

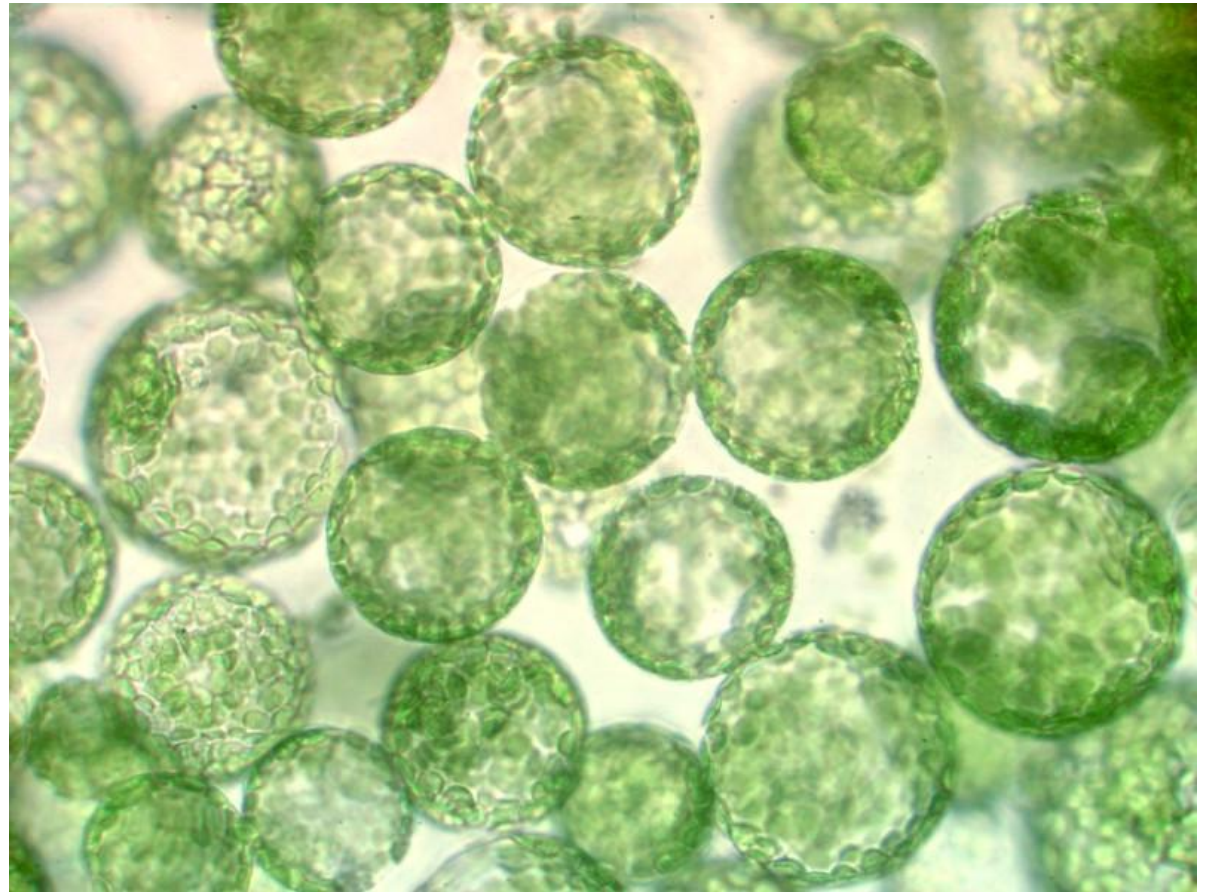
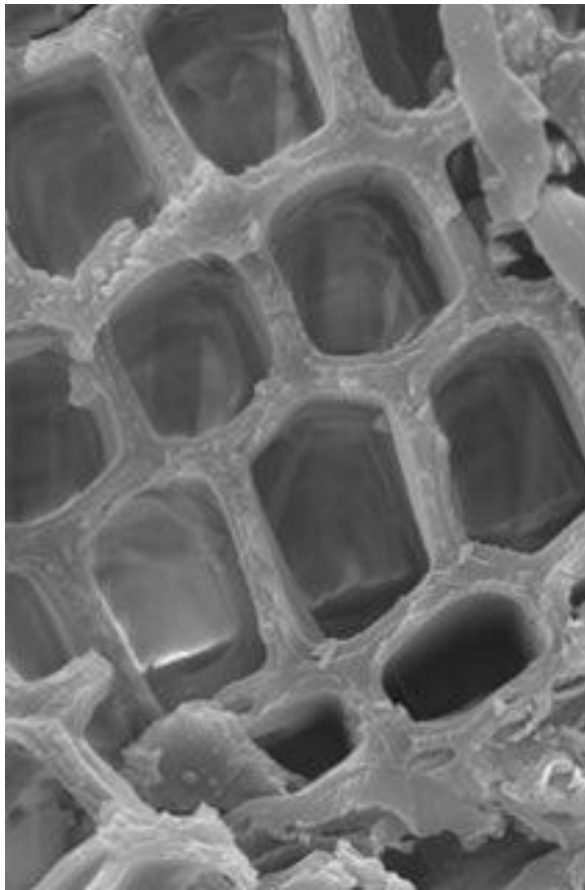
Трансгенные растения



