

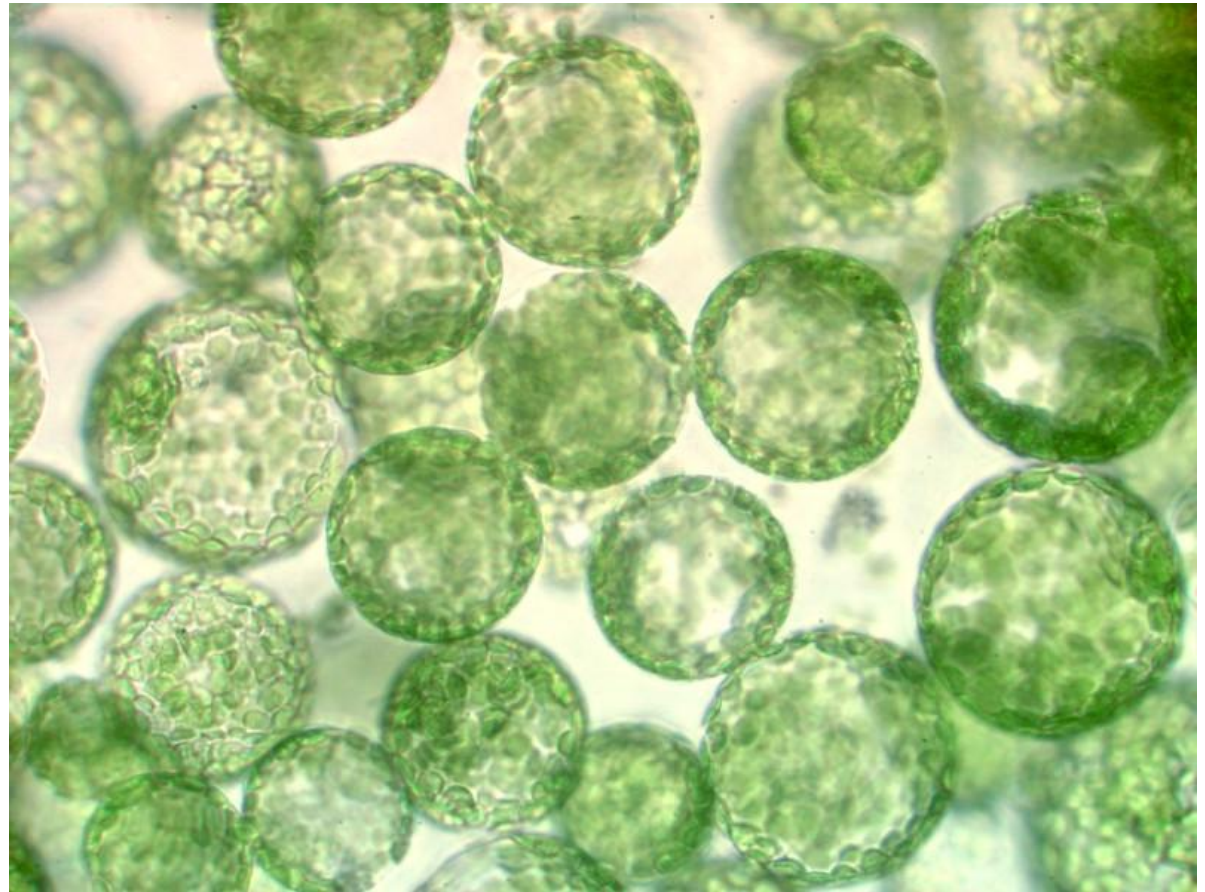
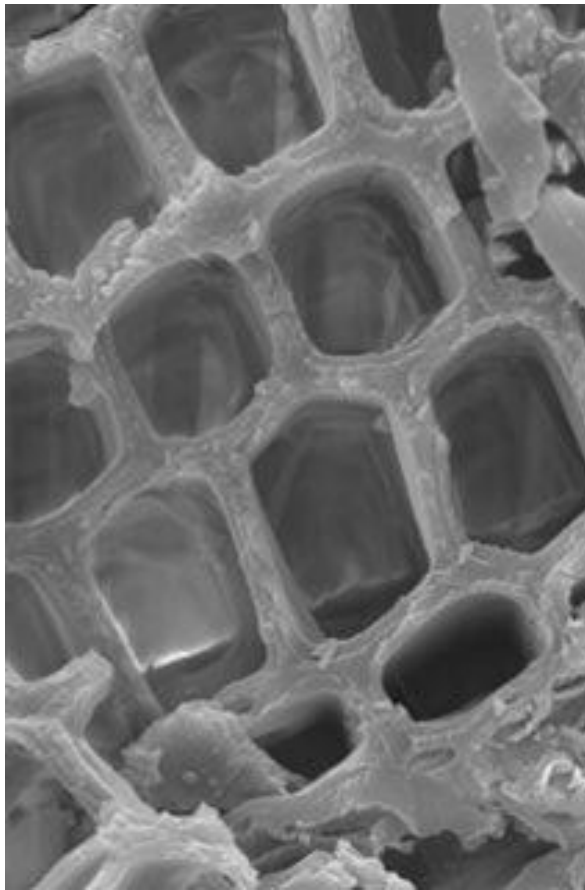
Трансгенные растения



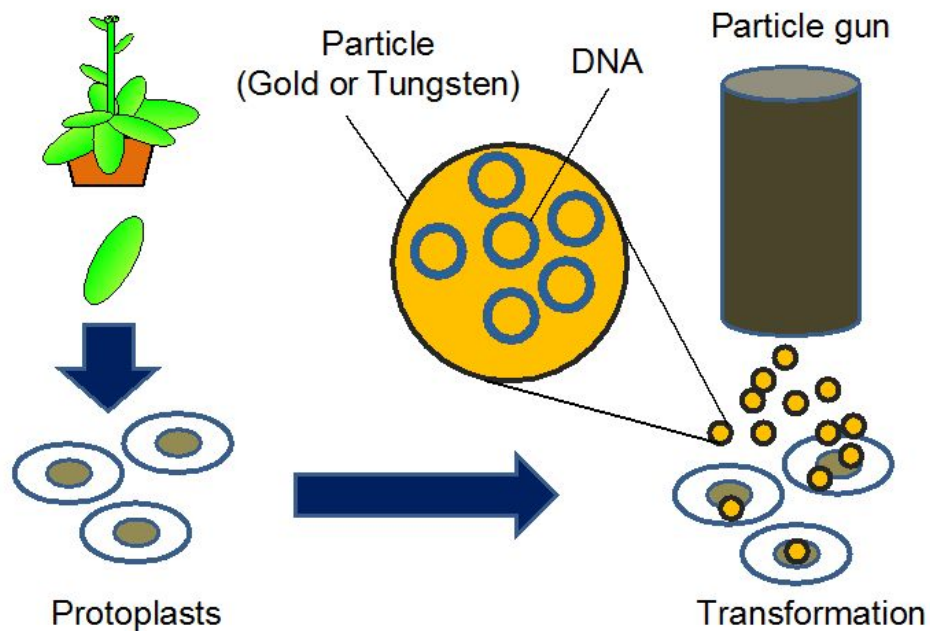
Что нужно, чтобы получить
трансгенное растение?

Чужеродная ДНК должна проникнуть в клетку

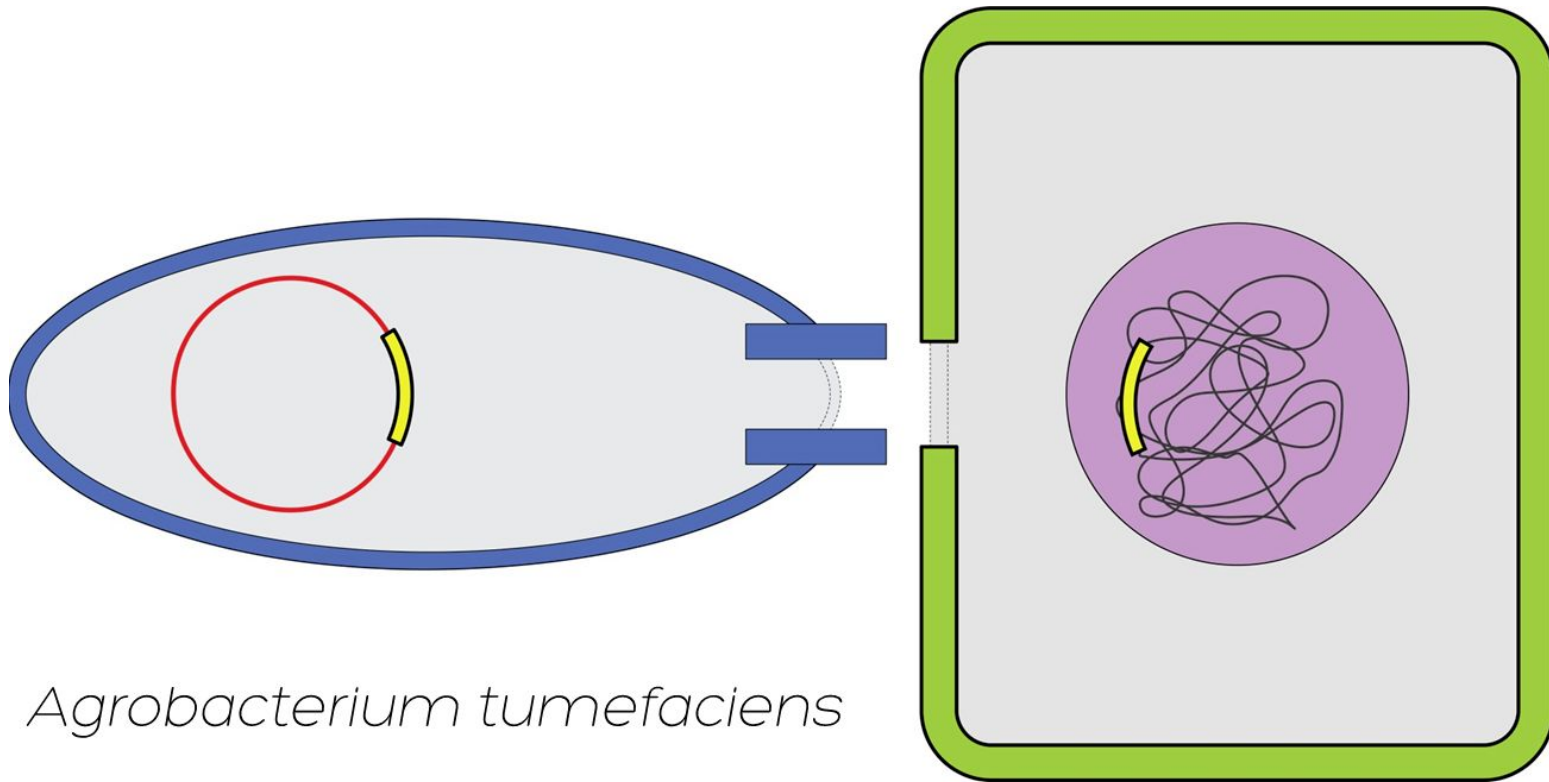
«Избавление» от клеточной стенки



Бомбардировка клеток



Использование потенциала фитопатогенов



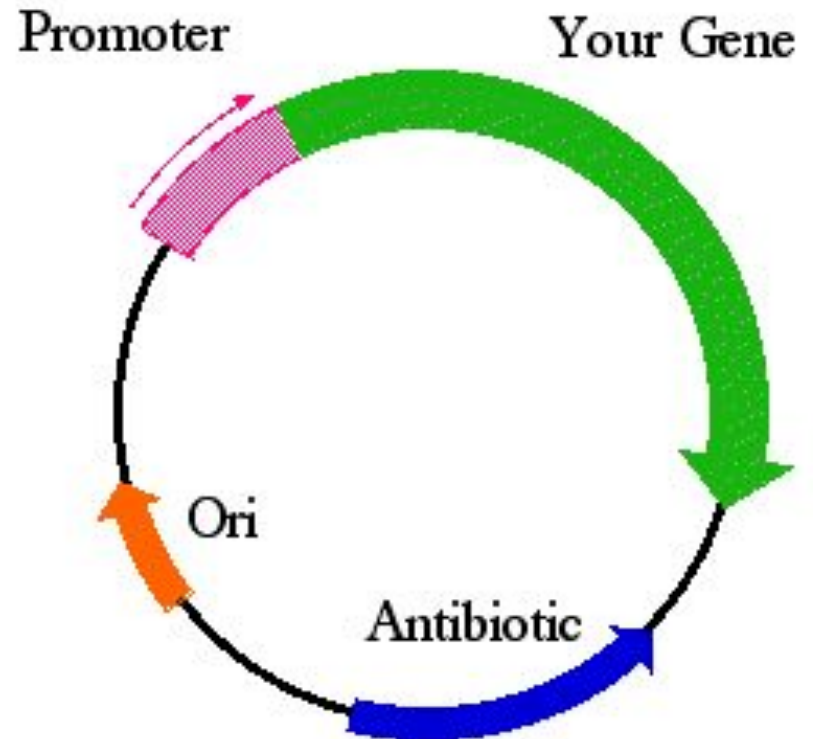
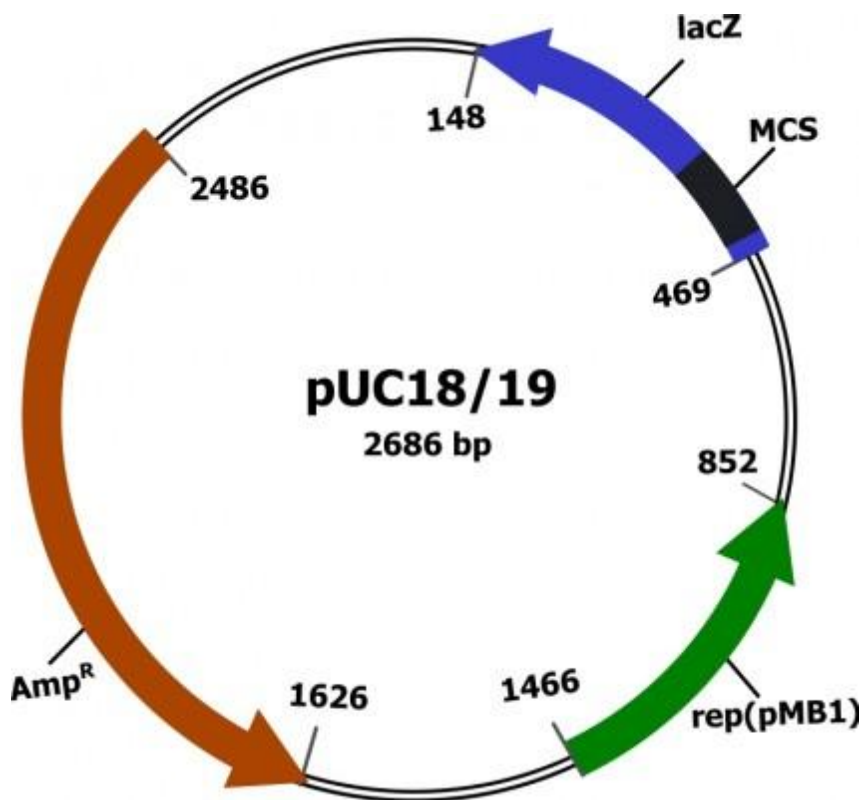
Agrobacterium tumefaciens

Нужно чтобы целевой участок ДНК:

Встроился в геном

Экспрессировался (чтобы генетическая информация считывалась)

Чужеродные гены находятся в составе векторных плазмид

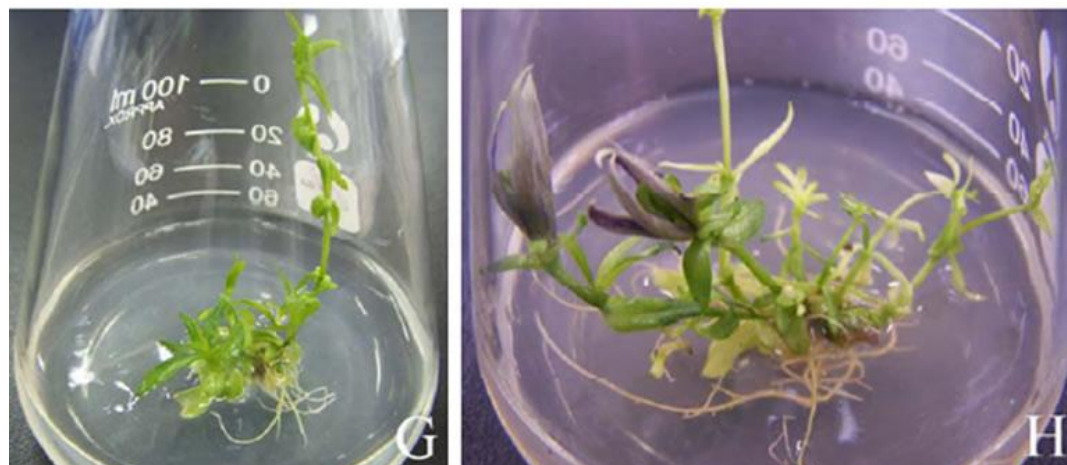
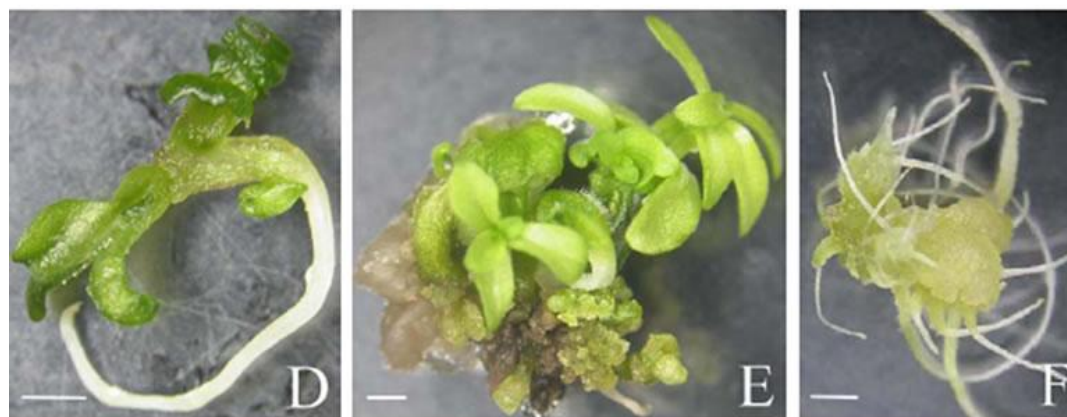
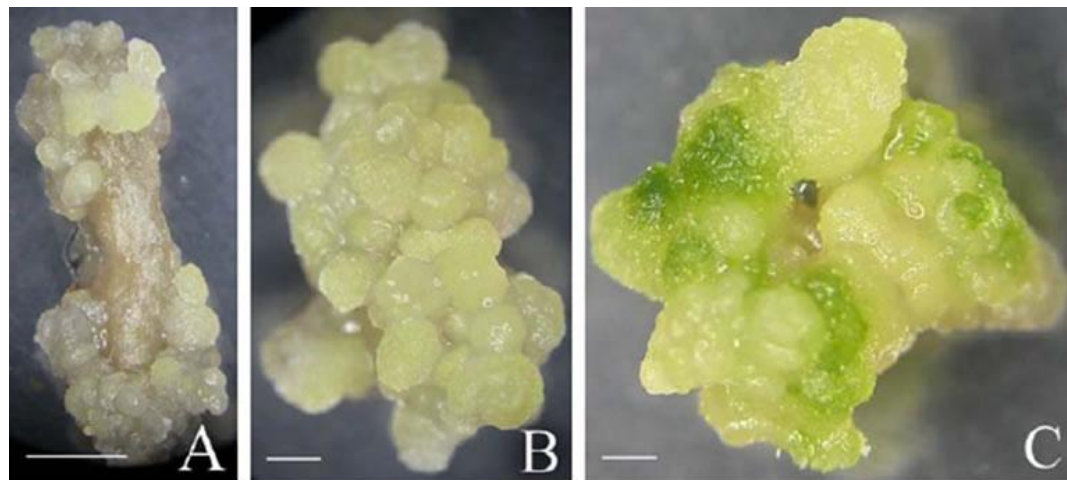


Введение векторных конструкций в клетки растений



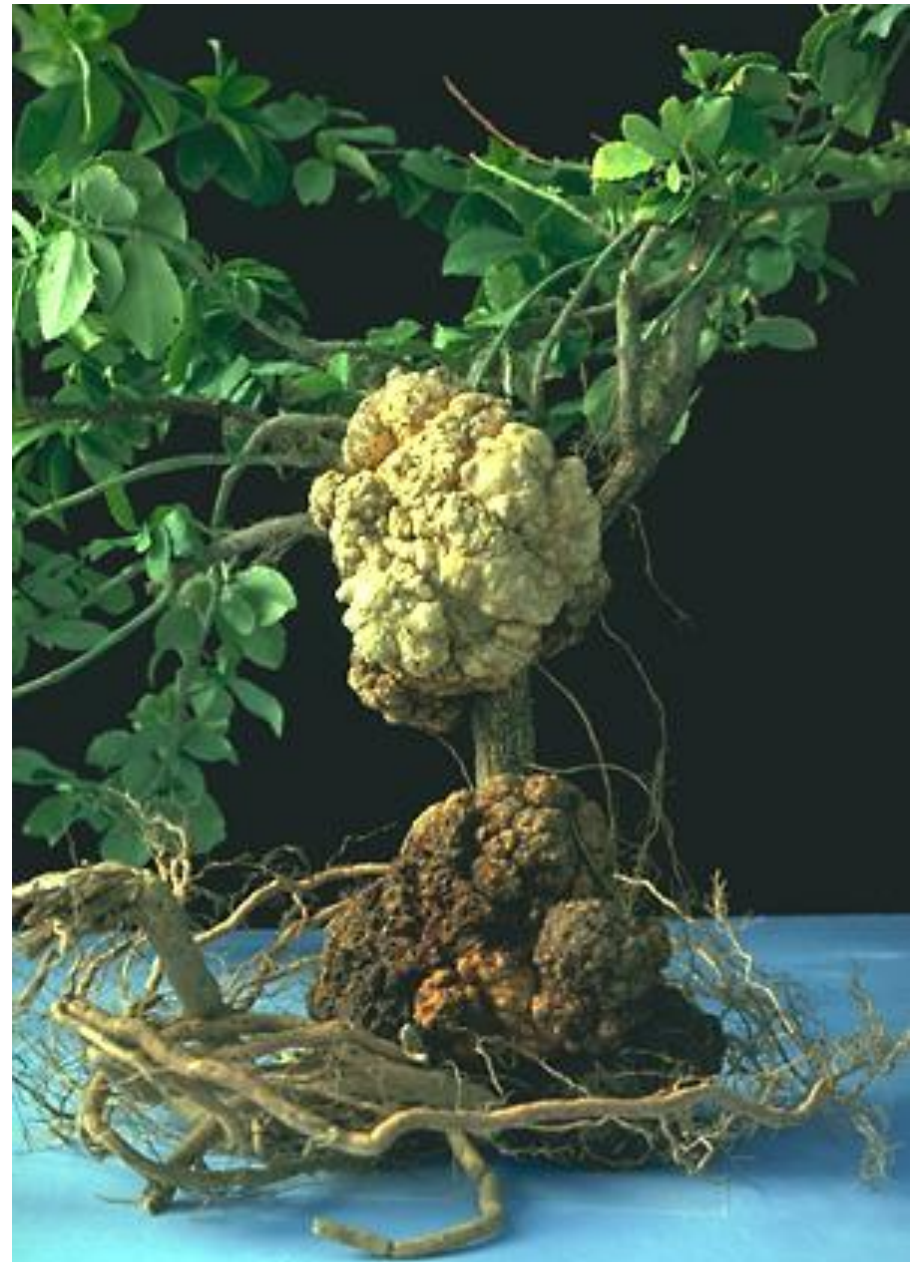
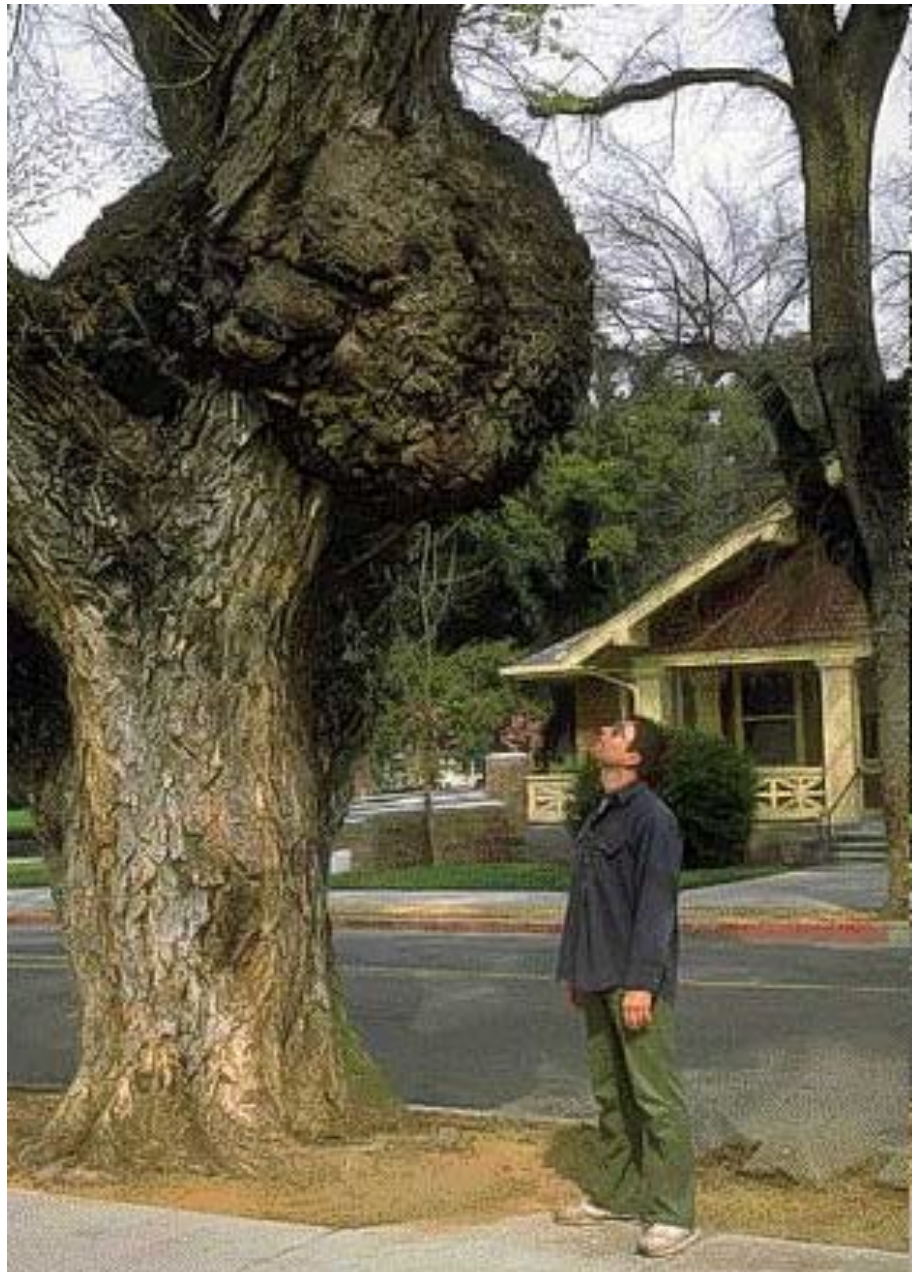
Станет ли после этого растение трансгенным?

