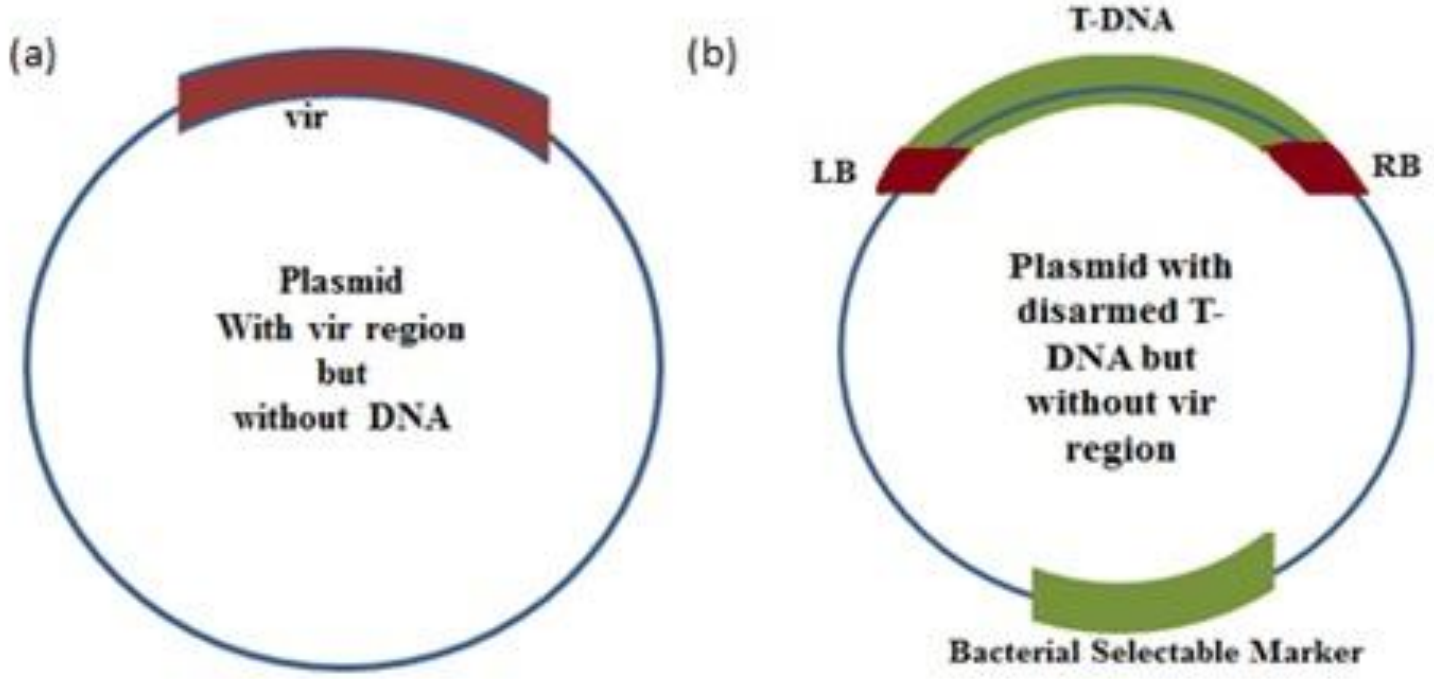
Модификация Ti – плазмиды для создания трансгенных растений

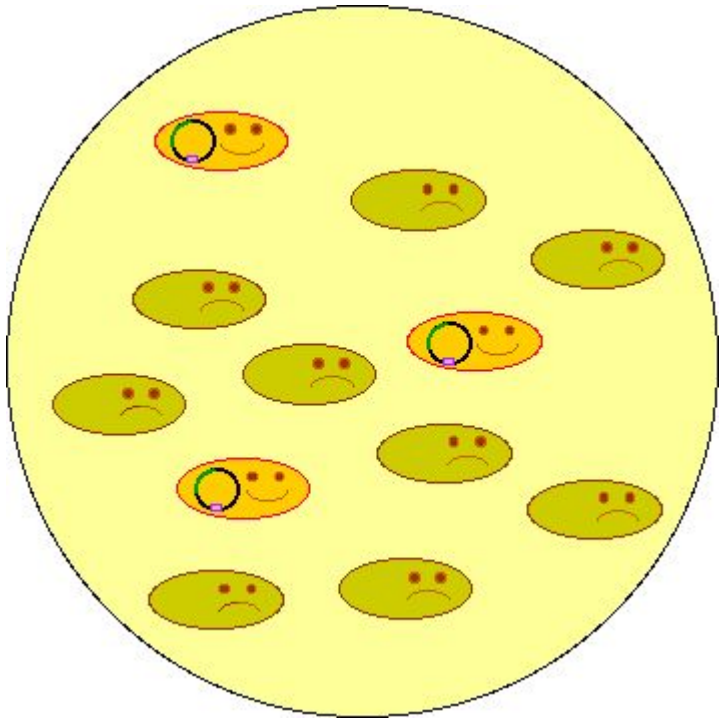


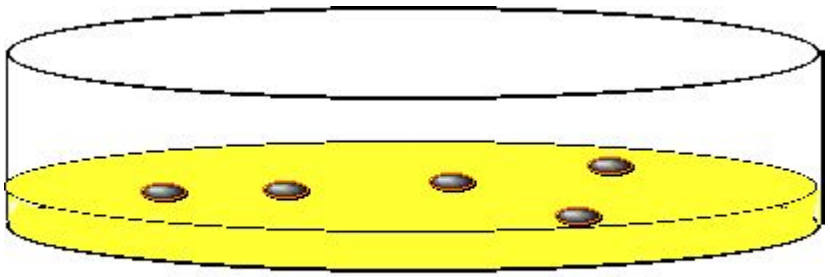
Ген, который хотят встроить

Селективный маркер

Бинарный вектор



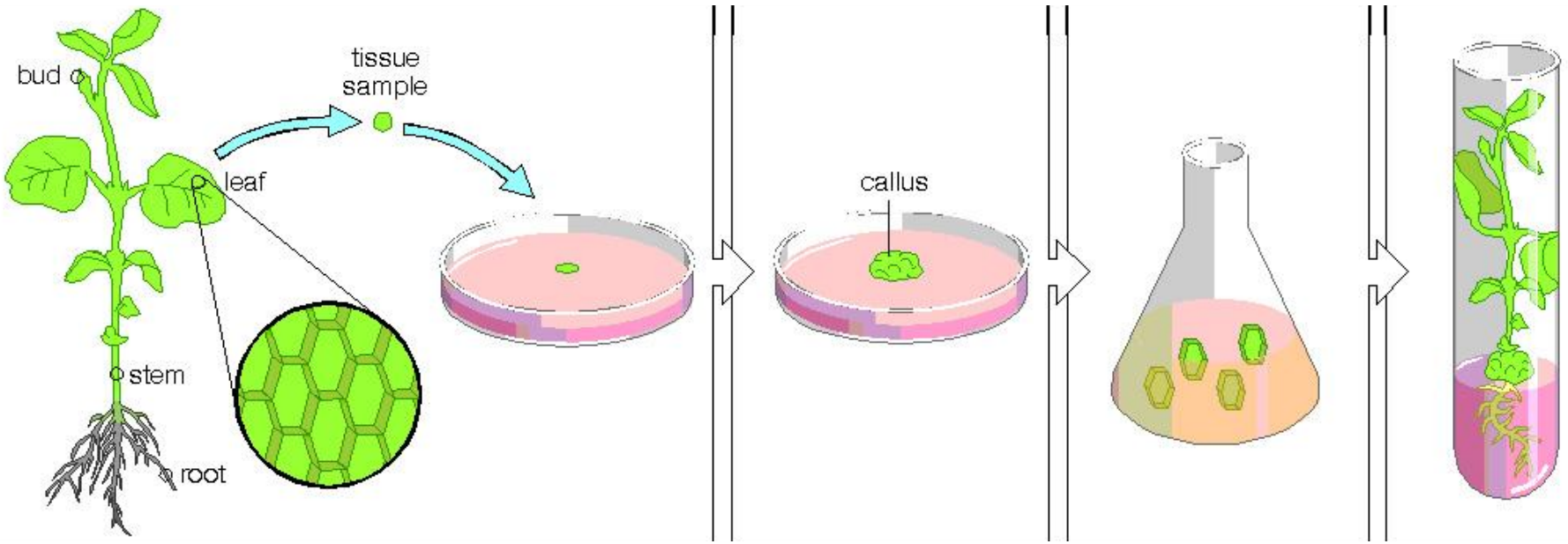




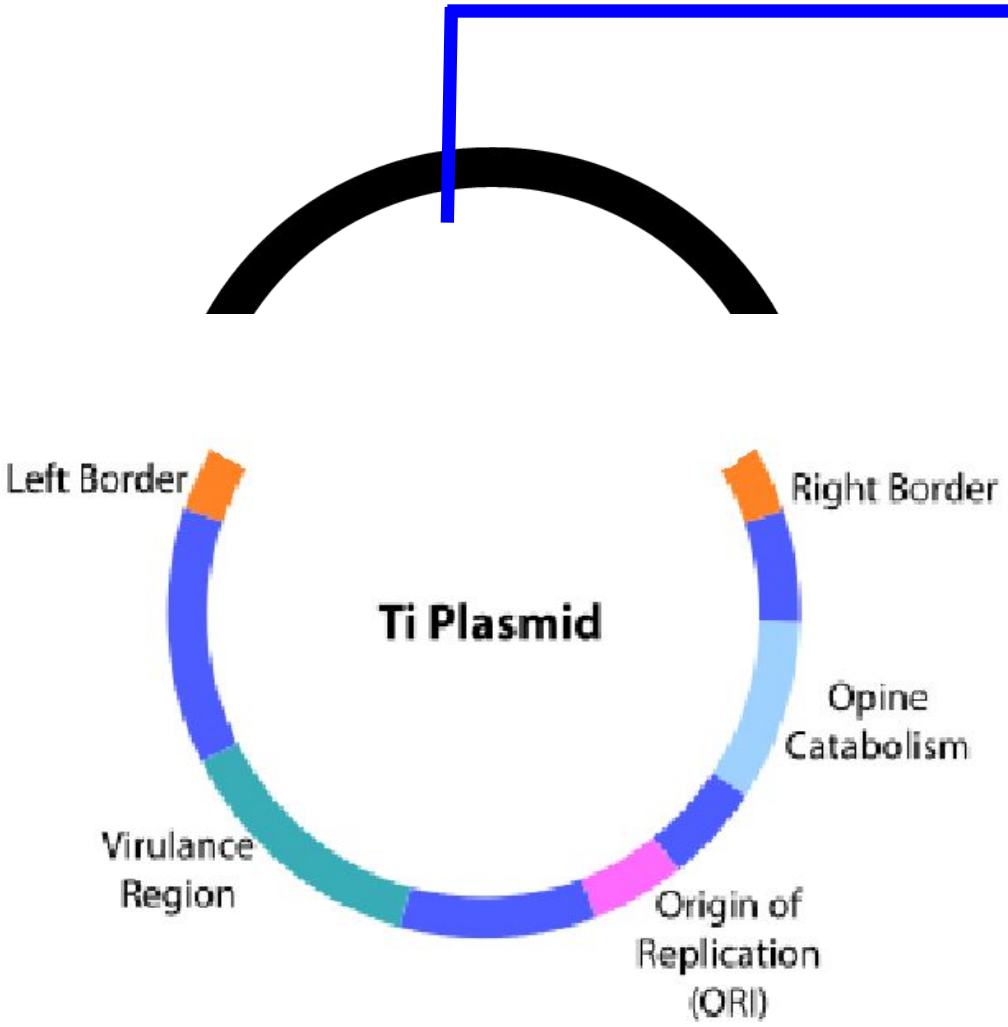
agar medium with antibiotic

Введение векторных конструкций в клетки растений

Регенерация целых растений из отдельных трансформированных клеток



Модификация Ti – плазмиды для создания трансгенных растений



Ген, который хотят встроить

Селективный маркер

Откуда берут целевой фрагмент ДНК, который хотят встроить в растительный геном?

ПЦР (полимеразная цепная реакция) – метод селективной амплификации участка ДНК *in vitro*

Компоненты реакции:

Вода

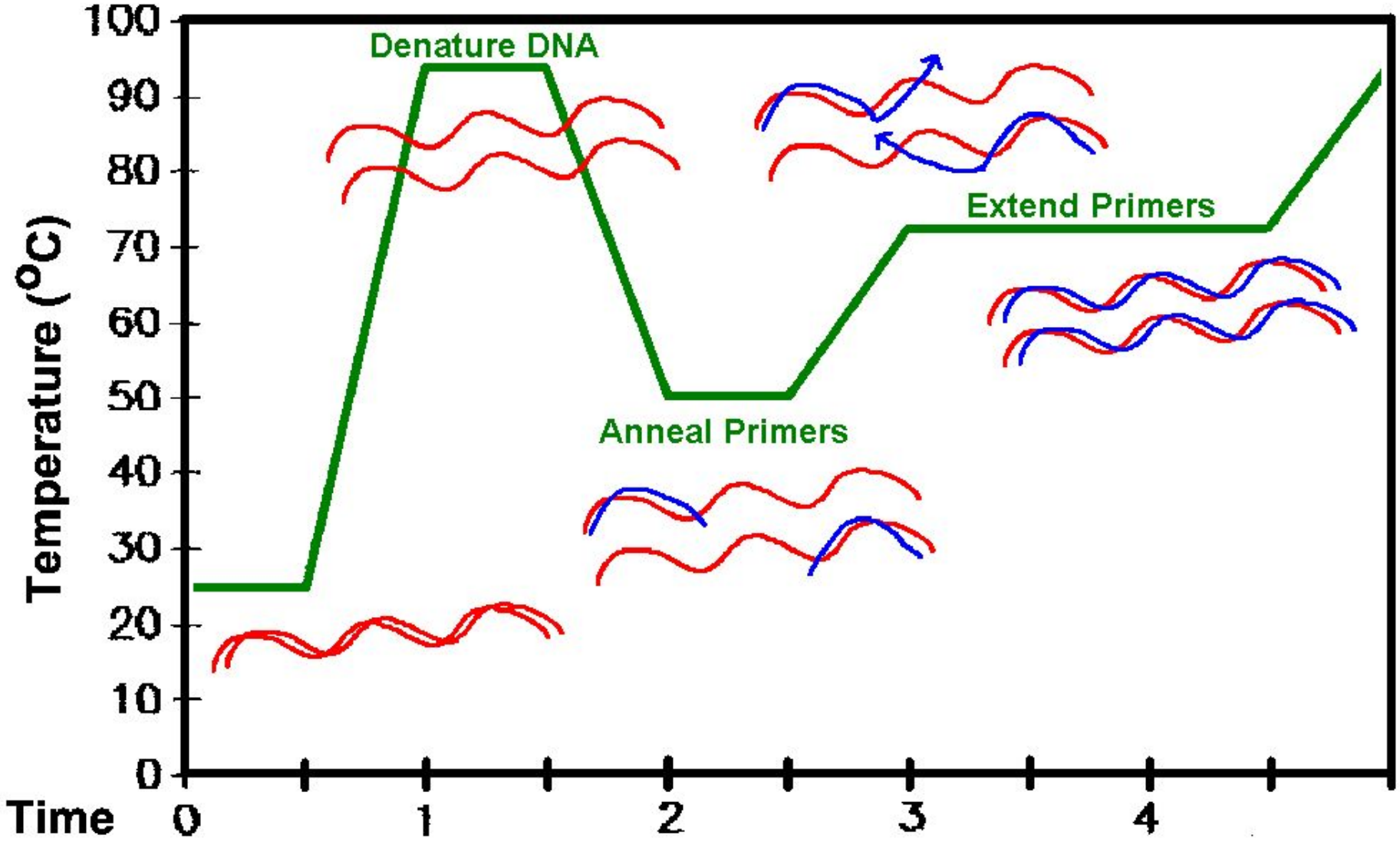
Буфер для фермента (Taq-полимеразы)

Дезоксинуклеотид фосфаты

Праймеры (прямой и обратный)

Фермент (Taq-полимераза)

ДНК-матрица


Denature DNA
Anneal Primers
Extend Primers
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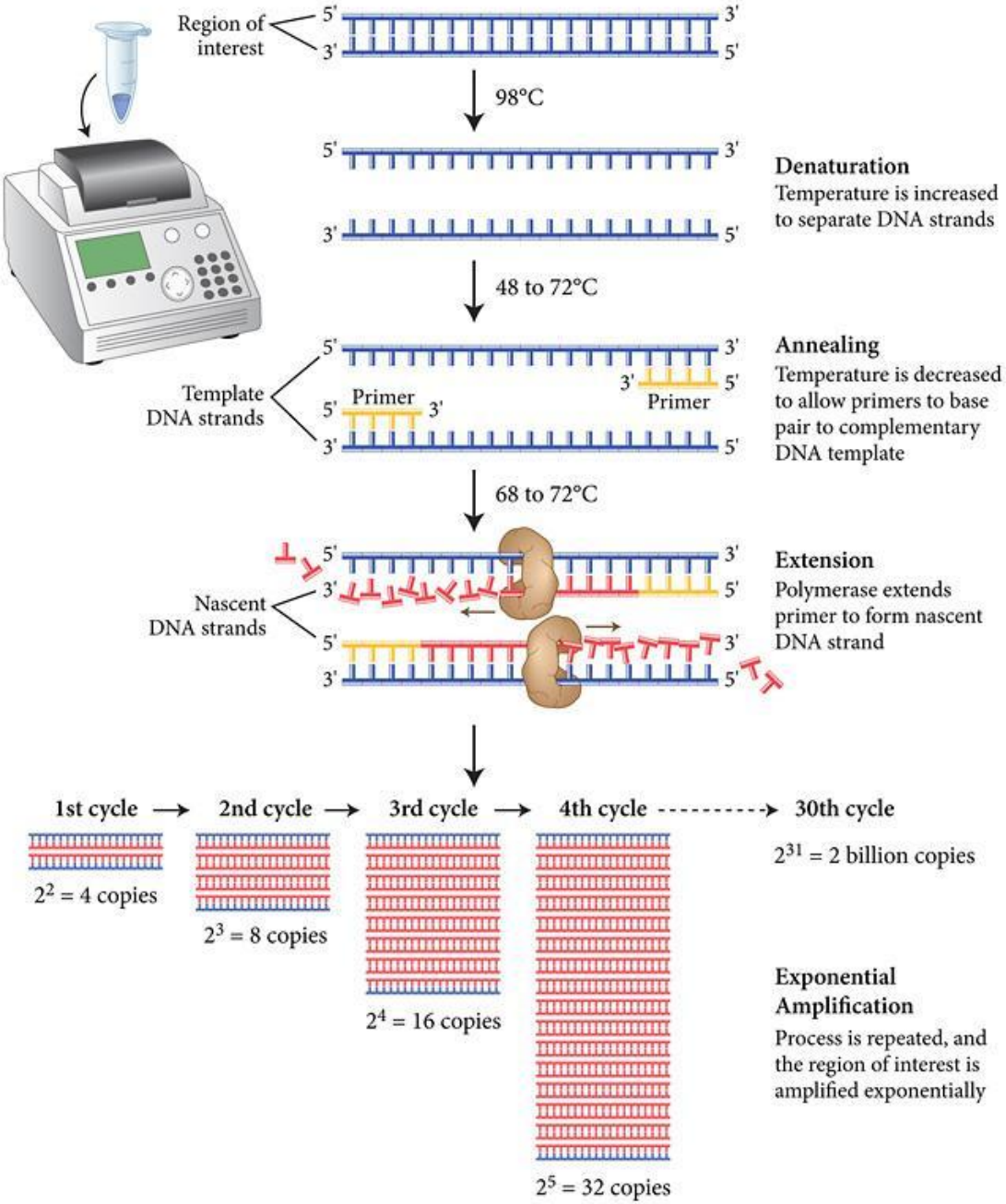
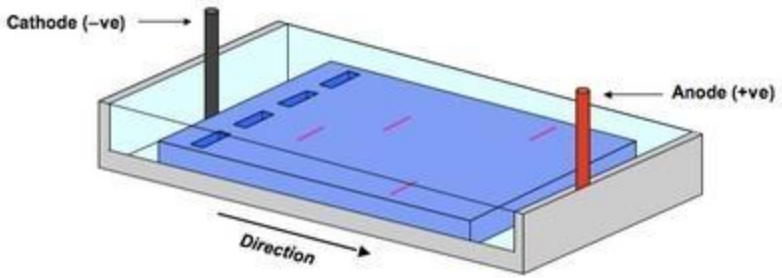
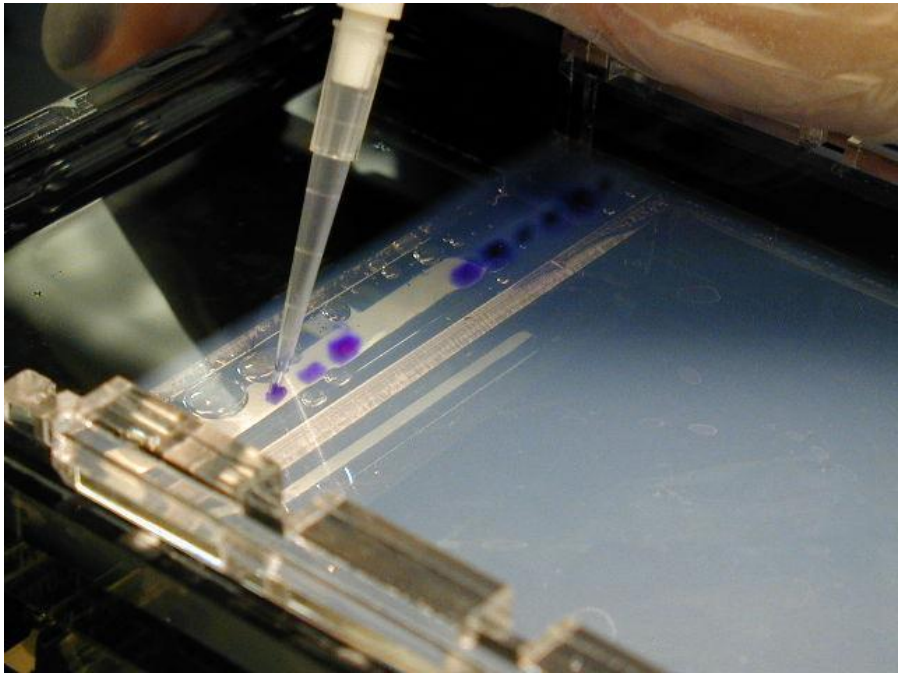
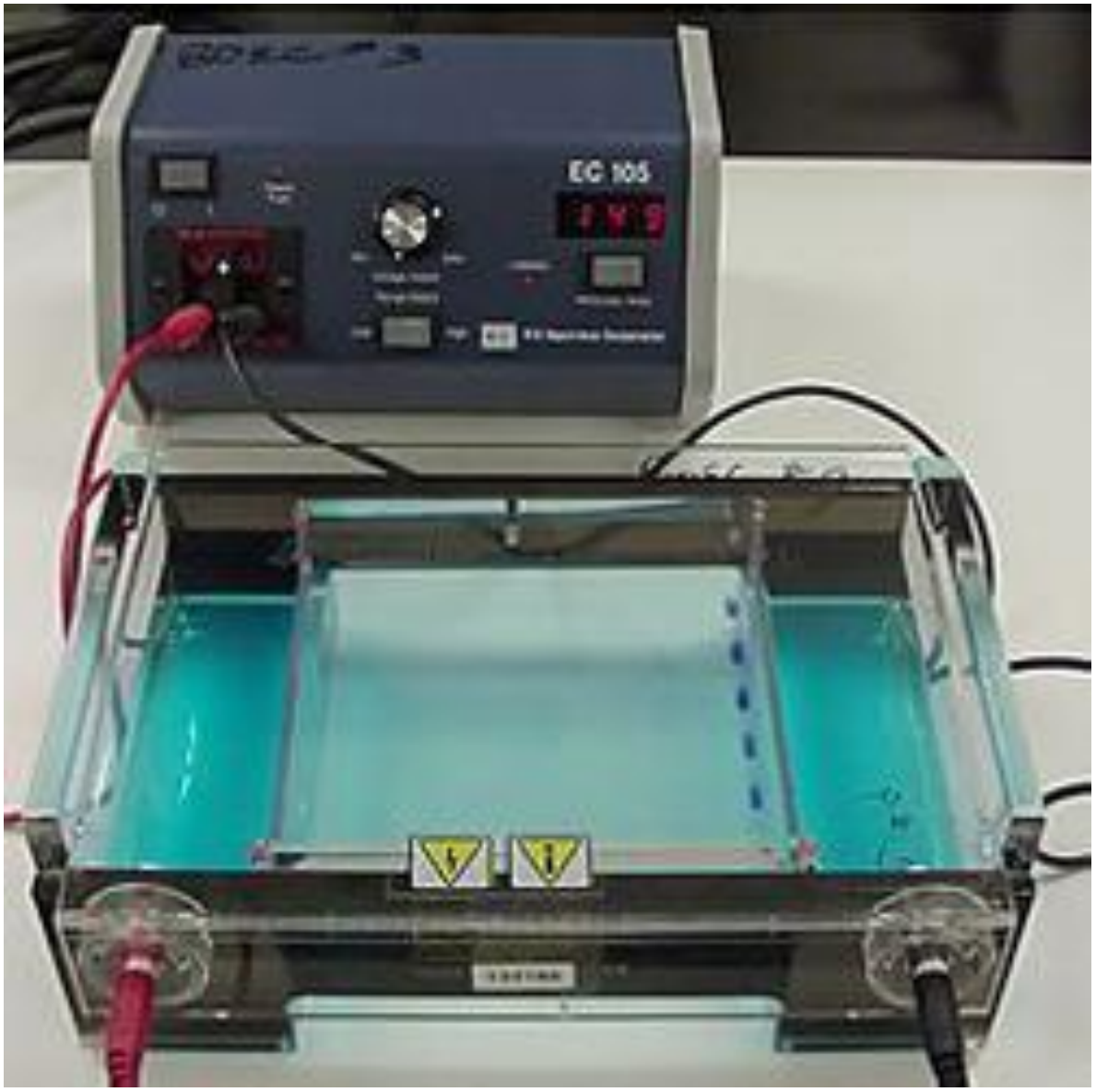
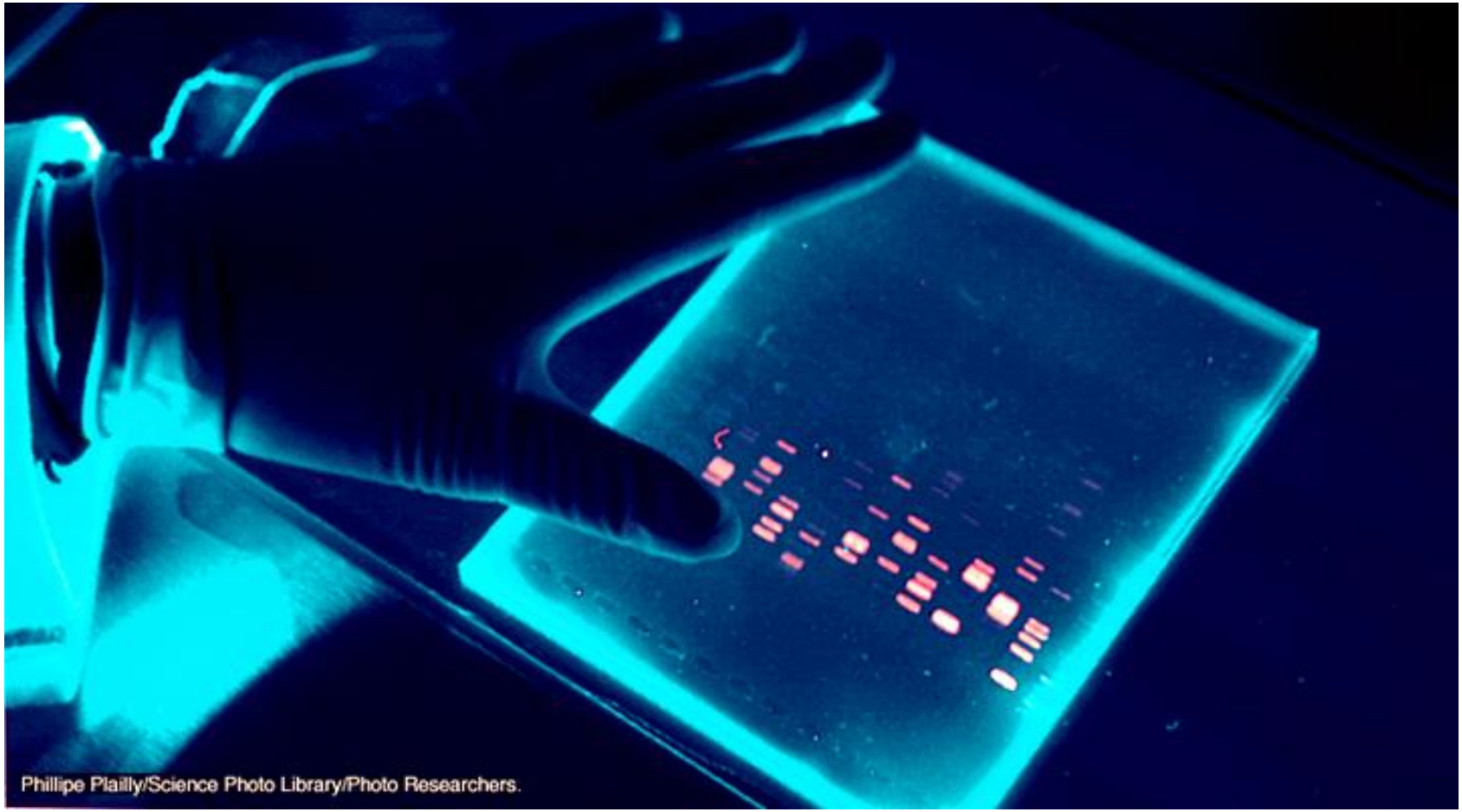
90
80
70
60
50
40
30
20
10
0
Time
0
1
2
3
4