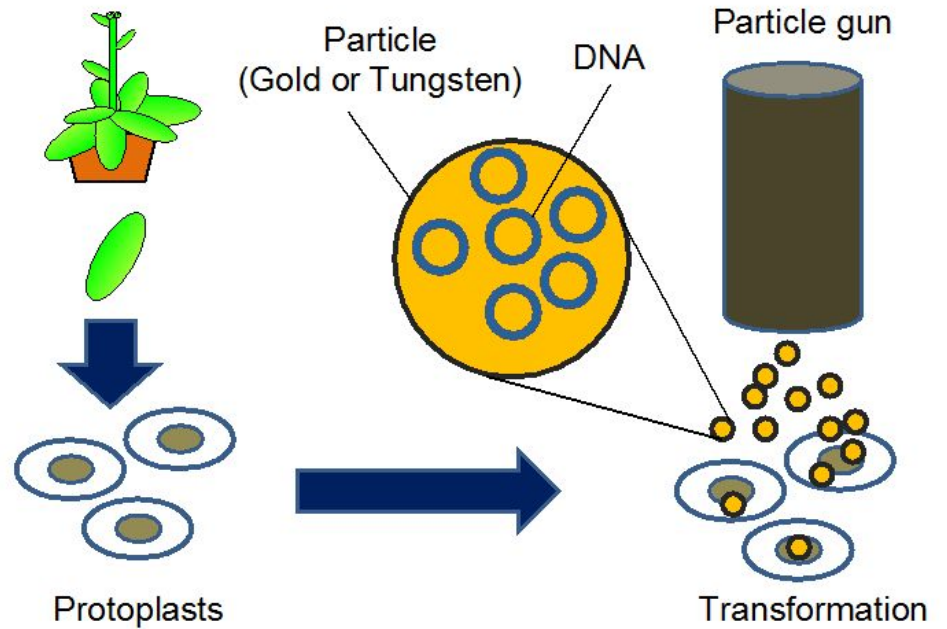
Что нужно, чтобы получить
трансгенное растение?