Регенерация
целых растений из
отдельных
трансформирован
ных клеток

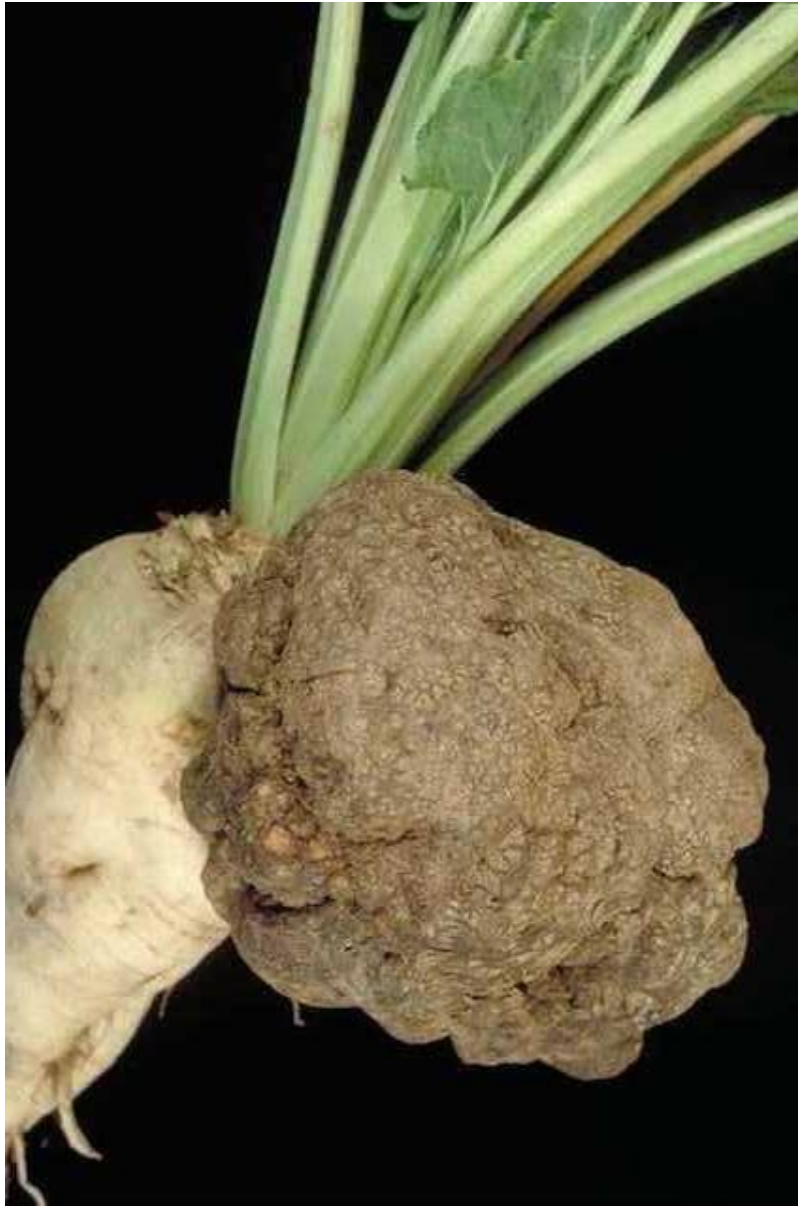


Основной подход для создания
трансгенных растений

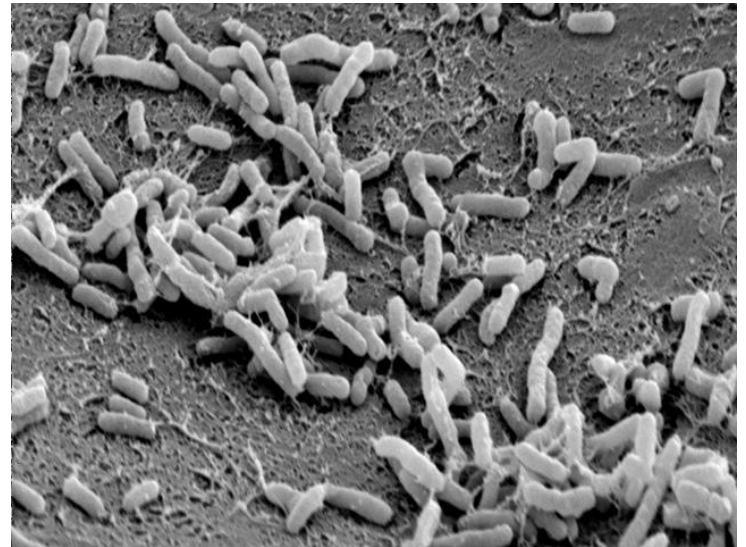
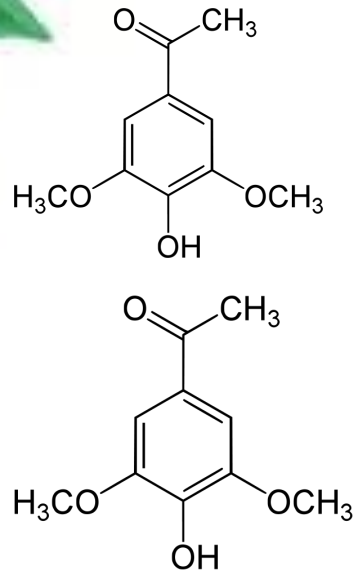
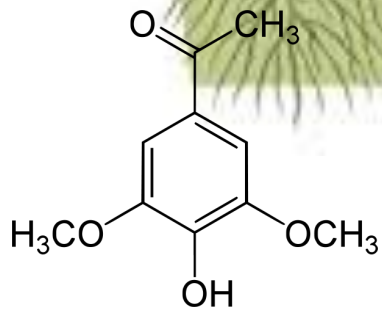
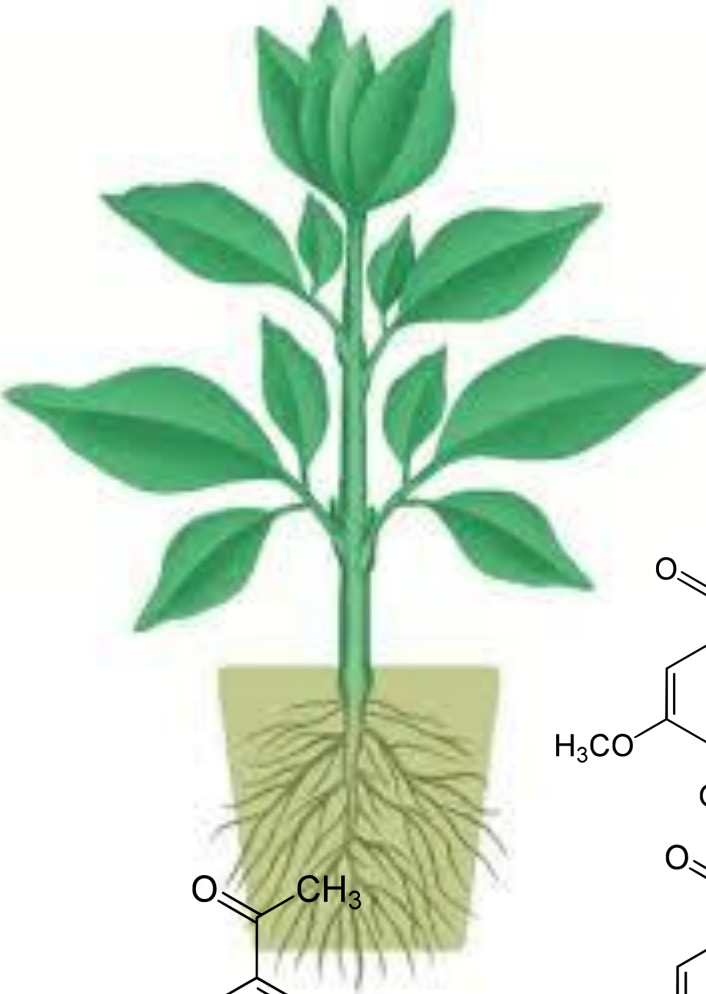
Корончатые галлы



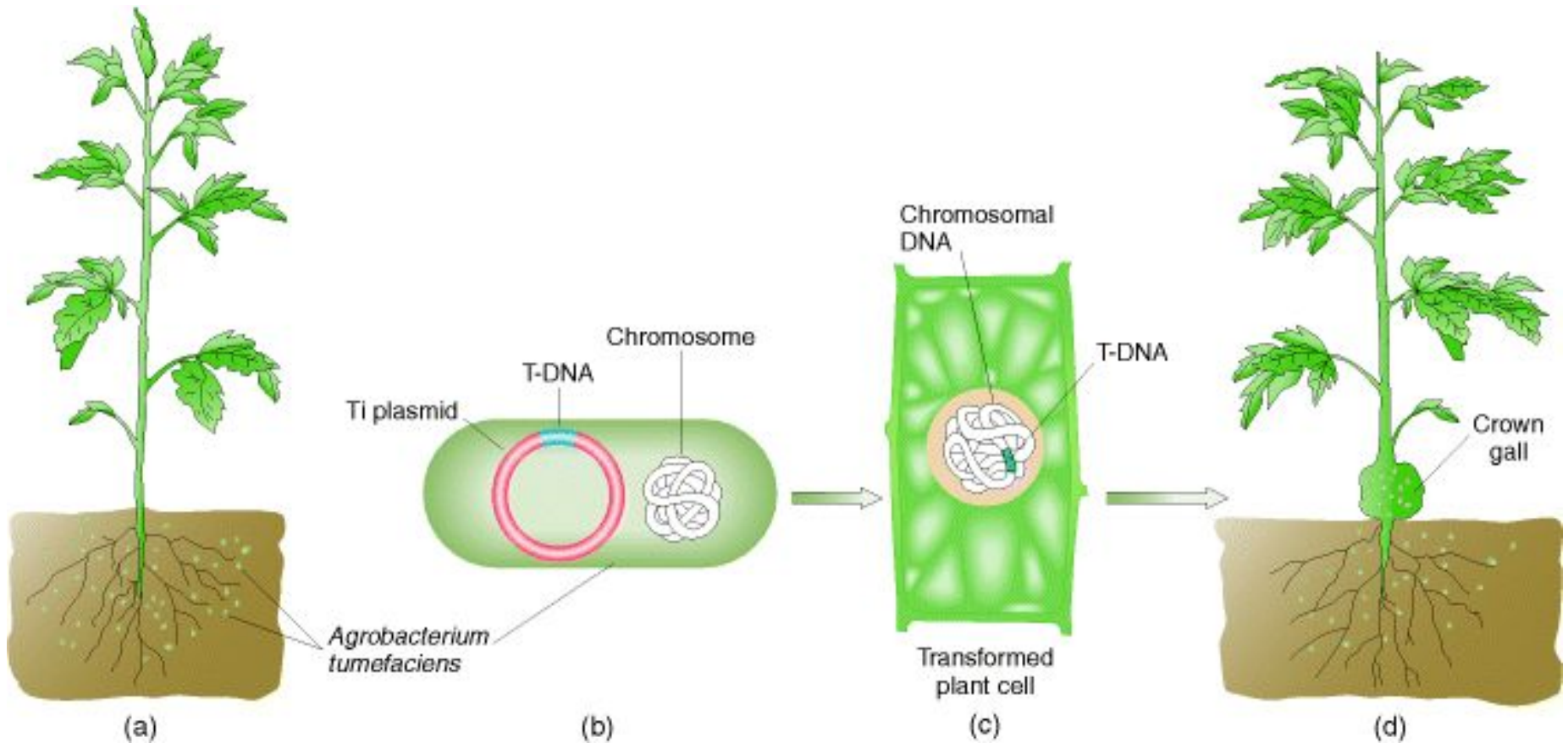
Корончатые галлы



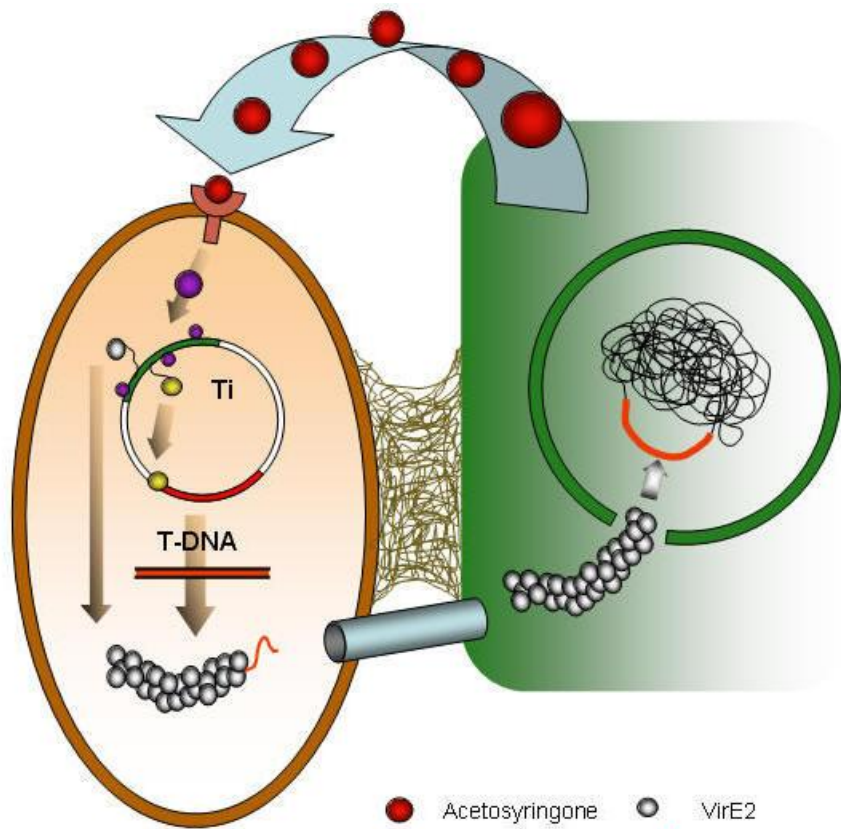
Agrobacterium tumefaciens



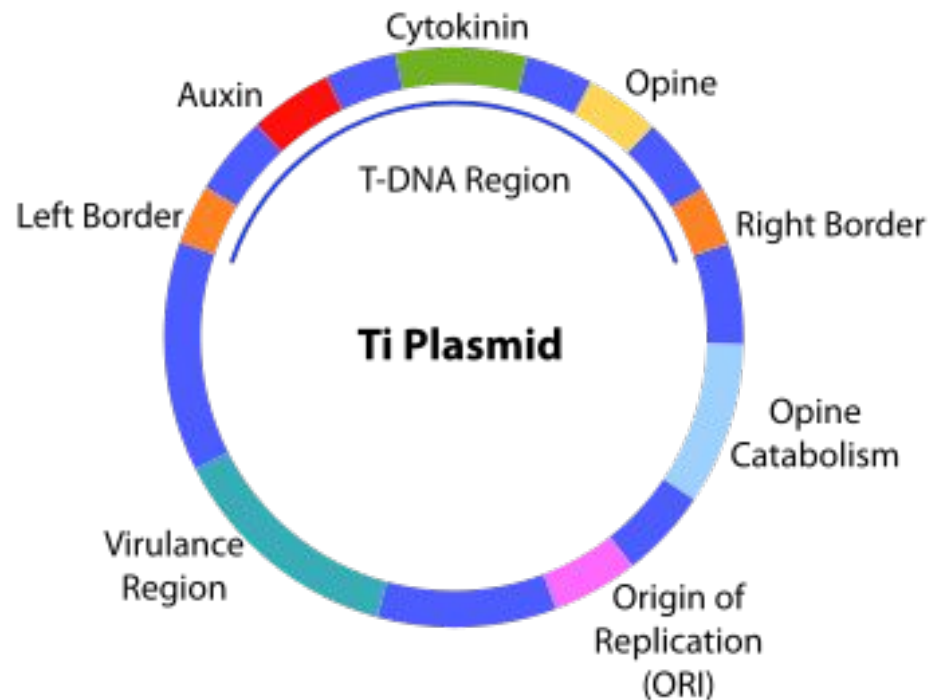
Agrobacterium tumefaciens



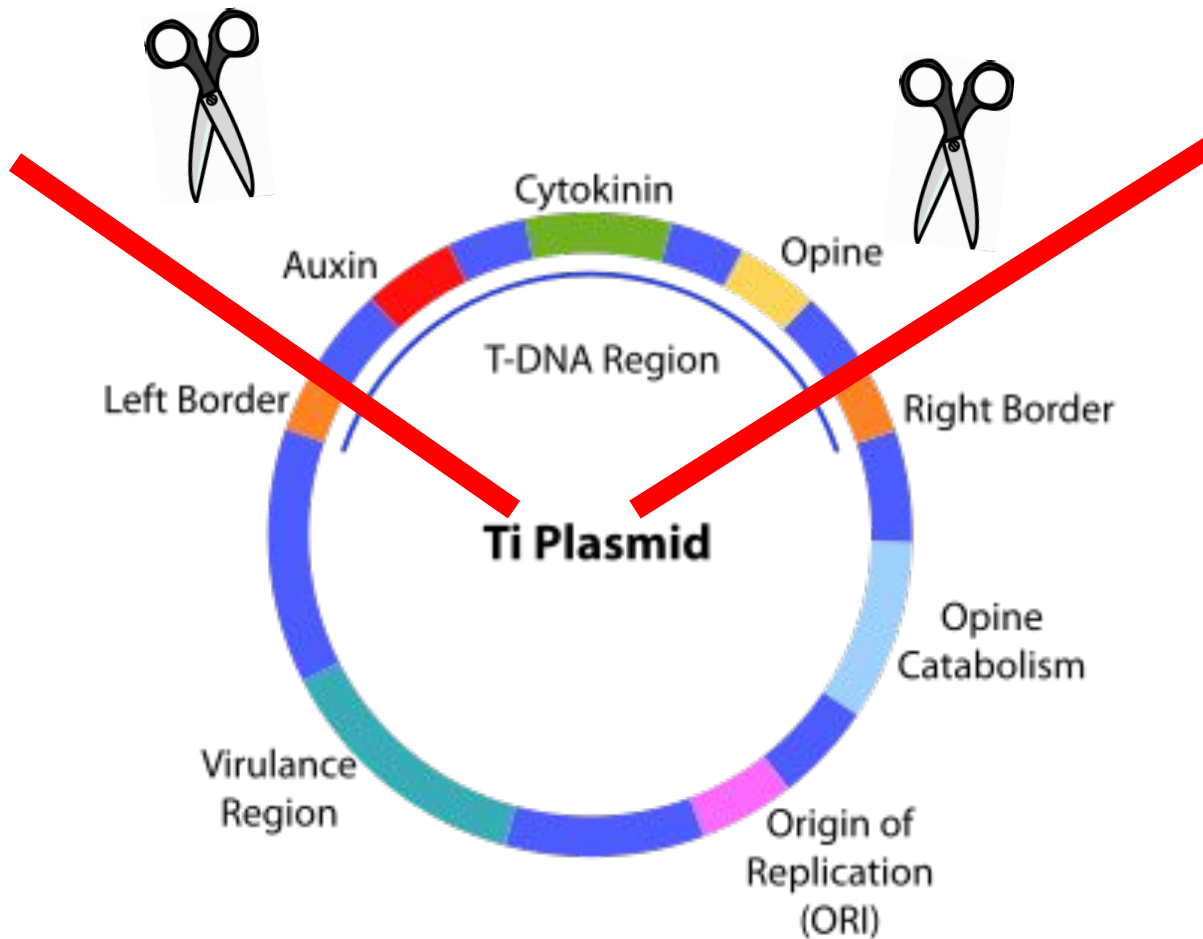
Интеграция тДНК в геном растения



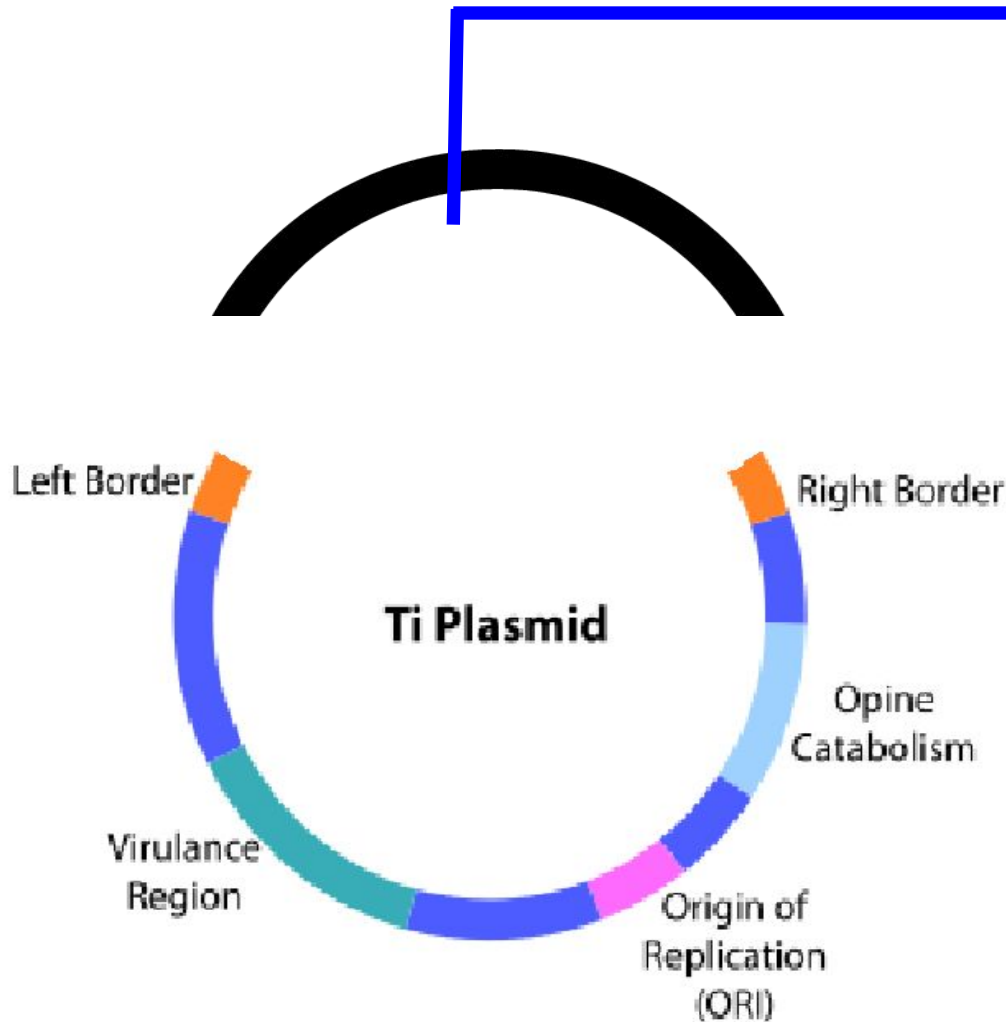
- Acetosyringone
- VirG
- VirC/VirD
- VirE2
- VirE2 + ssDNA
- T-Pilus (VirB2 – VirB11)



Модификация Ti – плазмиды для создания трансгенных растений



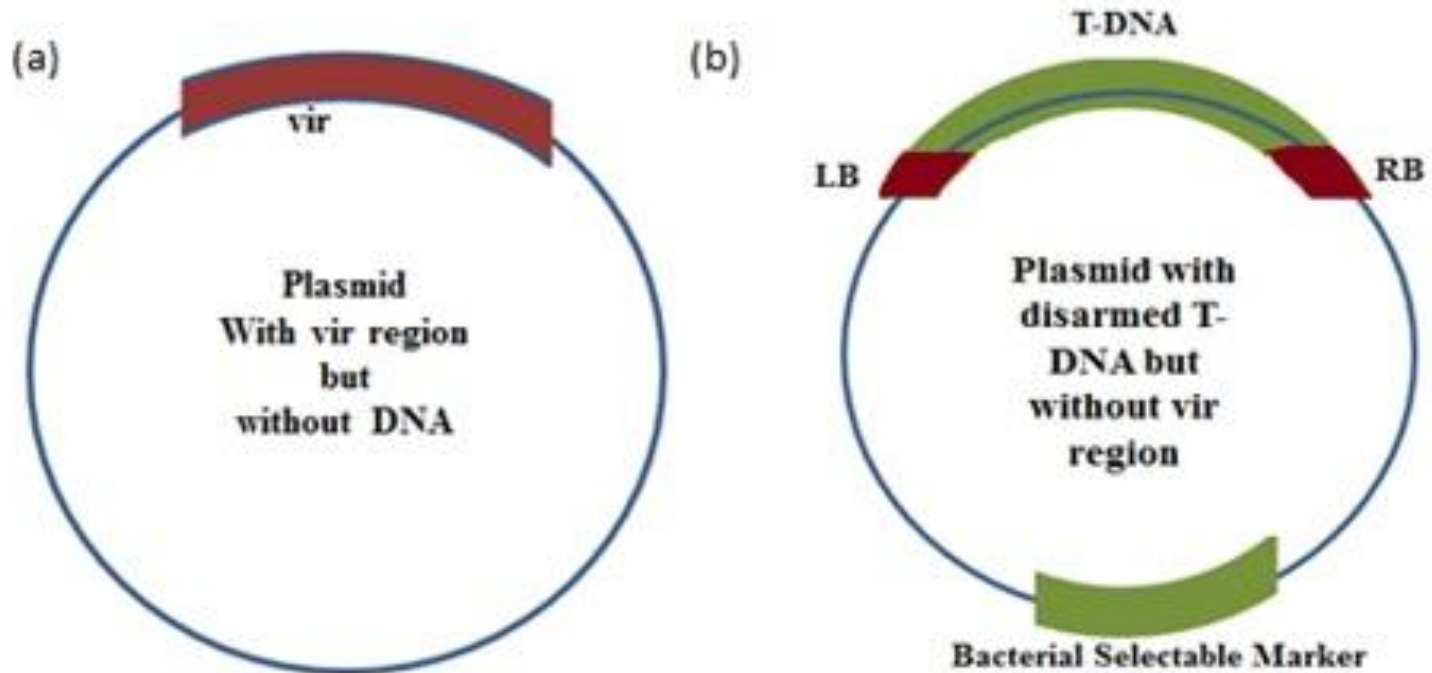
Модификация Ti – плазмиды для создания трансгенных растений

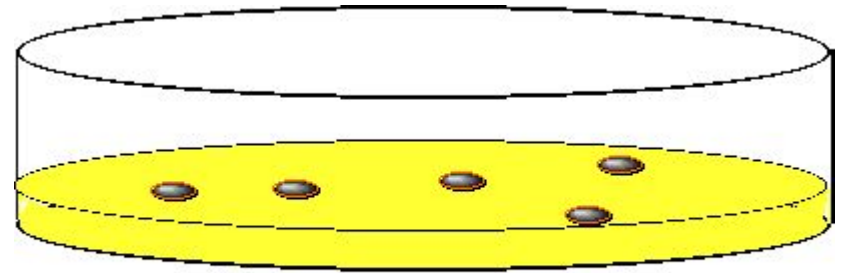
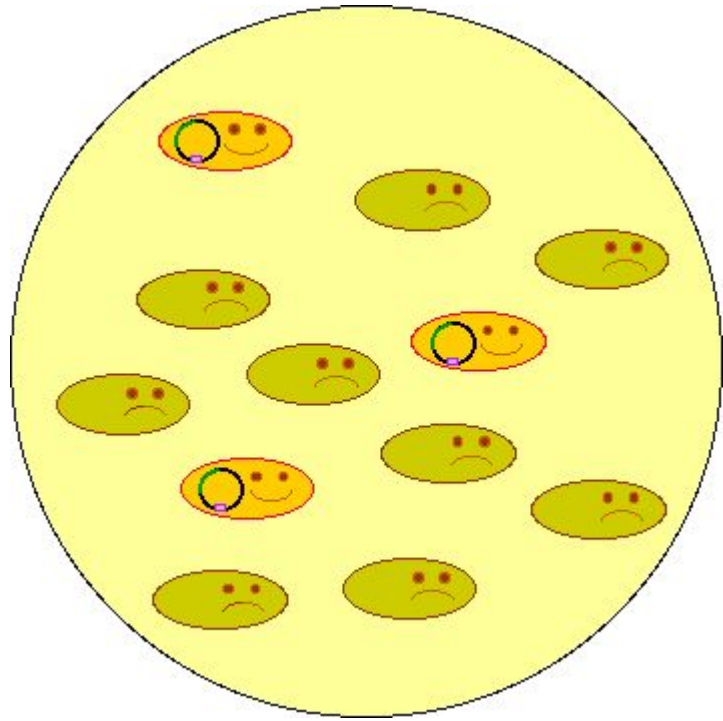