Region of interest
5'
3'
3'
5'
98°C
Denaturation
Temperature is increased to separate DNA strands
48 to 72°C
Annealing
Temperature is decreased to allow primers to base pair to complementary DNA template
Template DNA strands
Primer
Primer
68 to 72°C
Extension
Polymerase extends primer to form nascent DNA strand
Nascent DNA strands
1st cycle
2nd cycle
3rd cycle
4th cycle
30th cycle
2^2 = 4 copies
2^3 = 8 copies
2^4 = 16 copies
2^5 = 32 copies
2^{31} = 2 billion copies
Exponential Amplification
Process is repeated, and the region of interest is amplified exponentially

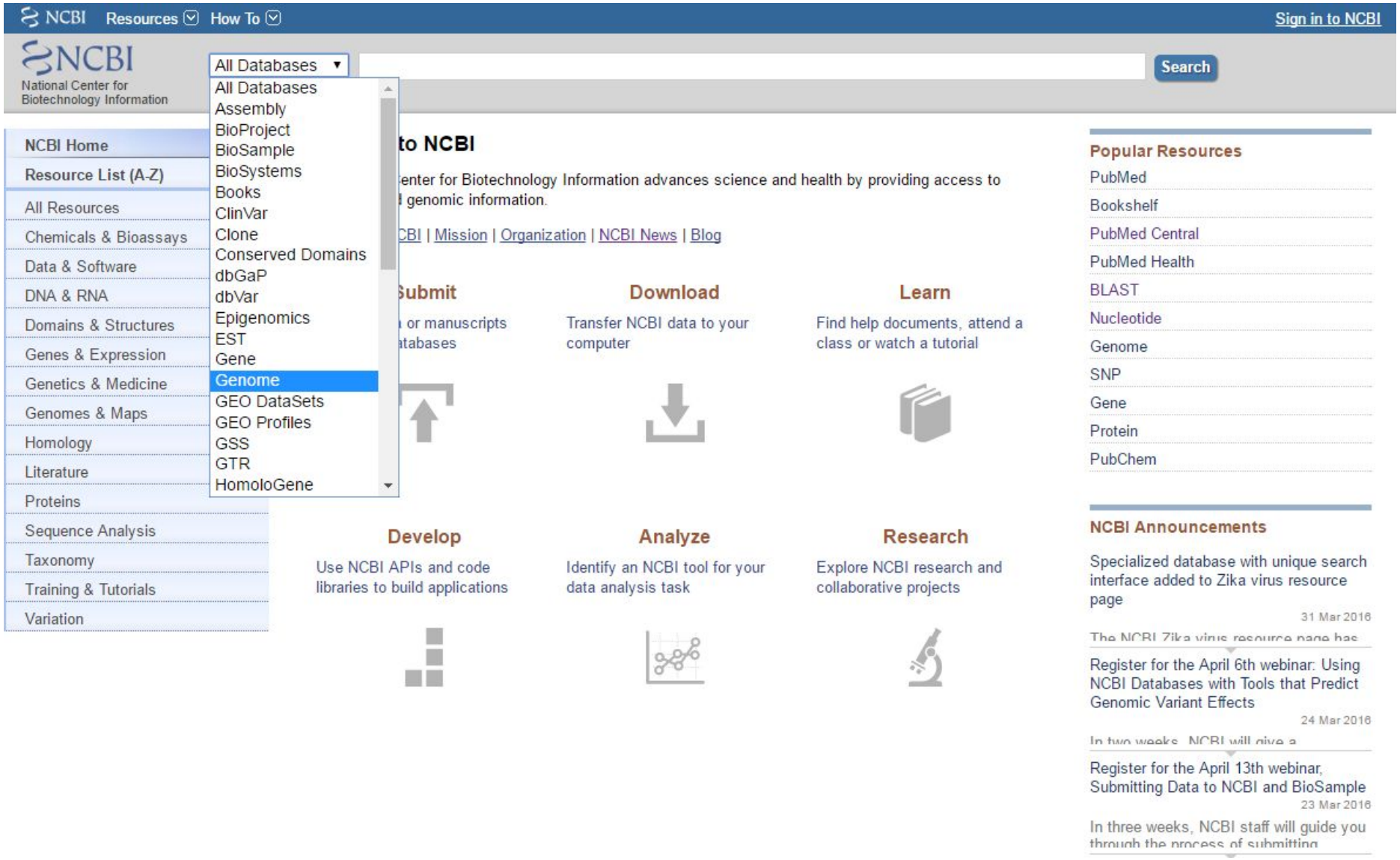

Cathode (–ve)
Anode (+ve)
Direction




EC 105


Phillipe Plailly/Science Photo Library/Photo Researchers.

Конструирование праймеров


NCBI
Resources
How To
Sign in to NCBI
NCBI
National Center for Biotechnology Information
All Databases
Search
All Databases
Assembly
BioProject
BioSample
BioSystems
Books
ClinVar
Clone
Conserved Domains
dbGaP
dbVar
Epigenomics
EST
Gene
Genome
GEO DataSets
GEO Profiles
GSS
GTR
HomoloGene
NCBI Home
Resource List (A-Z)
All Resources
Chemicals & Bioassays
Data & Software
DNA & RNA
Domains & Structures
Genes & Expression
Genetics & Medicine
Genomes & Maps
Homology
Literature
Proteins
Sequence Analysis
Taxonomy
Training & Tutorials
Variation
to NCBI
enter for Biotechnology Information advances science and health by providing access to
genomic information.
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PubMed Health
BLAST
Nucleotide
Genome
SNP
Gene
Protein
PubChem
NCBI Announcements
Specialized database with unique search interface added to Zika virus resource page
31 Mar 2016
Register for the April 6th webinar: Using NCBI Databases with Tools that Predict Genomic Variant Effects
24 Mar 2016
Register for the April 13th webinar, Submitting Data to NCBI and BioSample
23 Mar 2016
In three weeks, NCBI staff will guide you

FASTA Send:

Erwinia carotovora subsp. atroseptica SCRI1043, complete genome

NCBI Reference Sequence: NC_004547.2

GenBank Graphics

>gi|50118965|ref|NC_004547.2| Erwinia carotovora subsp. atroseptica SCRI1043, complete genome

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Change region shown

Customize view

Analyze this sequence

Run BLAST
Pick Primers
Highlight Sequence Features

Related information

Assembly
BioProject
BioSample
Components (Core)
Full text in PMC
Gene
Genome
Identical GenBank Sequence
Probe
Protein
PubMed (Weighted)
Taxonomy

LinkOut to external resources

SCRI 1043 (strain passport) [StrainInfo]
Order NAPG cDNA clone/Protein/Antibody/RNAi

GenBank ▾ Send: ▾

Erwinia carotovora subsp. atroseptica SCRI1043, complete genome

NCBI Reference Sequence: NC_004547.2

FASTA Graphics

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VERSION     NC_004547.2  GI:50118965
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            Assembly: GCF_000011605.1
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SOURCE      Pectobacterium atrospecticum SCRI1043
  ORGANISM  Pectobacterium atrospecticum SCRI1043
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REFERENCE   1  (bases 1 to 13677)
  AUTHORS   Bell,K.S., Sebaihia,M., Pritchard,L., Holden,M., Hyman,L.J.,
            Holeva,M.C., Thomson,N.R., Bentley,S.D., Churcher,C., Mungall,K.,
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            Norbertczak,H., Ormond,D., Price,C., Quail,M.A., Sanders,M.,
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            Barrell,B.G., Parkhill,J. and Toth,I.K.
  TITLE     The genome sequence of the enterobacterial phytopathogen Erwinia
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  JOURNAL   Unpublished
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  AUTHORS   Sebaihia,M.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-FEB-2004) Submitted on behalf of the Pathogen
            Sequencing Unit, Sanger Institute, Wellcome Trust Genome Campus,
            Hinxton, Cambridge CB10 1SA E-mail: ms5@sanger.ac.uk
COMMENT     REFSEQ INFORMATION: The reference sequence was derived from
            BX950851.
            On Jul 9, 2004 this sequence version replaced gi:28850470.
            Annotation was added by the NCBI Prokaryotic Genome Annotation
            Pipeline (released 2013). Information about the Pipeline can be
            found here: http://www.ncbi.nlm.nlm.nih.gov/genome/annotation_prok/
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Change region shown

- ○ Whole sequence (abbreviated view)
- ◉ Selected region

from: 3656910 to: 3670586

Update View

Customize view

Analyze this sequence

- Run BLAST
- Pick Primers
- Highlight Sequence Features

Related information

- Assembly
- BioProject
- BioSample
- Components (Core)
- Full text in PMC
- Gene
- Genome
- Identical GenBank Sequence
- Probe
- Protein
- PubMed (Weighted)
- Taxonomy

LinkOut to external resources

cpnDB: A Chaperonin Database

[cpnDB: A Chaperonin Database]

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ORIGIN

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Vector NTI - [DNA/RNA Molecule from document 2180]

File Edit View Analyses Cloning Gel List Align Assemble Tools Window Help

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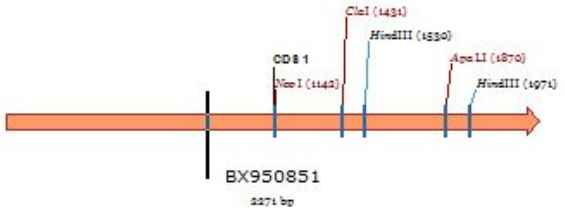
- Standard Fields
- References
- Comments
- Annotations
- Feature Map
- Restriction/Methylation Map

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   301  TGGGATAAGA TTGGGCATAG TCCATGTGAG AAAATAATTA GTGGTTCTTC TTTAATTGCT TTATTACCTC TTTCATTTAC TACTGGAAAA TCCTATAAAG
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   601  TATCTGAACA TAAATGAAAC TGATAATACC AATACTCAAT CAAACCCACA TATGGGGTTA GATTATTGGG AAAAAAATCT TACTGAAAGT CAAAAAAGAT
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  1001  TTGATAGCGA TGCTTTTGAA TCTAAGCAGT TACAGCTCTT ATATATAAAC GAAGCTCTTG AAGATTTTTT GATAAATGAT TACGCAGGAT CTGTCAACTT
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NcoI