Чужеродная ДНК должна проникнуть в клетку

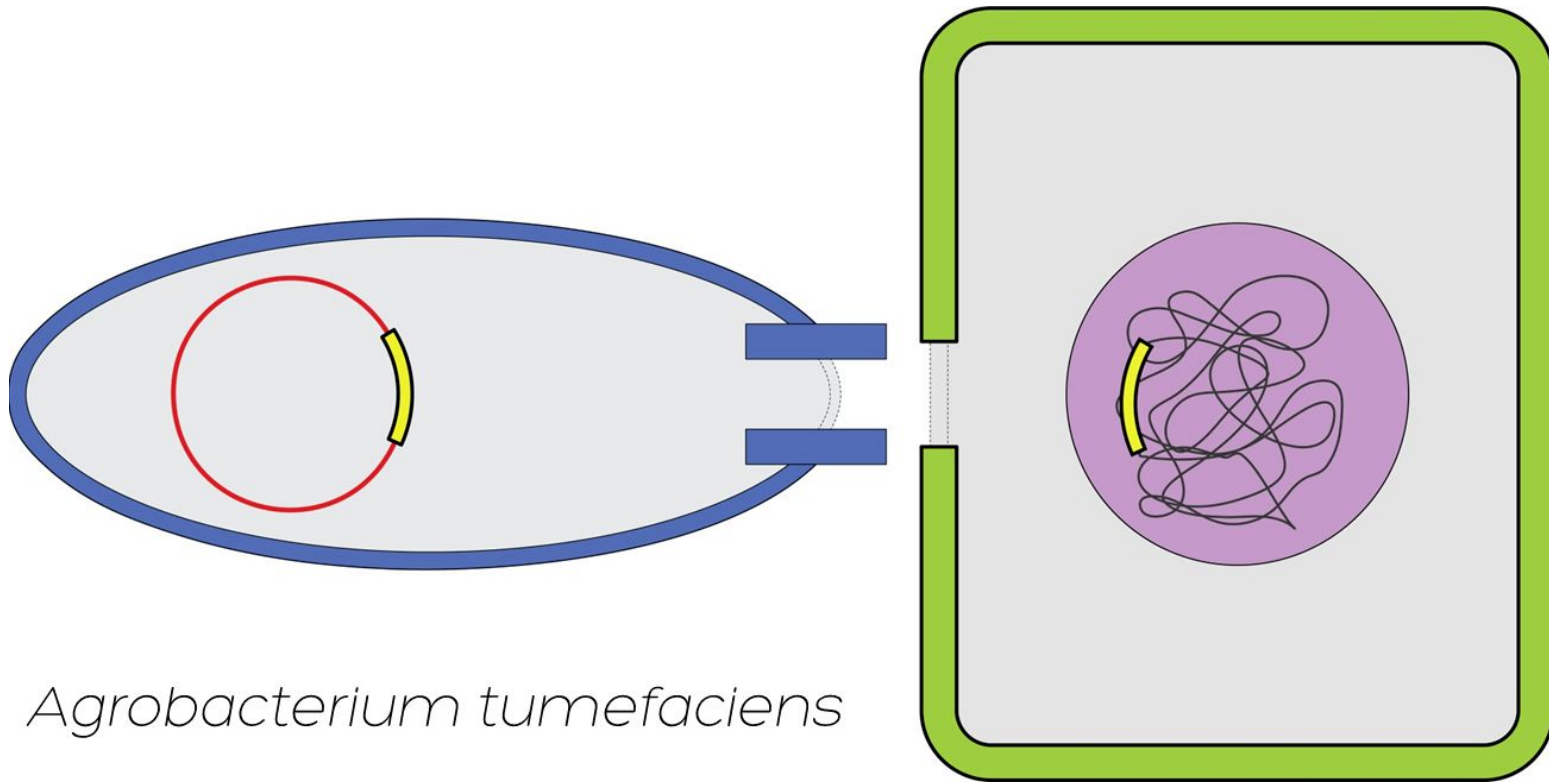
«Избавление» от клеточной стенки



Бомбардировка клеток



Использование потенциала фитопатогенов