Ген, который хотят встроить

Селективный маркер

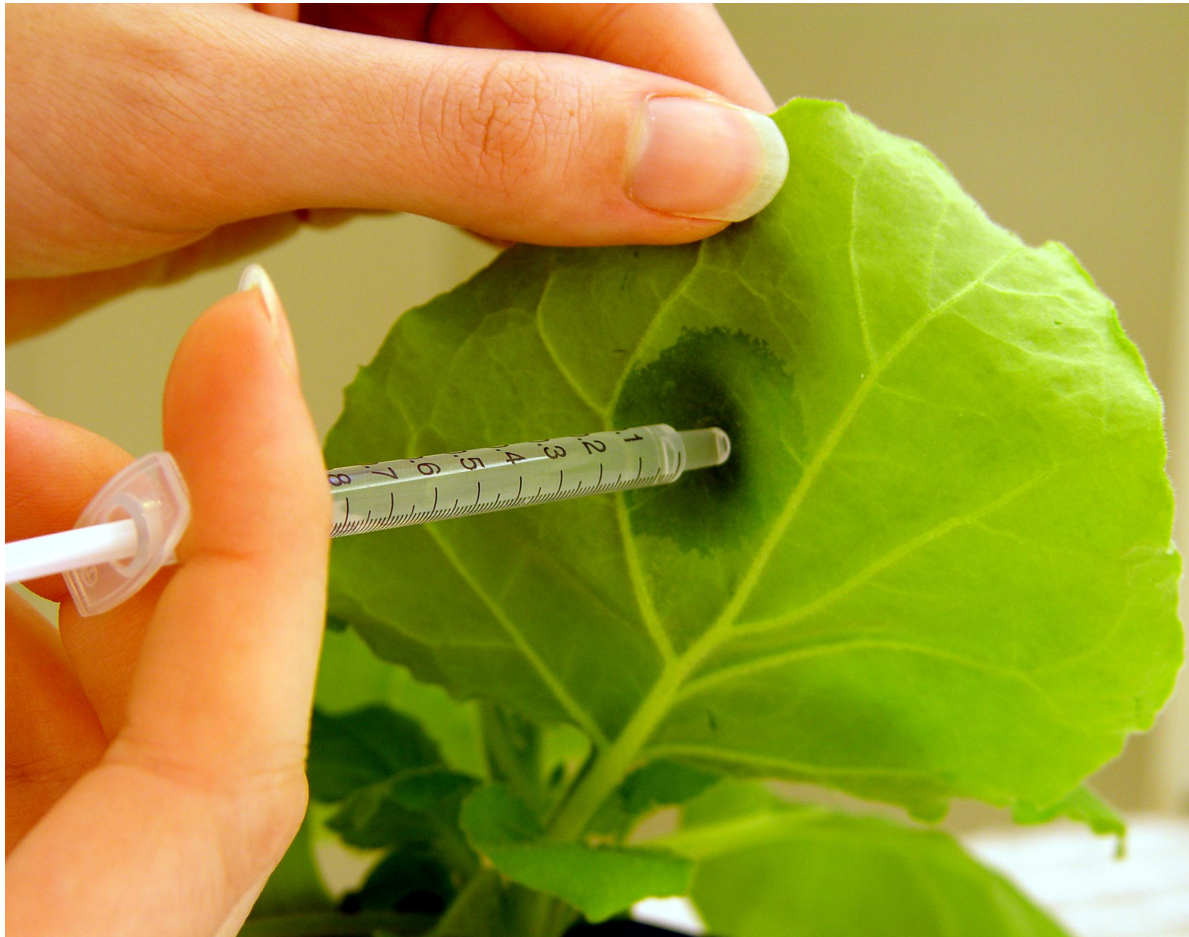
Бинарный вектор



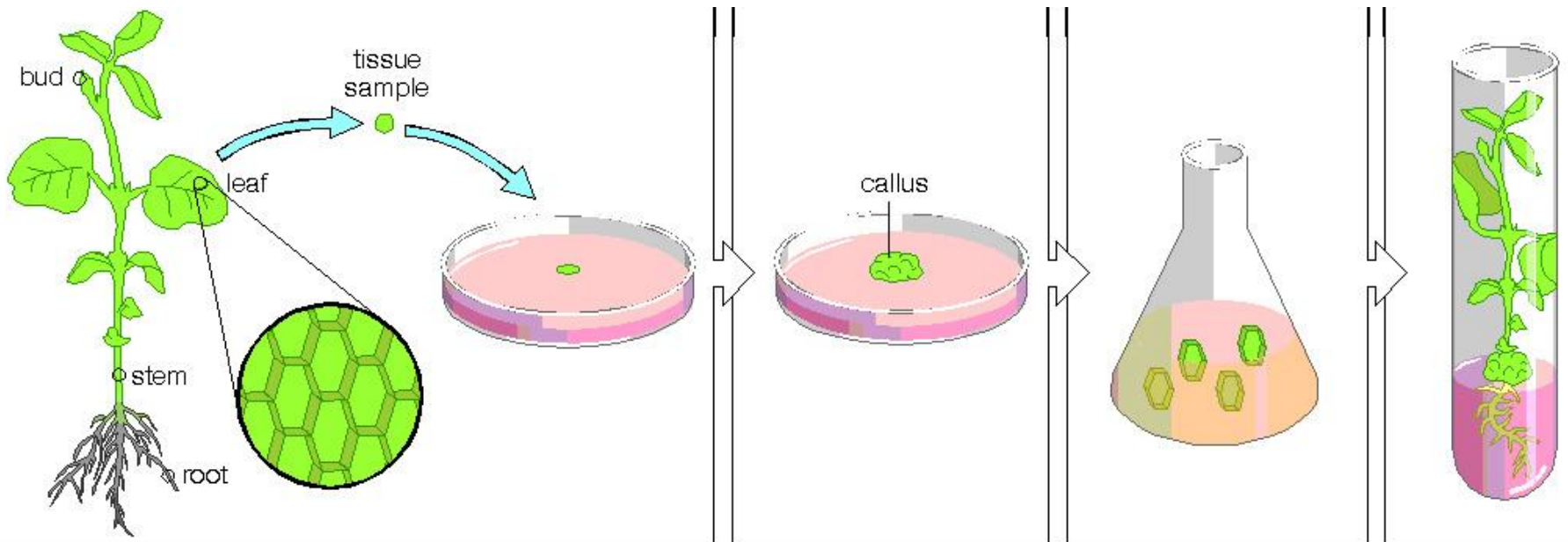


agar medium with antibiotic

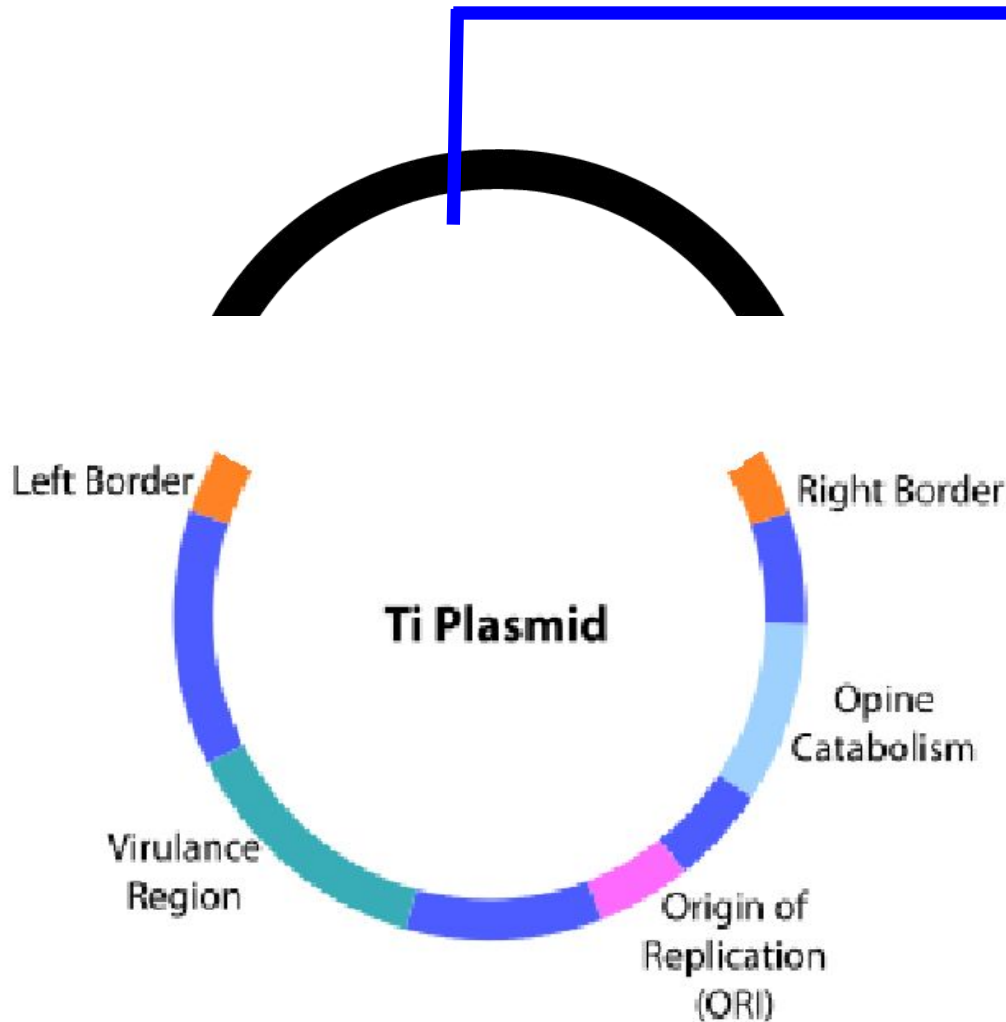
Введение векторных конструкций в клетки растений



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

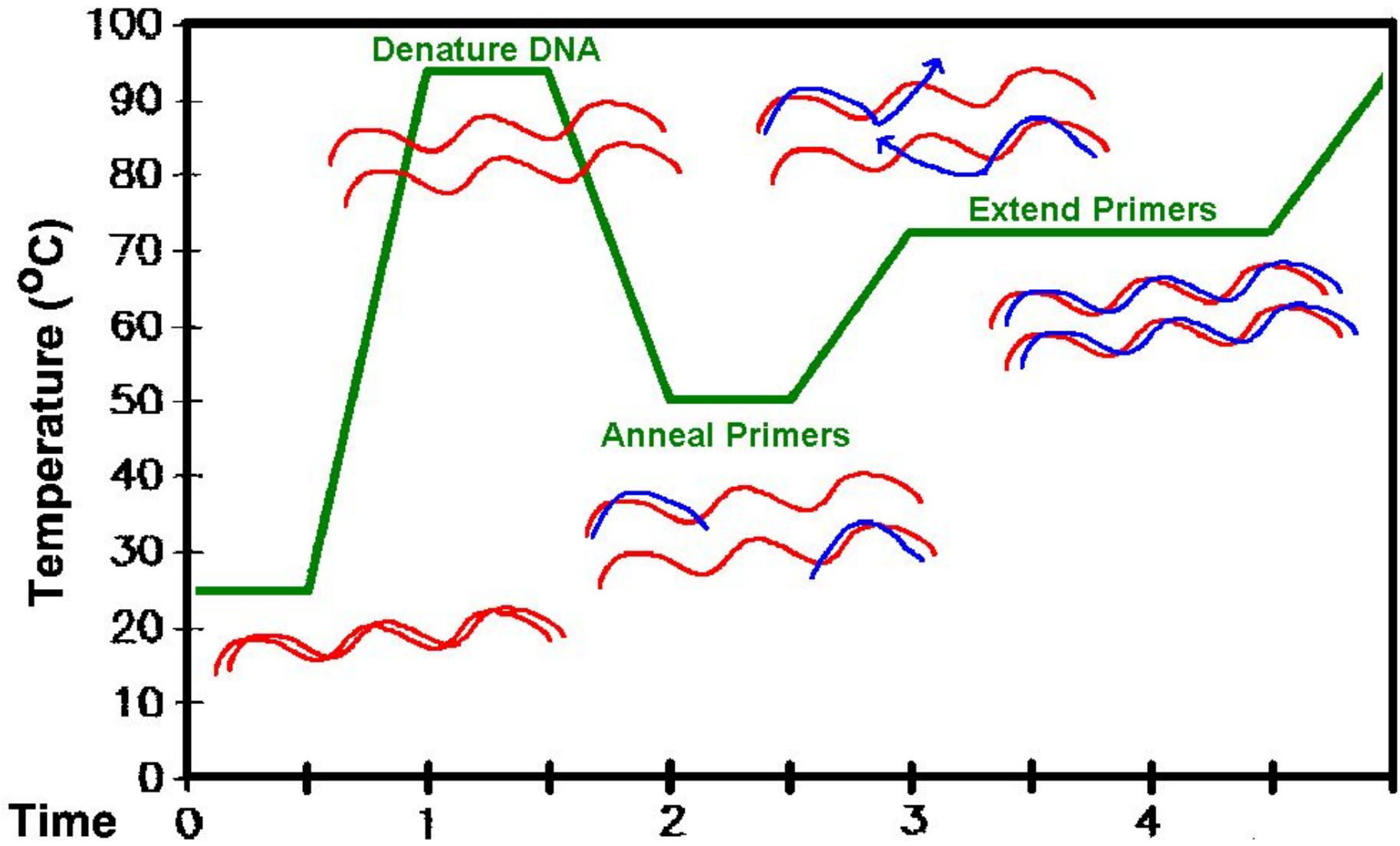
Буфер для фермента (Taq-полимеразы)

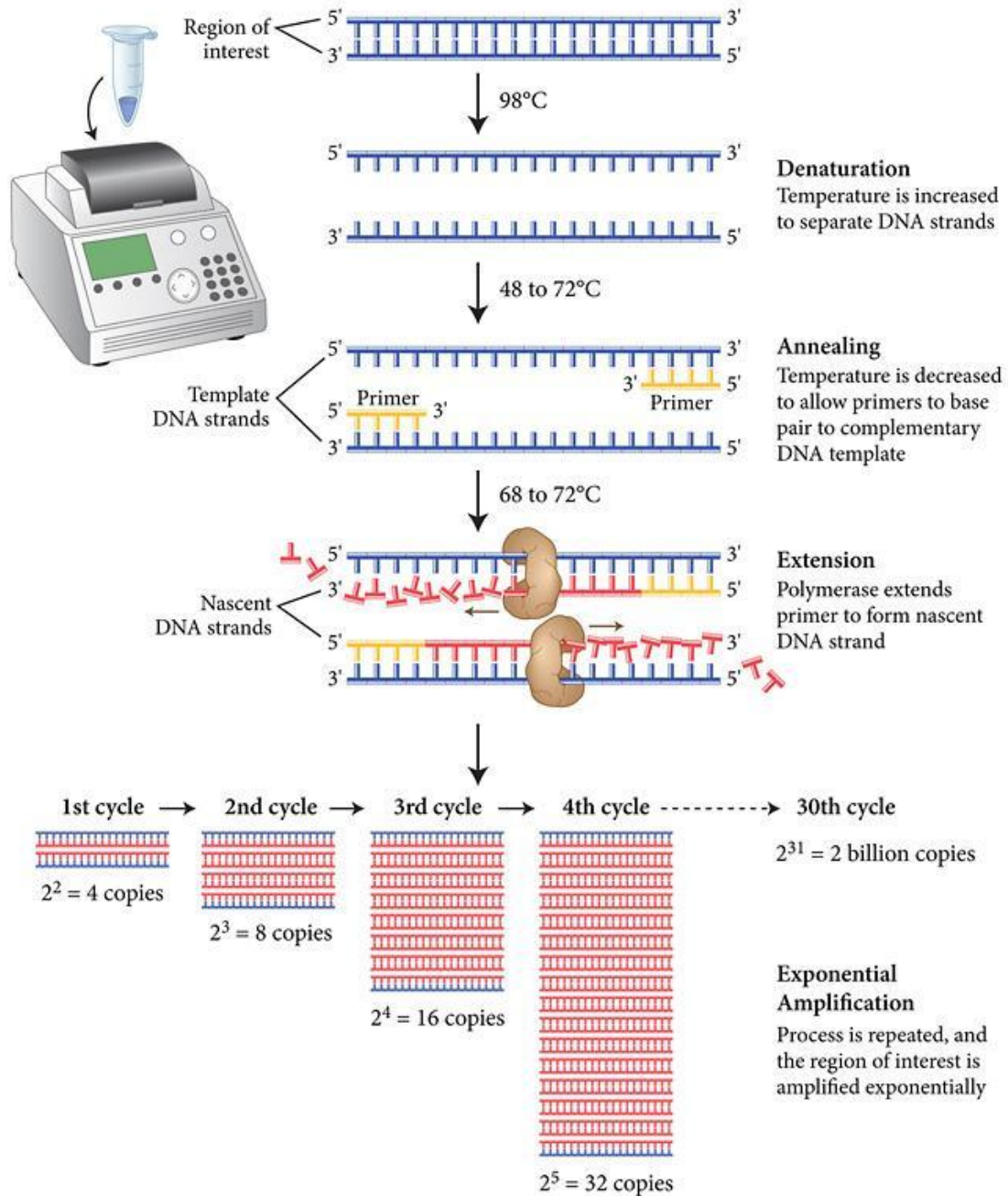
Дезоксинуклеотид фосфаты

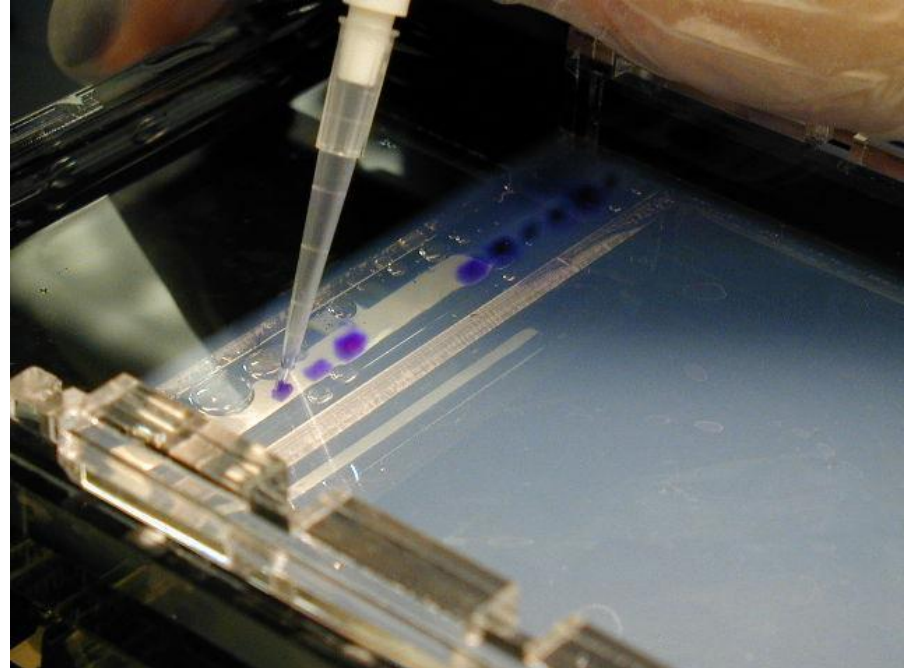
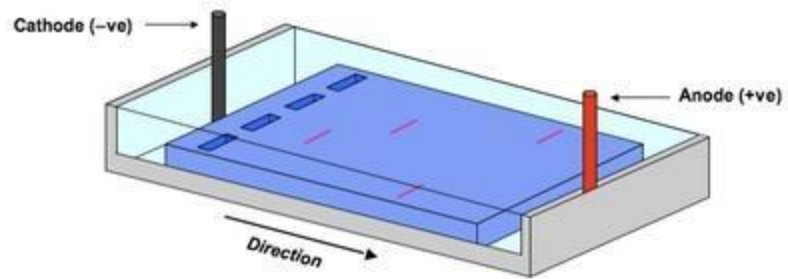
Праймеры (прямой и обратный)

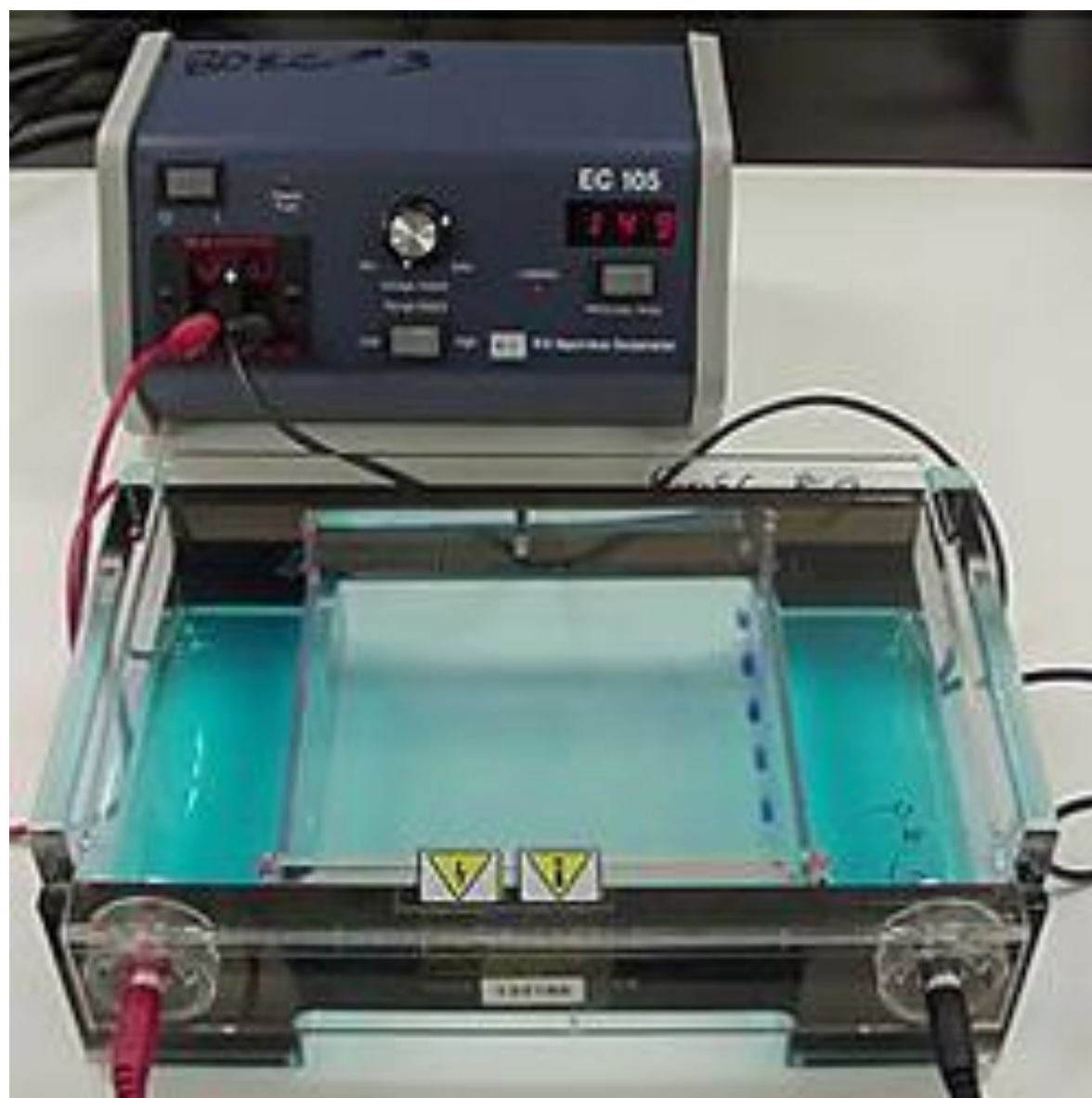
Фермент (Taq-полимераза)

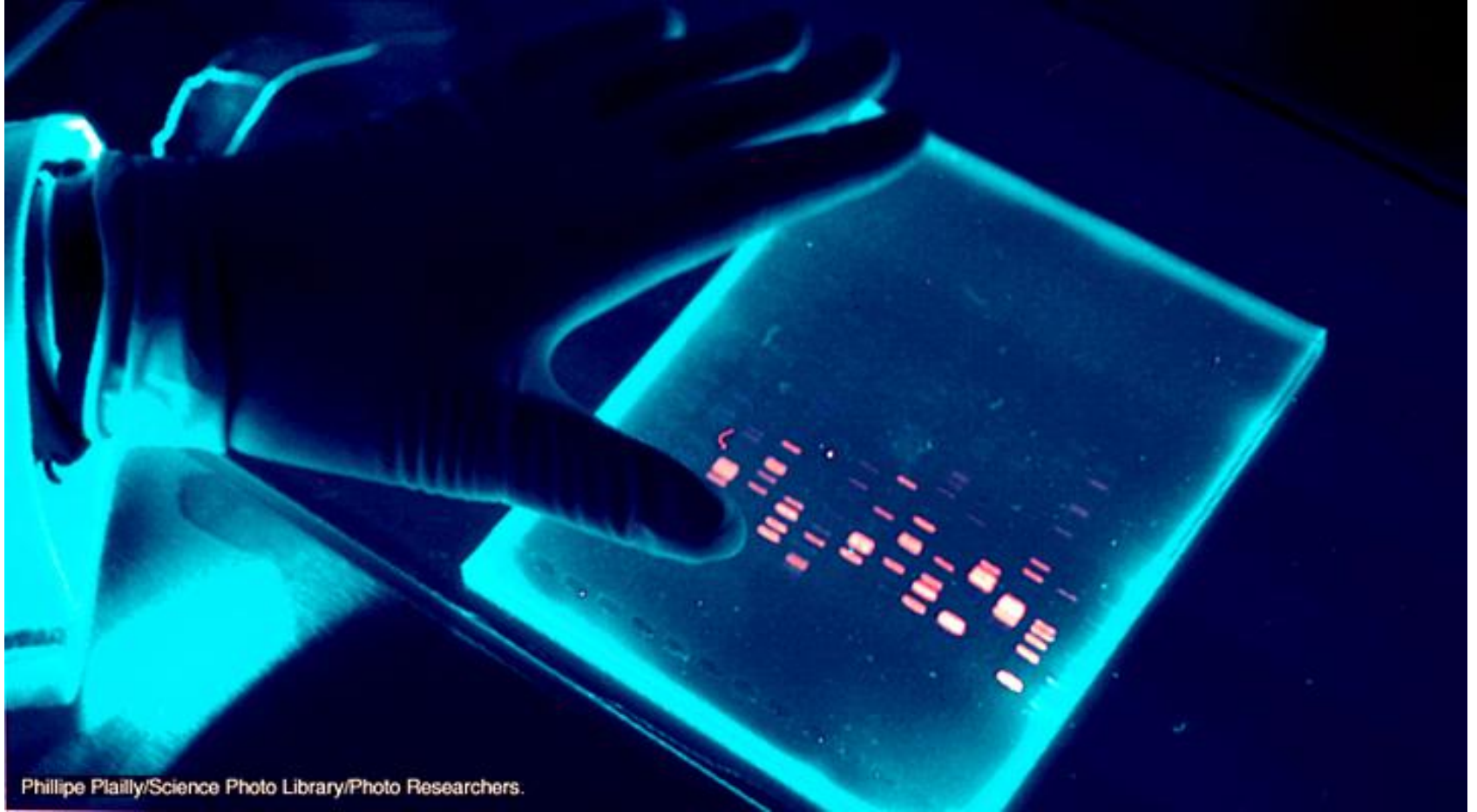
ДНК-матрица











Phillipe Plailly/Science Photo Library/Photo Researchers.