Характеристики праймеров

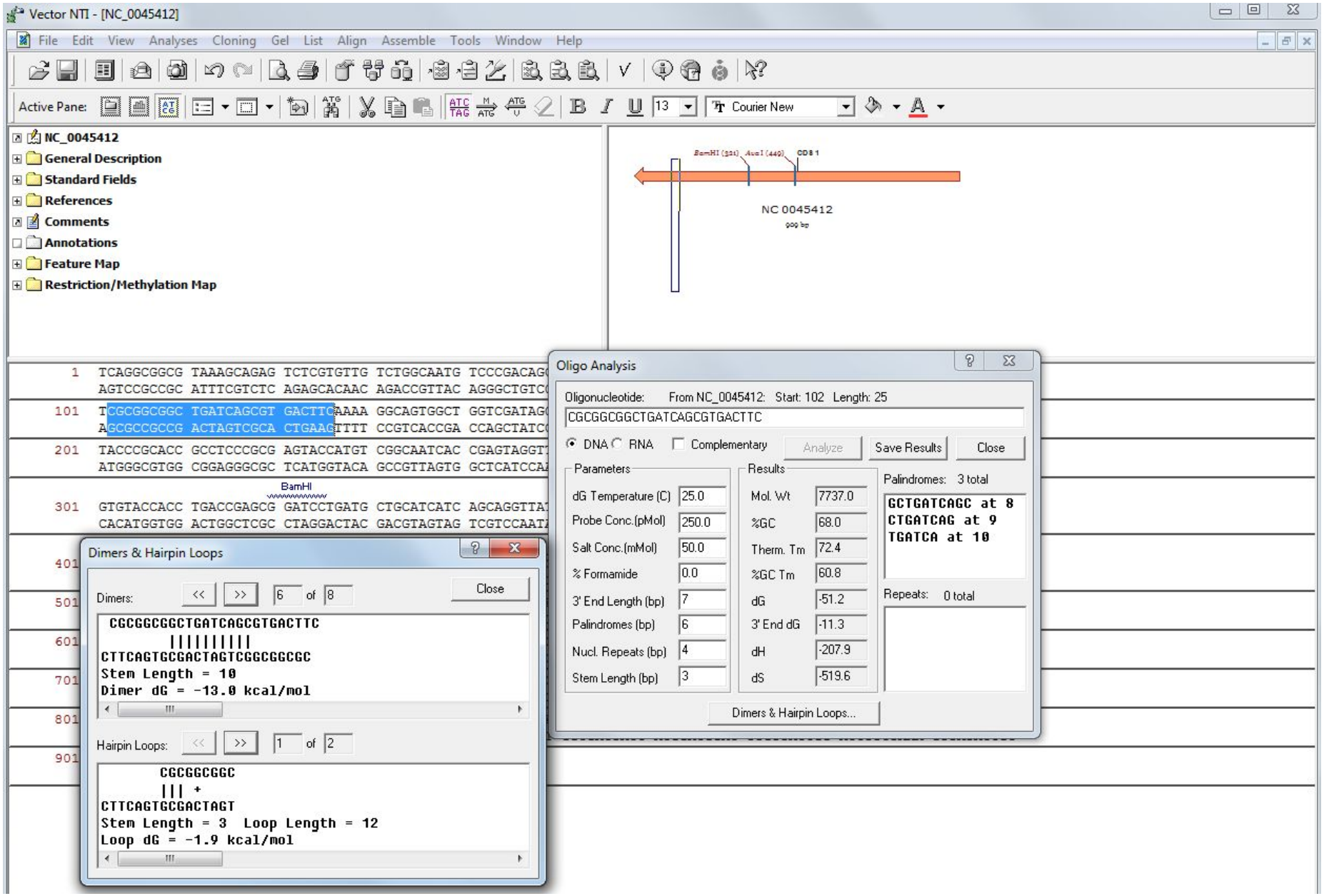
Комплементарность мишени

Высокое GC содержание

На 3’-конце праймера G или C

Температура отжига 55-60° C

Отсутствие вторичных структур (шпилек и димеров)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Comments
Annotations
Feature Map
Restriction/Methylation Map
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AvaI (449)
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BamHI
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501
601
701
801
901
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DNA RNA Complementary
Analyze
Save Results
Close
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Probe Conc.(pMol) 250.0
Salt Conc.(mMol) 50.0
% Formamide 0.0
3' End Length (bp) 7
Palindromes (bp) 6
Nucl. Repeats (bp) 4
Stem Length (bp) 3
Results
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%GC 68.0
Therm. Tm 72.4
%GC Tm 60.8
dG -51.2
3' End dG -11.3
dH -207.9
dS -519.6
Palindromes: 3 total
GCTGATCAGC at 8
CTGATCAG at 9
TGATCA at 10
Repeats: 0 total
Dimers & Hairpin Loops...
Dimers & Hairpin Loops
Dimers: << >> 6 of 8
Close
CGCGGCGGCTGATCAGCGTGACTTC
||||||||||
CTTCAGTGCGACTAGTCGGCGGCGC
Stem Length = 10
Dimer dG = -13.0 kcal/mol
Hairpin Loops: << >> 1 of 2
CGCGGCGGC
||| +
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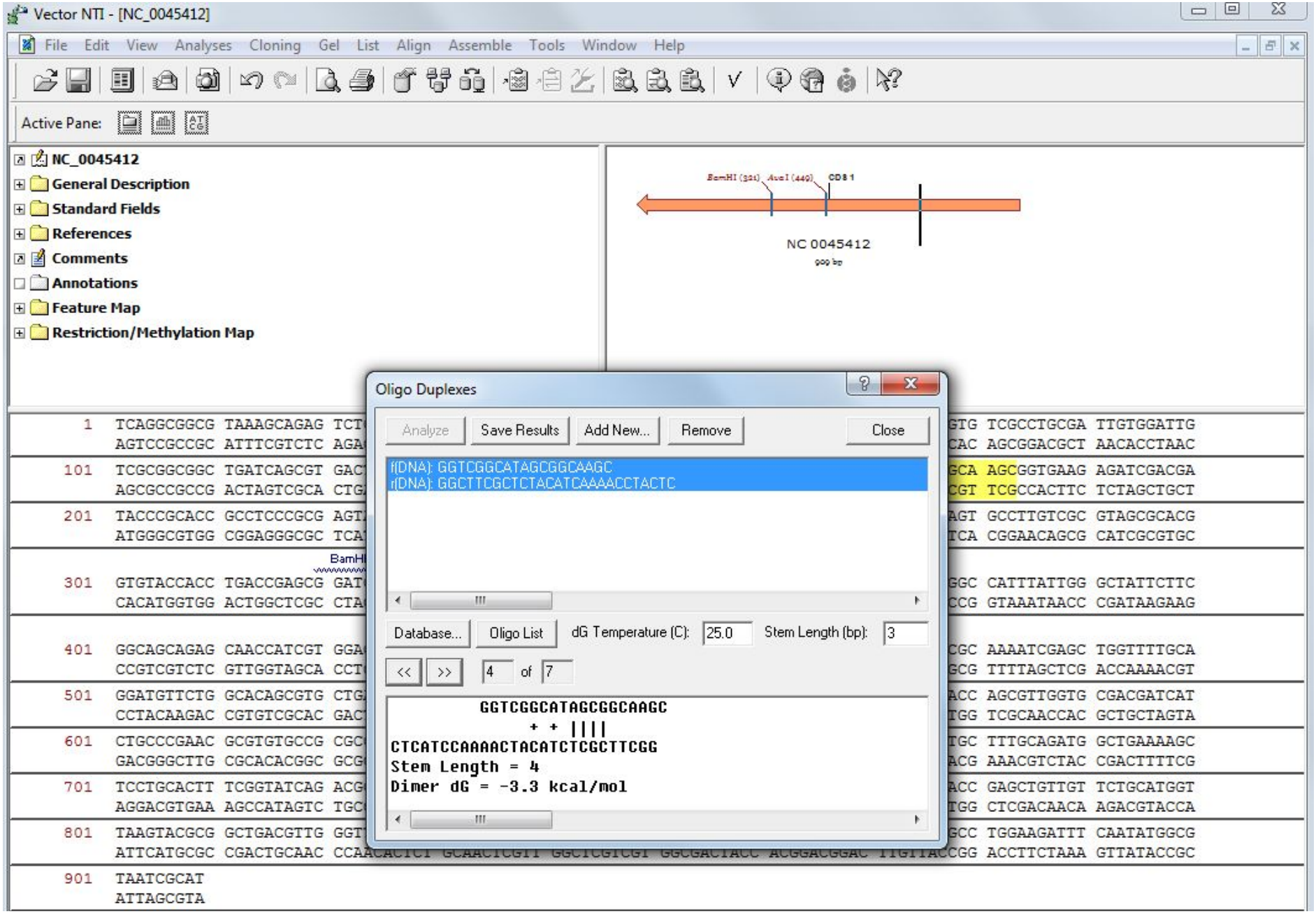
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Comments
Annotations
Feature Map
Restriction/Methylation Map
BamHI (321)
AvaI (440)
CDS 1
NC 0045412
Oligo Duplexes
Analyze
Save Results
Add New...
Remove
Close
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r(DNA): GGCTTCGCTCTACATCAAAACCTACTC
Database...
Oligo List
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Stem Length (bp):
3
<<
>>
4
of
7
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+ + ||||
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Dimer dG = -3.3 kcal/mol
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101
201
301
401
501
601
701
801
901
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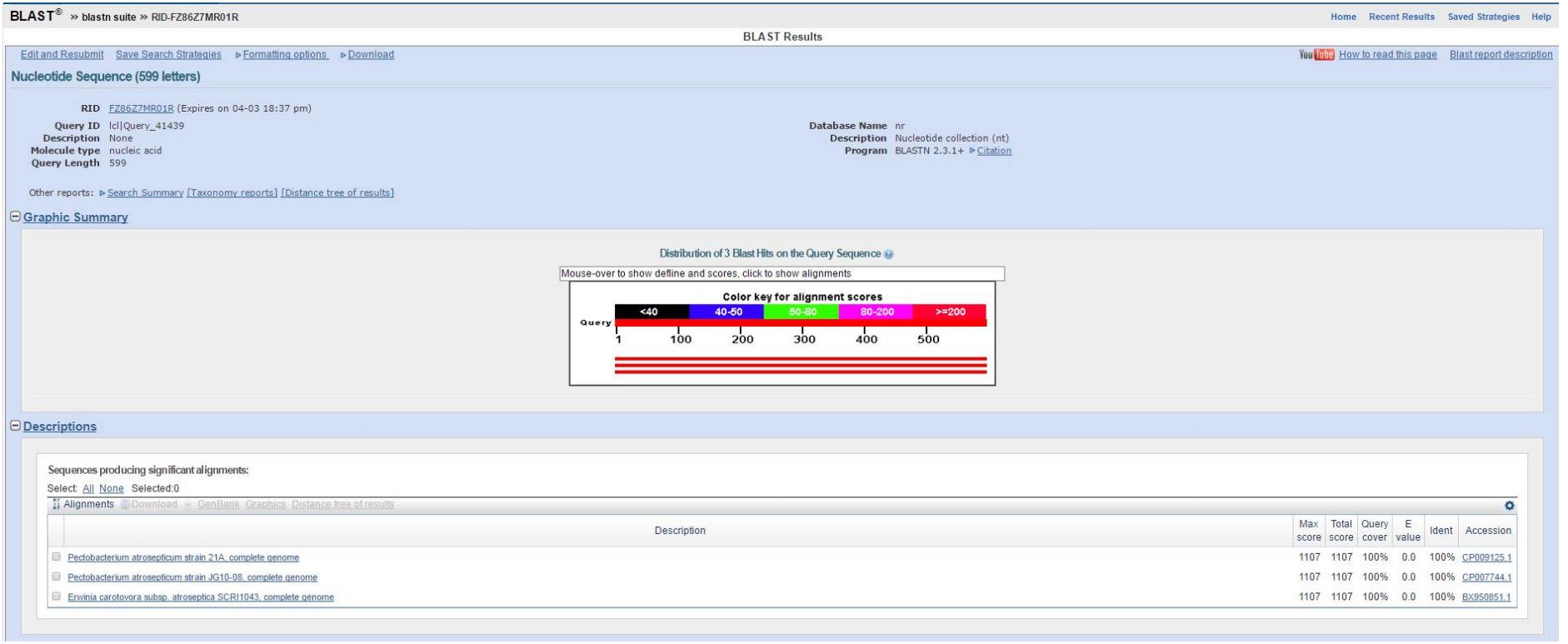
BLAST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300
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500
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Description
Max score
Total score
Query cover
E value
Ident
Accession
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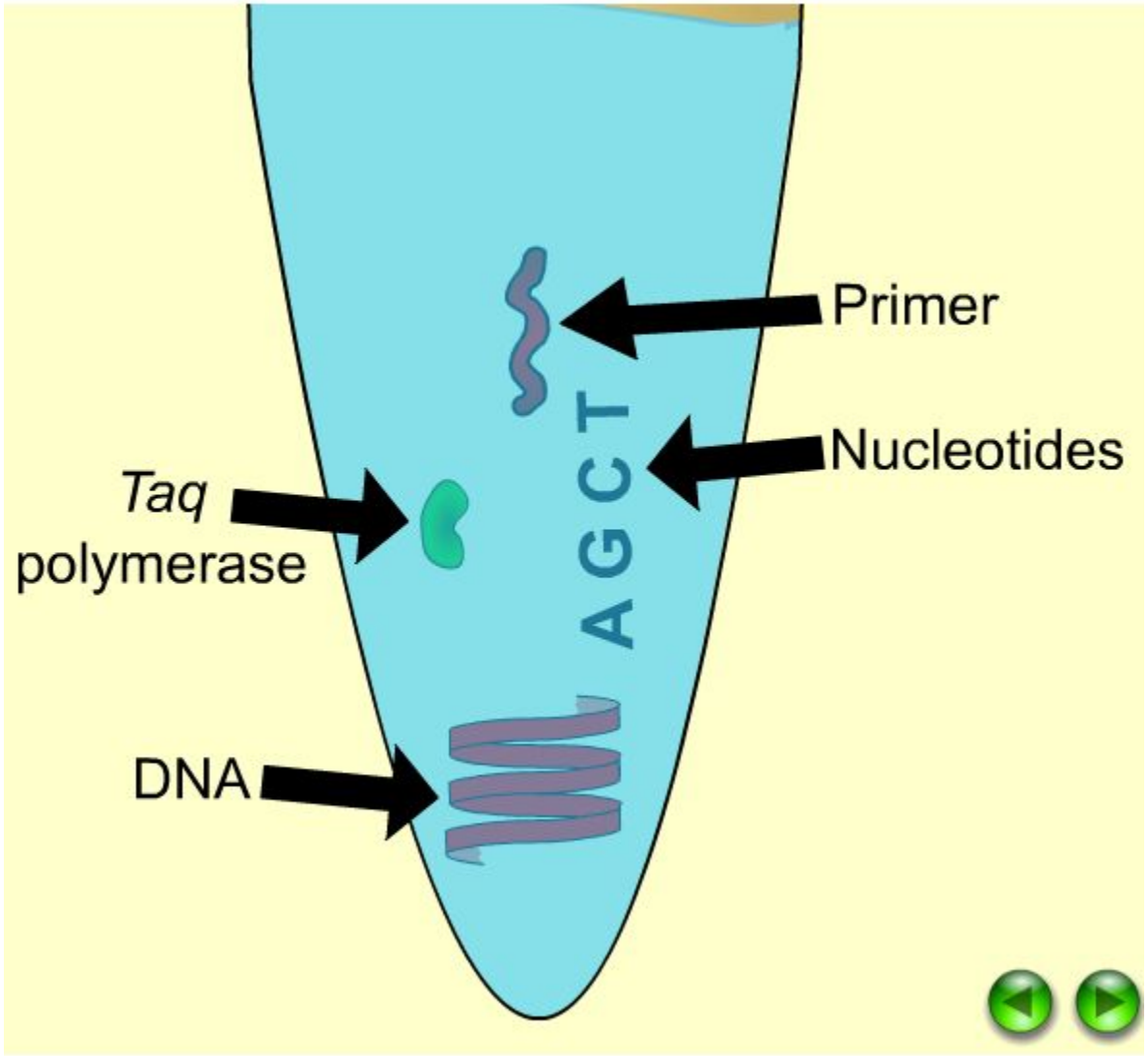
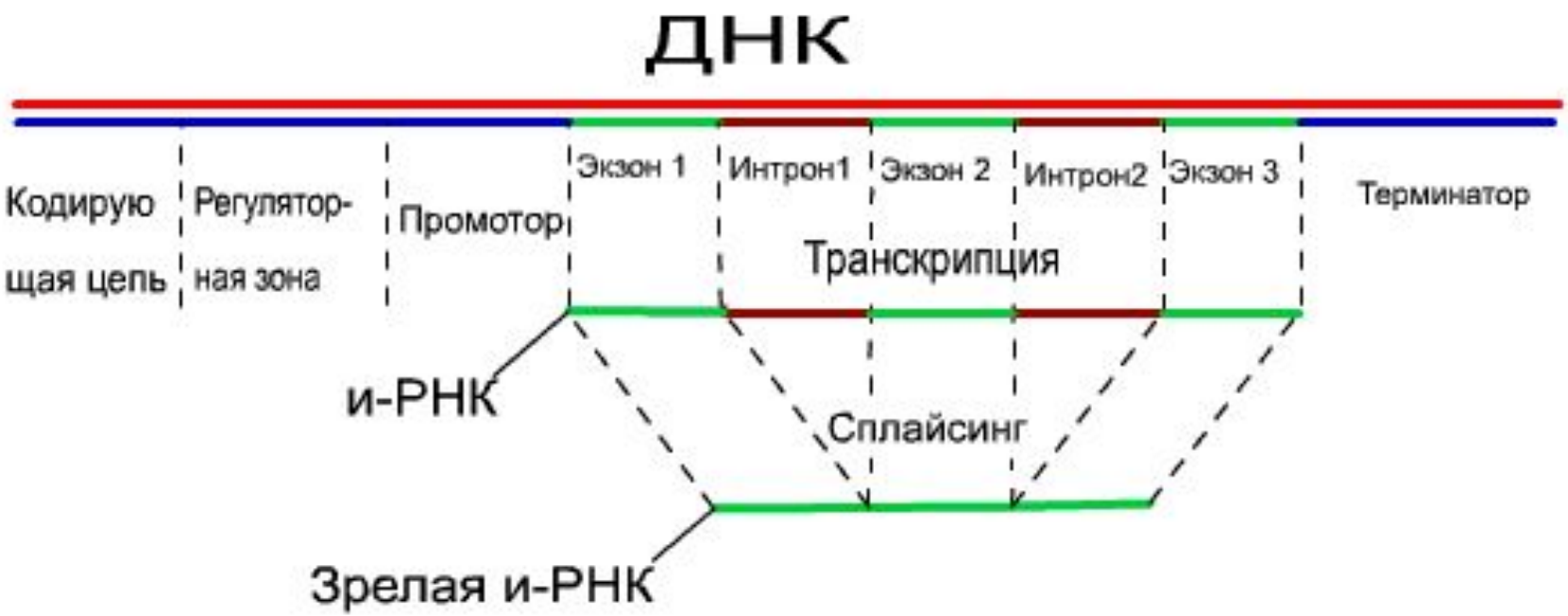

Primer
Nucleotides
Taq
polymerase
DNA
A G C T

Что обычно используется в качестве матрицы (основы) для амплификации «нужного» участка ДНК?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Кодирую
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Регулятор-
ная зона
Промотор
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Интрон1
Экзон 2
Интрон2
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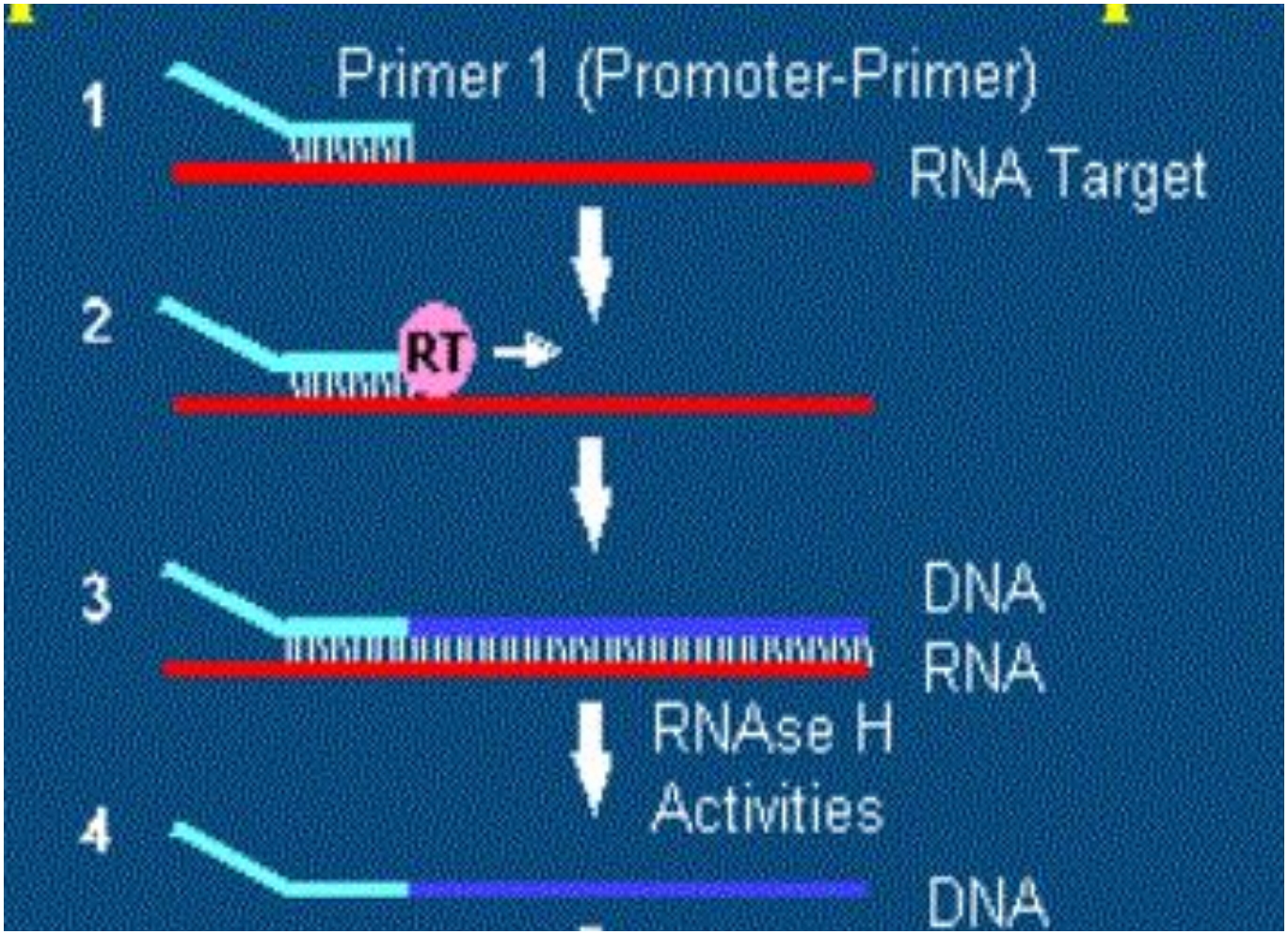
Терминатор
Транскрипция
и-РНК
Сплайсинг
Зрелая и-РНК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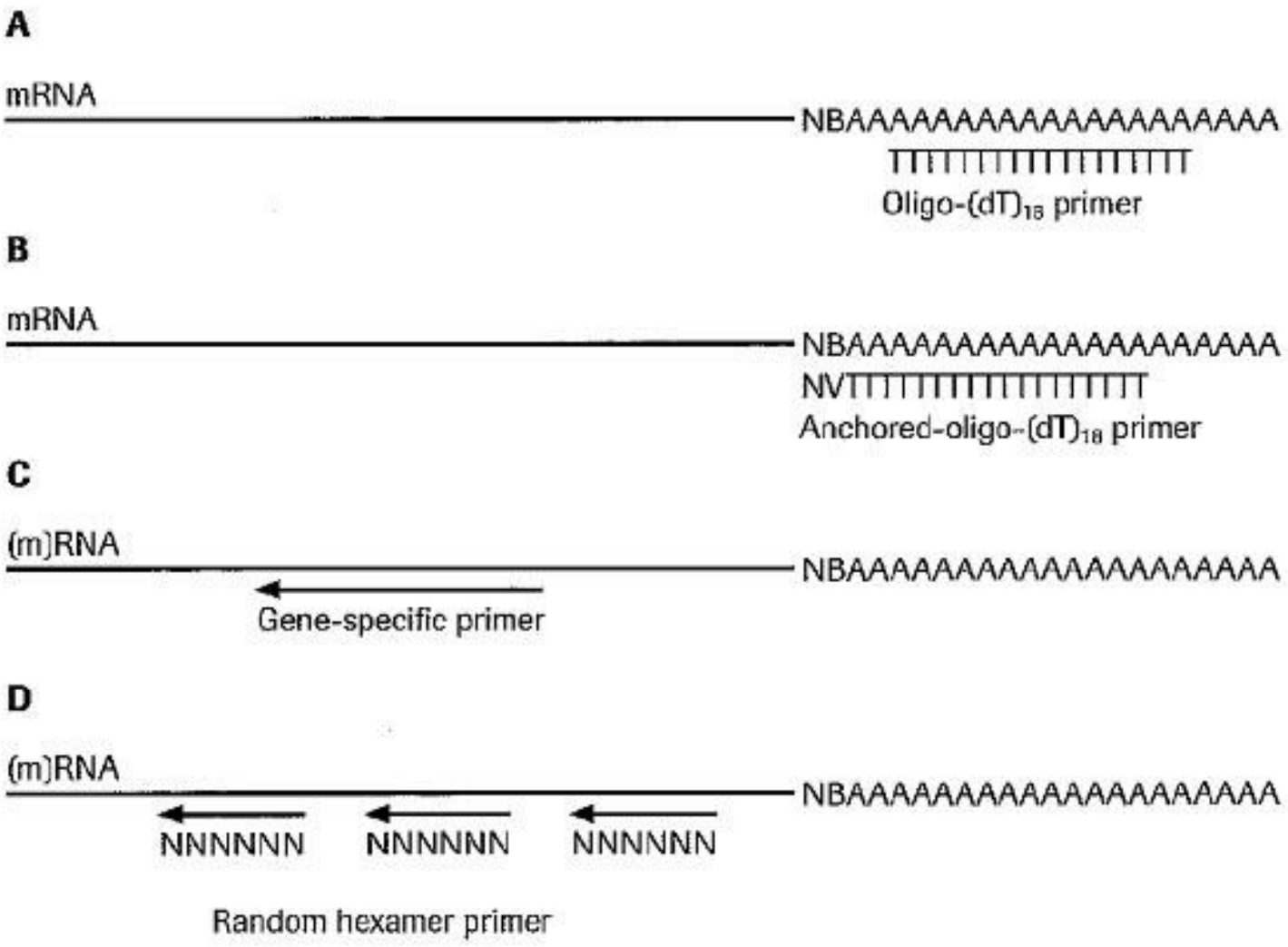
RNA Target
2
RT
3
DNA
RNA
RNAse H
Activities
4
DNA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mRNA
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C
(m)RNA
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Gene-specific primer
D
(m)RNA
NBAAAAAAAAAAAAAAAAAAA
NNNNNN
NNNNNN
NNNNNN
Random hexamer primer

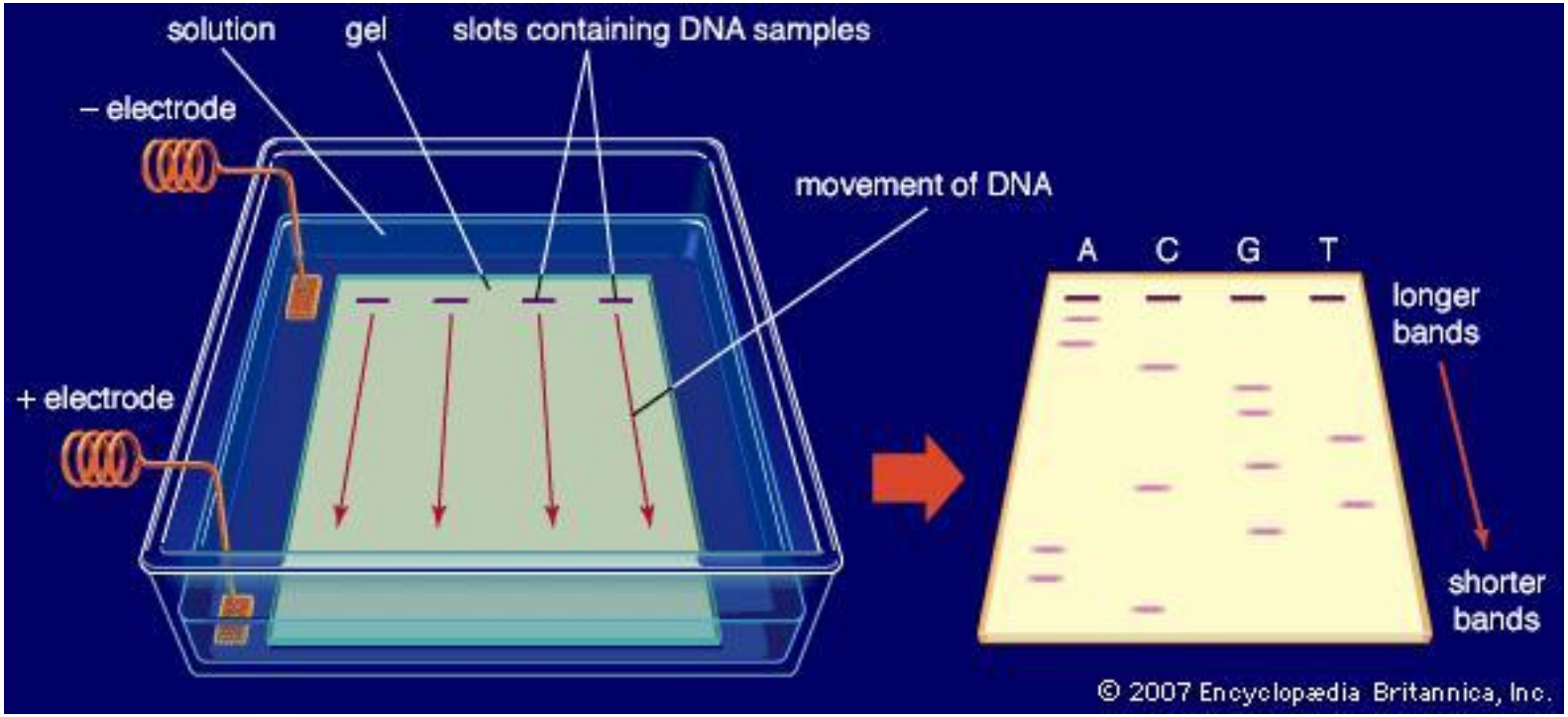
Мы провели ПЦР, чтобы получить целевой фрагмент ДНК