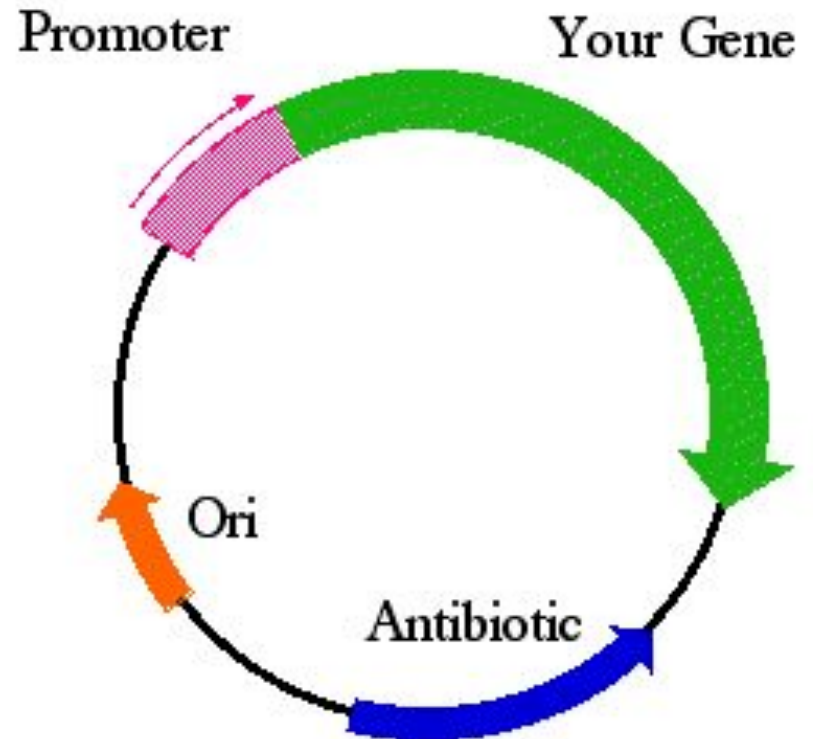
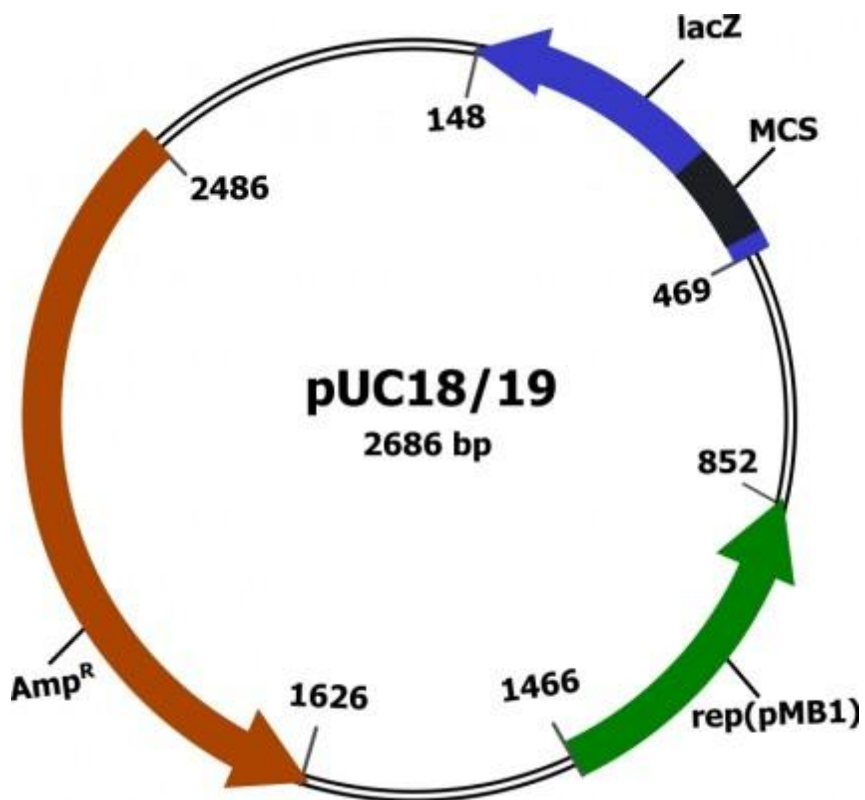
Agrobacterium tumefaciens

Нужно чтобы целевой участок ДНК:

Встроился в геном

Экспрессировался (чтобы генетическая информация считывалась)