Конструирование праймеров

- All Databases ▾
- 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
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- Sequence Analysis
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- PubMed Central
- 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

The NCBI Zika virus resource page has

Register for the April 6th webinar: Using NCBI Databases with Tools that Predict Genomic Variant Effects

24 Mar 2016

In two weeks, NCBI will give a

Register for the April 13th webinar, Submitting Data to NCBI and BioSample

23 Mar 2016

In three weeks, NCBI staff will guide you through the process of submitting

Nucleotide

Advanced

Help

NCBI is phasing out sequence GI numbers in September 2016. Please use accession.version! [Read more...](#)

FASTA

Send

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Customize view

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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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

Erwinia carotovora subsp. atroseptica SCRI1043, complete genome

NCBI Reference Sequence: NC_004547.2

[FASTA](#) [Graphics](#)

LOCUS NC_004547 13677 bp DNA linear CON 17-AUG-2015

DEFINITION Erwinia carotovora subsp. atroseptica SCRI1043, complete genome.

ACCESSION [NC_004547](#) REGION: 3656910..3670586

VERSION NC_004547.2 GI:50118965

DBLINK BioProject: [PRJNA224116](#)BioSample: [SAMEA1705920](#)Assembly: [GCF_000011605.1](#)

KEYWORDS RefSeq; complete genome.

SOURCE Pectobacterium atrosepticum SCRI1043

ORGANISM [Pectobacterium atrosepticum SCRI1043](#)

Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Pectobacterium.

REFERENCE 1 (bases 1 to 13677)

AUTHORS Bell,K.S., Sebahia,M., Pritchard,L., Holden,M., Hyman,L.J., Holeva,M.C., Thomson,N.R., Bentley,S.D., Churcher,C., Mungall,K., Atkin,R., Bason,N., Brooks,K., Chillingworth,T., Clark,K., Doggett,J., Fraser,A., Hance,Z., Hauser,H., Jagels,K., Moule,S., Norbertczak,H., Ormond,D., Price,C., Quail,M.A., Sanders,M., Walker,D., Whitehead,S., Salmond,G.P.C., Birch,P.R.J., Barrell,B.G., Parkhill,J. and Toth,I.K.

TITLE The genome sequence of the enterobacterial phytopathogen Erwinia carotovora subsp. atroseptica SCRI1043 and functional genomic identification of novel virulence factors

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 13677)

AUTHORS Sebahia,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.ukCOMMENT [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.nih.gov/genome/annotation_prok/**Change region shown** Whole sequence (abbreviated view) Selected regionfrom: to: **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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421 gccgaaaagg atgtcatccc catcgccgcc gttgaggatg tcatttcccc ctttggctga
481 cgagagatca aactcagcac tatgcgactc gatatagtta tgcatgtcct tcgtggctcg
541 tacgcccgtt tgcatagcta gctgcttacc gatgtagttt tgtaaggcag cgatgccgct
601 accatcggtg ttagggaacg acacgacatc gccgaacaga acatcatcgt tatacgtgcc
661 ggatagtagt tcattactcg ccgcaaccgac actcaatcca tactgctcag gccttccgt
721 cgaataacct gttgccgtat cgtgggtgcc tatttctgtc gatgttgctt gcgccacgac
781 attcaattta cccgcatcgg ctggcacctc aagcgtaatc ttcccggcca gcgtctgagc
841 attgtgcgca ttttgaatca gatacgaacc atcggcgccg acgggtatact tcacattggt
901 gatctggcca gtcagcaccg ttcccgtcgg gatccccgtc aacctaatac ccgacagcgt
961 ttgctaccg tcacggtcgg tcagtgccggc tgaacatcg agcgtatagg tctgttttcc
1021 cgccctgaccg ggtacttttt ccaccgtcgc gctattgggt ccgggggat acgtcgaacc
1081 atcgctgtaa atcacgcctt caatattggt gctaacctga ttcgtaaacg agcctgattt
1141 accactgcta tcgggtataat taacctcaa cccatccaga gtgttgaccg acgtgtctgc
1201 attgtgttgc gtactgttgg tcgctgtata ggtgtagccc gtttccctga tgacgaaat
1261 gtagtcagaa tgccggttgc tgccgttact ggtccgggtg ccgttgaatg aatccaaatc
1321 gcgggtgatc ttgcgatcgg ccgcccagtc gctctgccga taaaaatagc cgctgttaga
1381 gtgcacaacg aagatatcgc tgtaactacc gtcgccgctg gtattgccct gactgtaata
1441 cgccaccttt ccggtgccga ccacggtagg cgccgaatcc ccttcggtca gccagacgcg
1501 gacaccgttg ccgaccgca cgatttttcc gttctgcacg tcaaaaaccac cgtagcgt
1561 tccccattc accttaataa cttccgcaga ggctggcgtc gtggtaccgc tactggtgtt
1621 caggctcacg actggcgcac cggcaactgg cgtaatacca atatgcaacg cggctttatc
1681 gcccgtattc cctgcactat cgacaggtct gtaagcaatg tcaccagcg atgcaccaga
1741 gacattactc actggcgtaa agcgtagatc ggtgttacc gccgtaaagg tttggcctgc
1801 ggcgaccgct ttccaactcc cactatcggt aaaatagagc gtaccaacgc tggctggcgg
1861 cagagacgaa atcacgatgc ttgtggatc actcgatagc cccagatcgc tccaaccaat
1921 atgtagcggg gatcttccg caccgacac cgtattgtaa tgtgcaactg gcggcgtaat
1981 atccatgatc gcttggctgt tatcatgggc ttgattacct gccgcatcgg tcgccgttgc
2041 ccctgcggta atcttgcctt ccgctaactg gcttagatcg acattcgtct gccacttggc
2101 gtttgcact gacacgtcat gcagcgtaac cgacttaca ttcacatcag taaagaccg
2161 atcaaccgta ttctcgttgc tgggtgccact gactagggtg ttttctgact cactctggct
2221 gacatatcca tcgttacggg caaaattcgt aatatcaatt tctggagcga tggatccac
2281 agcgtatgca tgatgggtgt tcgccgtggt agcgttcccc gctgcatcgc gcgtggtcac
2341 gctggcgcta atatcactat tactcaccag cacagaacc ggaacattca cgctccagg
2401 ttttccgta gtattgacgg tggctctgga agtctcgggt ccaacgggta ccgtcaccgc
2461 atcaccggtc ttgacctcgt tgccgacctt tccagtaaca gggatcgtct gtttggattc
2521 gttcgcgtta atgacgttat cgctggtcac attatcgata gagatcgacg ccaactggcgc
2581 cacagtatcc acaccgtaag catggtgcga gtttagcggc gtgacgttgc ctgcccgtatc
2641 acgggtgggt accgtcgac tgacatcact gttcgcagcc agcaccgatc ccggcacatt

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

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

Active Pane: [Icons] [ATC TAG] [M ATC] [ATC U] [B] [I] [U] [13] [Courier New] [A]

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

BX950851
2271 bp

1	ATGAGTTTCA	TAGCCATTGA	AAGTAATGCA	GCACGAAAAA	TCGTTTCTGA	AAGAATGCIT	CAGTCAGCAG	ATTATGATAA	AGGTCAGATA	CTTGCTGATT
	TACTCAAAGT	ATCGGTAACT	TTCATTACGT	CGTGCTTTTT	AGCAAAGACT	TTCTTACGAA	GTCAGTCGTC	TAATACTATT	TCCAGTCTAT	GAACGACTAA
101	TATTGAAAGA	CAGAAAAATA	CTTGATTTC	GCCTCGAAGT	TAAAGCTGTC	TCTATCAATA	CTGGTATATA	TATATTAAGC	AAAAACCCAA	ATAAAAAGAA
	ATAACTTTCT	GTCCTTTTTAT	GAACATAAAGT	CGGAGCTTCA	ATTTTCGACAG	AGATAGTTAT	GACCATATAT	ATATAATTCG	TTTTTGGGTT	TATTTTTCTT
201	TATGTTAATA	TTCGACACAA	CGACCTTTGA	TGGTTTTAAA	AAAAACAGTA	ATGAAATTAT	CACGATAAAT	CAGAAATCTT	GCAGGCTGGC	GATAAAATTG
	ATACAATTAT	AAGCTGTGTT	GCTGGAAACT	ACCAAAATTT	TTTTTGTTCAT	TACTTTAATA	GTGCTATTAA	GTCTTTAGAA	CGTCCGACCG	CTATTTTAAAC
301	TGGGATAAGA	TGGGCATAG	TCCATGTGAG	AAAATAATTA	GTGGTTCTTC	TTAATTGCT	TTATTACCTC	TTTCATTTAC	TACTGGAAAA	TCCTATAAAG
	ACCCTATTCT	AACCCGTATC	AGGTACACTC	TTTTATTAAT	CACCAAGAAG	AAATTAACGA	AATAATGGAG	AAAGTAAATG	ATGACCTTTT	AGGATATTTT
401	TCATACTTGA	TAAATCTCCT	GATAAGGAGC	GACAAGAAAA	AAGGAACGAA	AGTAGTTTTT	TAATATTTCA	TGACGGGTAT	GACACTACCG	ATGCGGTGCC
	AGTATGAACT	ATTTAGAGGA	CTATTCTCTG	CTGTTCTTTT	TTCTTGCTT	TCATCAAAAA	ATTATAAAGT	ACTGCCATA	CTGTGATGGC	TACGCCACGG
501	AAAATTAGCT	AATTTCCGCA	AAGCTAAAGA	AGGGATACAT	GATATTGATA	CGCTTTCTCT	GTTTAAAAATA	CCCACATAG	AAGAGGAGAA	TGTCACTACA
	TTTTAATCGA	TTAAAGGCGT	TTCGATTCT	TCCCTATGTA	CTATAACTAT	GCGAAAGAGA	CAAATTTTAT	GGGCTGTATC	TTCTCTCTT	ACAGTGATGT
601	TATCTGAACA	TAAATGAAAC	TGATAATACC	AATACTCAAT	CAAACCCACA	TATGGGGTTA	GATTATTGGG	AAAAAAATCT	TACTGAAAGT	CAAAAAAGAT
	ATAGACTTGT	ATTTACTTTG	ACTATTATGG	TTATGAGTTA	GTTTGGGTGT	ATACCCCAAT	CTAATAACCC	TTTTTTTAGA	ATGACTTTCA	GTTTTTTCTA
701	TTGTTTATAG	CGAAAGCTAT	GGGCCGGATA	TTCTTAAAGG	GGCAGCAGGA	ACAGGAAAAA	CTTTAAGTTT	AATATTGAGA	TGTGTAGTTC	AGCTTCAAAA
	AACAAATATC	GCTTTCGATA	CCCGGCCTAT	AAGAATTTCC	CCGTCGTCCT	TGTCCTTTTT	GAAATTCAAA	TTATAACTCT	ACACATCAAG	TCGAAGTTTT
801	TTCAAAAAAA	ACAGACAATC	TGCTACGAGC	TATTTTTATA	ACCCACAGCA	TAGCAACTAA	GACTAATATA	GAAAACTTA	TAGCATCTAA	TGGTGGCTCT
	AAGTTTTTTT	TGCTGTTAG	ACGATGCTCG	ATAAAAAATAT	TGGGTGTCGT	ATCGTTGAT	CTGATTATAT	CTTTTGGAAAT	ATCGTAGATT	ACCACCGAGA
901	GAATTTATTG	ACTCAGAACA	TCAGCAGTCT	GTTGAAGTGA	CAACTTTACA	GGAATGGTGT	ATCAAAAATT	TAGGTAACAG	AATAGAAGCA	ACAGAGTATC
	CTTAAATAAC	TGAGTCTTGT	AGTCGTCAGA	CAACTTCACT	GTTGAAATGT	CCTTACCACA	TAGTTTTTAA	ATCCATTGTC	TTATCTTCGT	TGTCTCATAG
1001	TTGATAGCGA	TGCTTTTGAA	TCTAAGCAGT	TACAGCTCTT	ATATATAAAC	GAAAGCTCTG	AAGATTTTTT	GATAAATGAT	TACGCAGGAT	CTGTCAACTT
	AACTATCGCT	ACGAAAACCT	AGATTCGTCA	ATGTCGAGAA	TATATATTTG	CTTCGAGAAC	TTCTAAAAAA	CTATTTACTA	ATGCGTCCTA	GACAGTTGAA

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)

Vector NTI - [NC_0045412]

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

Active Pane: ATG TAG ATG M ATG U **B** / U 13 Courier New

- NC_0045412
 - General Description
 - Standard Fields
 - References
 - Comments
 - Annotations
 - Feature Map
 - Restriction/Methylation Map

BamHI (521) XbaI (449) ODS 1
 NC_0045412
 999 bp

```

1 TCAGGCGGG TAAAGCAGAG TCTCGTGTG TCTGGCAATG TCCCACAGAG
  AGTCCGCCGC ATTCGTCTC AGAGCACAA CAGACCGTAC AGGGCTGTC
101 TCGCGCGGGC TGATCAGCGT GACTTCAAAA GGCAGTGGCT GGTCGATAG
  AGCGCCGCCG ACTAGTCGCA CTGAAGTTTT CCGTCACCGA CCAGCTATC
201 TACCCGCACC GCCTCCCGCG AGTACCATGT CGGCAATCAC CGAGTAGGT
  ATGGCGTGG CGGAGGCGC TCATGGTACA GCGGTAGTG GCTCATCCA
  BamHI
301 GTGTACCACC TGACCGAGCG GATCCTGATG CTGCATCATC AGCAGGTTA
  CACATGGTGG ACTGGCTCGC CTAGGACTAC GACGTAGTAG TCGTCCAAT
  
```

Dimers & Hairpin Loops

Dimers: << >> 6 of 8 Close

CGCGGCGGCTGATCAGCGTGACTTC
 |||
 CTTCAGTGGGACTAGTCCGCGCGGC
 Stem Length = 10
 Dimer dG = -13.0 kcal/mol

Hairpin Loops: << >> 1 of 2

CGCGGCGGC
 ||| +
 CTTCAGTGGGACTAGT
 Stem Length = 3 Loop Length = 12
 Loop dG = -1.9 kcal/mol

Oligo Analysis

Oligonucleotide: From NC_0045412: Start: 102 Length: 25
 CGCGGCGGCTGATCAGCGTGACTTC

DNA RNA Complementary Analyze Save Results Close

Parameters		Results	
dG Temperature (C)	25.0	Mol. Wt	7737.0
Probe Conc. (pMol)	250.0	%GC	68.0
Salt Conc. (mMol)	50.0	Therm. T _m	72.4
% Formamide	0.0	%GC T _m	60.8
3' End Length (bp)	7	dG	-51.2
Palindromes (bp)	6	3' End dG	-11.3
Nucl. Repeats (bp)	4	dH	-207.9
Stem Length (bp)	3	dS	-519.6

Palindromes: 3 total
GCTGATCAGC at 8
CTGATCAG at 9
TGATCA at 10

Repeats: 0 total

Dimers & Hairpin Loops...

Vector NTI - [NC_0045412]

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

Active Pane: [Icons]

NC_0045412

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

NC 0045412
900 bp

1	TCAGGCGGCG	TAAAGCAGAG	TCT	AGTCCGCCGC	ATTCGTCTC	AGA
101	TCGCGGCGGC	TGATCAGCGT	GAC	AGCGCCGCCG	ACTAGTCGCA	CTG
201	TACCCGCACC	GCCTCCCGCG	AGT	ATGGGCGTGG	CGGAGGGCGC	TCA
301	GTGTACCACC	TGACCGAGCG	GAT	CACATGGTGG	ACTGGCTCGC	CTA
401	GGCAGCAGAG	CAACCATCGT	GGA	CCGTCGTCTC	GTTGGTAGCA	CCT
501	GGATGTTCTG	GCACAGCGTG	CTG	CCTACAAGAC	CGTGTGCGAC	GAC
601	CTGCCCGAAC	GCGTGTGCCG	CGC	GACGGGCTTG	CGCACACGGC	GCG
701	TCCTGCACCT	TCGGTATCAG	ACG	AGGACGTGAA	AGCCATAGTC	TGC
801	TAAGTACGCG	GCTGACGTTG	GGT	ATTATGCGC	CGACTGCAAC	CCAACACTCT
901	TAATCGCAT	ATTAGCGTA				

BamHI (321) AclI (449) O38 I

WTG TCGCCTGCGA TTGTGGATTG
CAC AGCGGACGCT AACACCTAAC
GCA AGCGGTGAAG AGATCGACGA
CGT TCGCCACTTC TCTAGCTGCT
AGT GCCTTGTGCG GTAGCGCACG
TCA CGGAACAGCG CATCGCGTGC
GGC CATTATTGG GCTATTCTTC
CCG GTAAATAACC CGATAAGAAG
CGC AAAATCGAGC TGGTTTTGCA
GCG TTTTAGCTCG ACCAAAACGT
ACC AGCGTTGGTG CGACGATCAT
TGG TCGCAACCAC GCTGCTAGTA
TGC TTTGCAGATG GCTGAAAAGC
ACG AAACGTCTAC CGACTTTTCG
ACC GAGCTGTTGT TCTGCATGGT
TGG CTCGACAACA AGACGTACCA
GCC TGGAAGATT CAATATGGCG
ACCTTCTAAA GTTATACCGC

Oligo Duplexes

Analyze Save Results Add New... Remove Close

```
{(DNA): GGTCCGCATAGCGGCAAGC
{(DNA): GGCTTCGCTCTACATCAAAACCTACTC
```

Database... Oligo List dG Temperature (C): 25.0 Stem Length (bp): 3

<< >> 4 of 7

```
GGTCCGCATAGCGGCAAGC
+ + ||||
CTCATCAAAACTACATCTCGCTTCGG
Stem Length = 4
Dimer dG = -3.3 kcal/mol
```

BLAST

BLAST® » blastn suite » RID-FZ86Z7MR01R

[Home](#) [Recent Results](#) [Saved Strategies](#) [Help](#)

BLAST Results

[Edit and Resubmit](#) [Save Search Strategies](#) [Formatting options](#) [Download](#)

[YouTube](#) [How to read this page](#) [Blast report description](#)

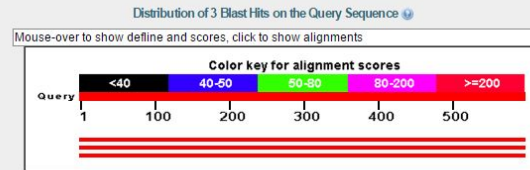
Nucleotide Sequence (599 letters)

RID [FZ86Z7MR01R](#) (Expires on 04-03 18:37 pm)
Query ID |c|Query_41439
Description None
Molecule type nucleic acid
Query Length 599

Database Name nr
Description Nucleotide collection (nt)
Program BLASTN 2.3.1+ [Citation](#)

Other reports: [Search Summary](#) [Taxonomy reports](#) [Distance tree of results](#)

Graphic Summary



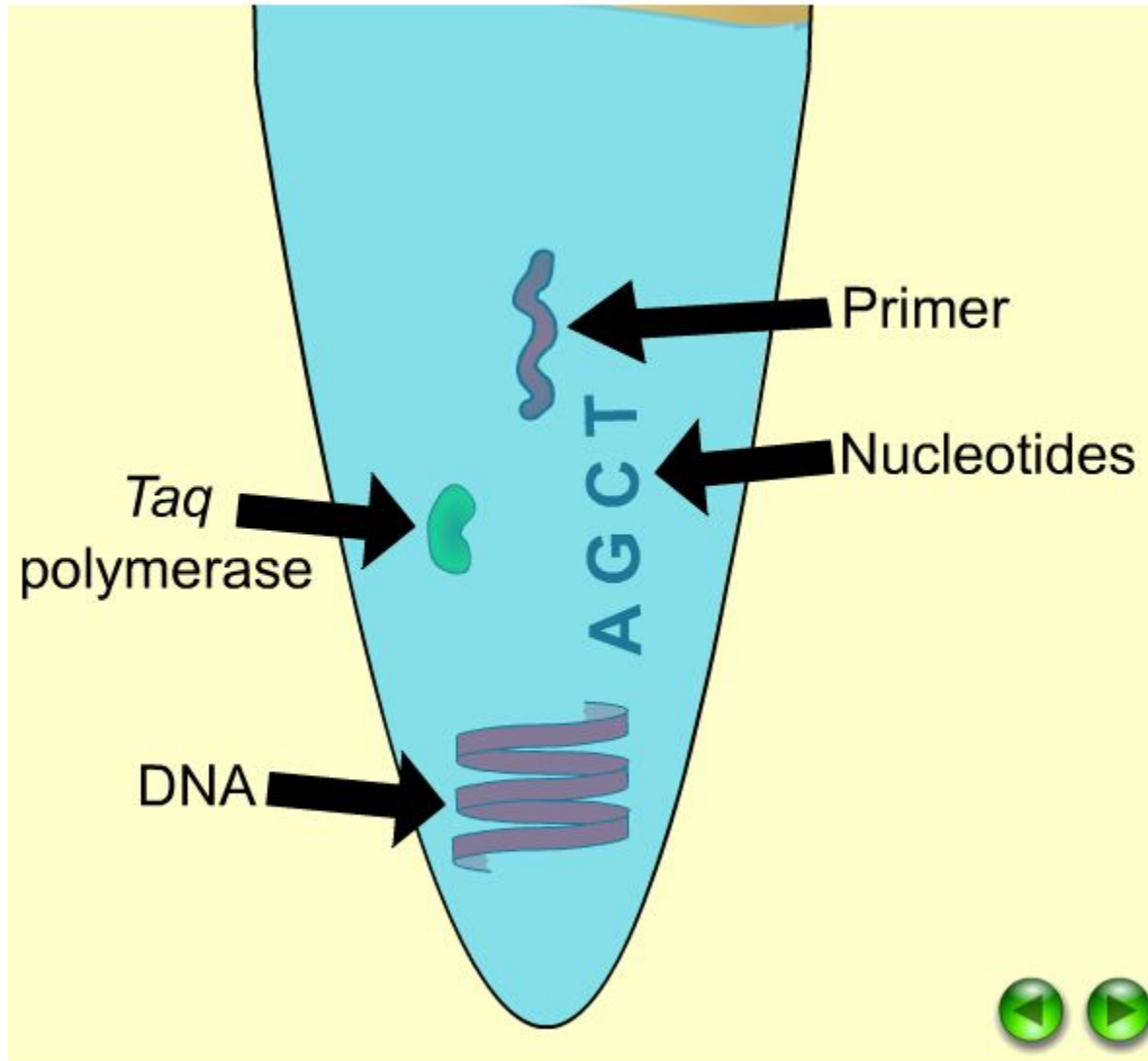
Descriptions

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

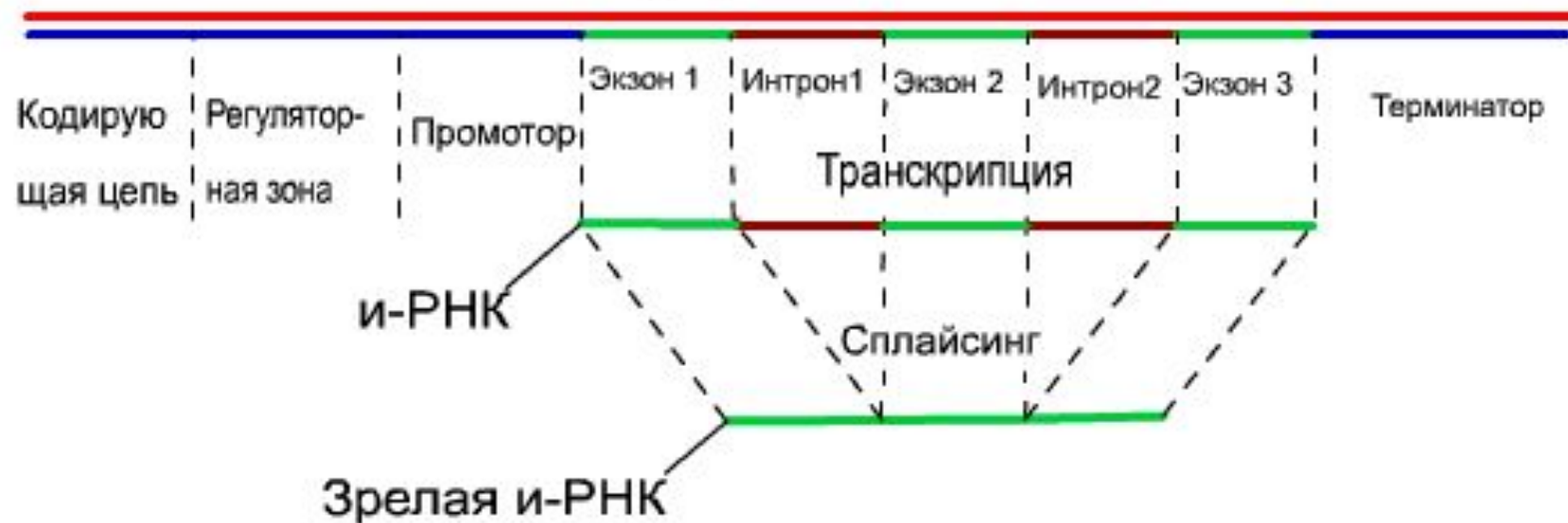
[Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)

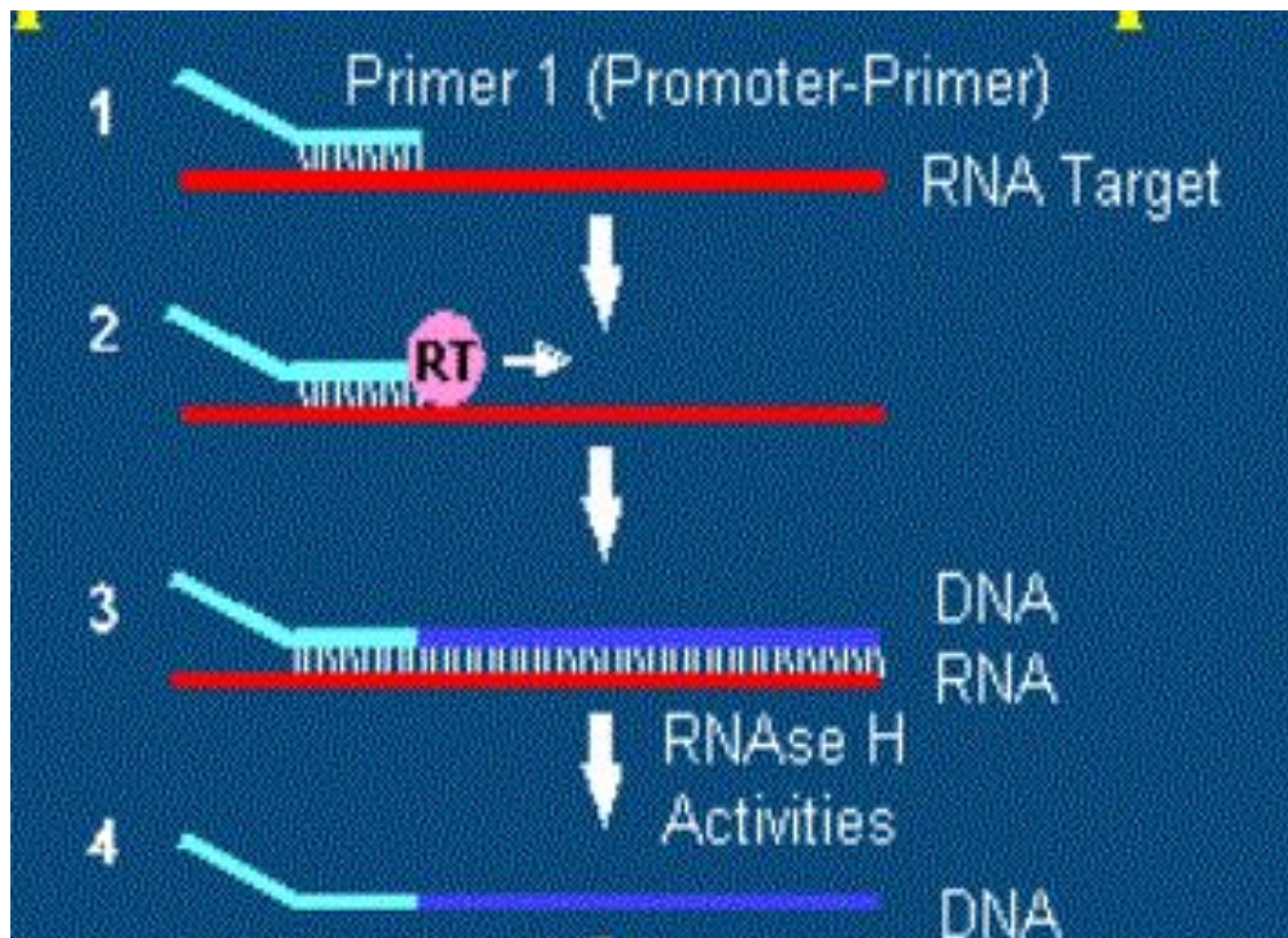
	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Pectobacterium atrosepticum strain 21A, complete genome	1107	1107	100%	0.0	100%	CP009125.1
<input type="checkbox"/>	Pectobacterium atrosepticum strain JG10-08, complete genome	1107	1107	100%	0.0	100%	CP007744.1
<input type="checkbox"/>	Erwinia carotovora subsp. atroseptica SCRI1043, complete genome	1107	1107	100%	0.0	100%	BX950851.1



Что обычно используется в качестве матрицы (основы) для амплификации «нужного» участка ДНК?