Нужно выяснить получился ли у нас ожидаемый продукт

Электрофорез

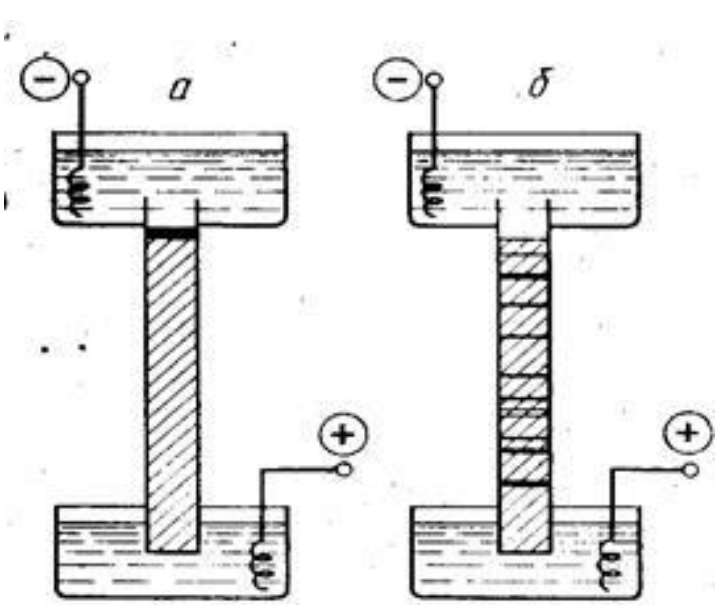
Электрофоретическое разделение нуклеиновых кислот

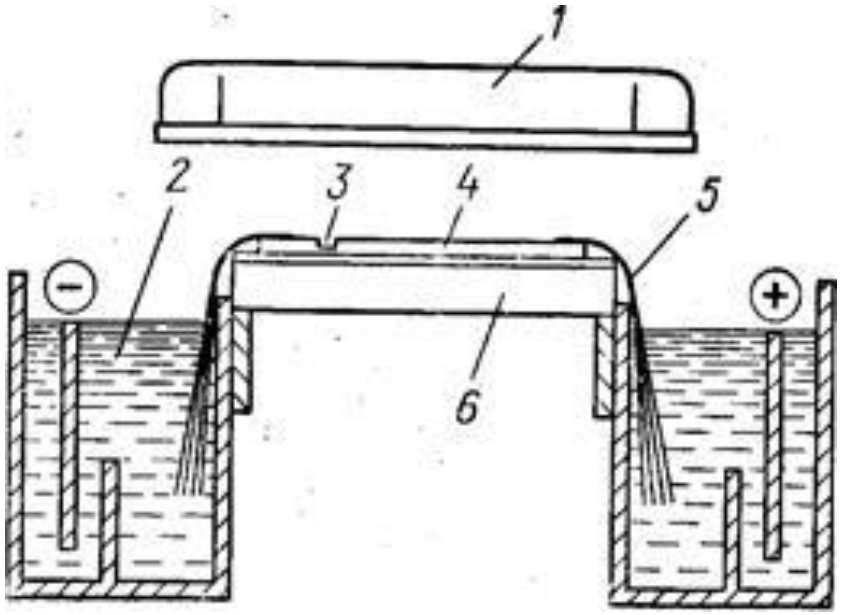
Электрофорез ДНК в агарозном геле



Электрофорез белков

Разделение молекул в электрическом поле





Разделение макромолекул в зависимости от:

размера,

пространственной конфигурации,

электрического заряда

Разделение проводят в полиакриламидном геле

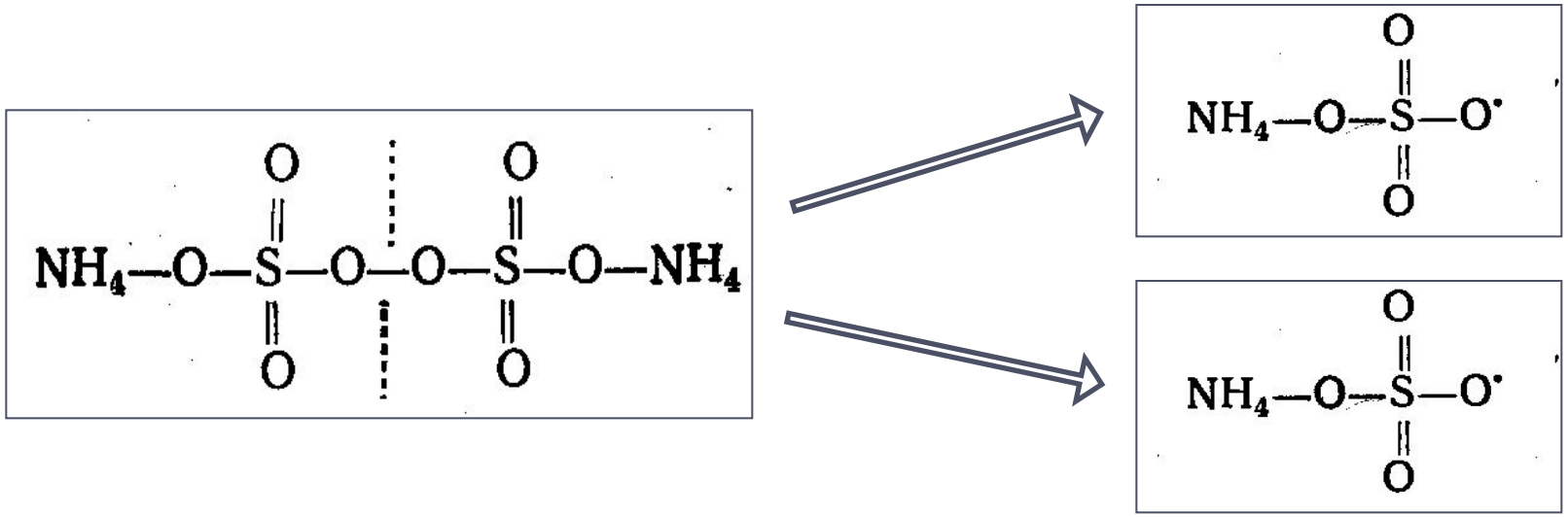
Акриламид
(CH2 = CH — CONH2)

NN'-Метиленбисакриламид
(CH2 = CH — CONH)2 – CH2

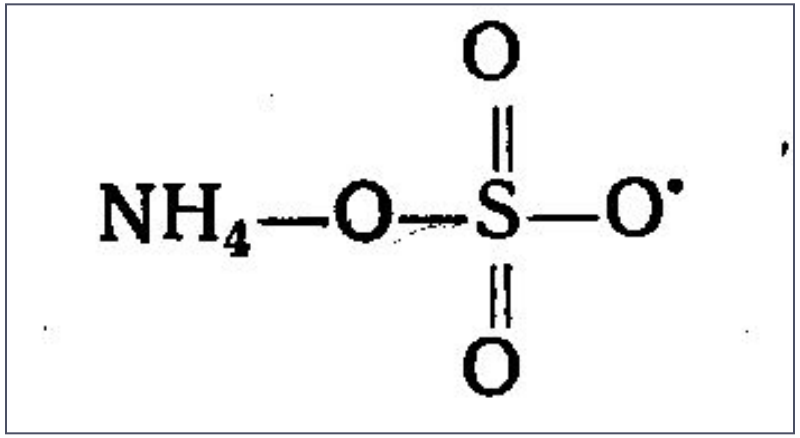
Буферные растворы

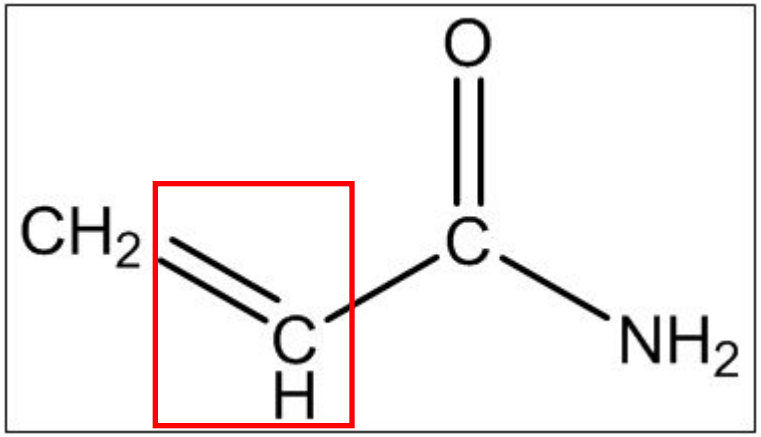
Индукция полимеризации акриламида

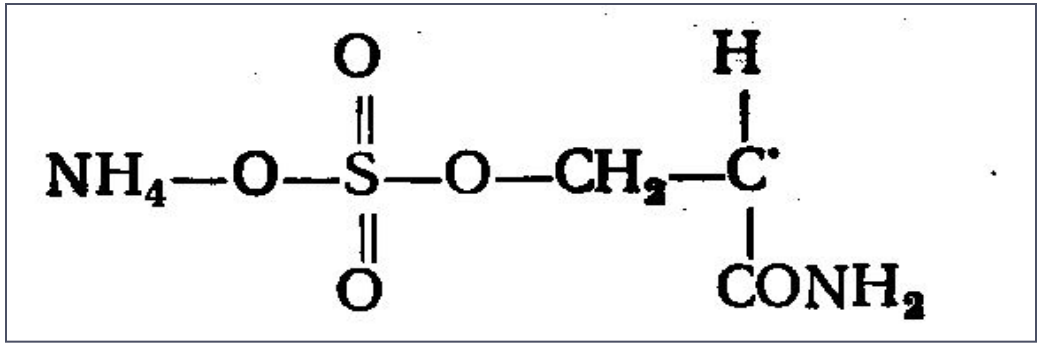
Персульфат аммония



Полимеризация акриламида



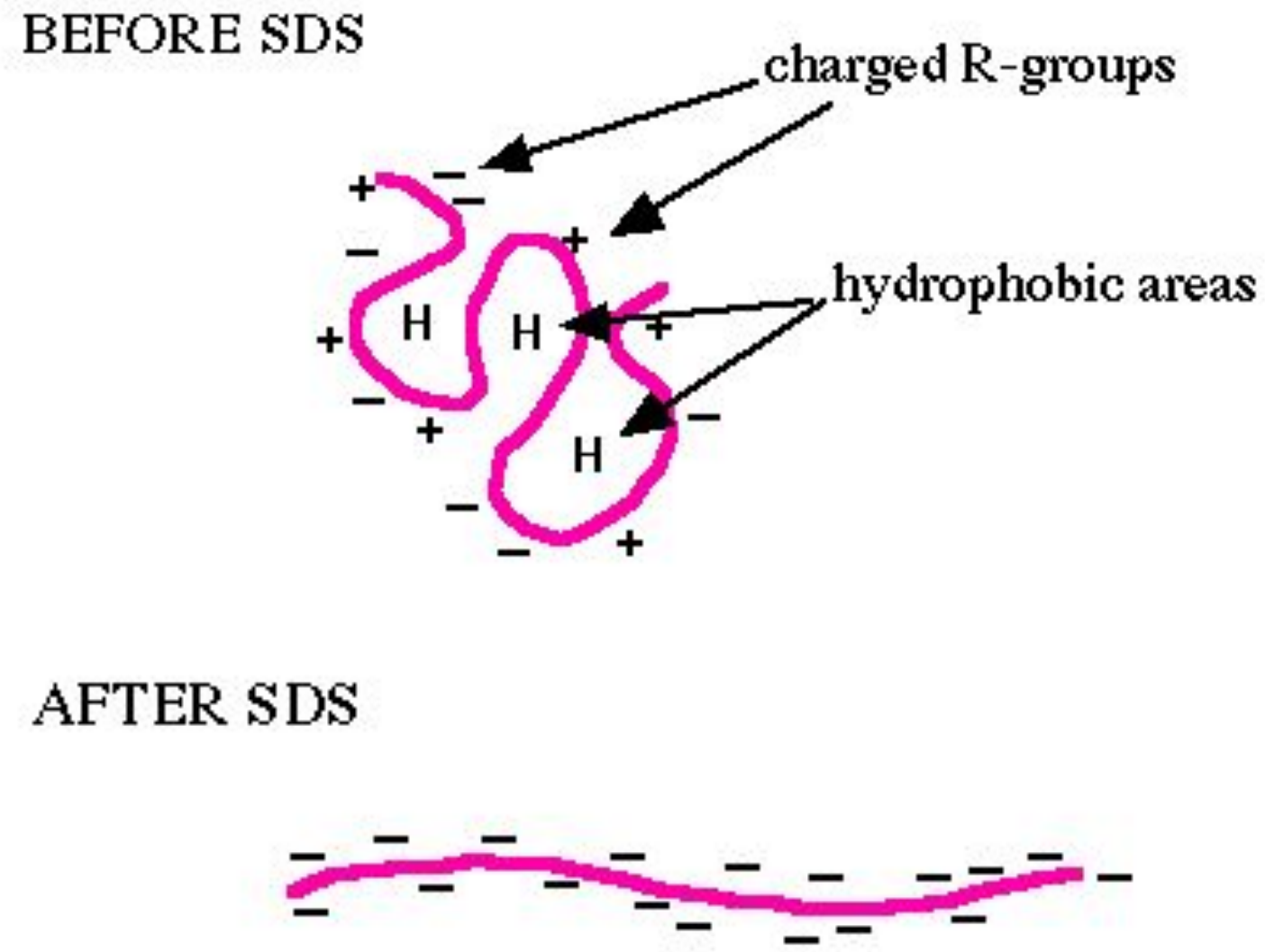


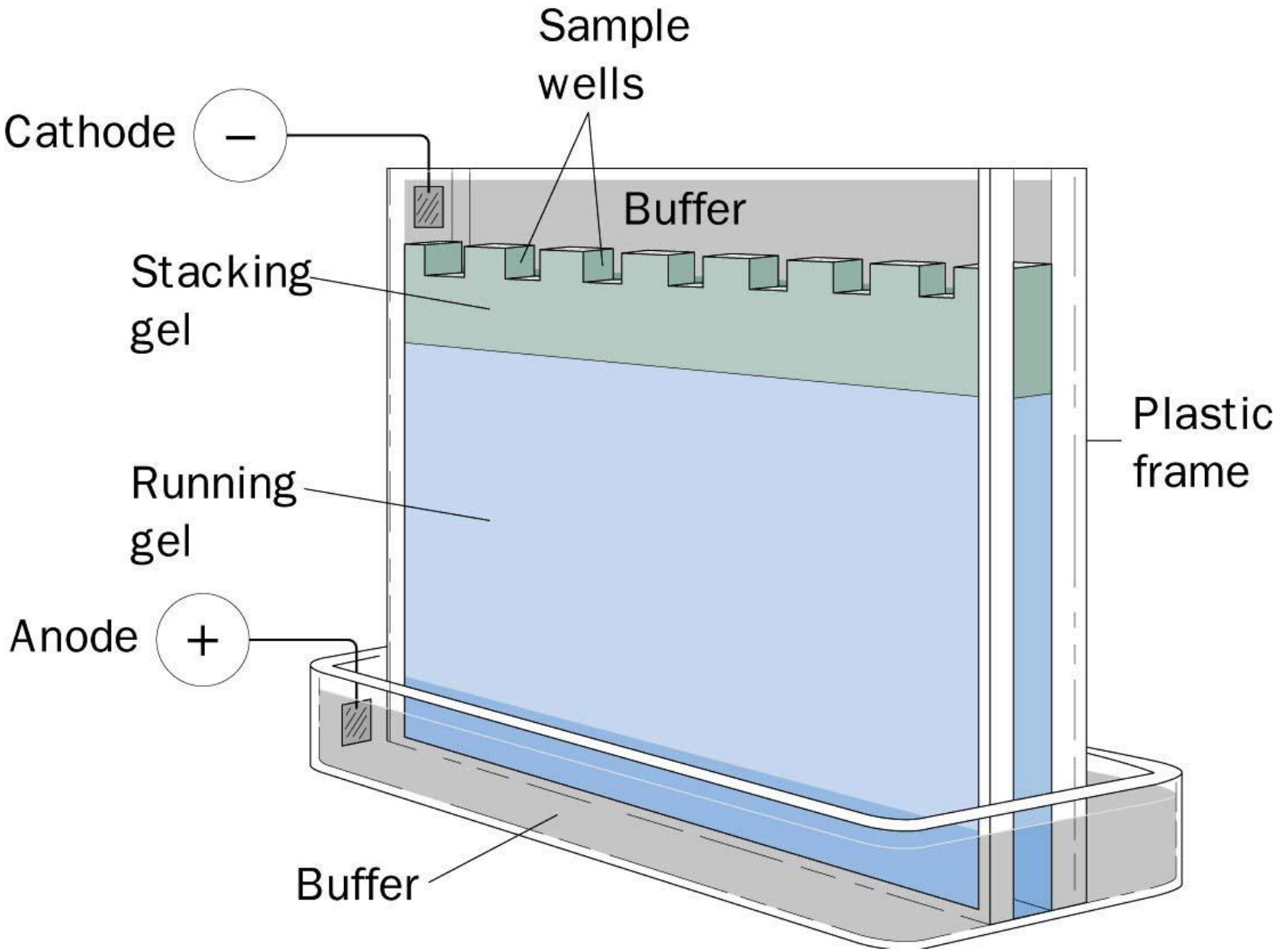


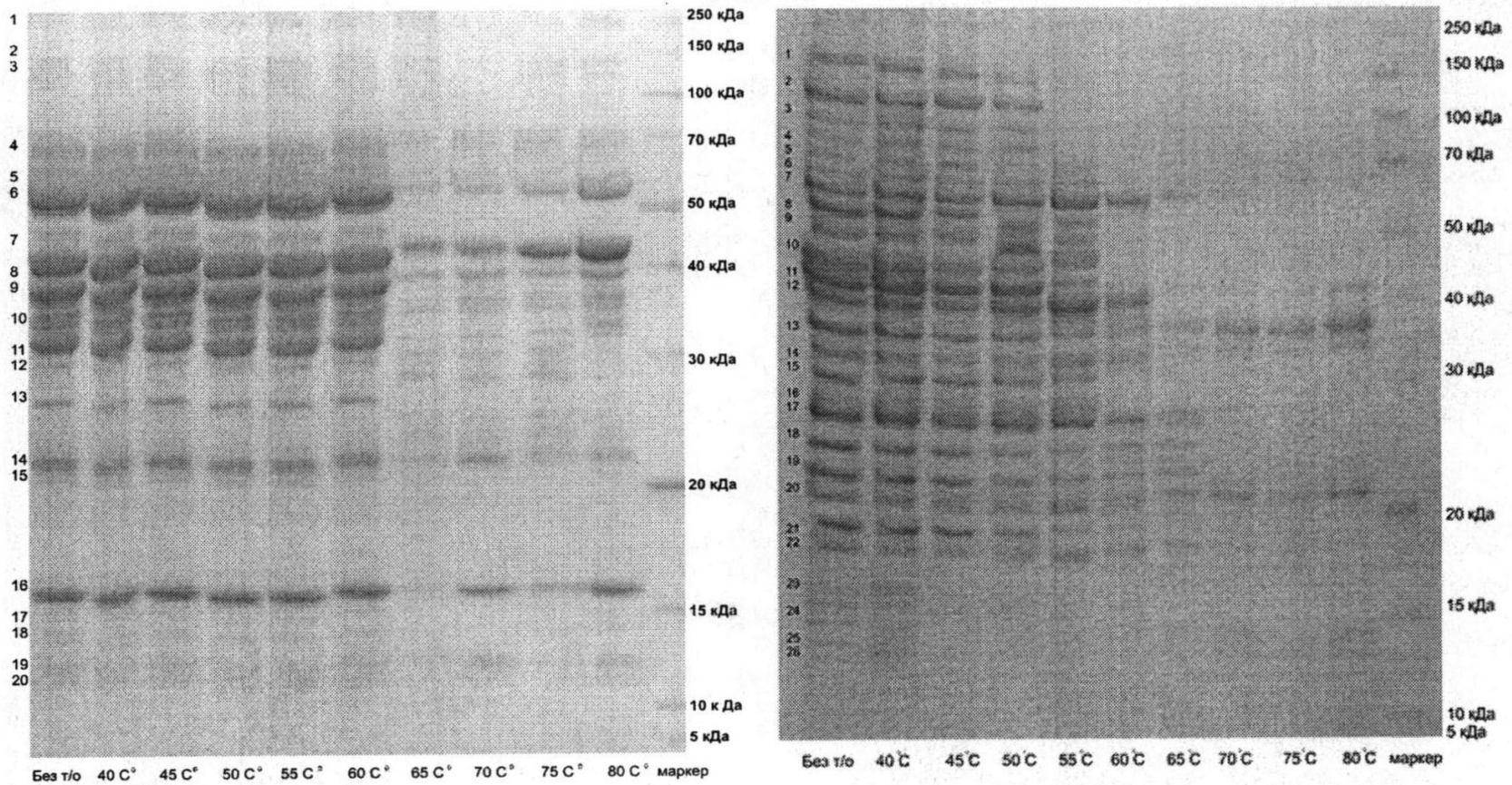
Катализатор процесса полимеризации

Тетраметилэтилендиамин (ТЕМЕД) — (CH3)2N — CH2 — CH2 — N(CH3)2

Денатурация белка с помощью ДДС

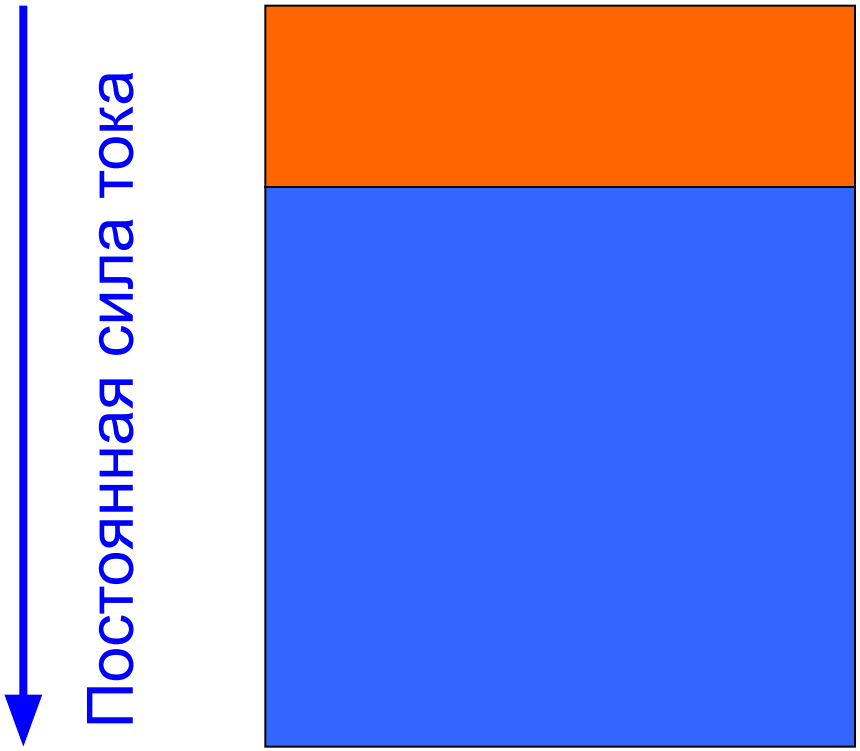



Sample
wells
Cathode
–
Buffer
Stacking
gel
Plastic
frame
Running
gel
Anode
+
Buffer