Чужеродные гены находятся в составе векторных плазмид

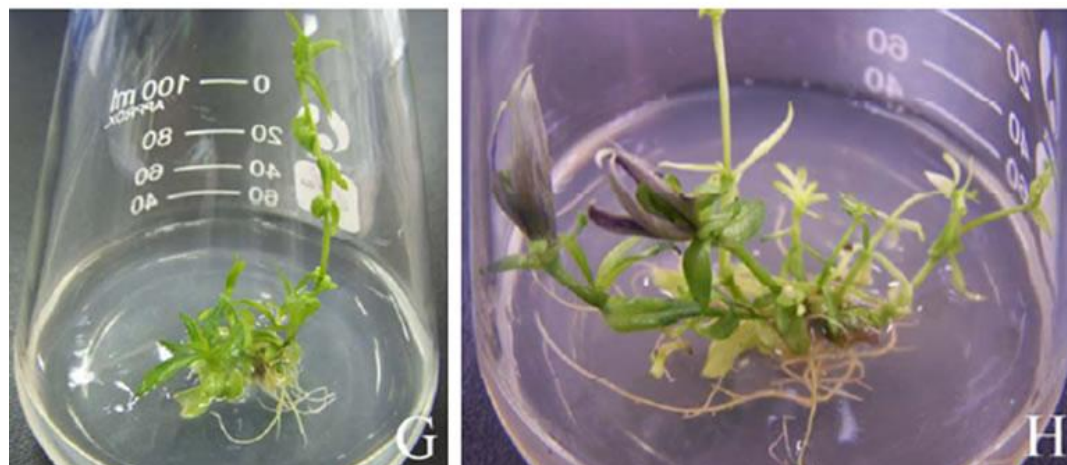
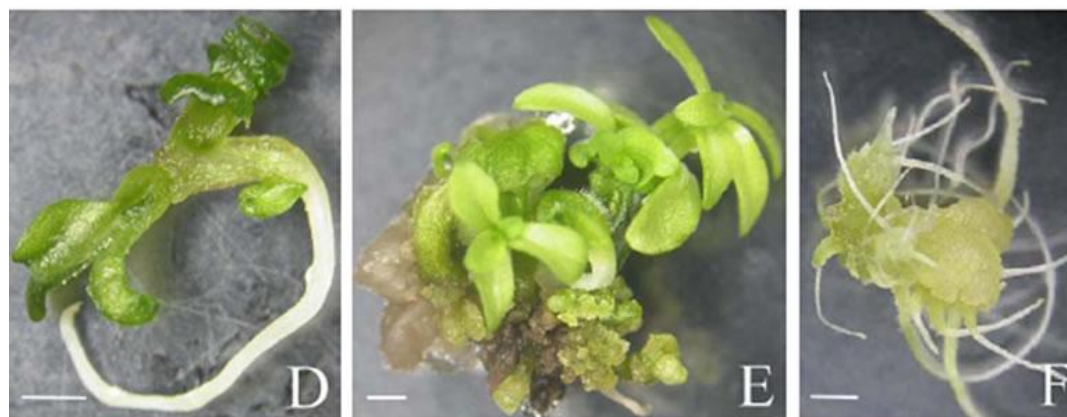
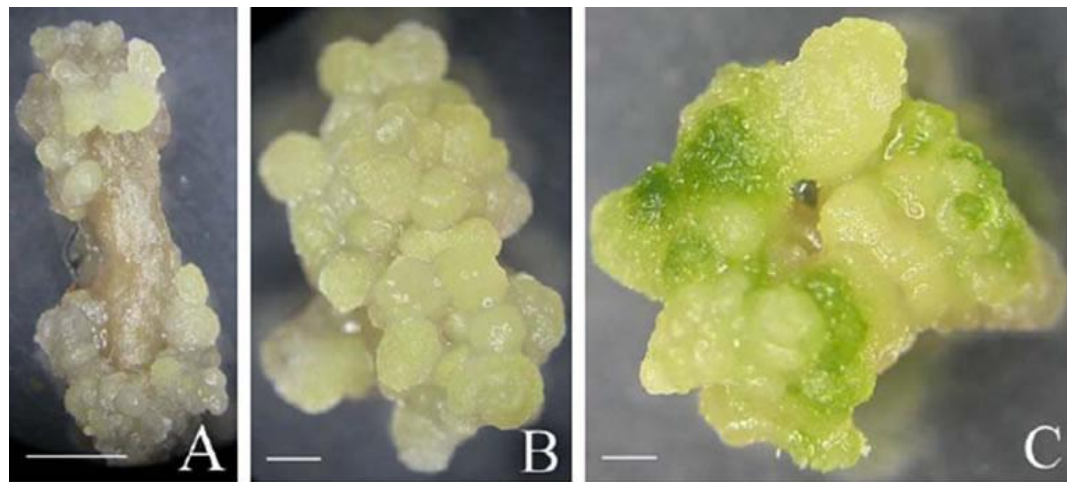