ДНК





A



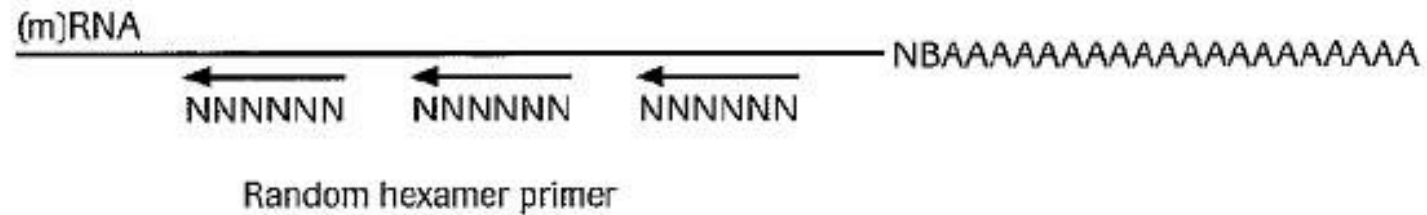
B



C



D



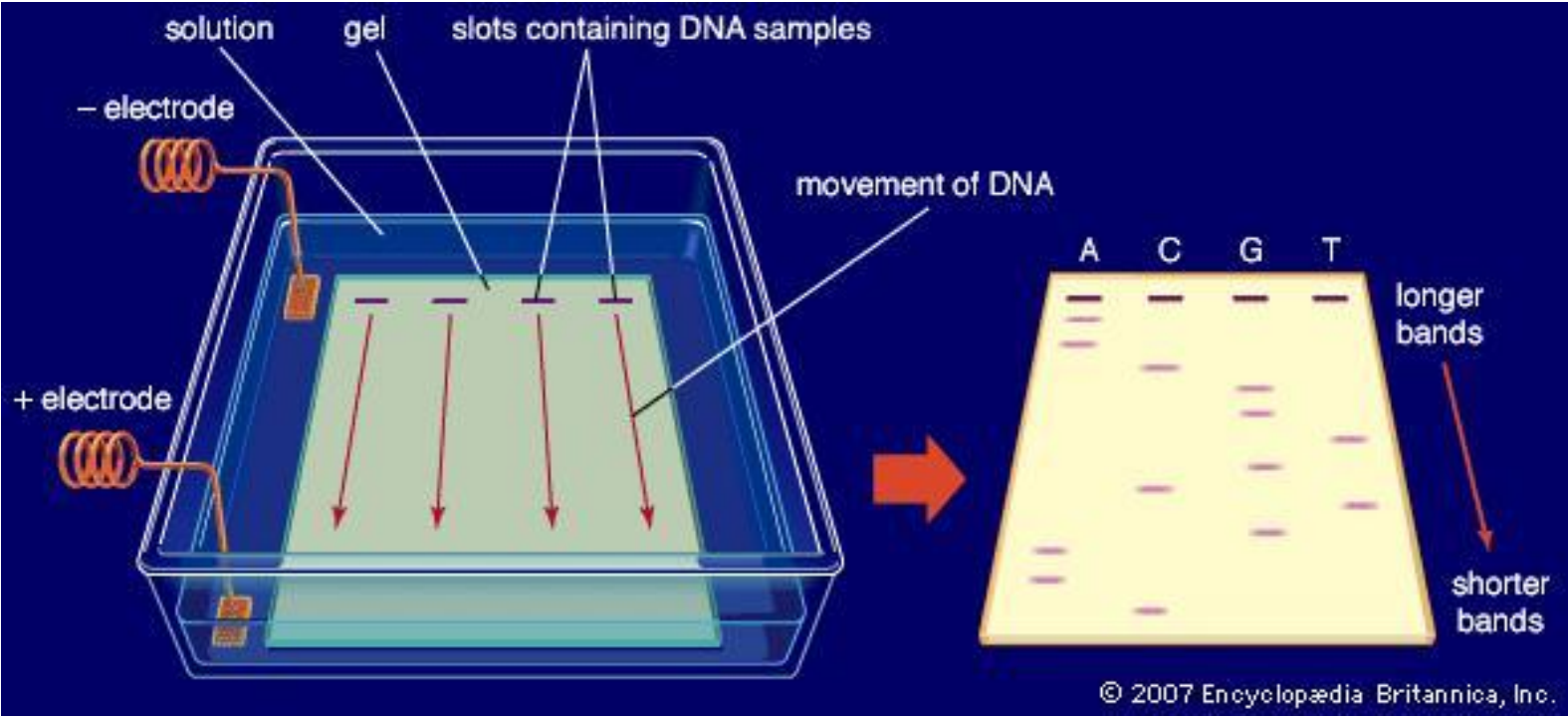
Мы провели ПЦР, чтобы получить целевой фрагмент ДНК

Нужно выяснить получился ли у нас ожидаемый продукт

Электрофорез

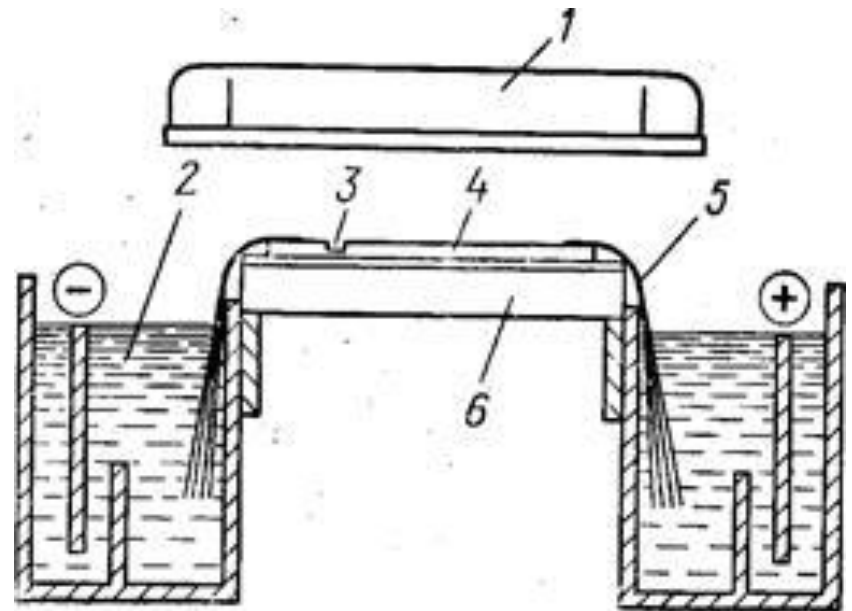
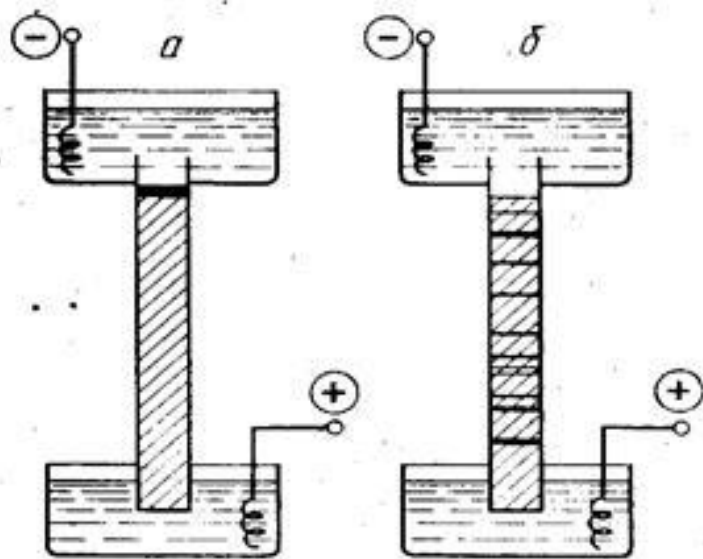
Электрофоретическое разделение нуклеиновых кислот

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

$(\text{CH}_2 = \text{CH} - \text{CONH}_2)$

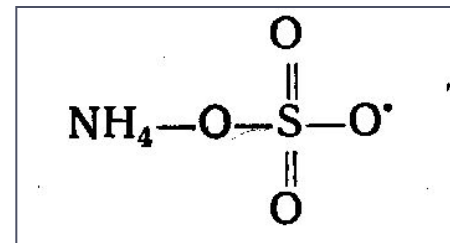
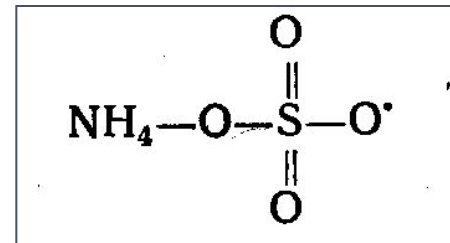
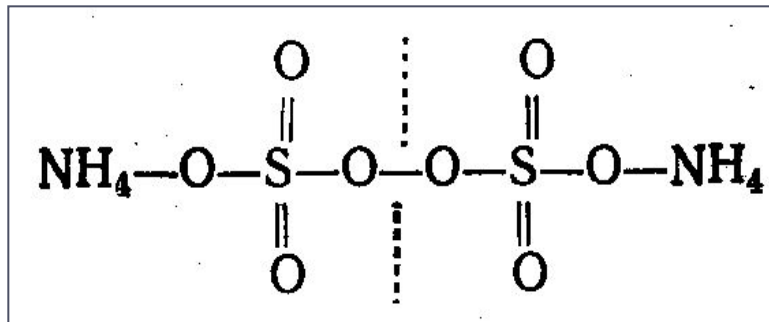
NN'-Метиленабисакриламид

$(\text{CH}_2 = \text{CH} - \text{CONH})_2 - \text{CH}_2$

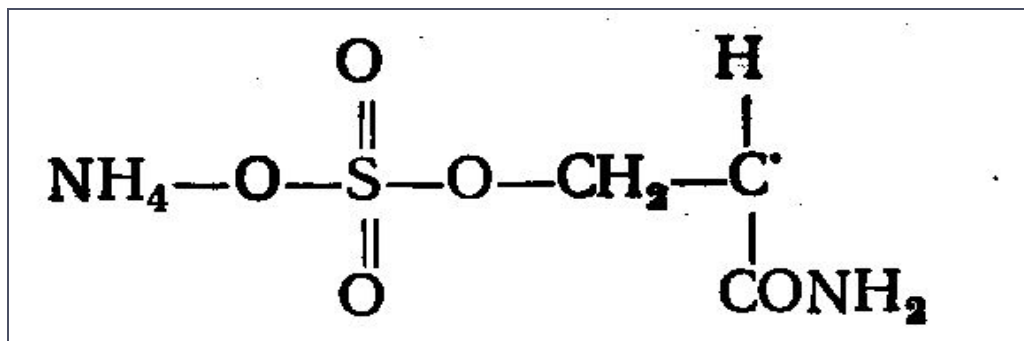
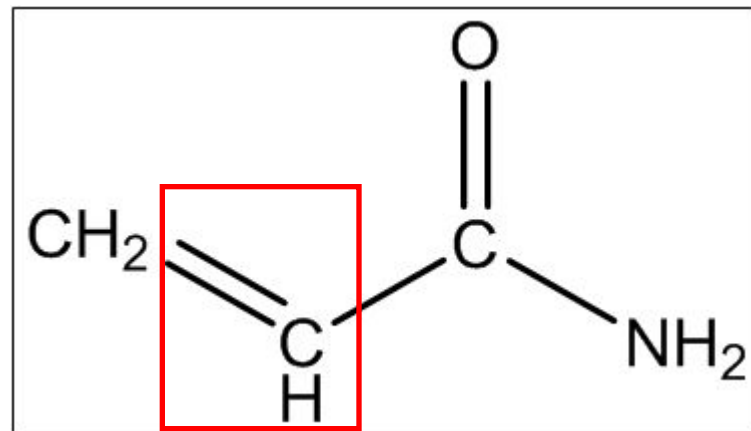
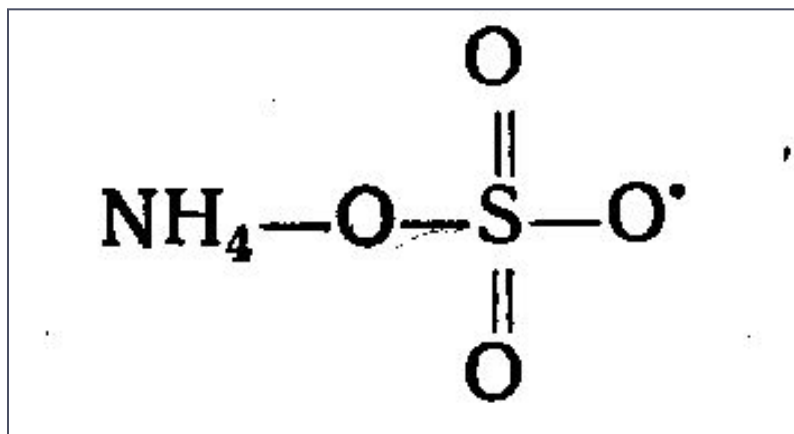
Буферные растворы

Индукция полимеризации акриламида

Персульфат аммония



Полимеризация акриламида

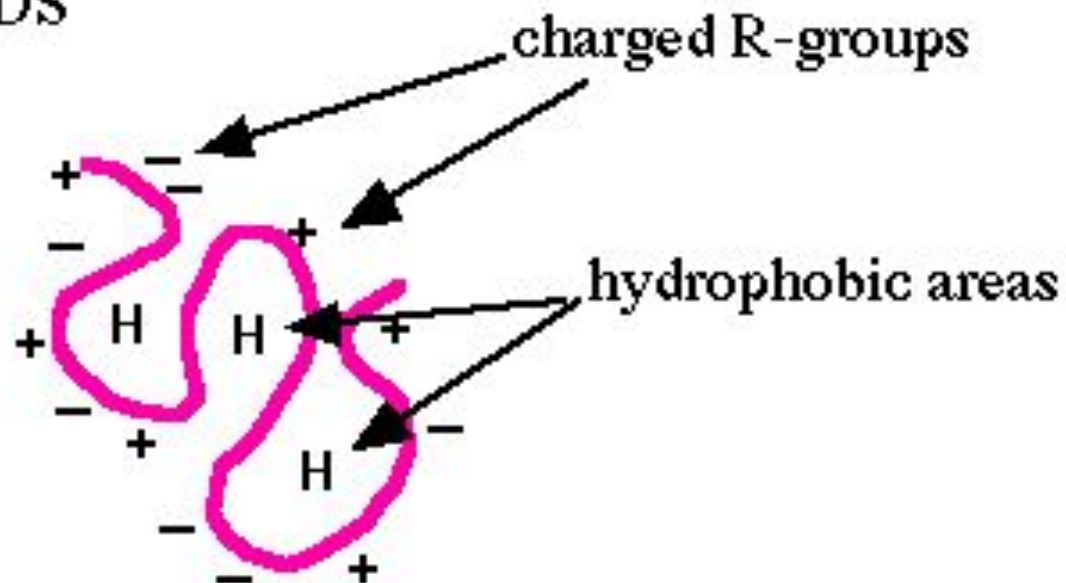


Катализатор процесса полимеризации

Тетраметилэтилендиамин (ТЕМЕД) — $(\text{CH}_3)_2\text{N} - \text{CH}_2 - \text{CH}_2 - \text{N}(\text{CH}_3)_2$

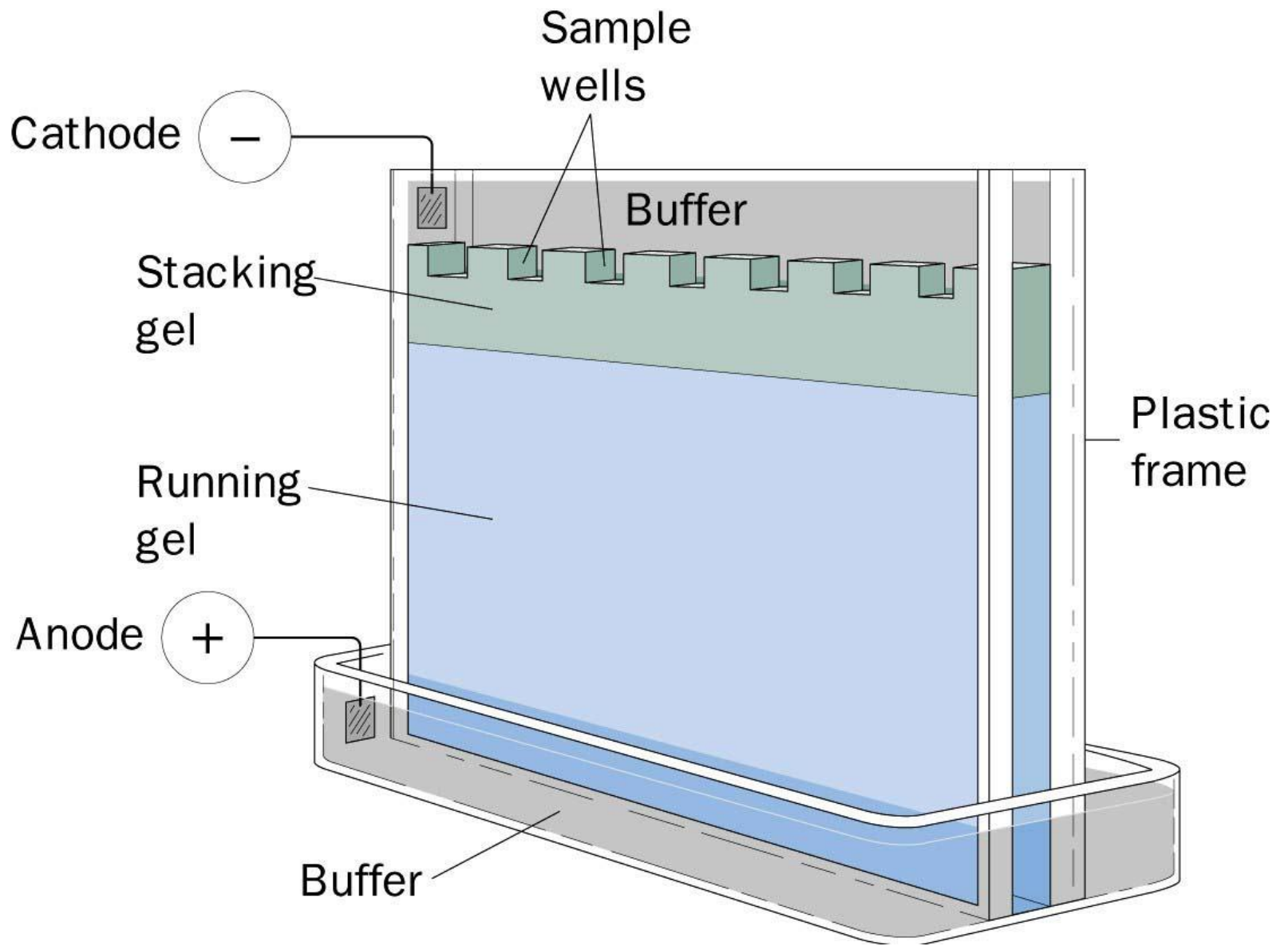
Денатурация белка с помощью ДДС

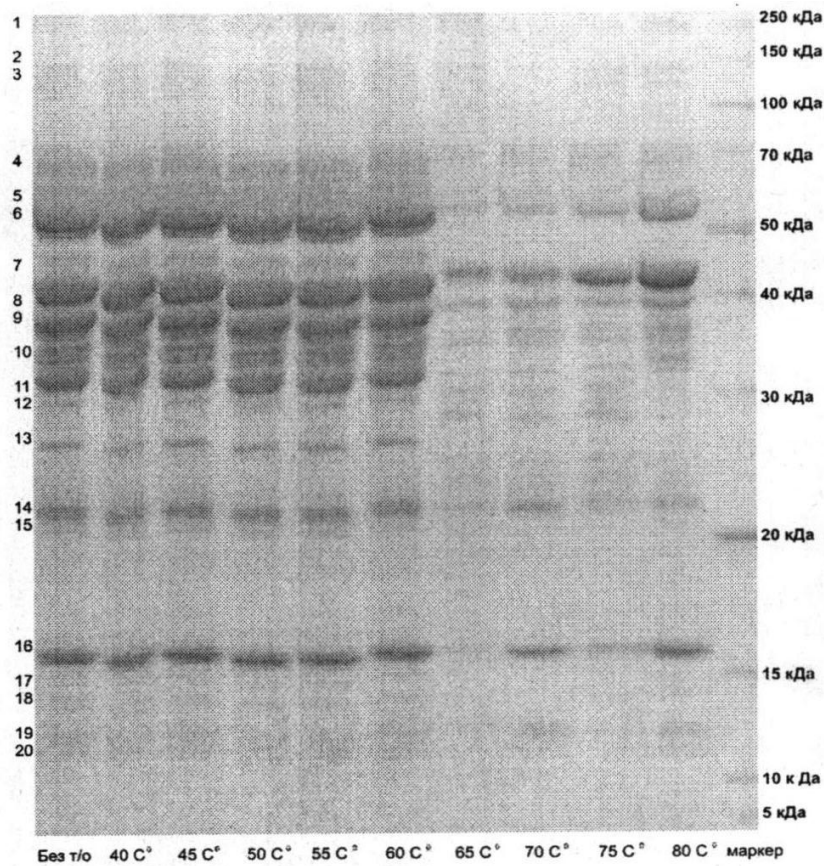
BEFORE SDS



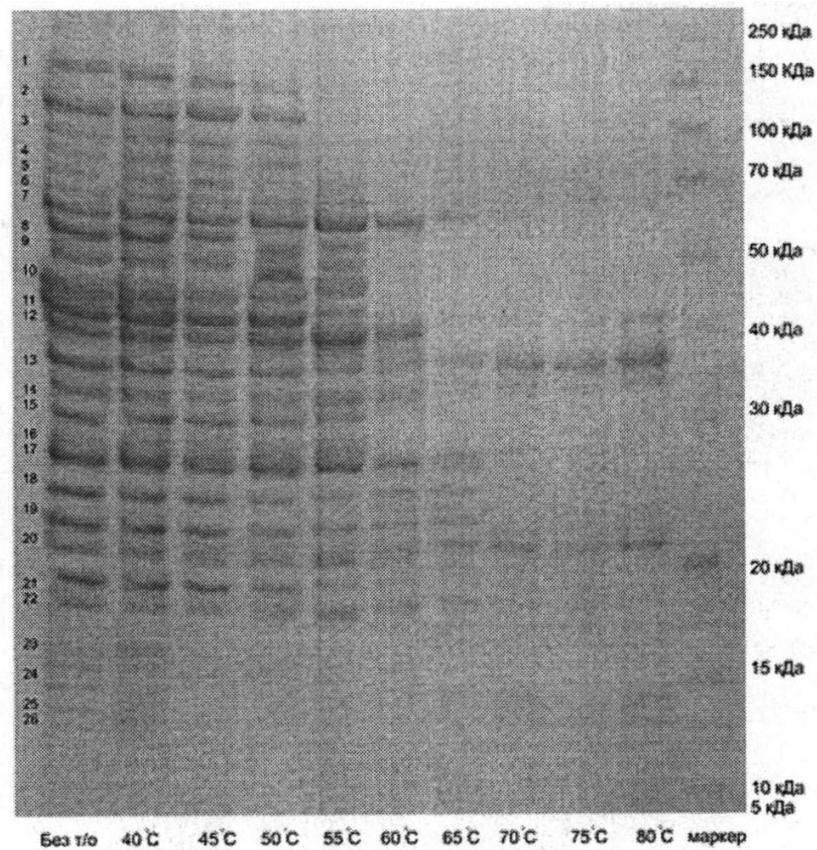
AFTER SDS







а)

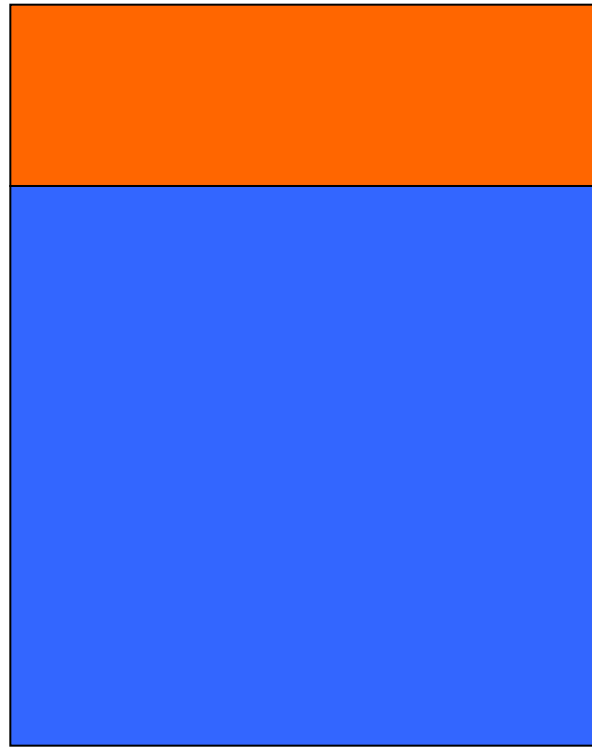


б)