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
250 кДа
150 кДа
100 кДа
70 кДа
50 кДа
40 кДа
30 кДа
20 кДа
15 кДа
10 к Да
5 кДа
Без т/о
40 С°
45 С°
50 С°
55 С°
60 С°
65 С°
70 С°
75 С°
80 С°
маркер
250 кДа
150 КДа
100 кДа
70 кДа
50 кДа
40 кДа
30 кДа
20 кДа
15 кДа
10 кДа
5 кДа
Без т/о
40 С
45 С
50 С
55 С
60 С
65 С
70 С
75 С
80 С
маркер

а) б)

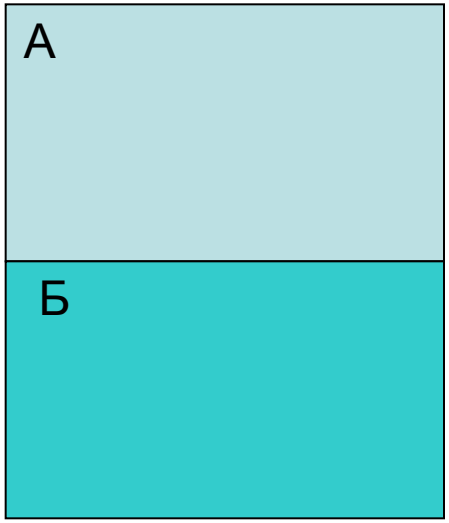
Фиг. 2



Подвижность ионов

Заряд ионов

Изоэлектрическая точка

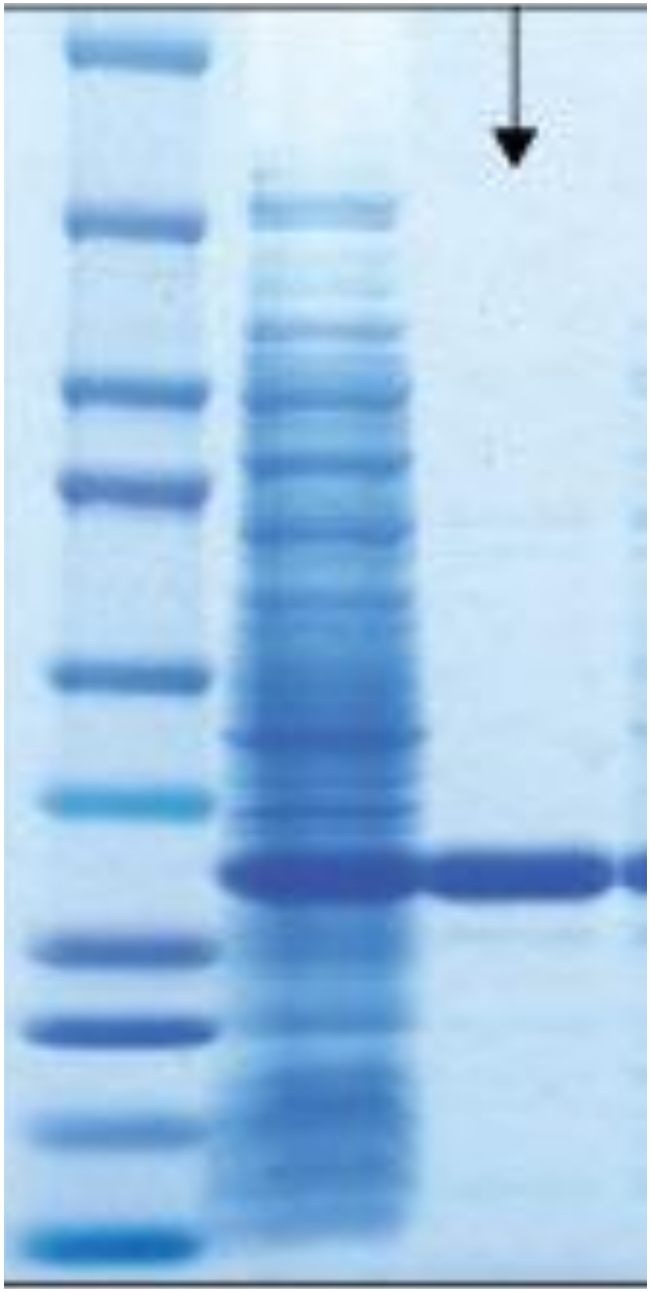


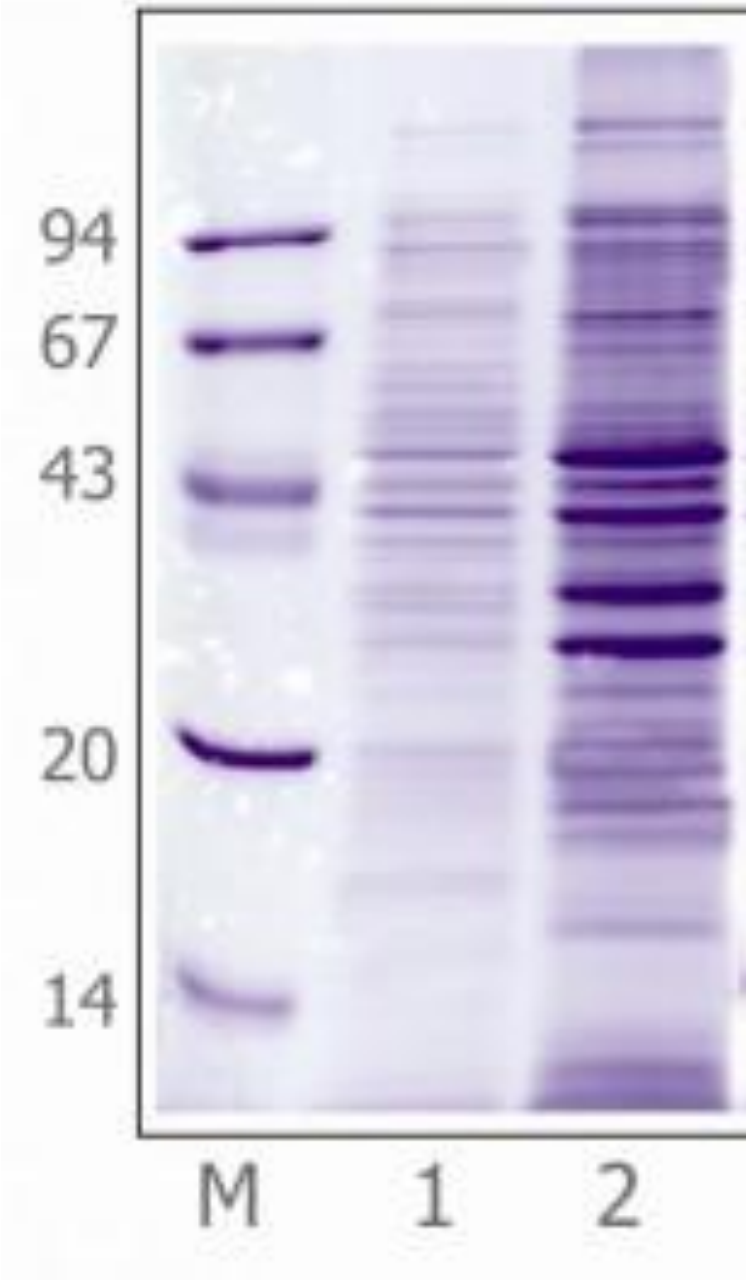
Глицин – малоподвижный ион. Высокое сопротивление. Высокая напряженность

Хлор – подвижный ион. Низкое сопротивление. Низкая напряженность

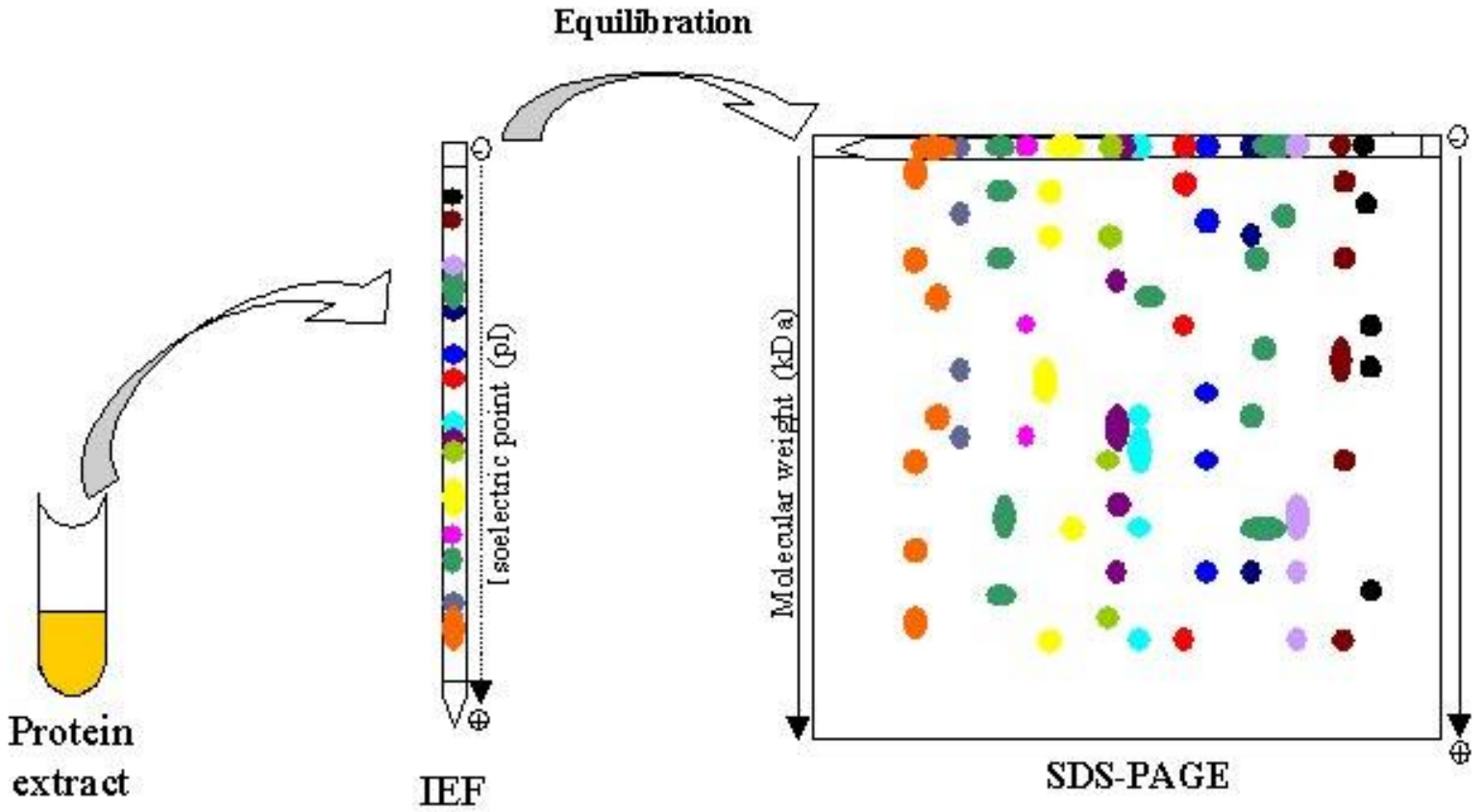
Одинаковая сила тока

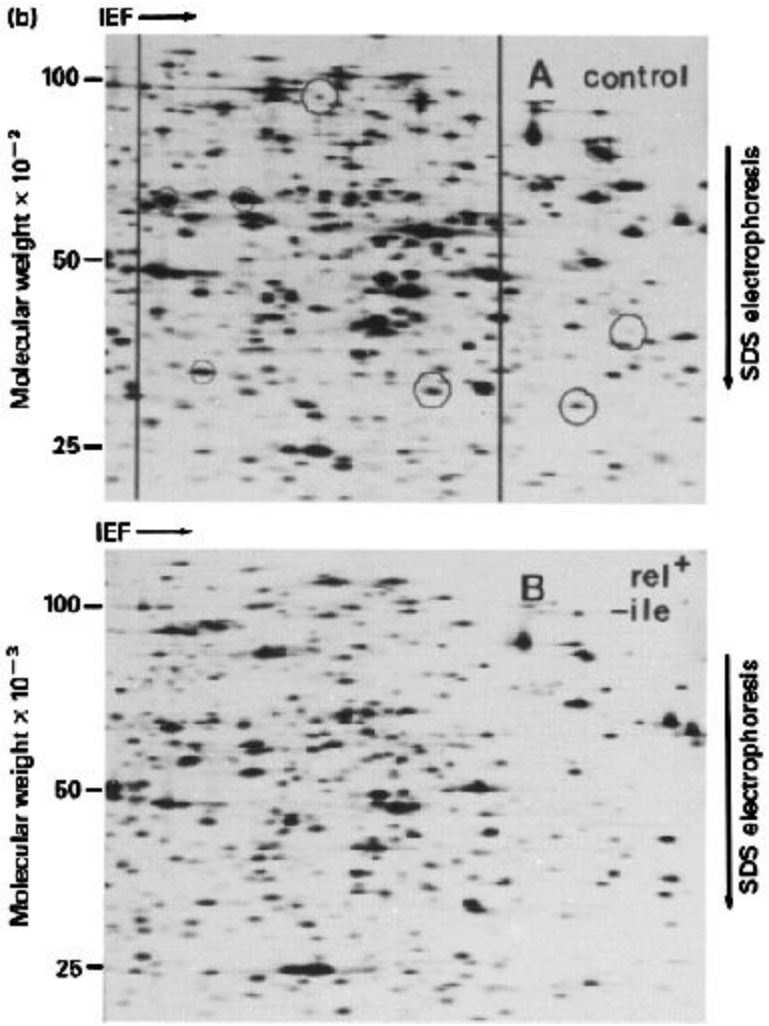
Суммарное напряжение распределится между участками А и Б так, что напряженность поля А будет выше, причем настолько, чтобы скорость миграции ионов глицина стала точно такой же как у ионов хлора. Этого требует условие неизменности величины тока вдоль всего геля (постоянство тока во всем геле).

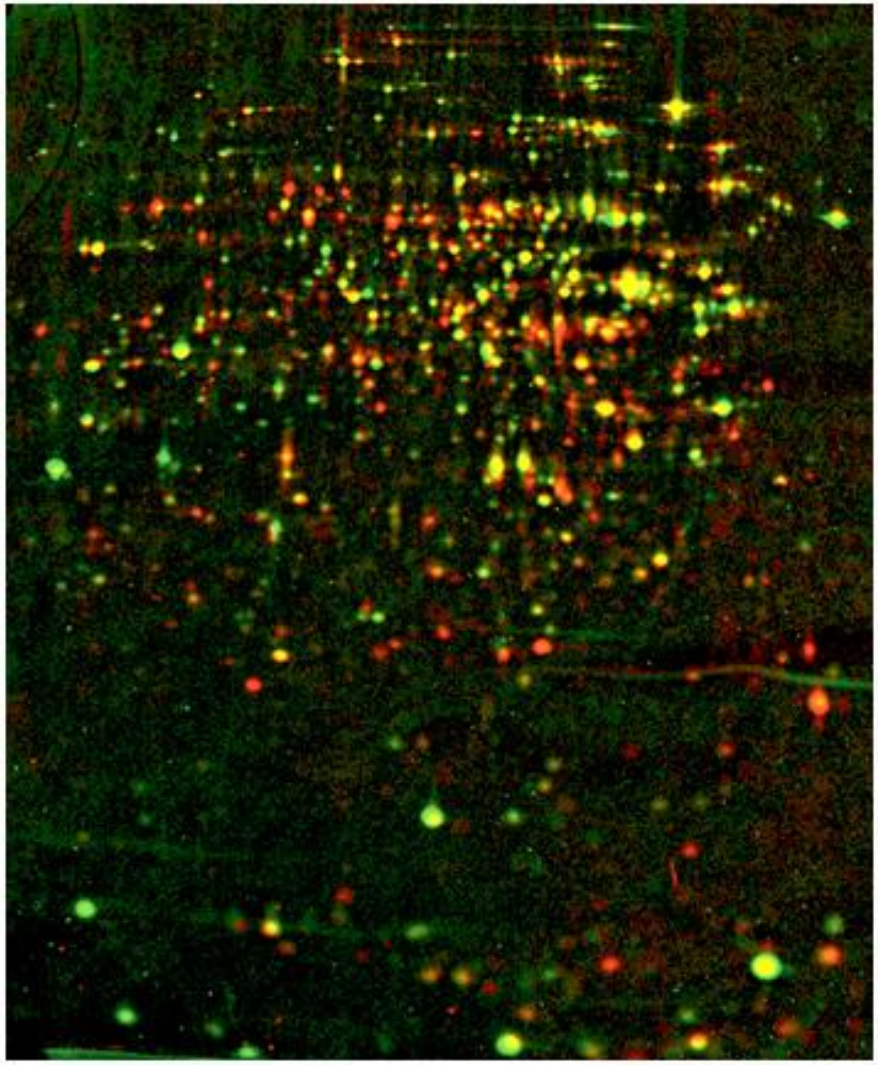



94
67
43
20
14
M
1
2

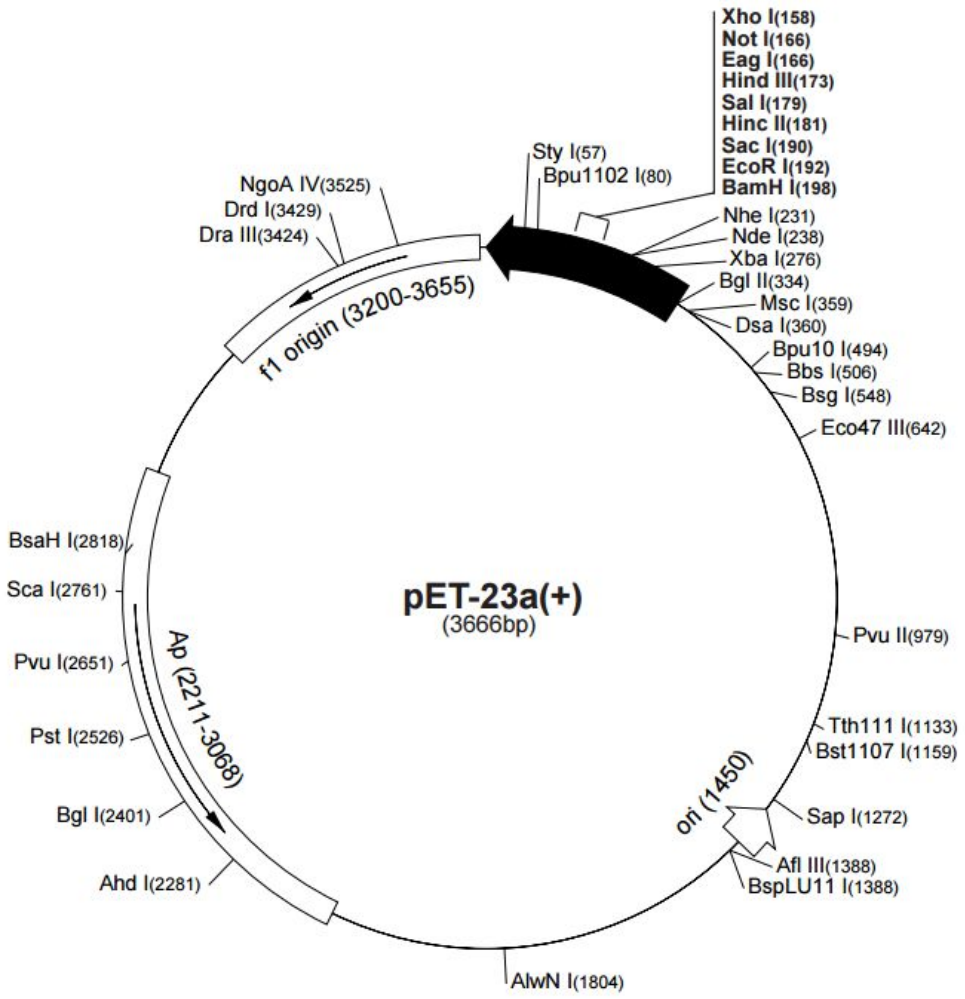
Двумерный электрофорез белков



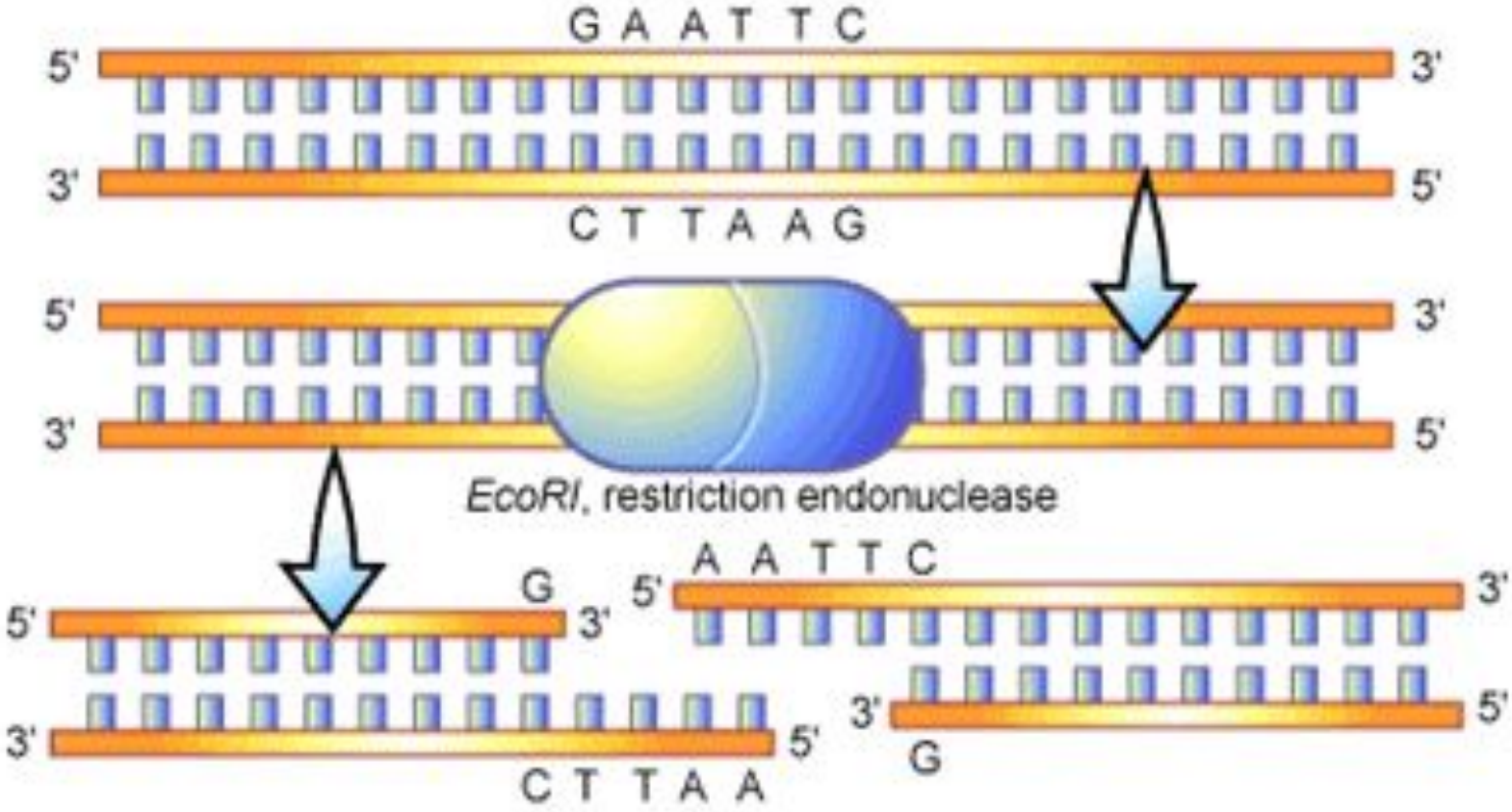

(b)
IEF
100
50
25
Molecular weight x 10^{-2}
A control
SDS electrophoresis
IEF
100
50
25
Molecular weight x 10^{-3}
B
rel^{+}
-ile
SDS electrophoresis