Фиг. 2



Постоянная сила тока



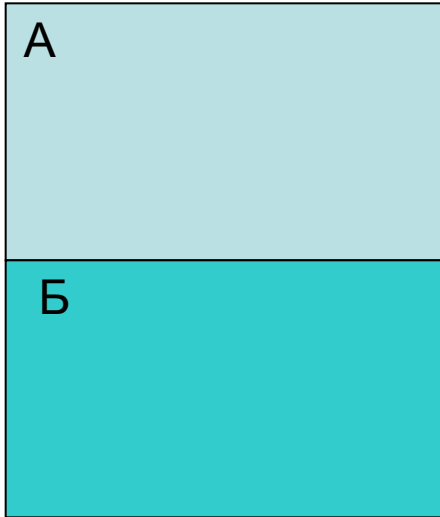
Высокая напряженность

Низкая напряженность

Подвижность ионов

Заряд ионов

Изоэлектрическая точка

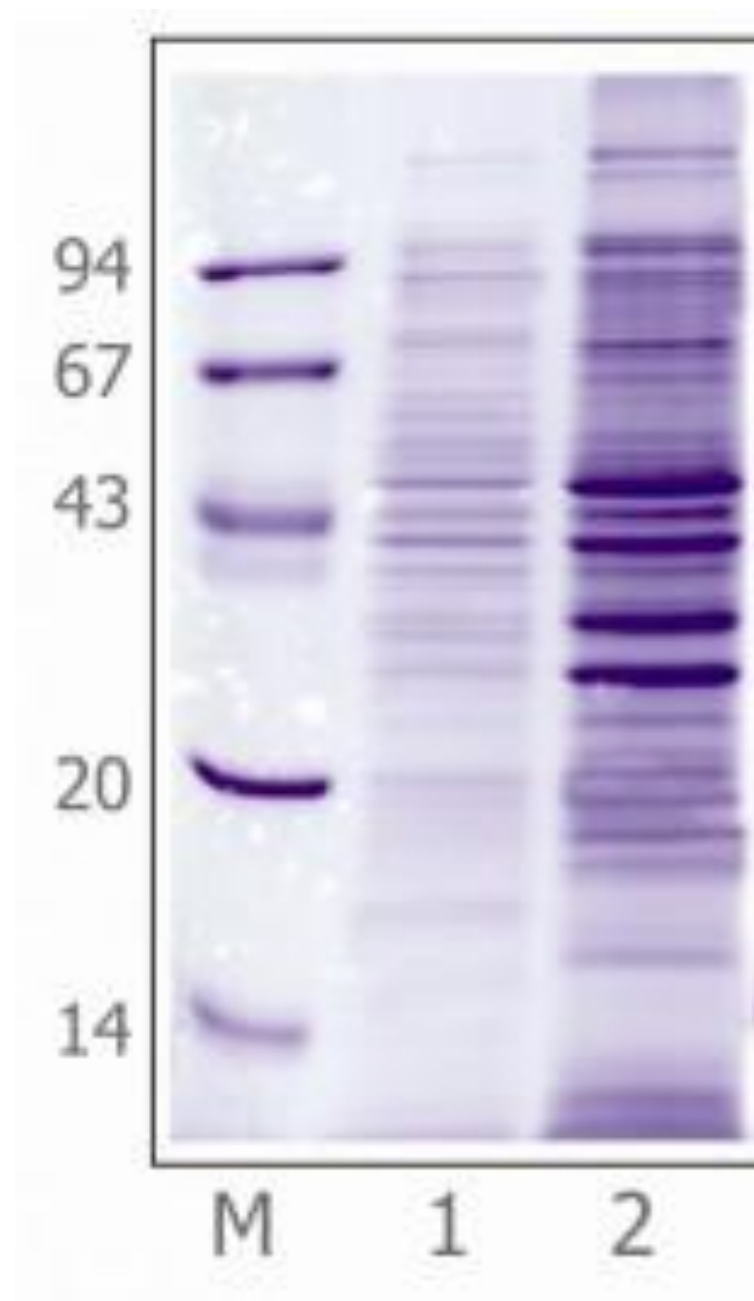
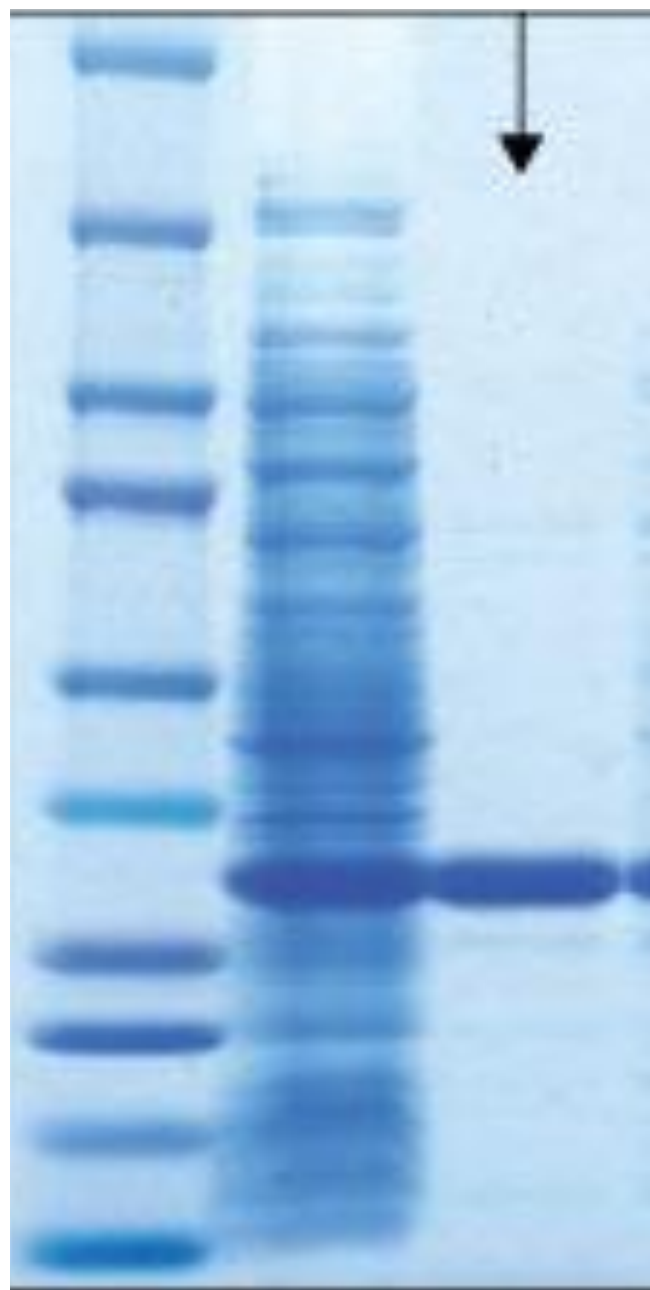


Глицин – малоподвижный ион. Высокое сопротивление. Высокая напряженность

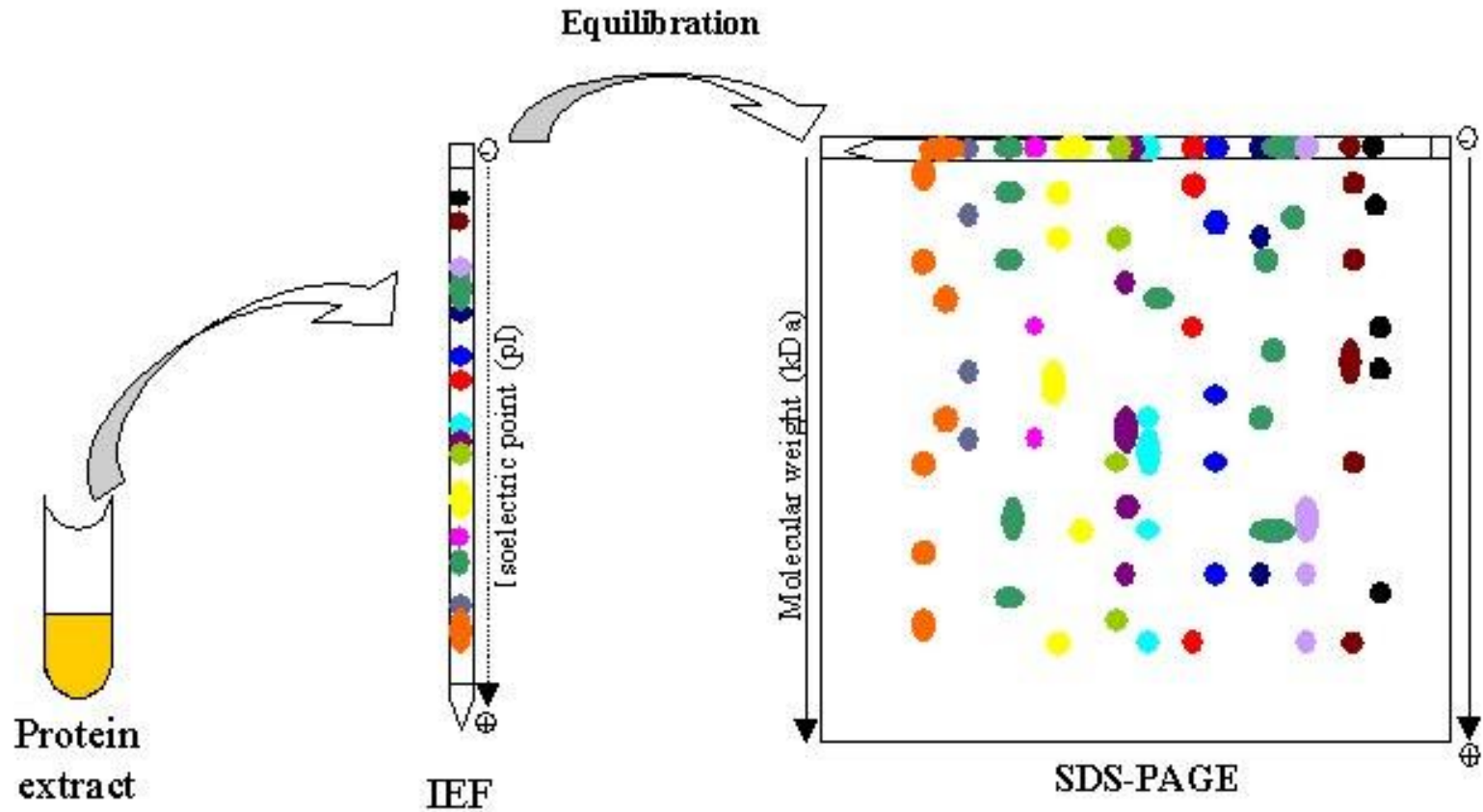
Хлор – подвижный ион. Низкое сопротивление. Низкая напряженность

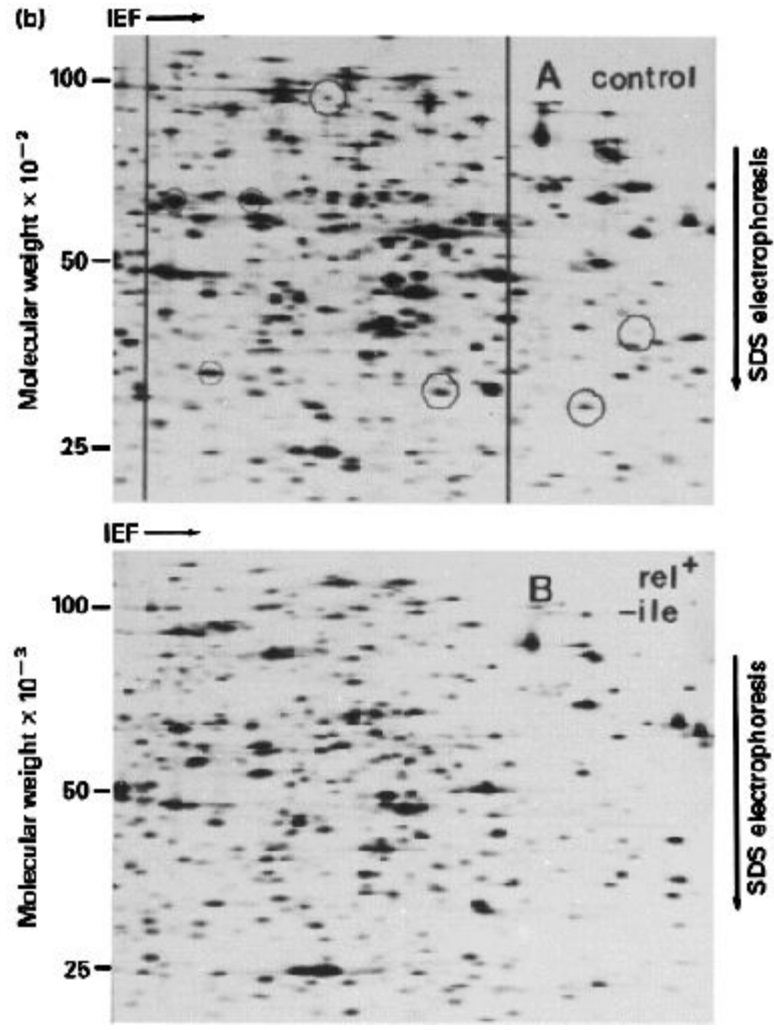
Одинаковая сила тока

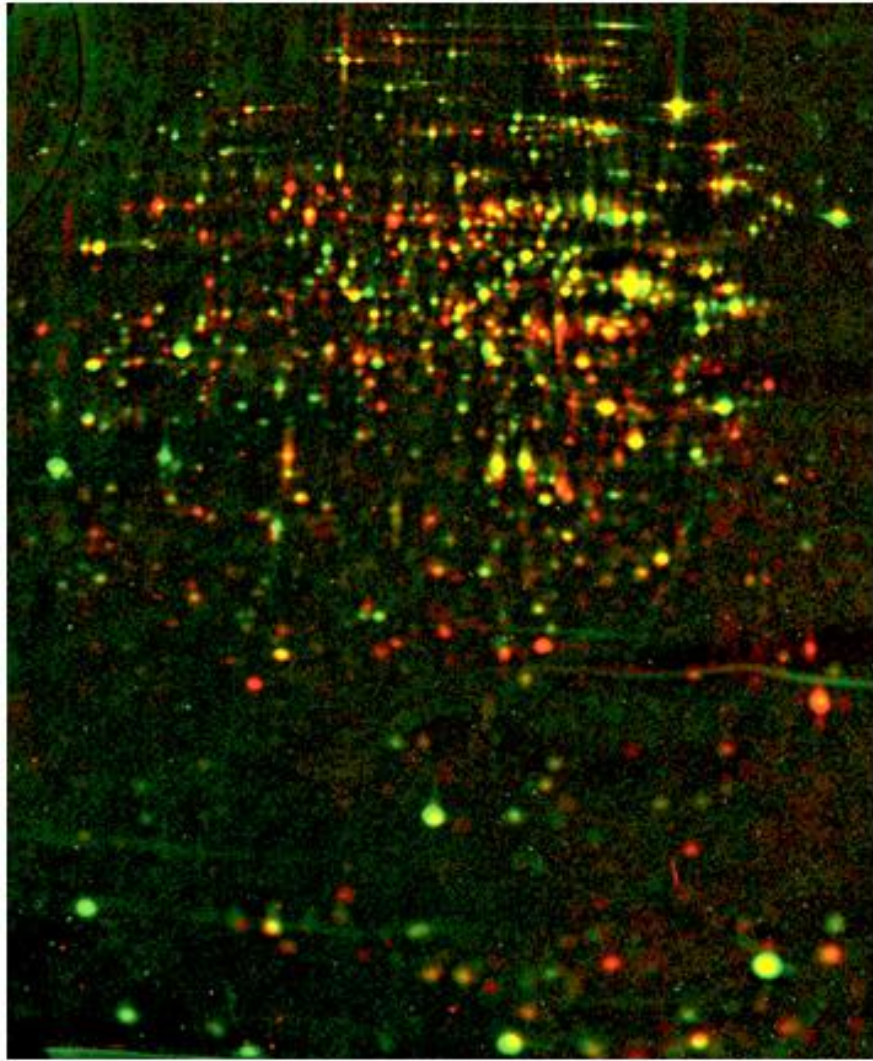
Суммарное напряжение распределится между участками А и Б так, что напряженность поля А будет выше, причем настолько, чтобы скорость миграции ионов глицина стала точно такой же как у ионов хлора. Этого требует условие неизменности величины тока вдоль всего геля (постоянство тока во всем геле).



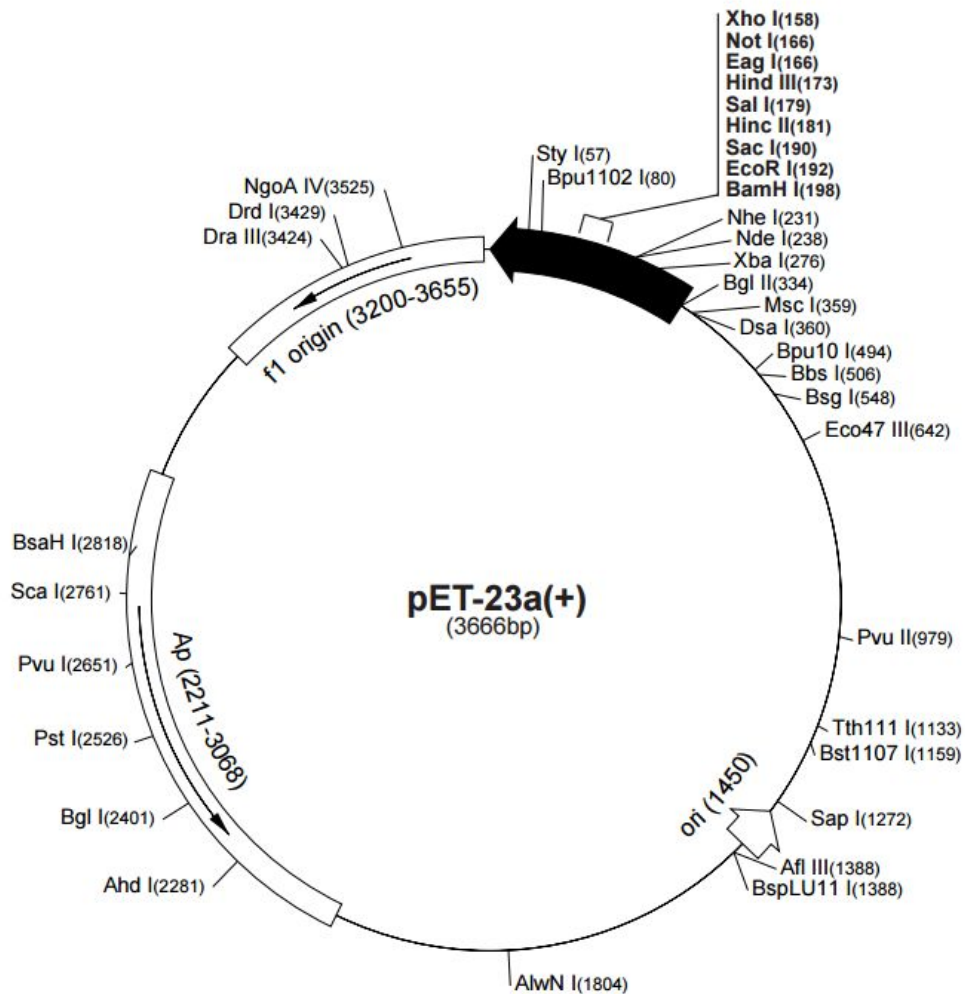
Двумерный электрофорез белков



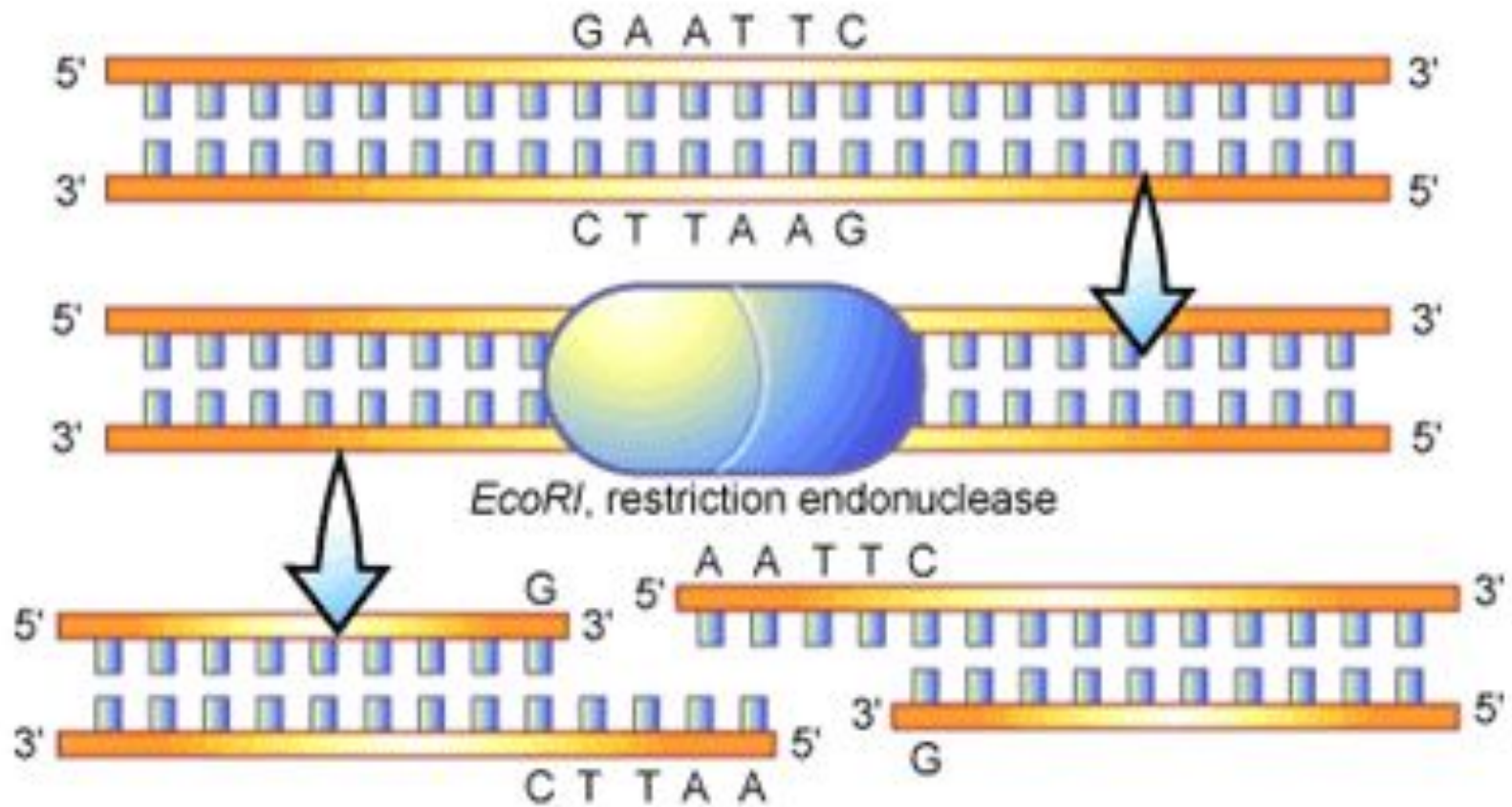




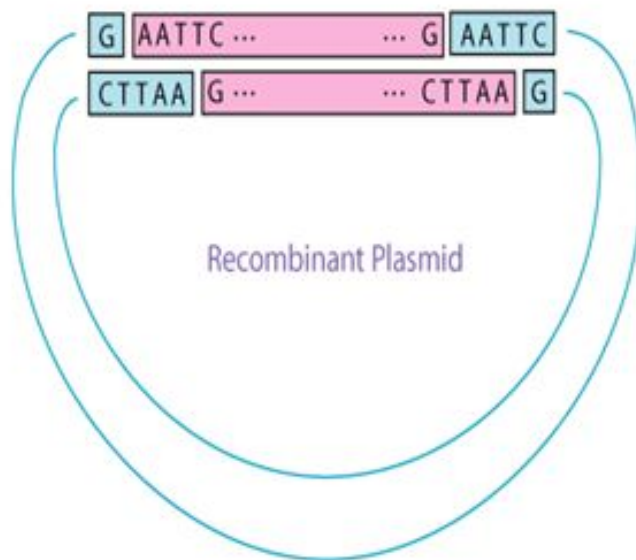
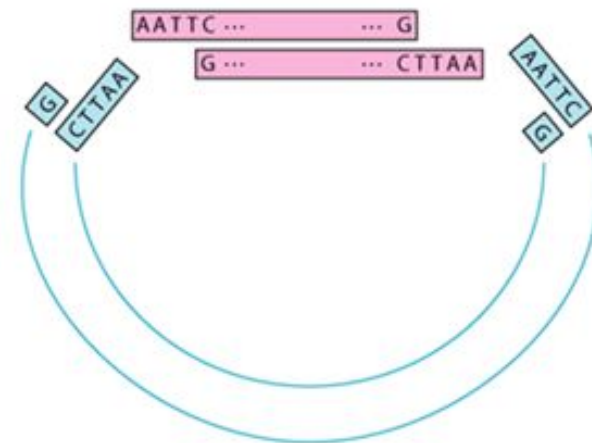
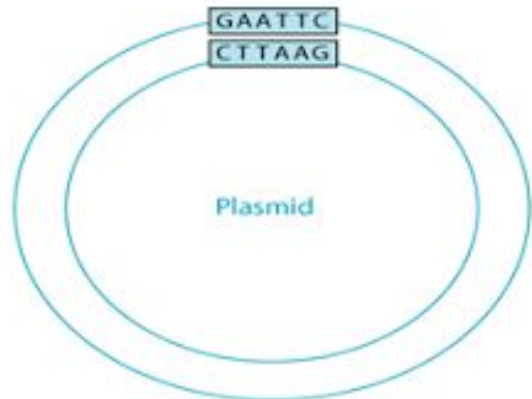
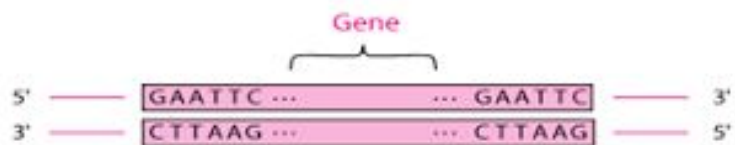
Векторные конструкции

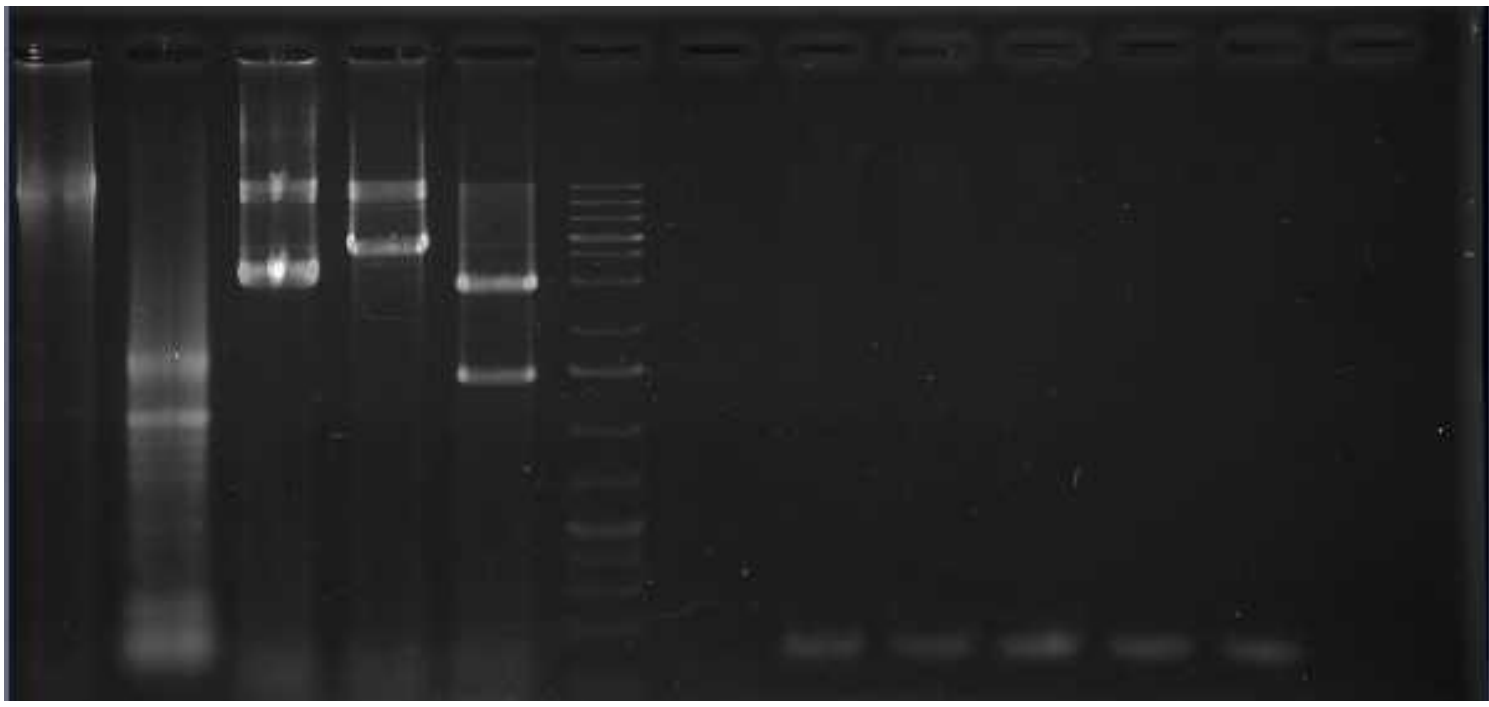


Рестриктазы



Сборка плазмиды. Рестрикция-лигирование.