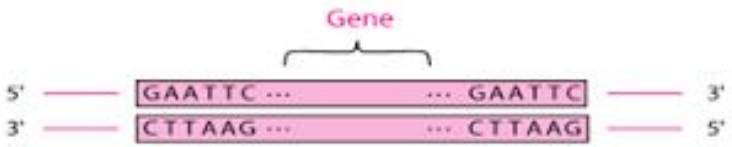
Векторные конструкции

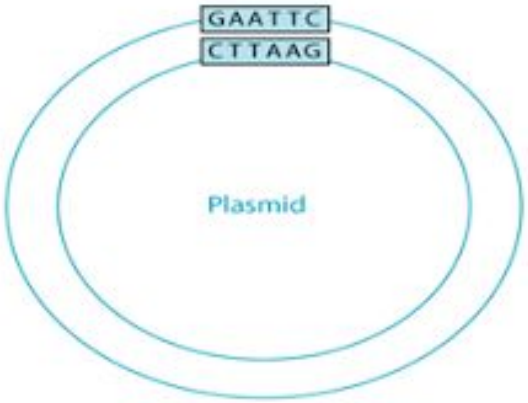


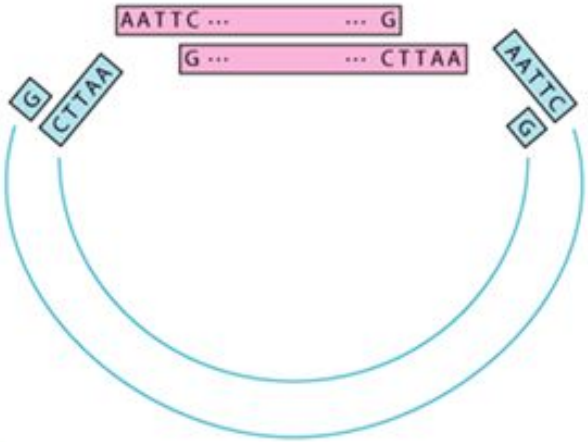
Рестриктазы

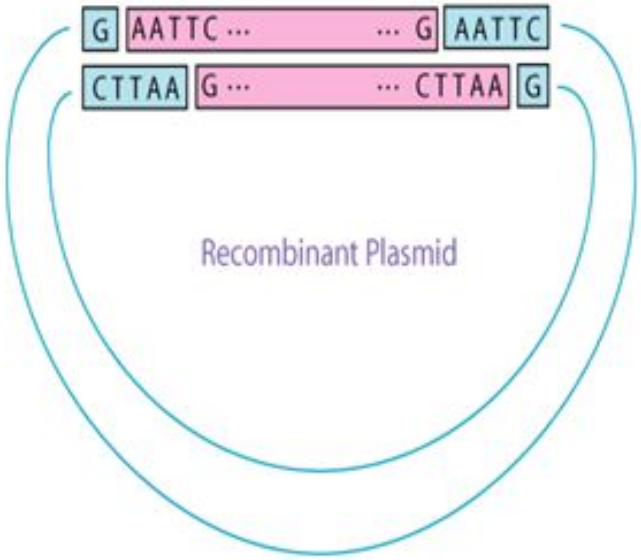


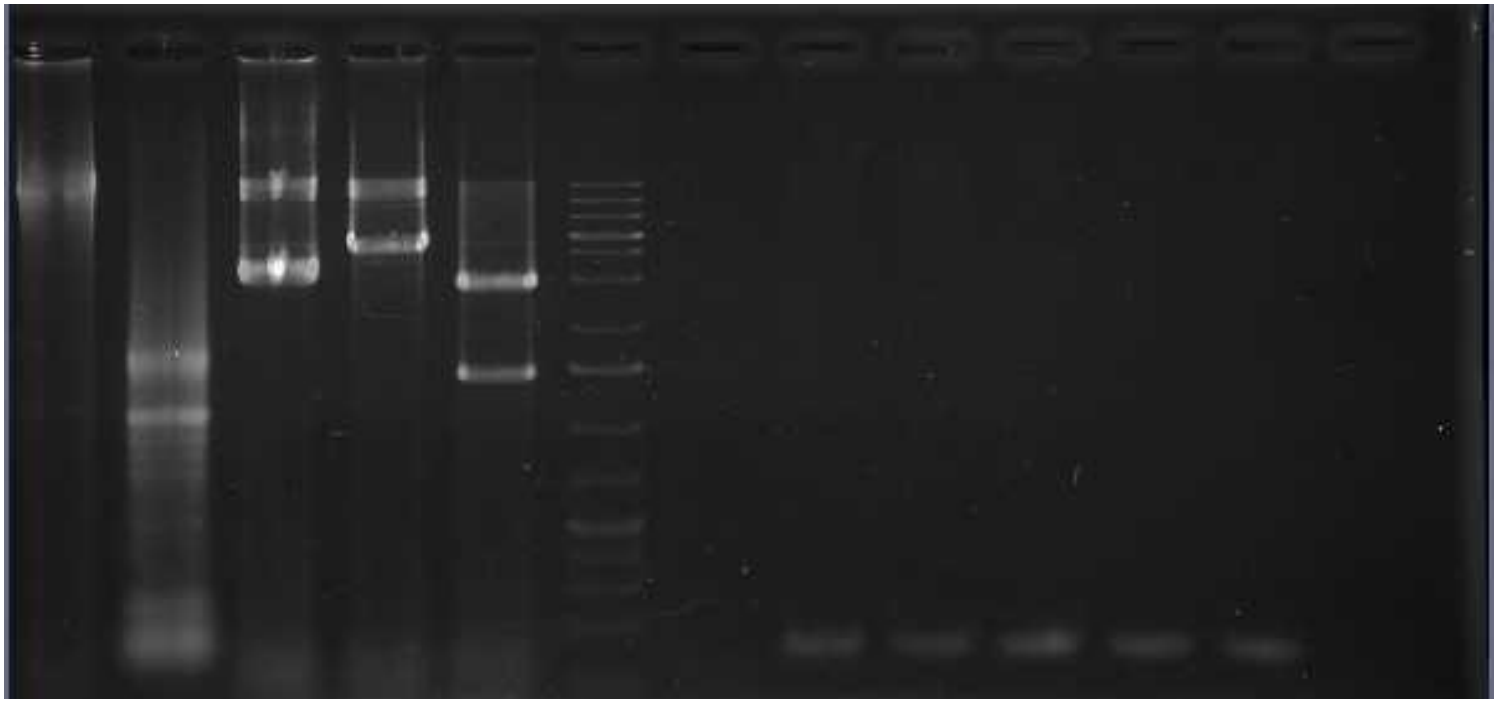
Сборка плазмиды. Рестрикция-лигирование.

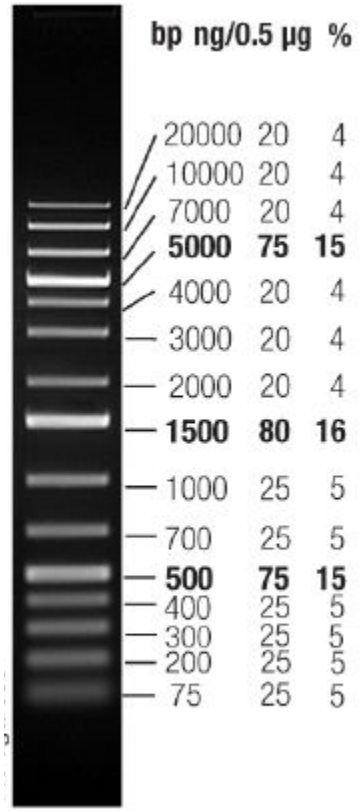






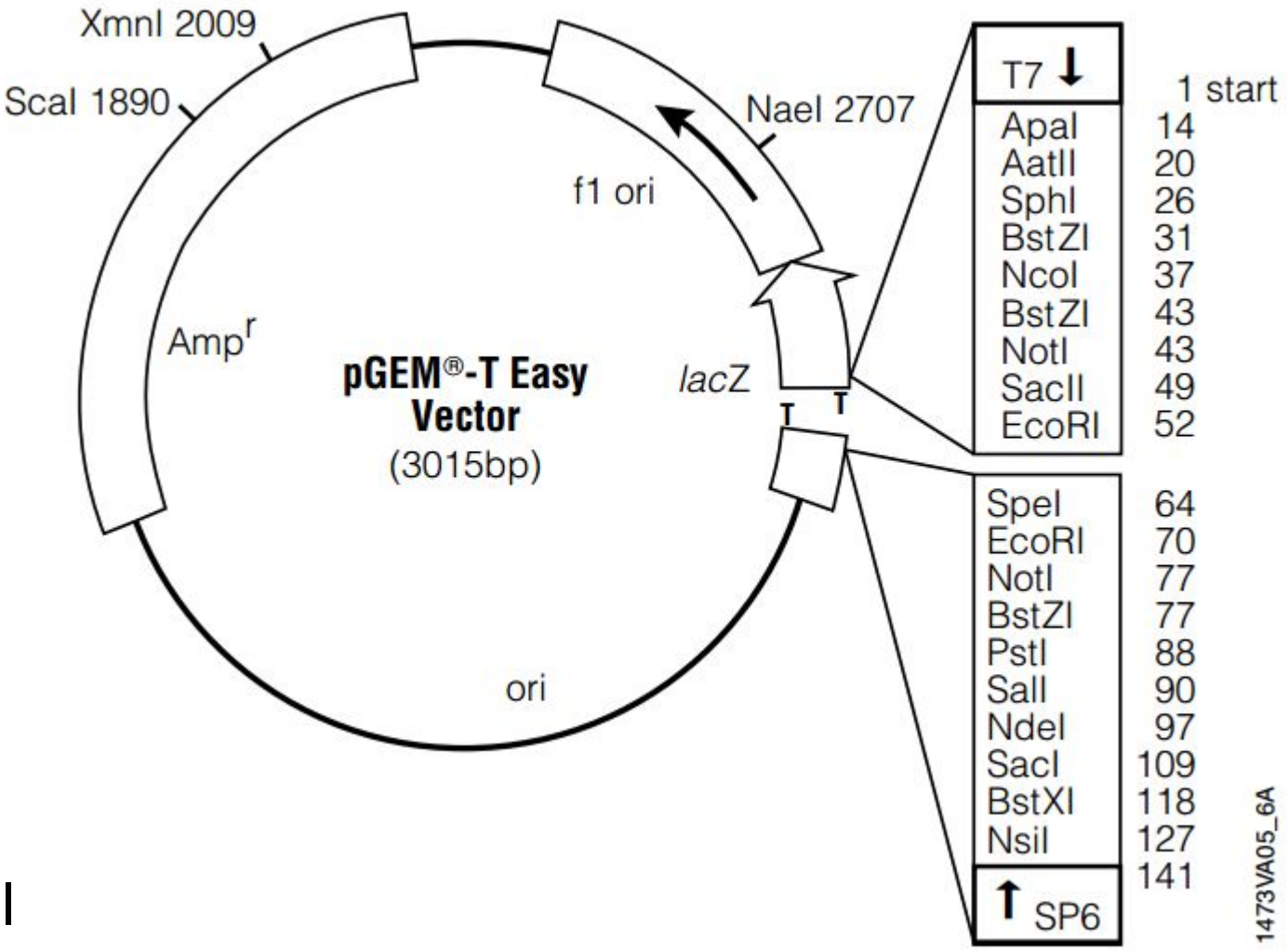






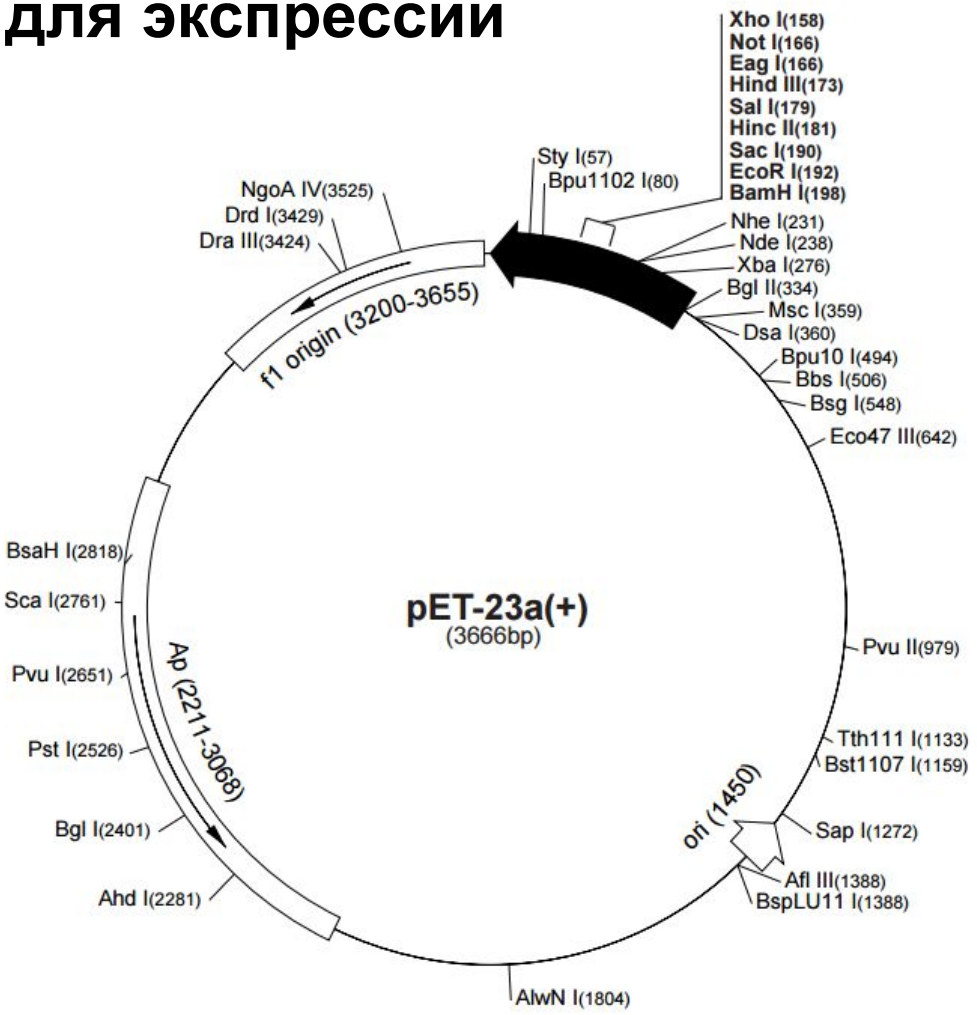
0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

Векторы для клонирования



X-Gal

Векторы для экспрессии



T7 promoter primer #69348-3

T7 promoter

Bgl II

rbs

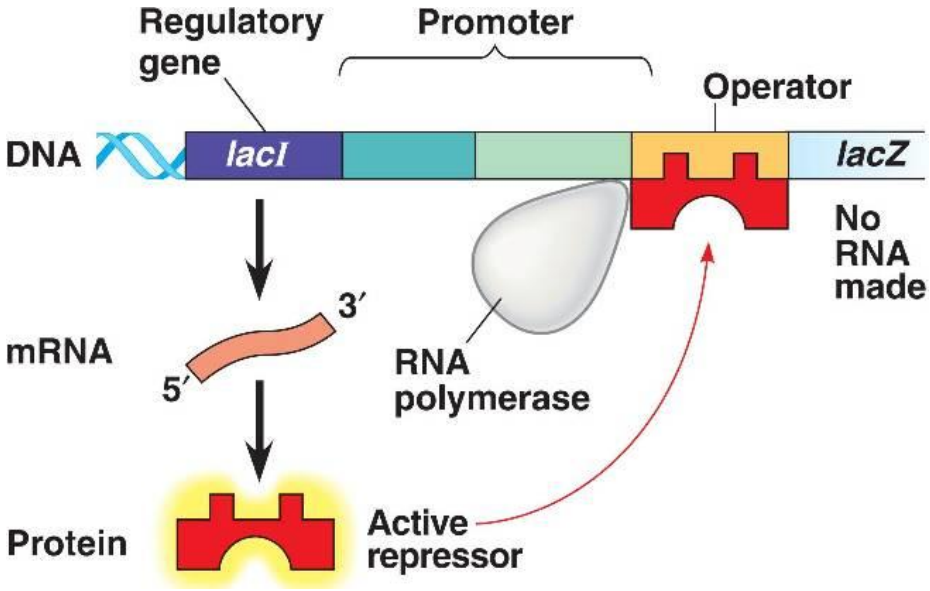
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA

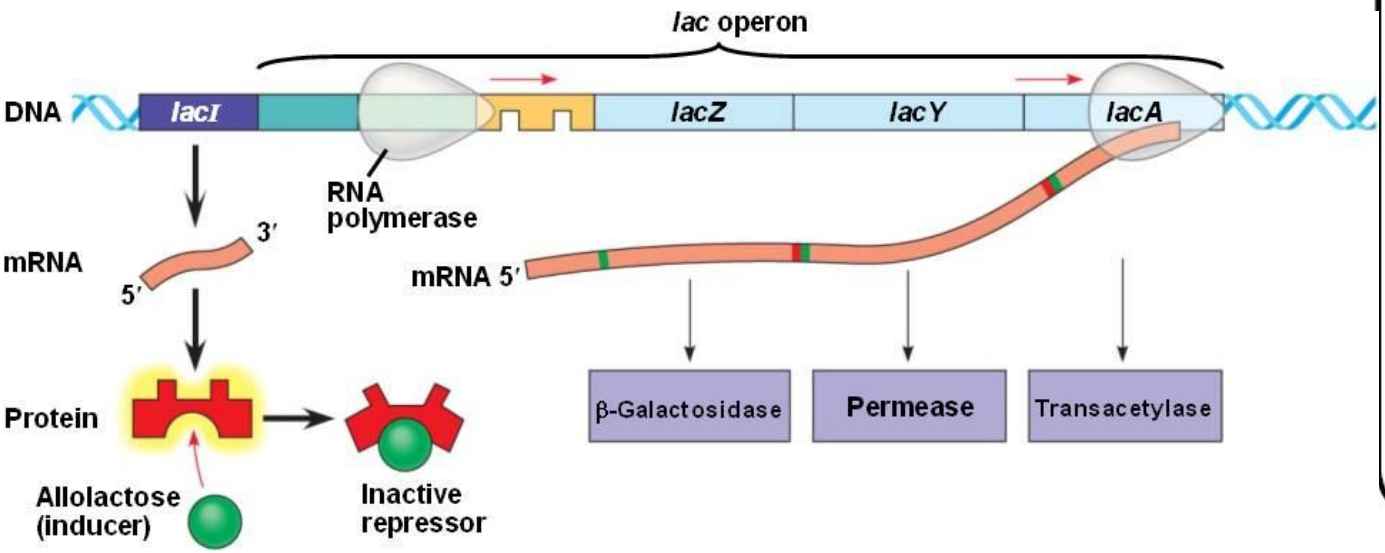
pET-23a

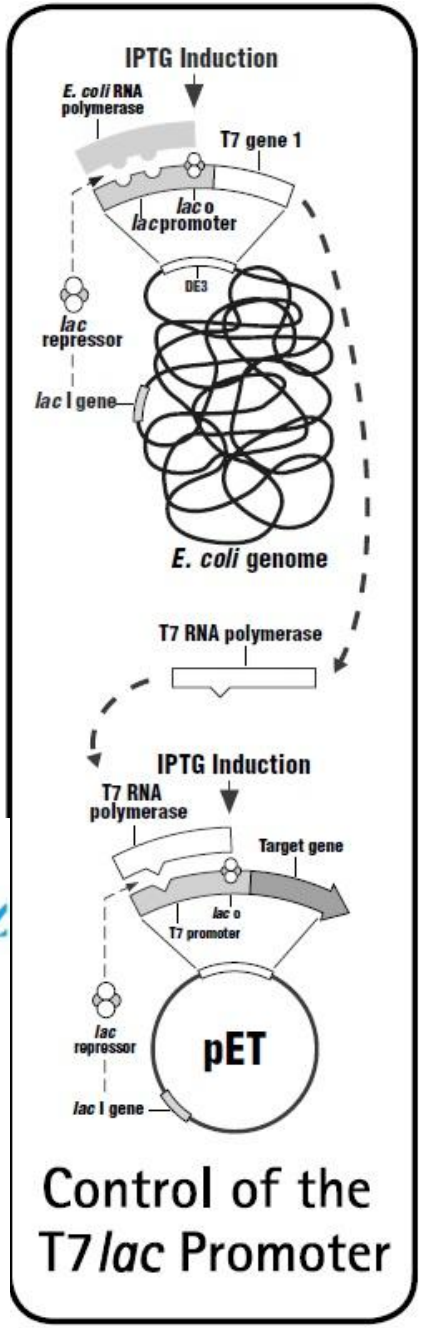
*Bam*H I *Eco*R I *Sac* I *Hinc* II *Sal* I *Hind* III *Eag* I *Not* I *Xho* I His•Tag

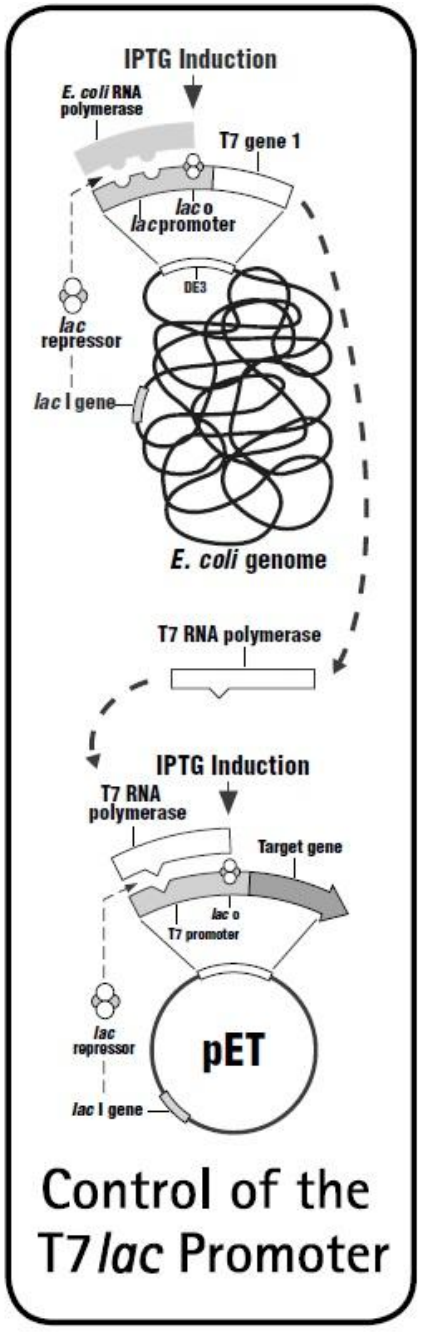
TATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGA

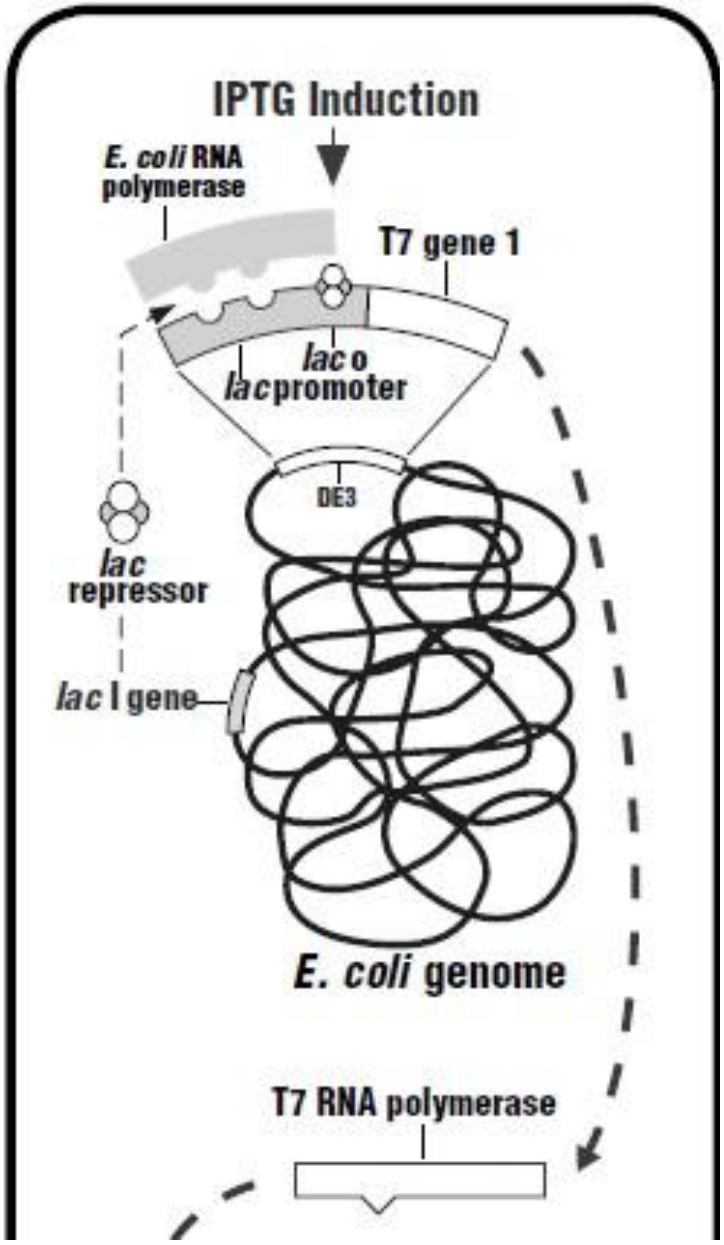
MetAlaSerMetThrGlyGlyGlnGlnMetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProProLeu

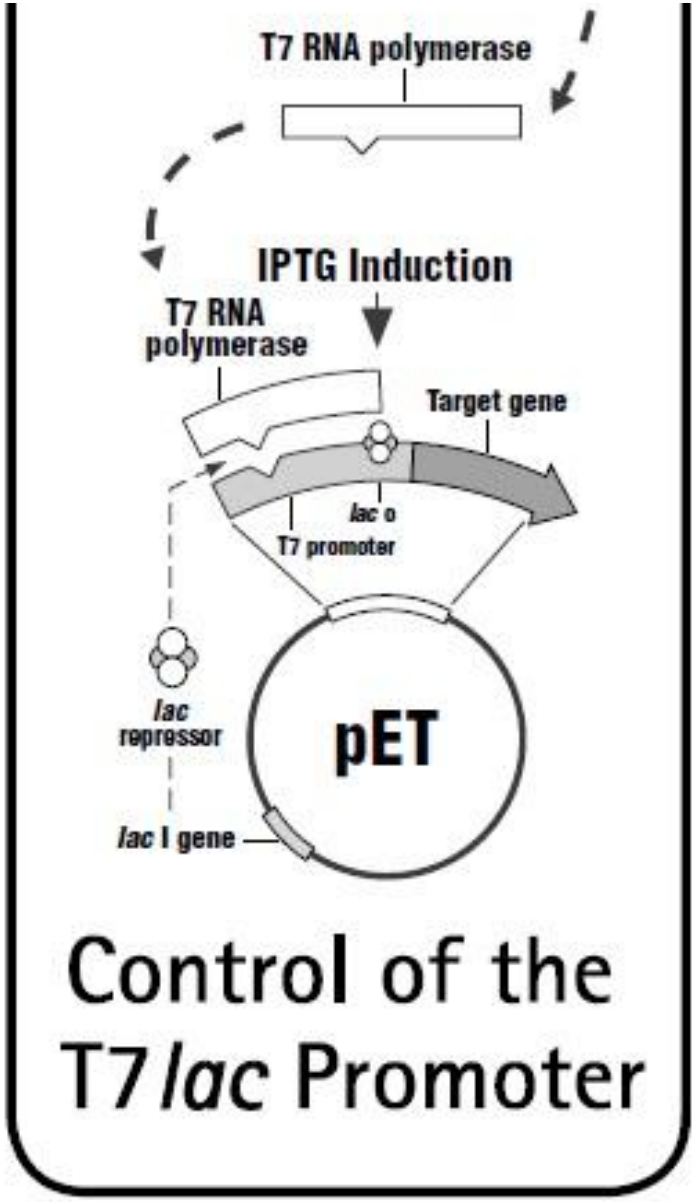

Regulatory gene
Promoter
Operator
DNA
lacI
lacZ
No RNA made
mRNA
3′
5′
RNA polymerase
Protein
Active repressor


lac operon
DNA
lacI
lacZ
lacY
lacA
RNA polymerase
mRNA
3′
5′
mRNA 5′
Protein
β-Galactosidase
Permease
Transacetylase
Allolactose (inducer)
Inactive repressor

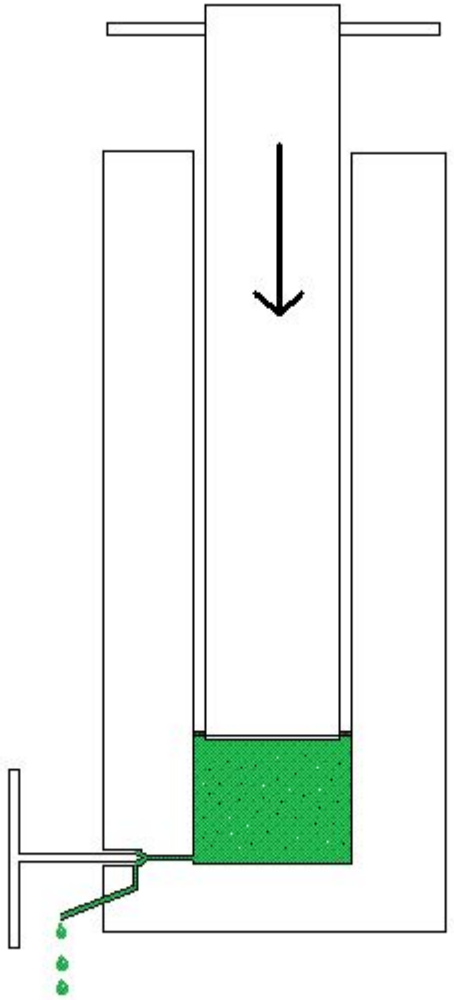

IPTG Induction
E. coli RNA polymerase
T7 gene 1
lac o
lac promoter
DE3
lac repressor
lac I gene
E. coli genome
T7 RNA polymerase
IPTG Induction
T7 RNA polymerase
Target gene
lac o
T7 promoter
lac repressor
pET
lac I gene
Control of the T7lac Promoter


IPTG Induction
E. coli RNA polymerase
T7 gene 1
lac o
lac promoter
DE3
lac repressor
lac I gene
E. coli genome
T7 RNA polymerase
IPTG Induction
T7 RNA polymerase
Target gene
lac o
T7 promoter
lac repressor
pET
lac I gene
Control of the T7 lac Promoter


IPTG Induction
E. coli RNA polymerase
T7 gene 1
lac o
lac promoter
DE3
lac repressor
lac I gene
E. coli genome
T7 RNA polymerase


T7 RNA polymerase
IPTG Induction
T7 RNA polymerase
Target gene
lac o
T7 promoter
lac repressor
pET
lac I gene
Control of the T7 lac Promoter

Лизис клеток



Очистка целевого белка

Хроматография

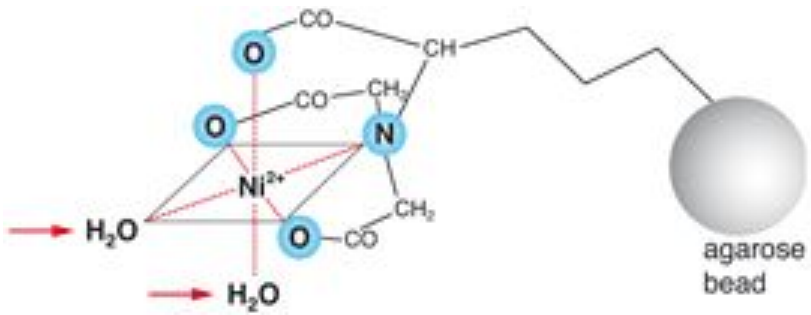
column containing stationary phase

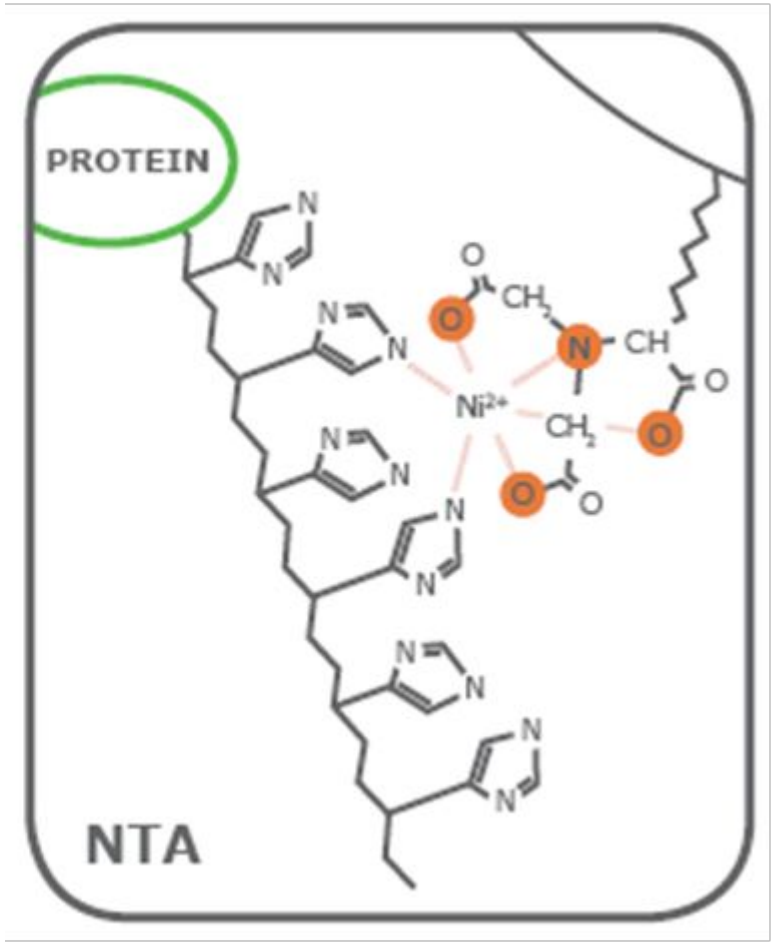
load sample

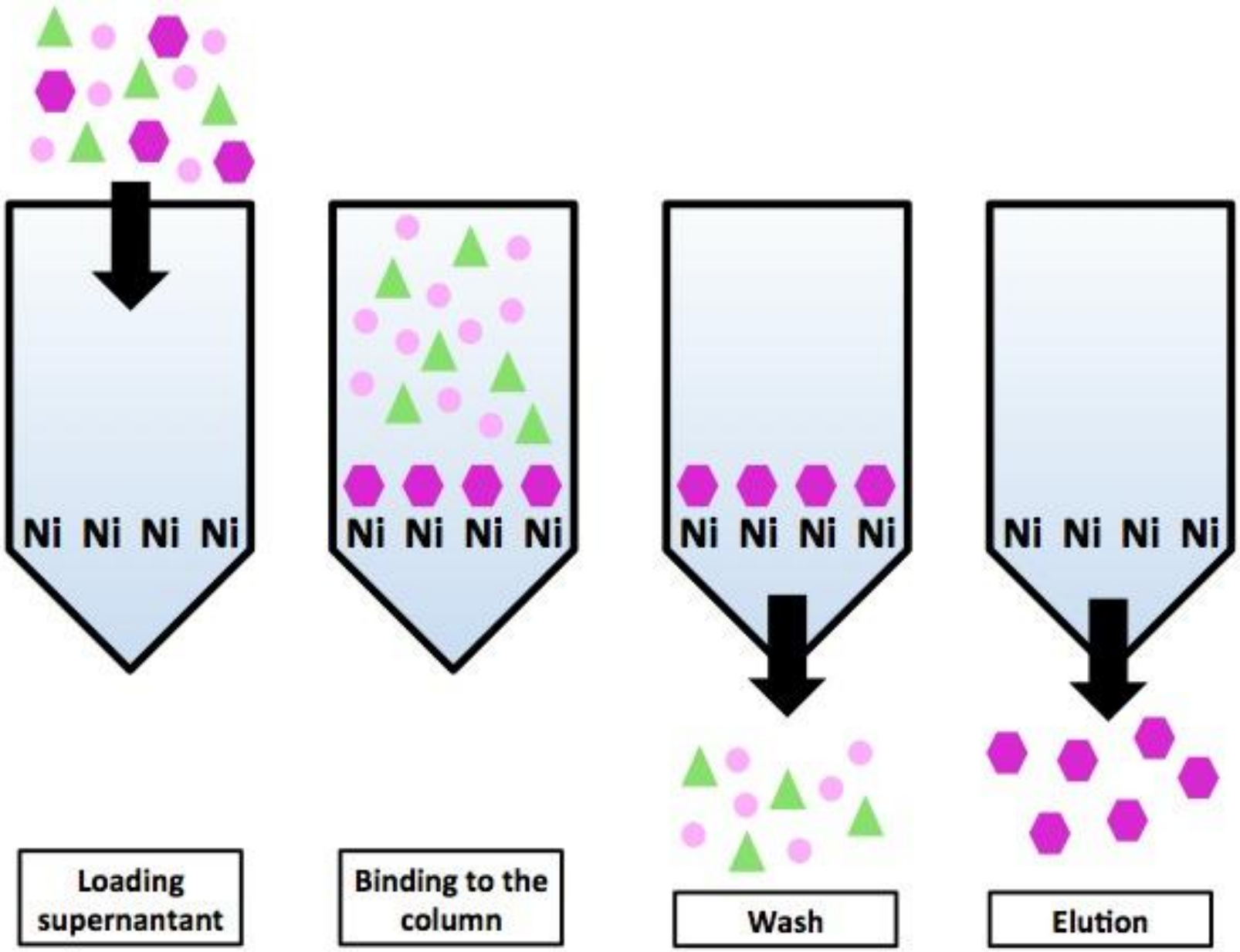
add solvent

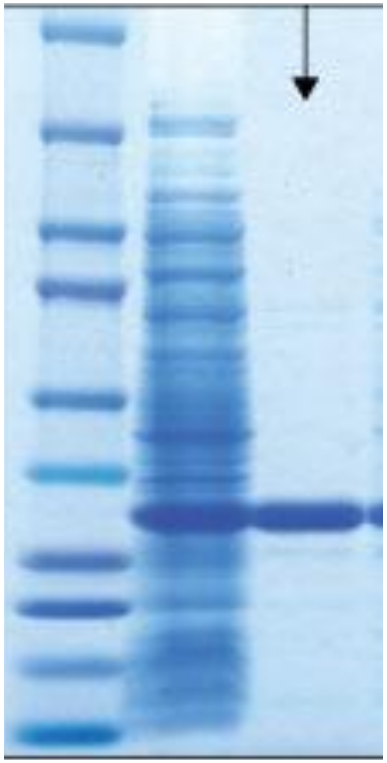
collect components

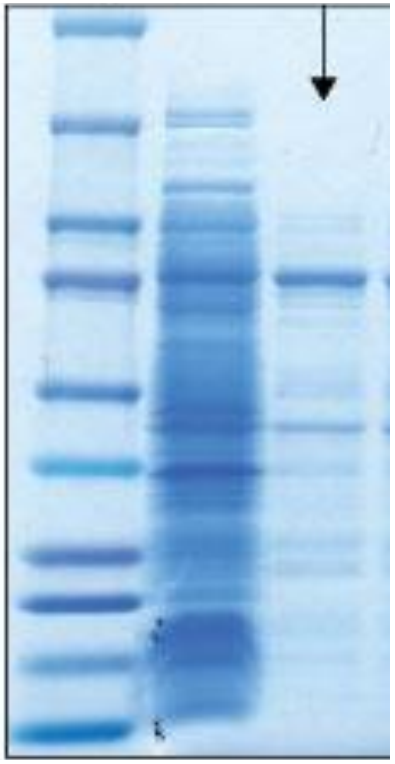

HisTrap™ HP
5 ml
HisTrap™ HP
1 ml


CO
CH
O
CO
CH2
O
N
Ni2+
CH2
H2O
O
CO
H2O
agarose
bead

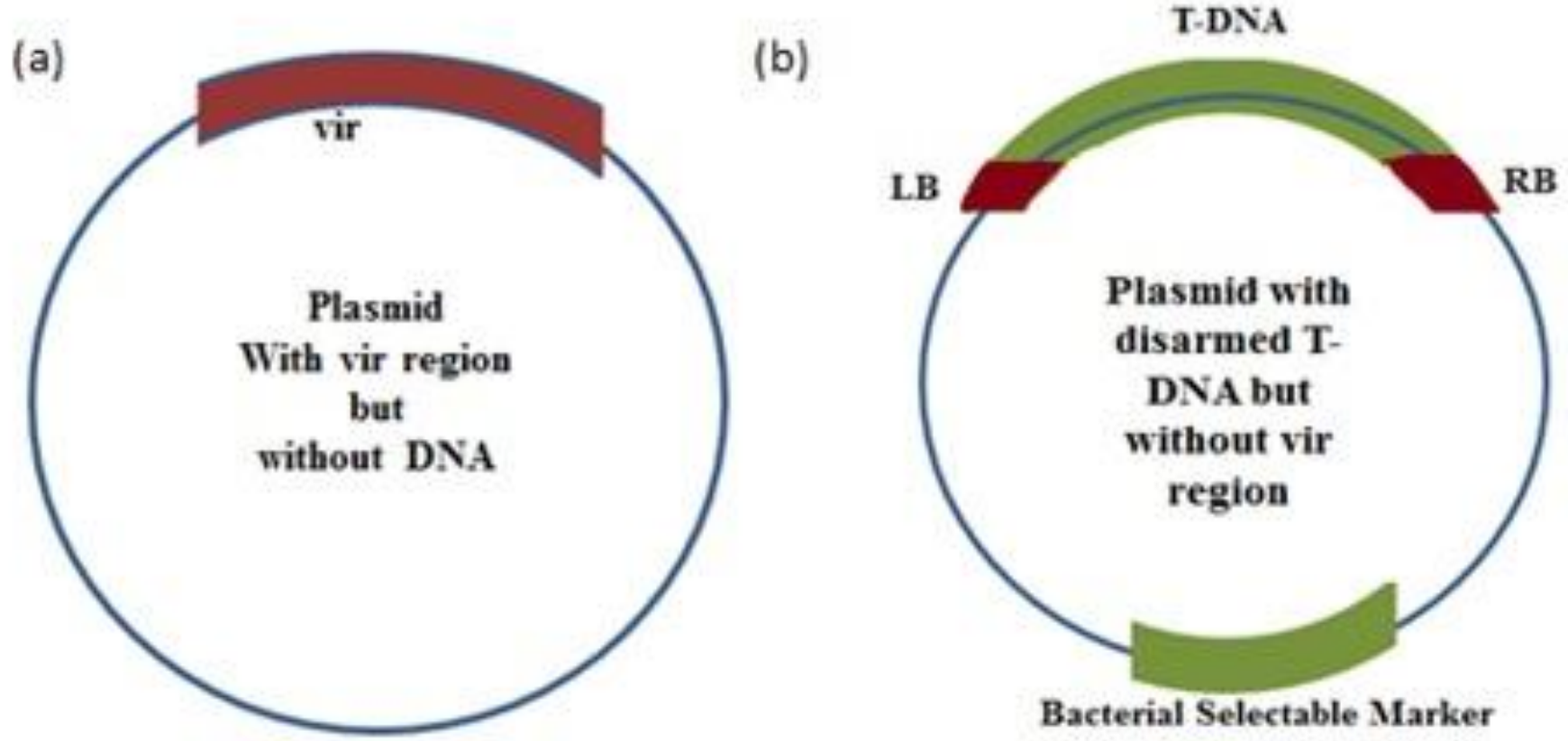

PROTEIN
NTA
Ni2+
CH2
CH
O
N

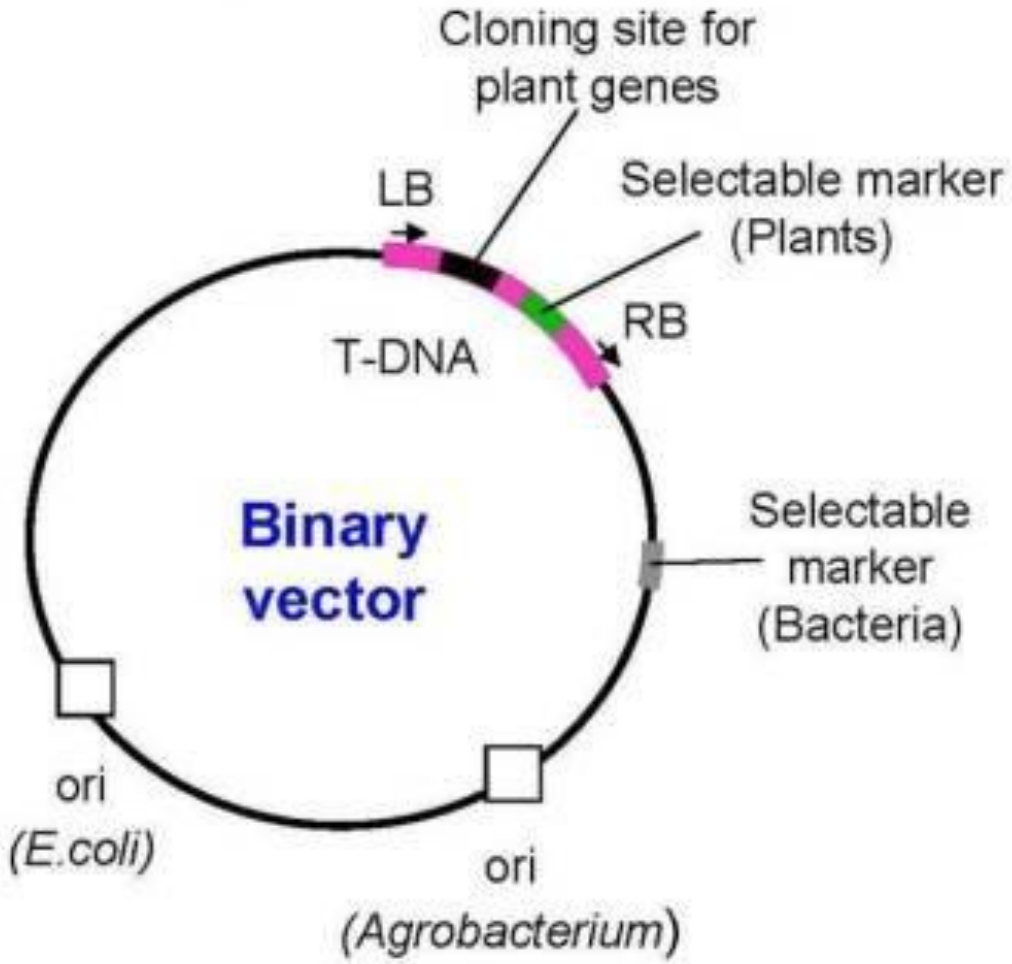

Ni Ni Ni Ni
Ni Ni Ni Ni
Ni Ni Ni Ni
Ni Ni Ni Ni
Loading supernantant
Binding to the column
Wash
Elution