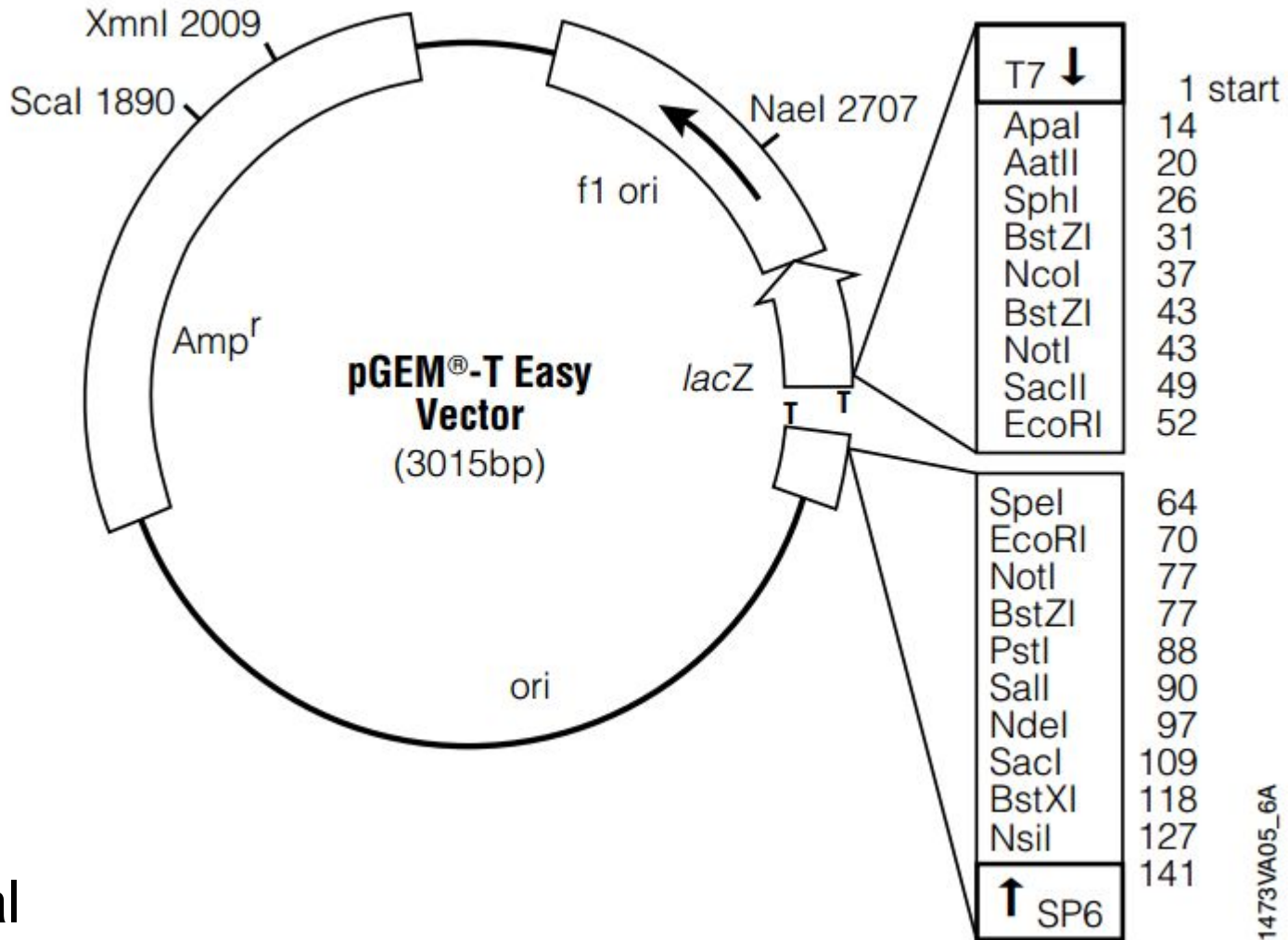




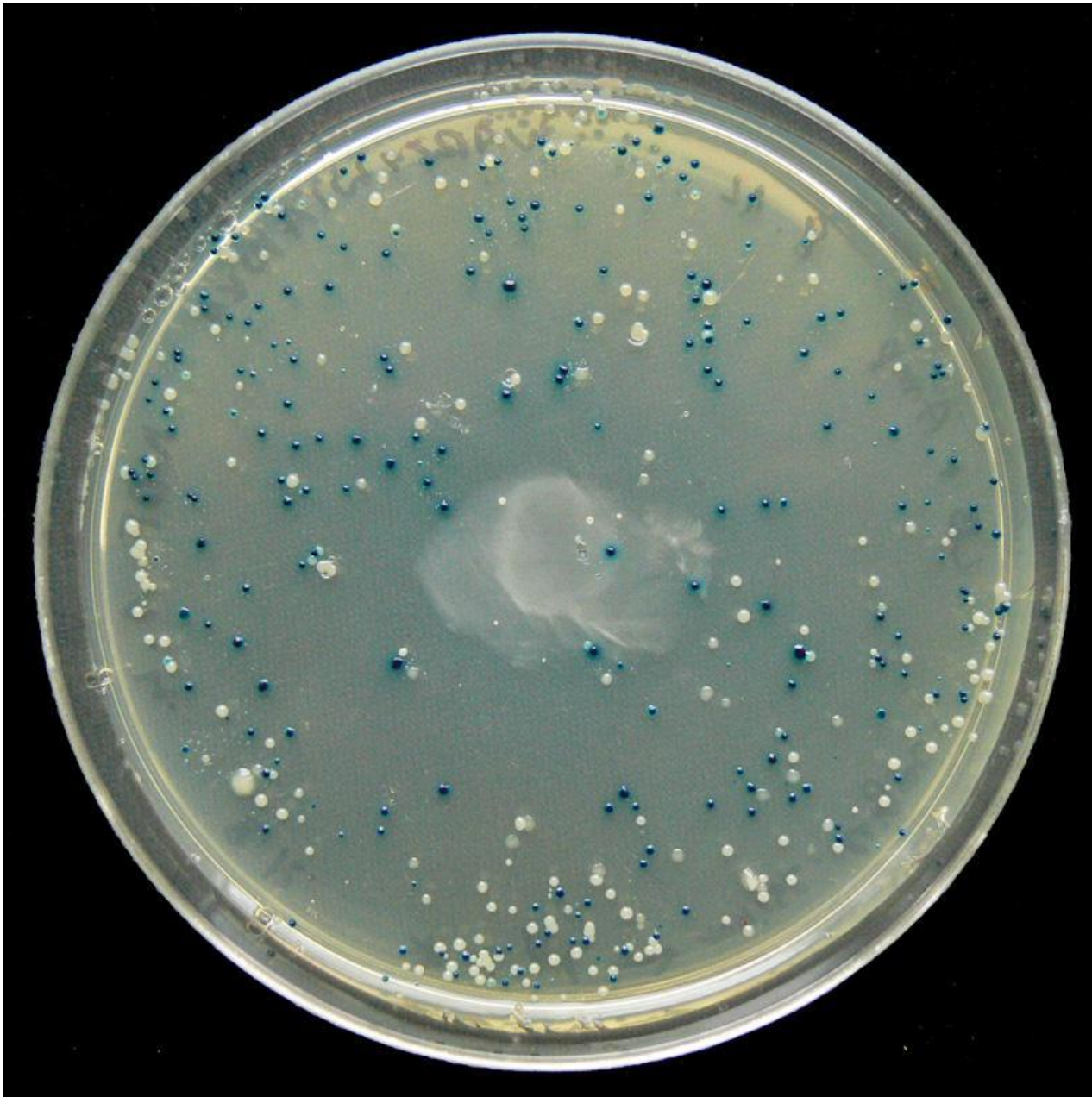
bp	ng/0.5 µg	%
20000	20	4
10000	20	4
7000	20	4
5000	75	15
4000	20	4
3000	20	4
2000	20	4
1500	80	16
1000	25	5
700	25	5
500	75	15
400	25	5
300	25	5
200	25	5
75	25	5

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

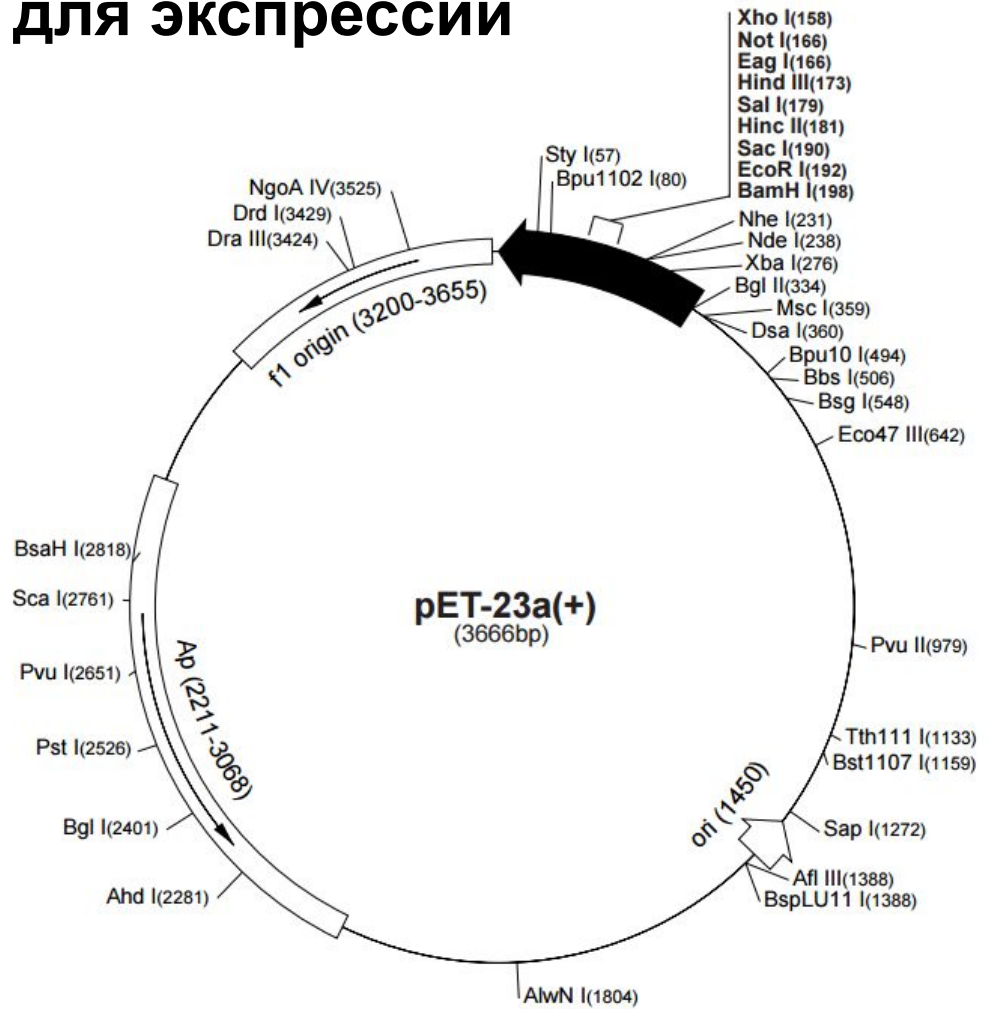
Векторы для клонирования



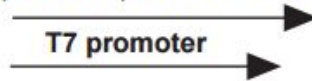
X-Gal



Векторы для экспрессии



T7 promoter primer #69348-3



Bgl II

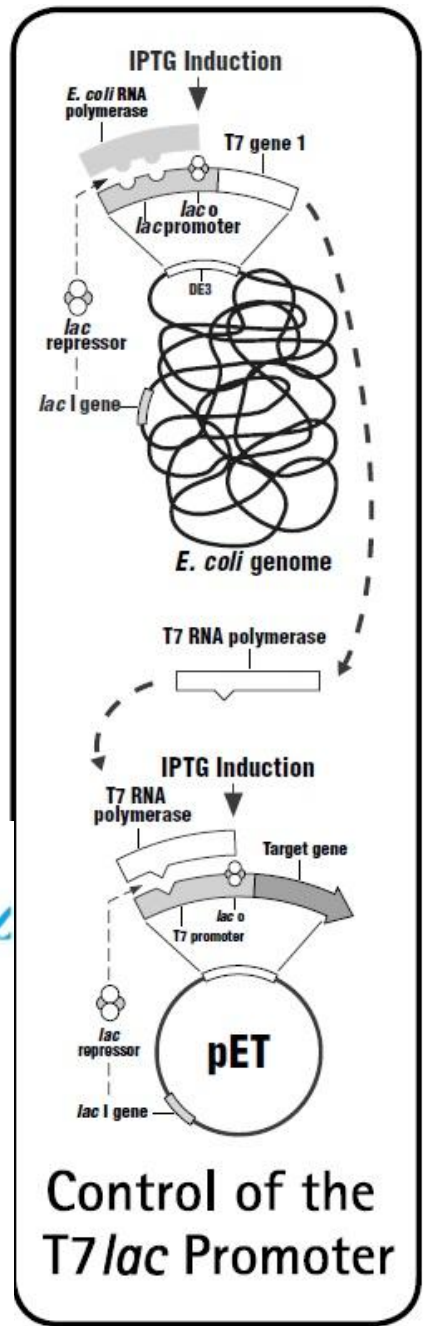
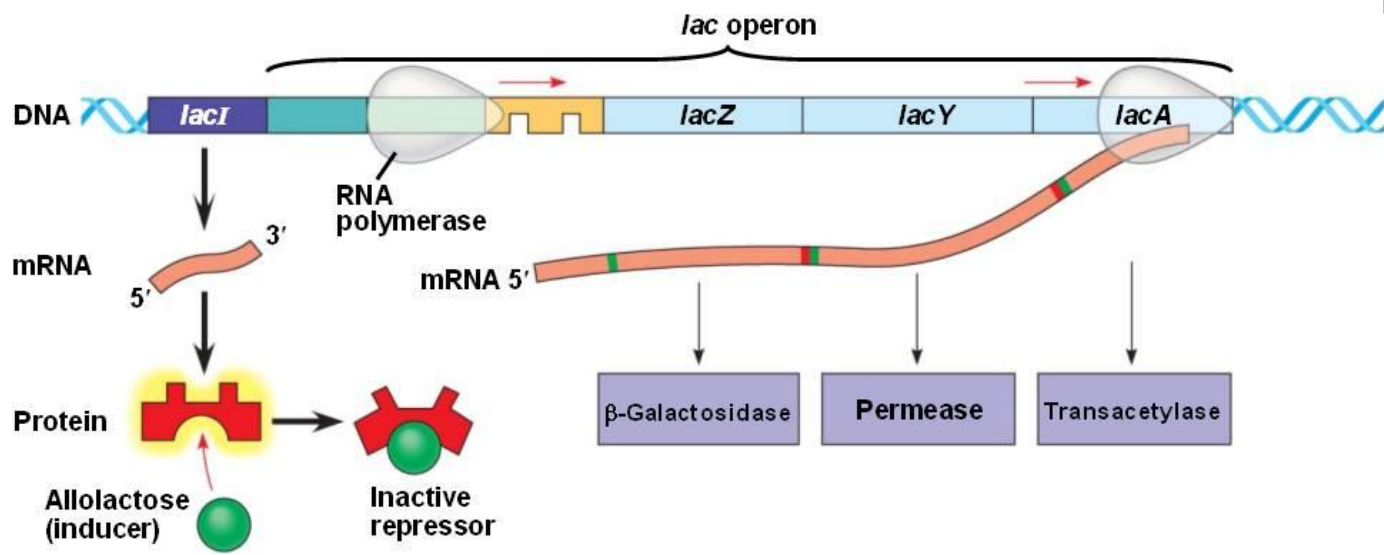
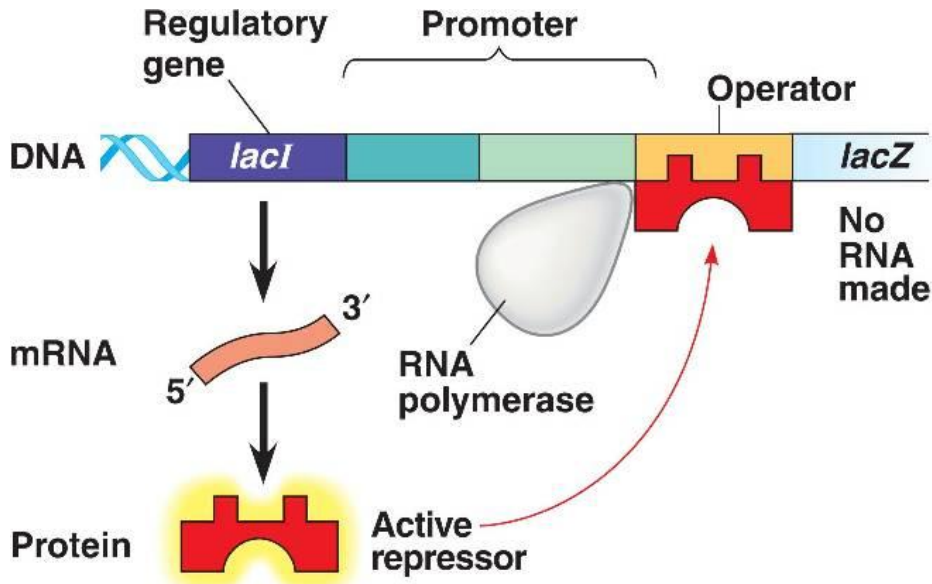
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA

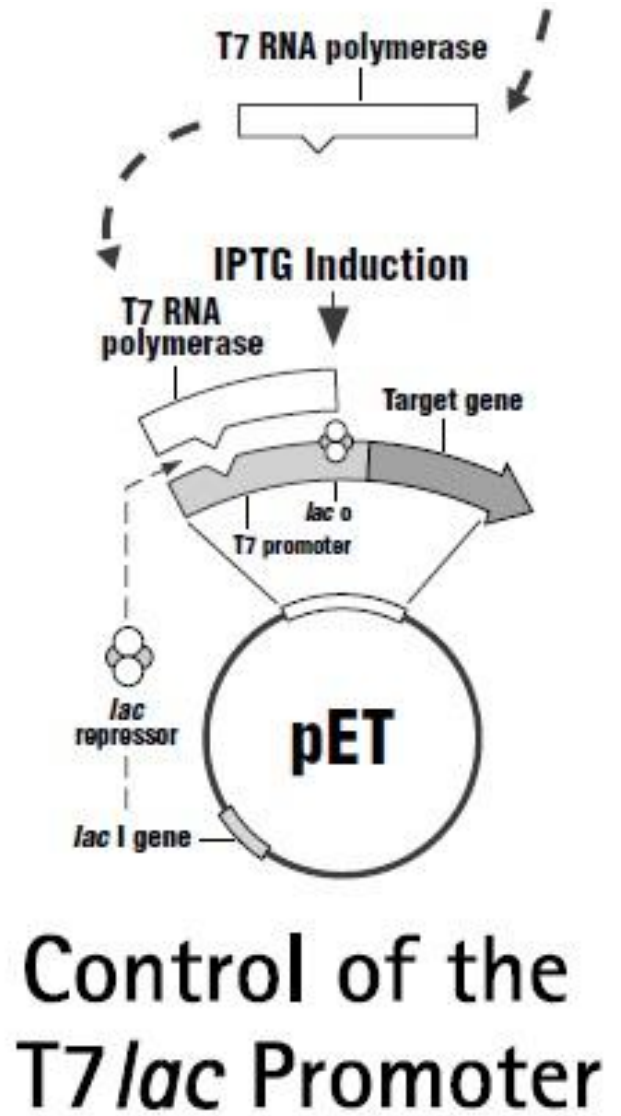
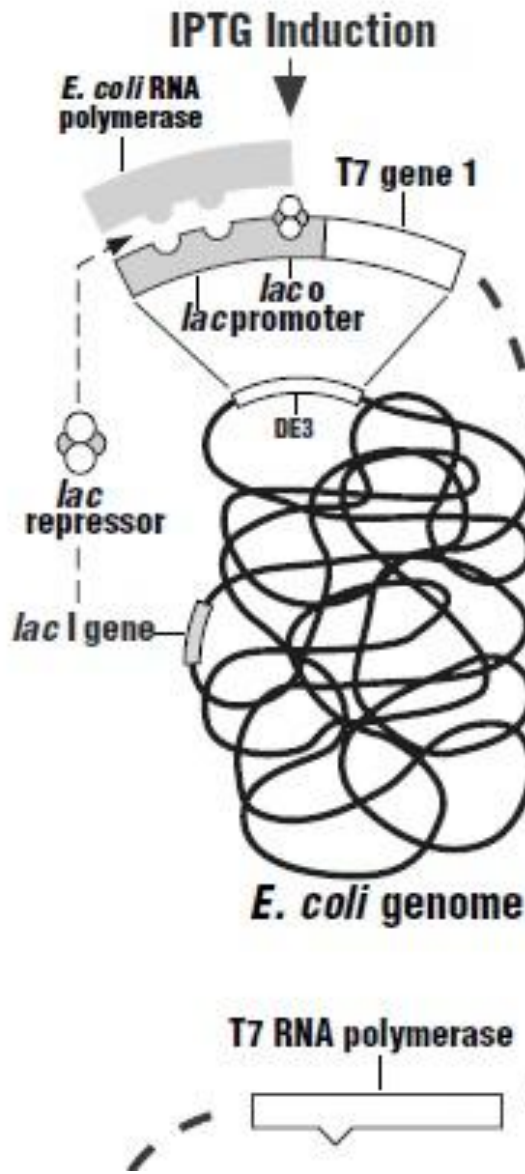
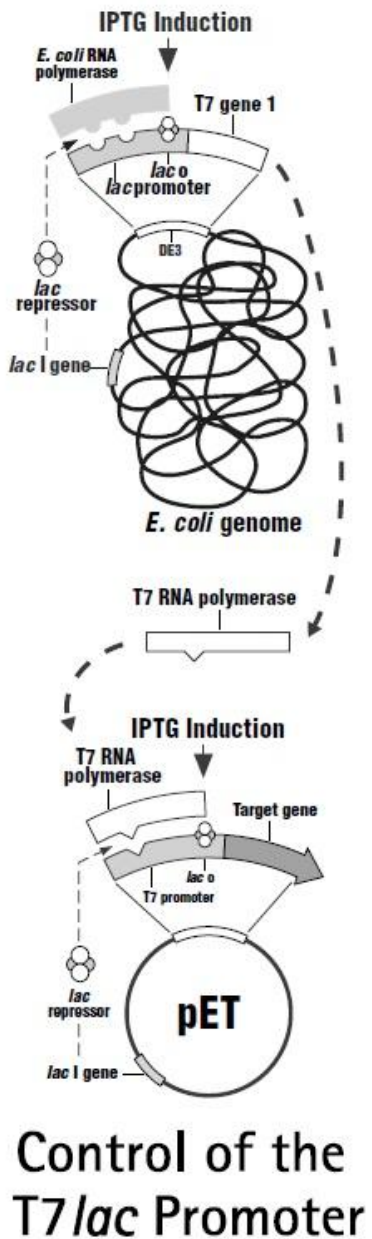
rbs

pET-23a

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

TATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGC GGCCGCACTCGAGCACCACCACCACCACCCTGA
MetAlaSerMetThrGlyGlyGlnGlnMetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProLeu