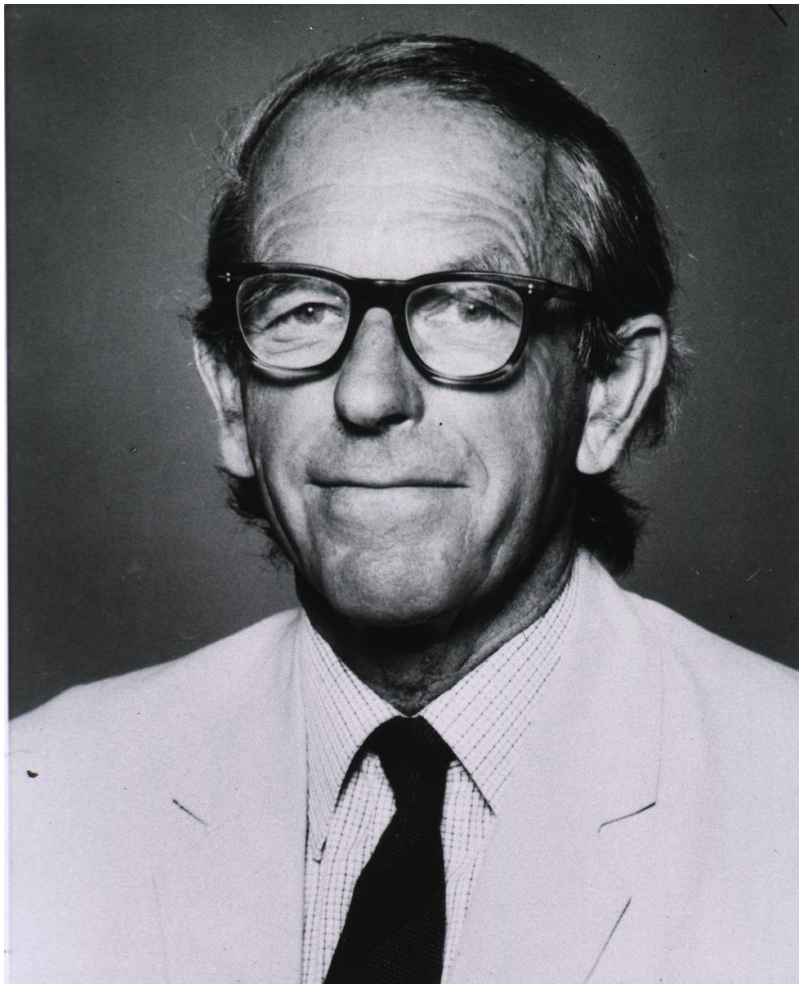


Векторы для трансформации растений

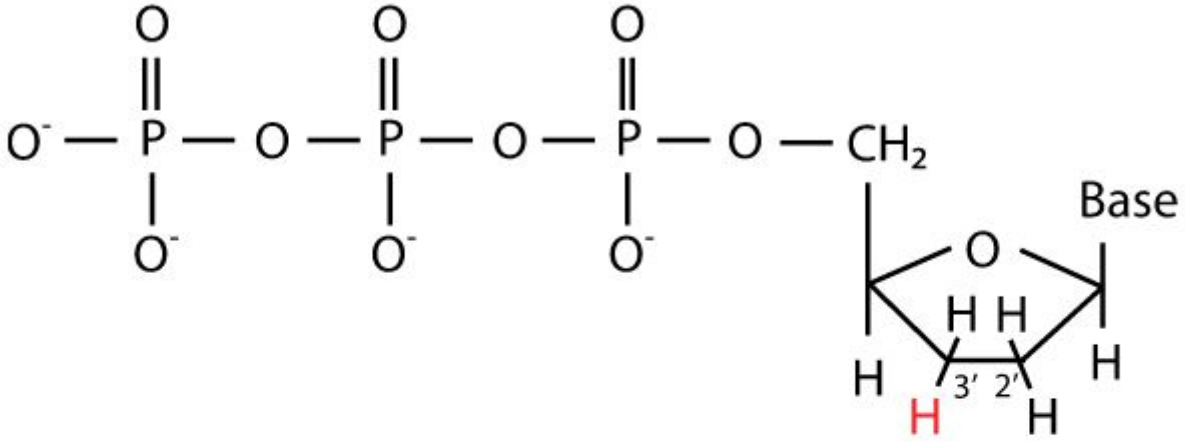



Cloning site for
plant genes
LB
Selectable marker
(Plants)
RB
T-DNA
Binary
vector
Selectable
marker
(Bacteria)
ori
(E.coli)
ori
(Agrobacterium)

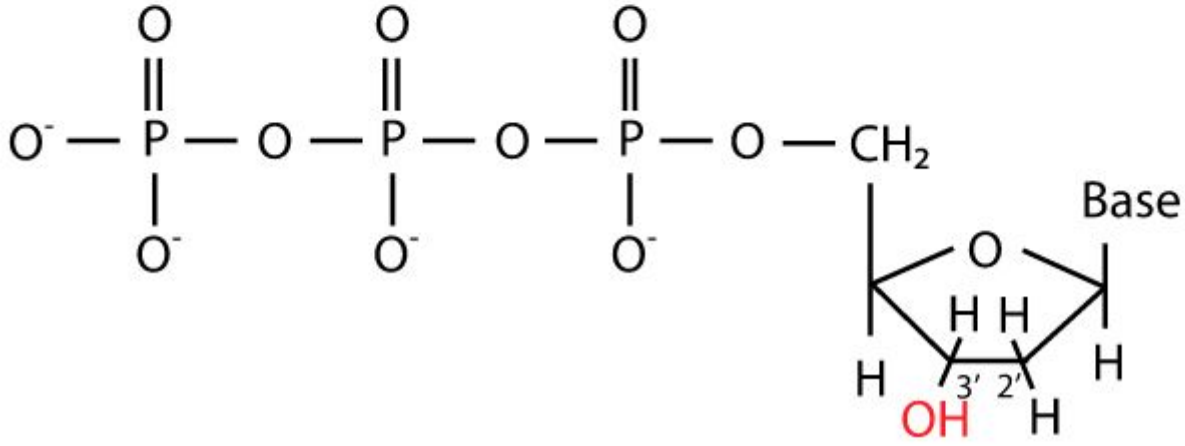
Определение первичной структуры ДНК

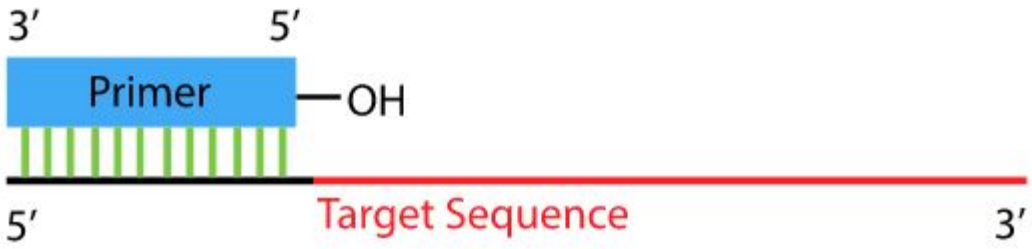


Dideoxynucleotide (ddNTP)



Deoxynucleotide (dNTP)



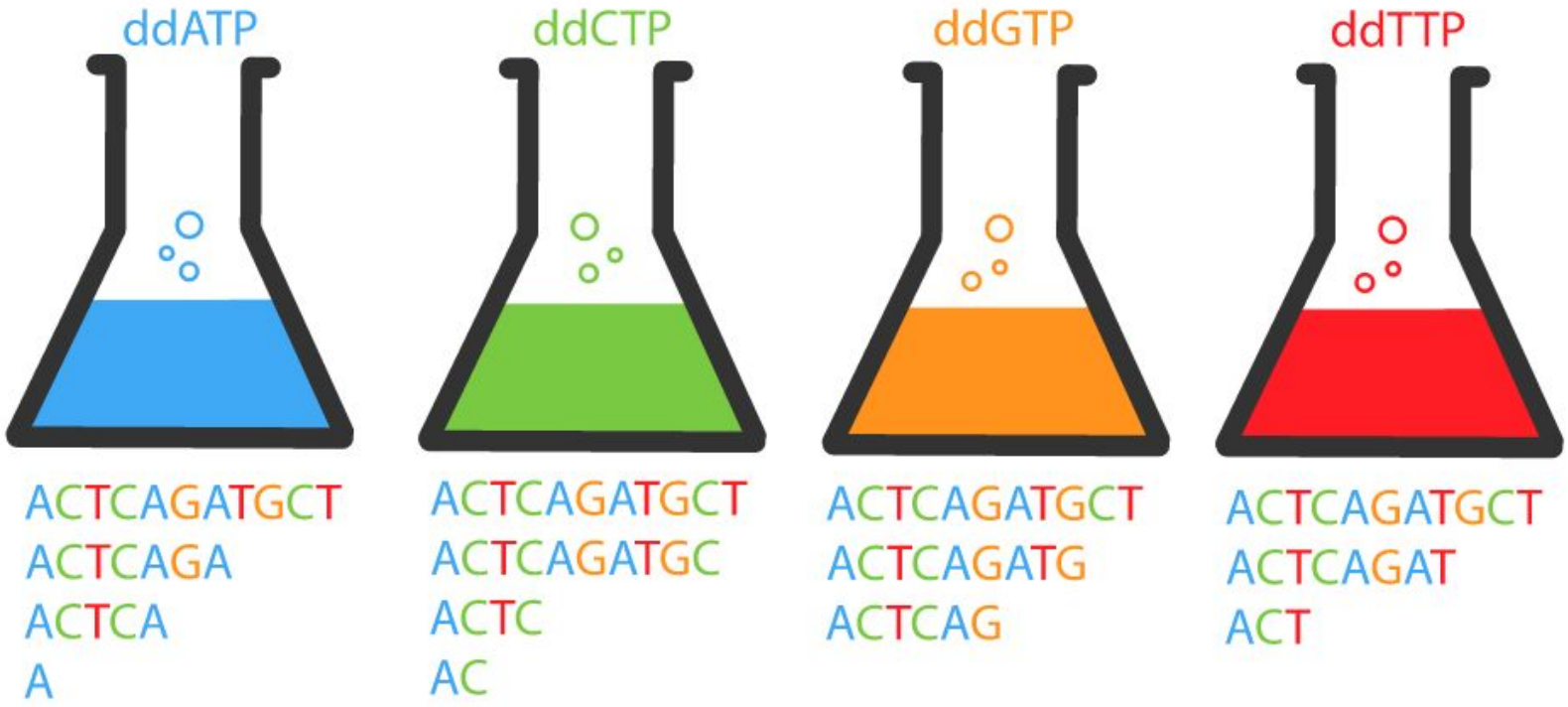

3′
5′
Primer
OH
5′
Target Sequence
3′

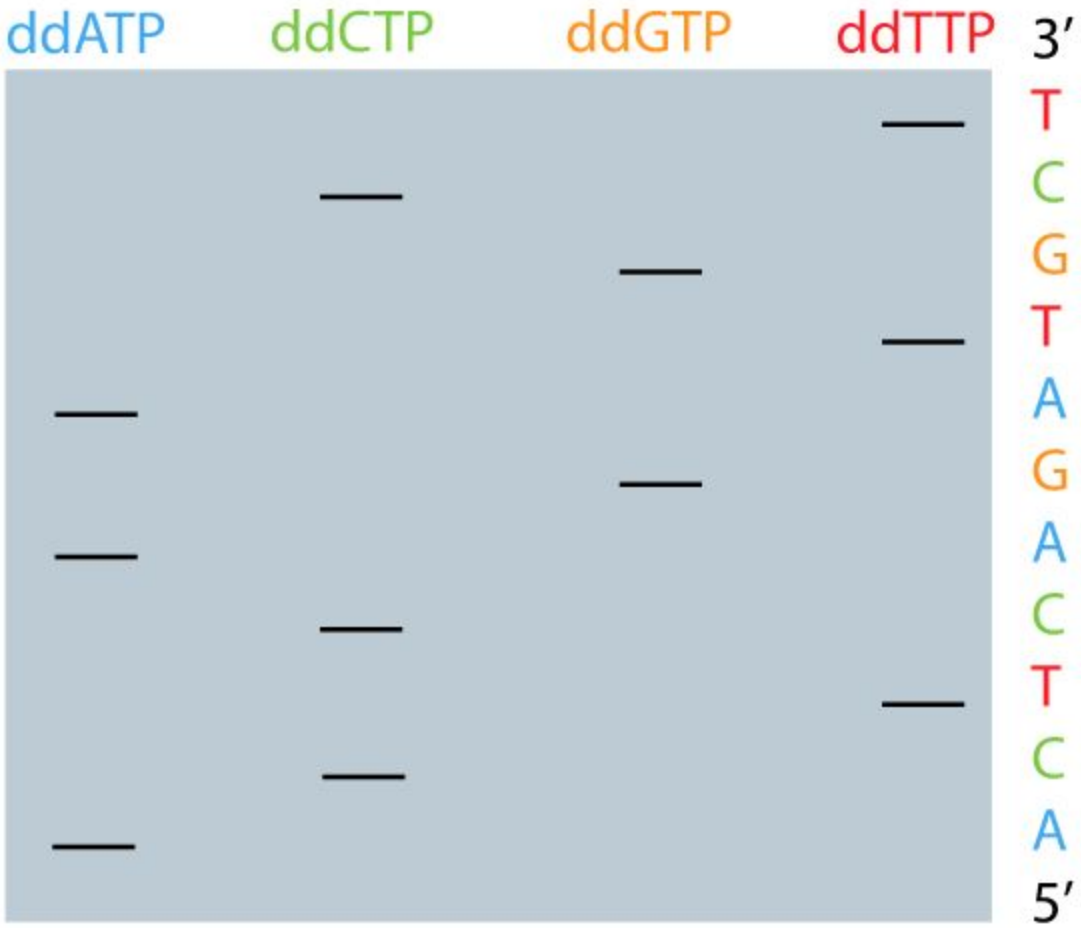
dATP + dCTP + dGTP + dTTP

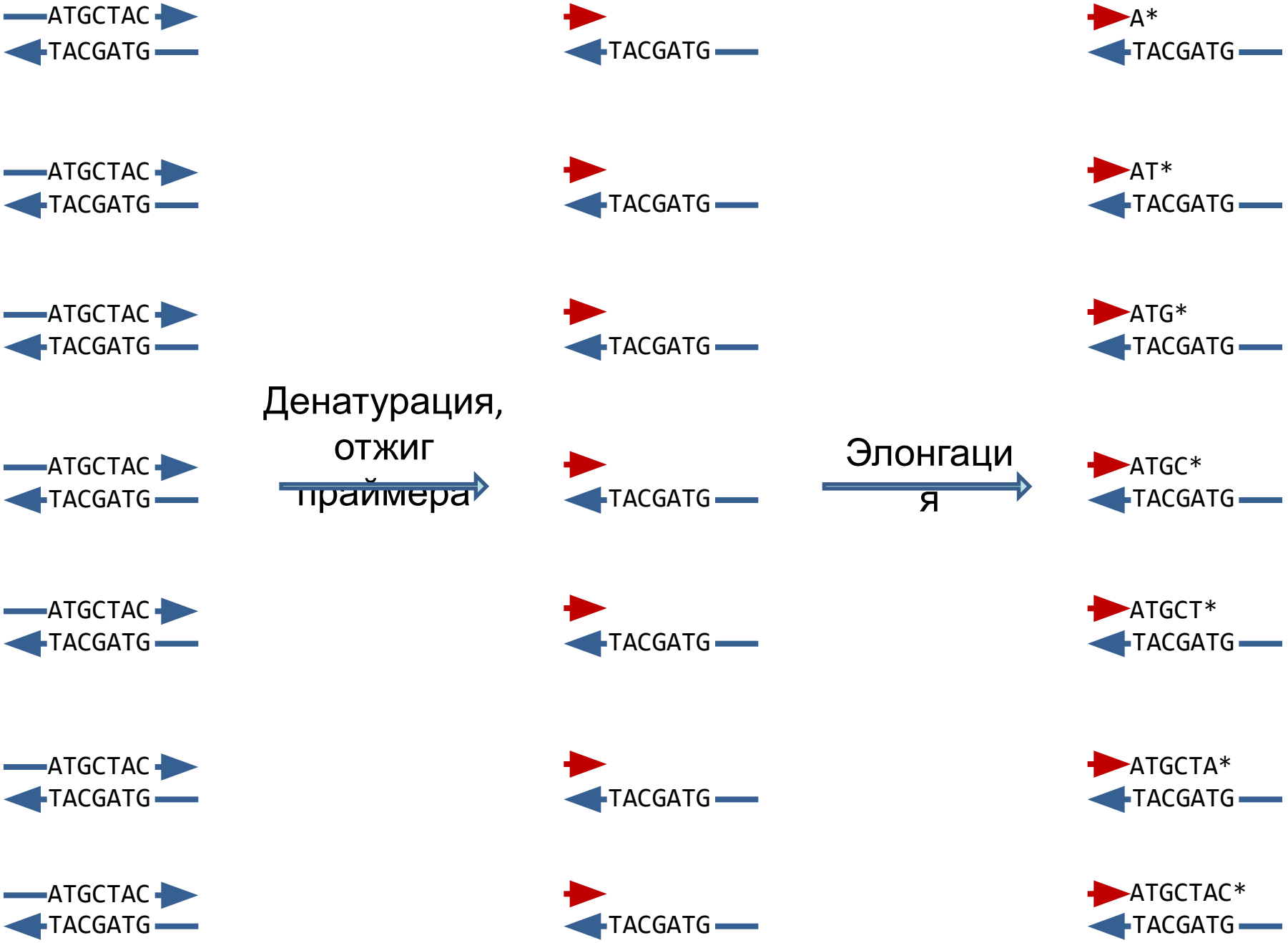
DNA Polymerase

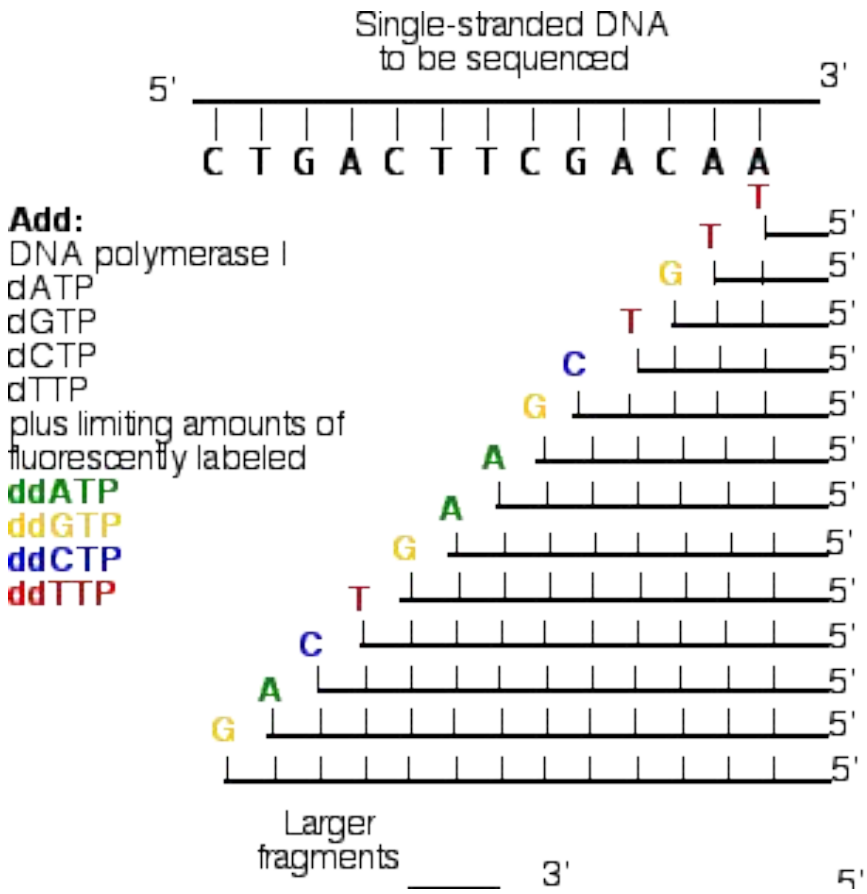
Template DNA

Primer




ddATP
ddCTP
ddGTP
ddTTP
3′
T
C
G
T
A
G
A
C
T
C
A
5′


ATGCTAC
TACGATG
TACGATG
A*
AT*
ATG*
ATGC*
ATGCT*
ATGCTA*
ATGCTAC*
Денатурация, отжиг праймера
Элонгация


Single-stranded DNA
to be sequenced
5'
3'
C T G A C T T C G A C A A
Add:
DNA polymerase I
dATP
dGTP
dCTP
dTTP
plus limiting amounts of
fluorescently labeled
ddATP
ddGTP
ddCTP
ddTTP
T
T
G
T
C
G
A
A
G
T
C
A
G
5'
Larger
fragments
3'
5'

A*
TACGATG

AT*
TACGATG

ATG*
TACGATG

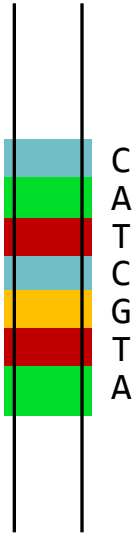
ATGC*
TACGATG

ATGCT*
TACGATG

ATGCTA*
TACGATG

ATGCTAC*
TACGATG

Капиллярный электрофорез



Анализ