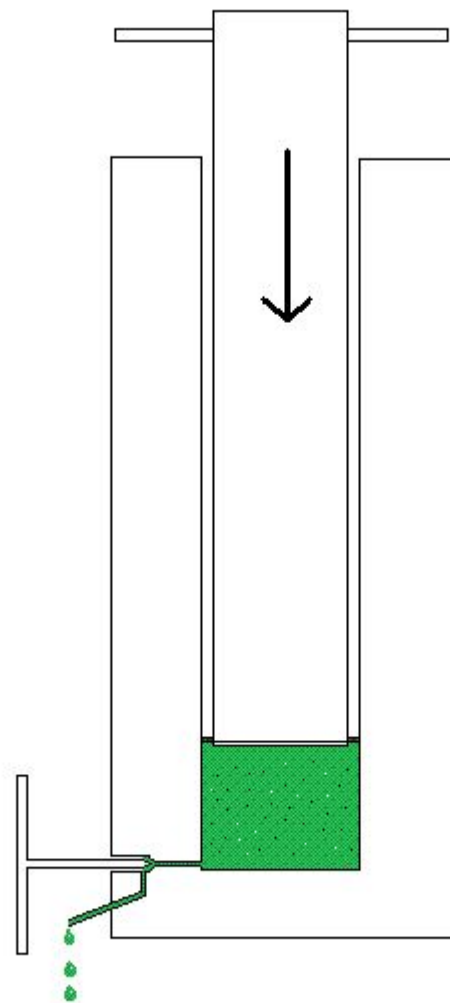




Лизис клеток



Pressure Cells



Очистка целевого белка

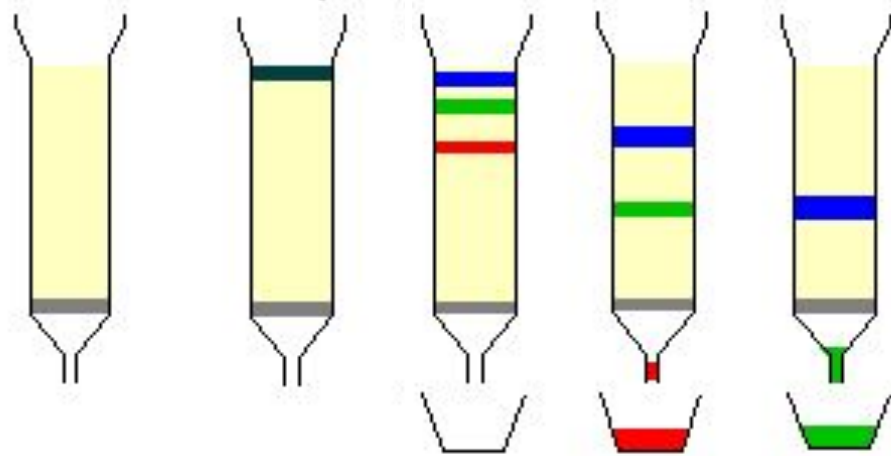
Хроматография



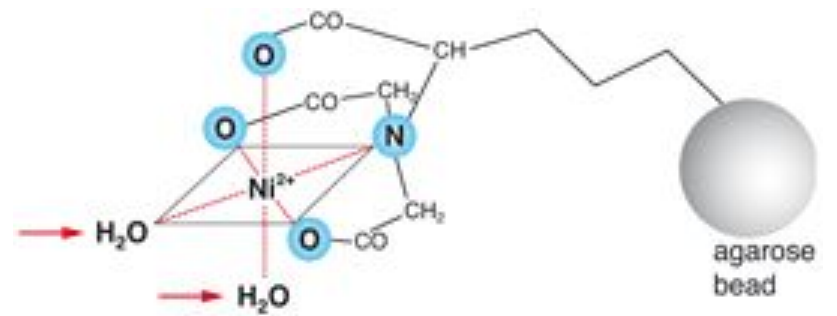
column
containing
stationary
phase

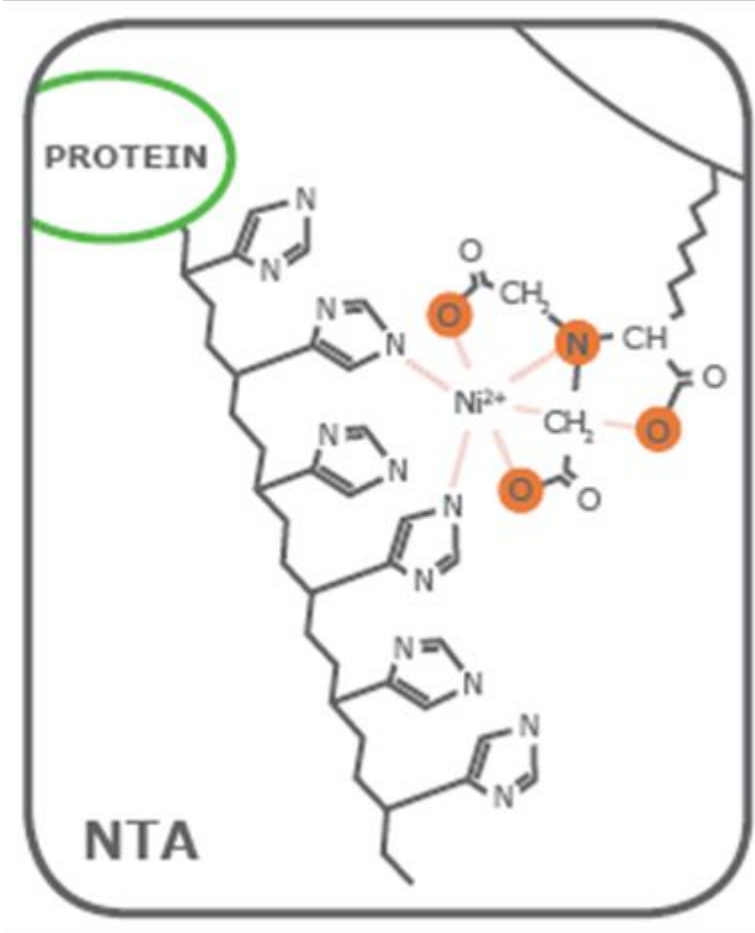
load
sample

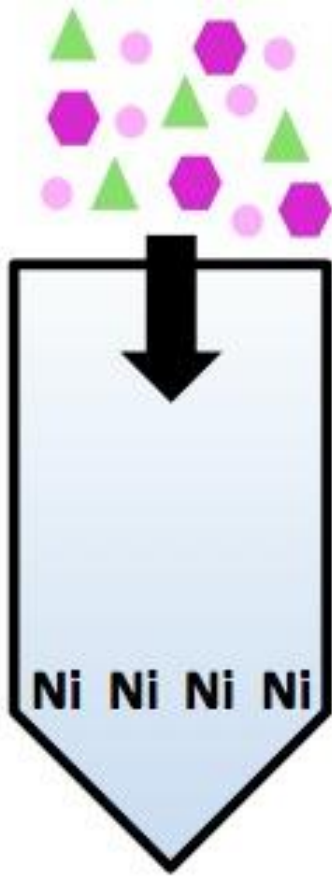
add
solvent



collect
components







Loading
supernatant



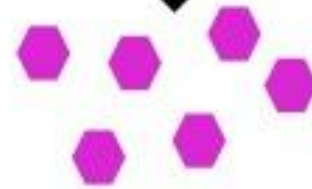
Binding to the
column

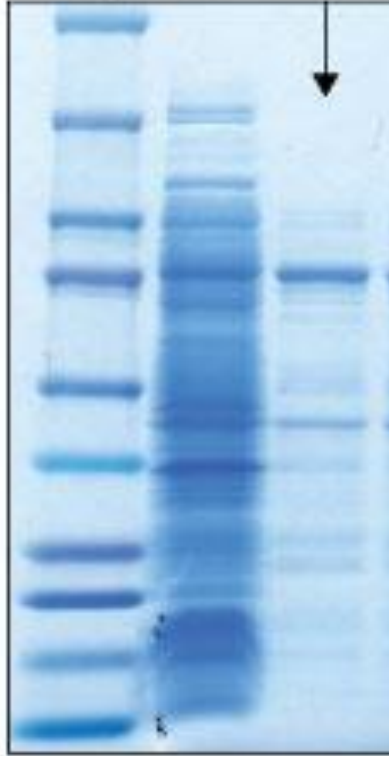
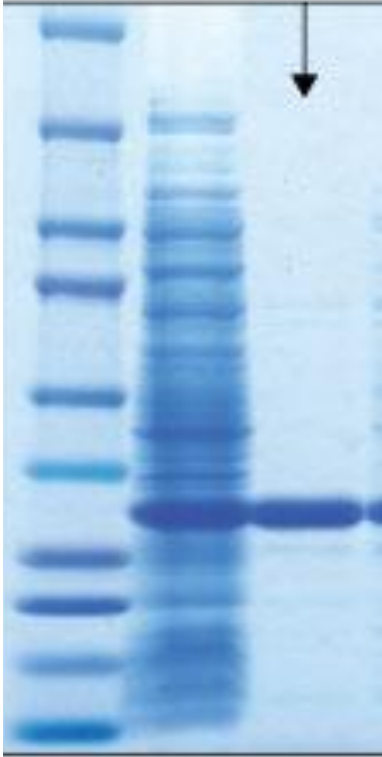


Wash

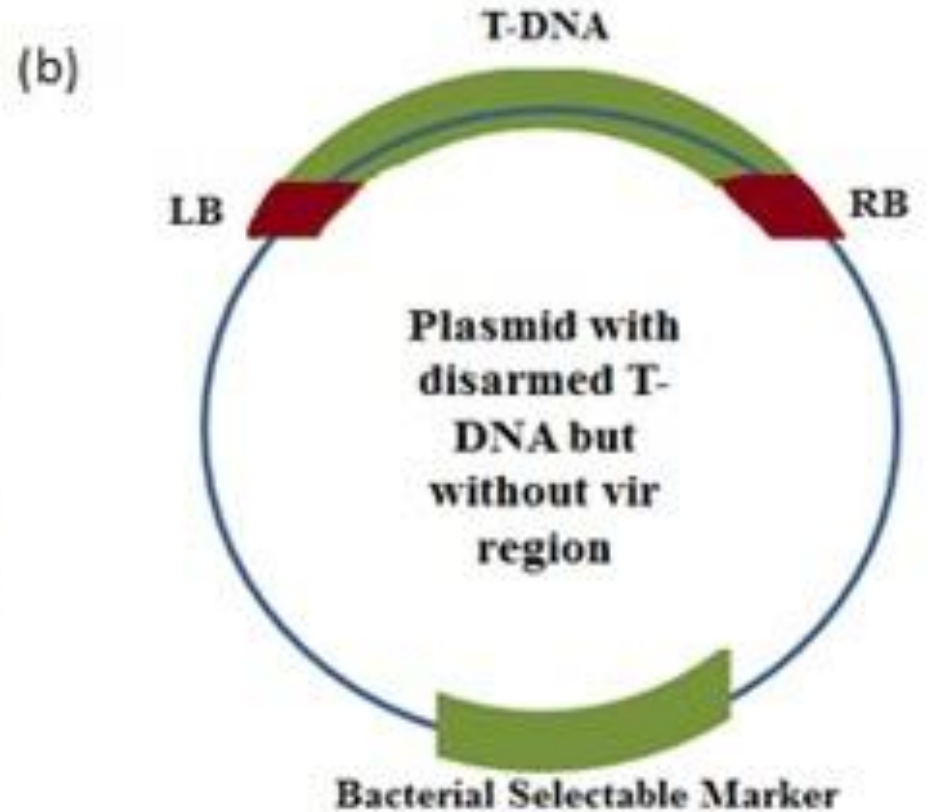
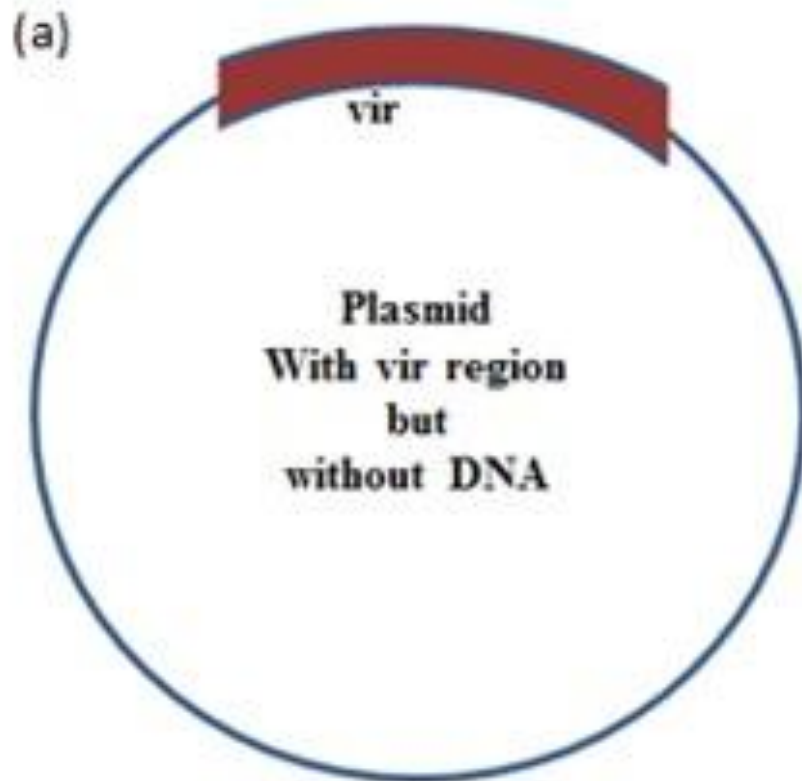


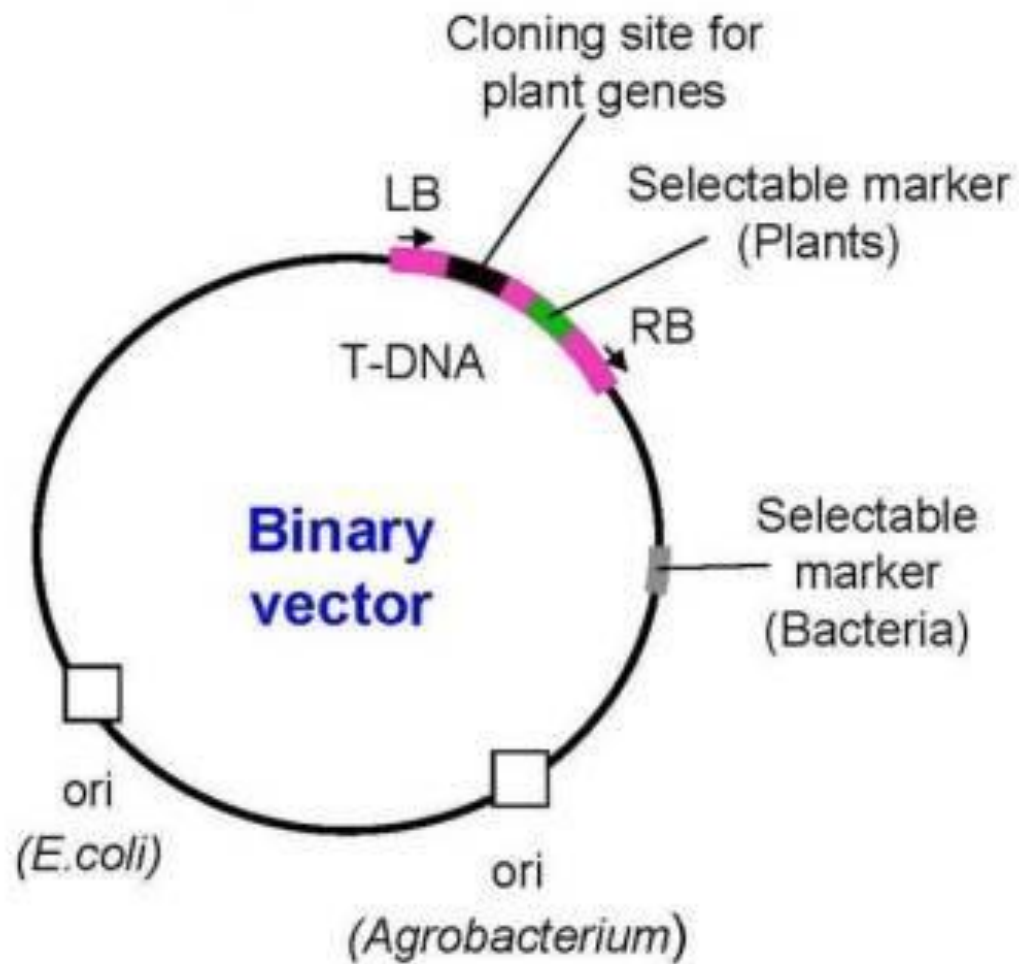
Elution



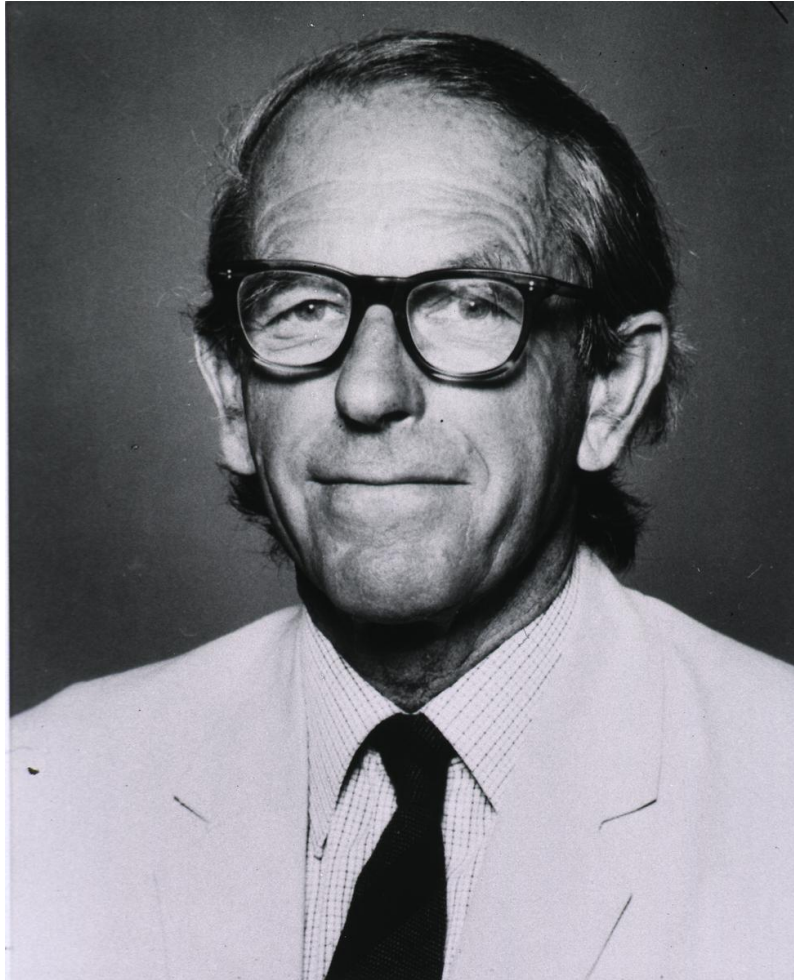


Векторы для трансформации растений

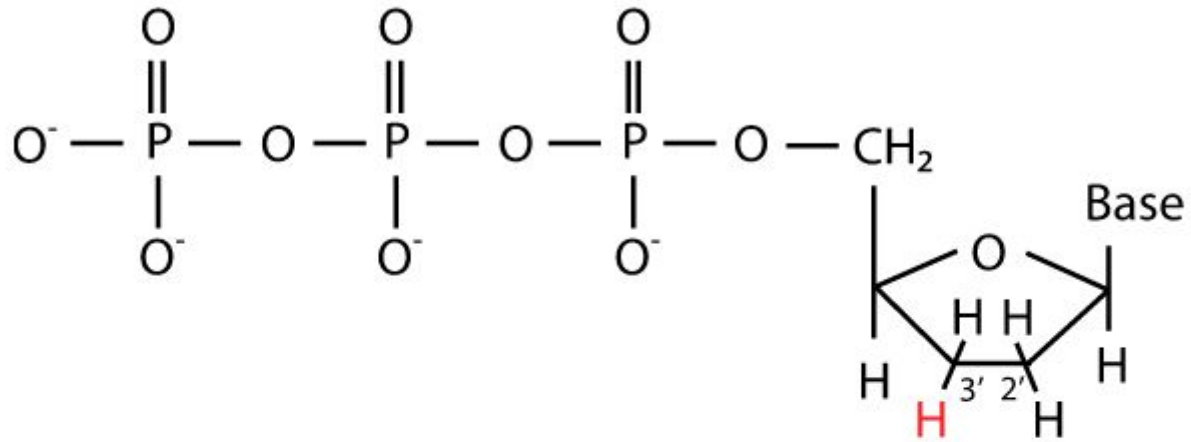




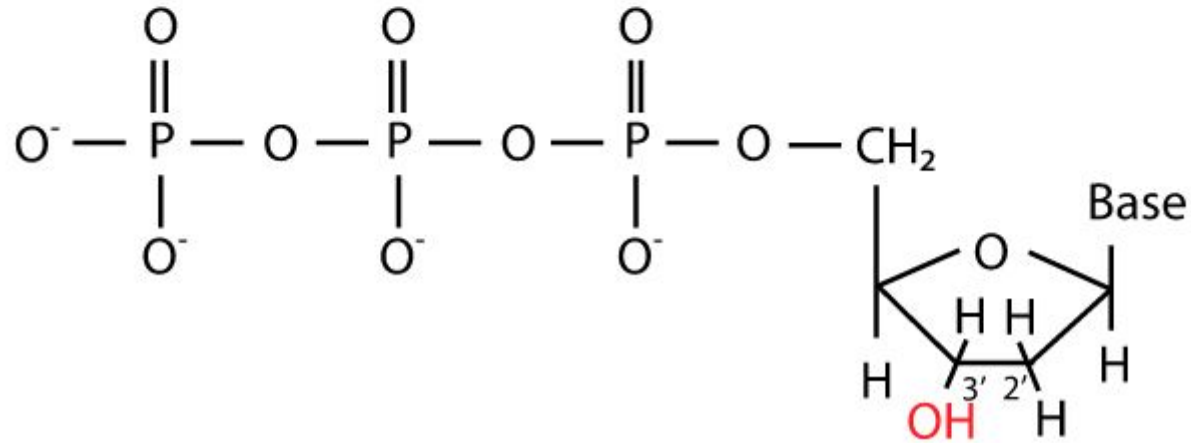
Определение первичной структуры ДНК



Dideoxynucleotide (ddNTP)



Deoxynucleotide (dNTP)





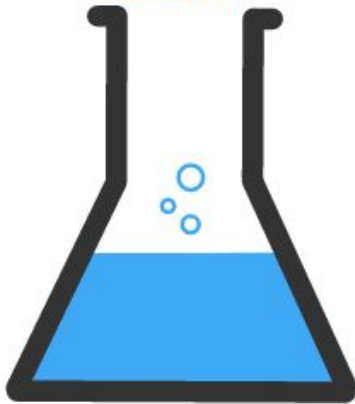
dATP + dCTP + dGTP + dTTP

DNA Polymerase

Template DNA

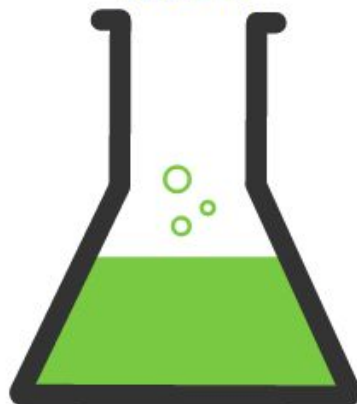
Primer

ddATP



ACTCAGATGCT
ACTCAGA
ACTCA
A

ddCTP



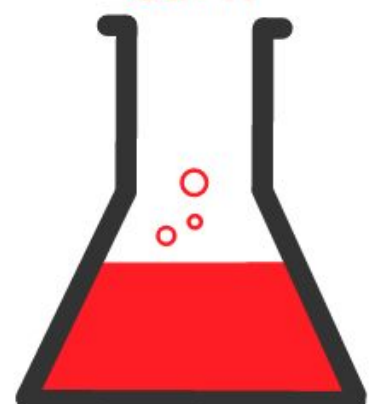
ACTCAGATGCT
ACTCAGATGC
ACTC
AC

ddGTP

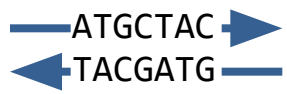
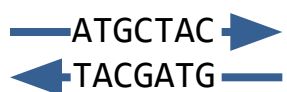
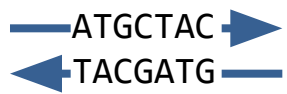
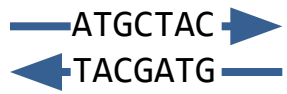
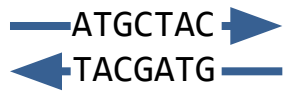
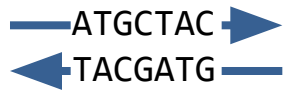
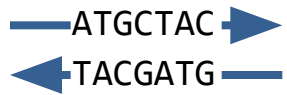


ACTCAGATGCT
ACTCAGATG
ACTCAG

ddTTP



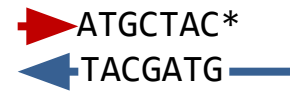
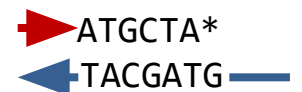
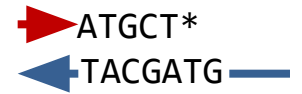
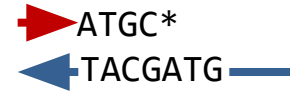
ACTCAGATGCT
ACTCAGAT
ACT

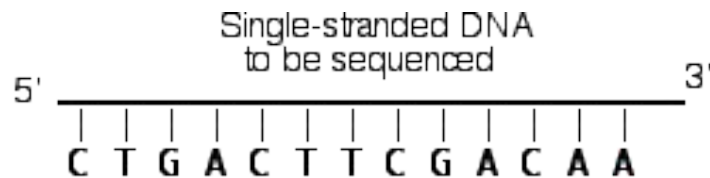


Денатурация,
отжиг
праймера →

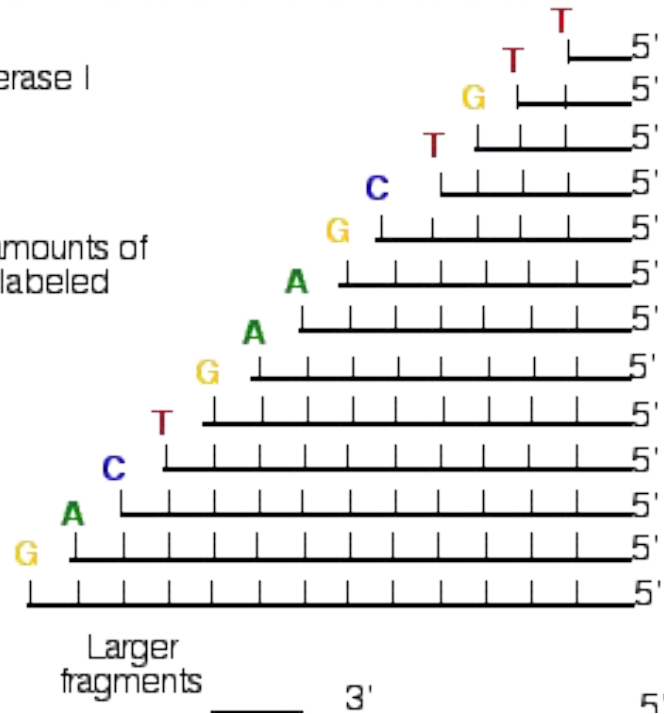


Элонгаци
я →

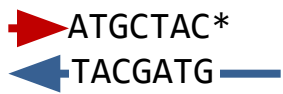
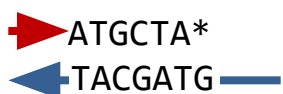
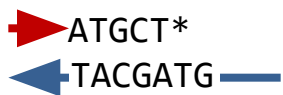
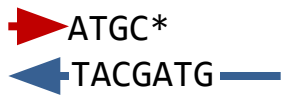
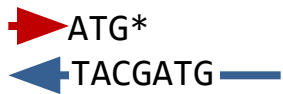




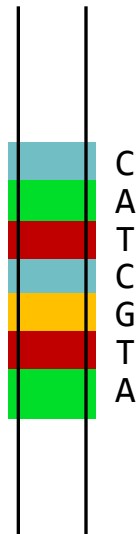
Add:
 DNA polymerase I
 dATP
 dGTP
 dCTP
 dTTP
 plus limiting amounts of
 fluorescently labeled
ddATP
ddGTP
ddCTP
ddTTP







Капиллярный
 электрофорез



Анали
 з