Введение векторных конструкций в клетки растений



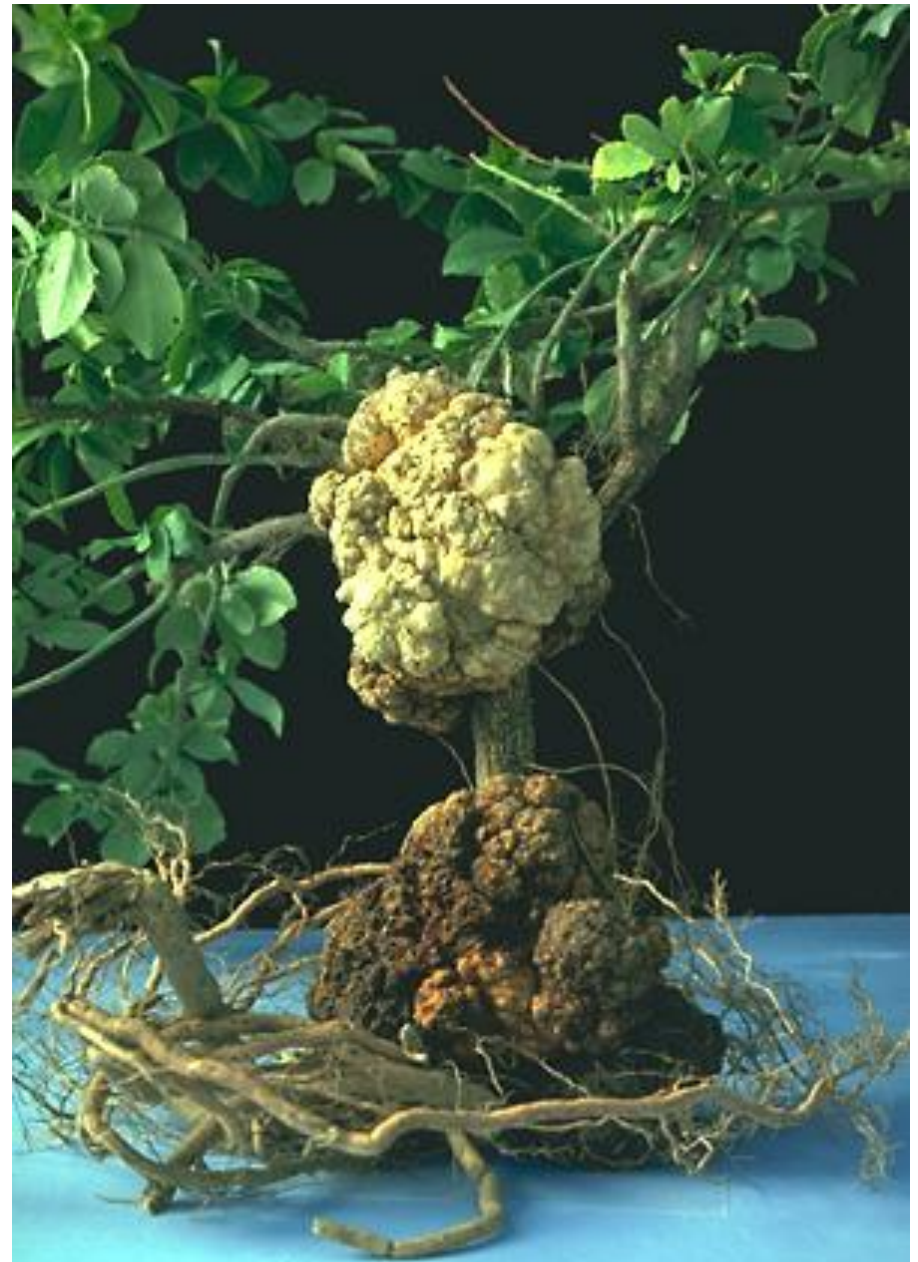
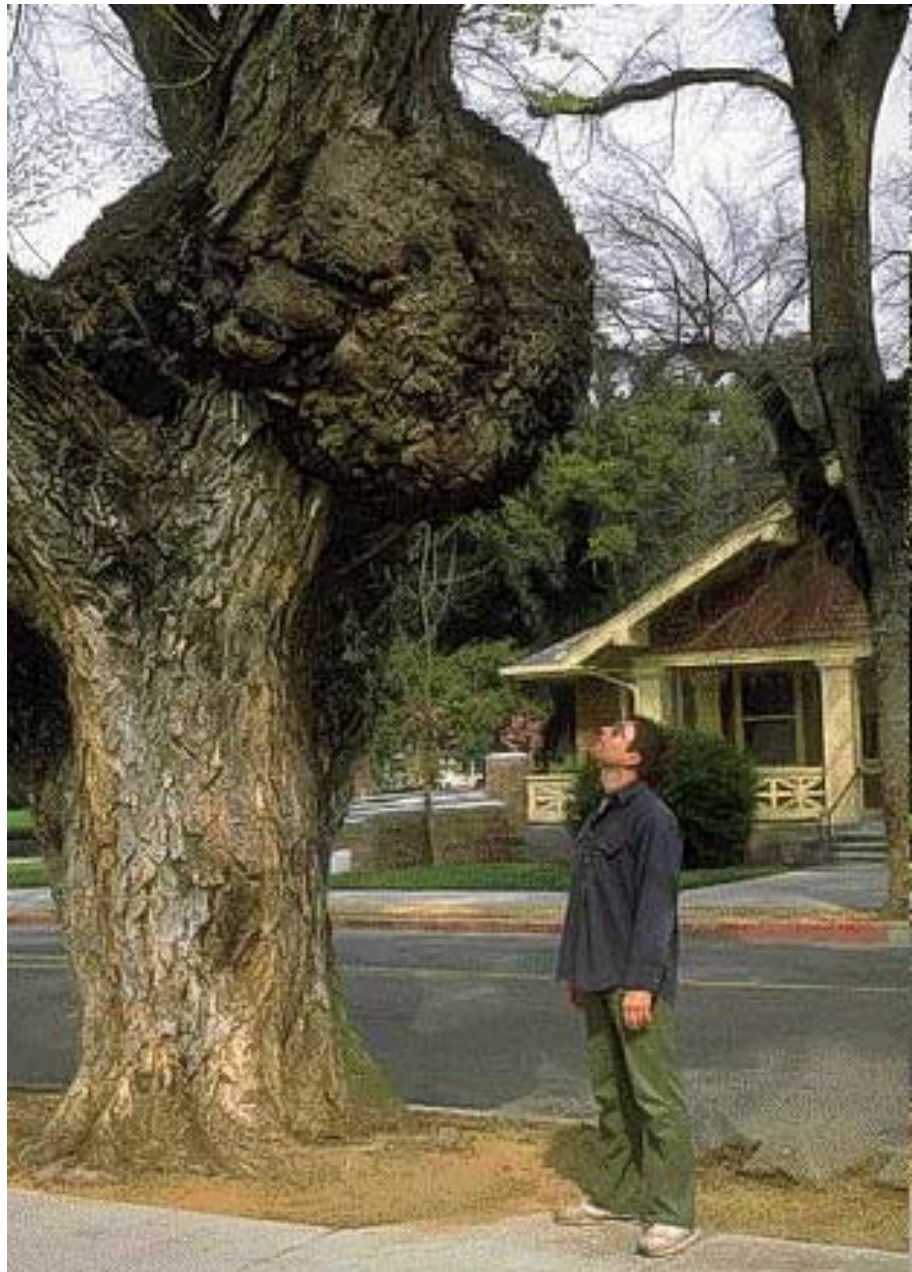
Станет ли после этого растение трансгенным?

Регенерация
целых растений из
отдельных
трансформирован
ных клеток

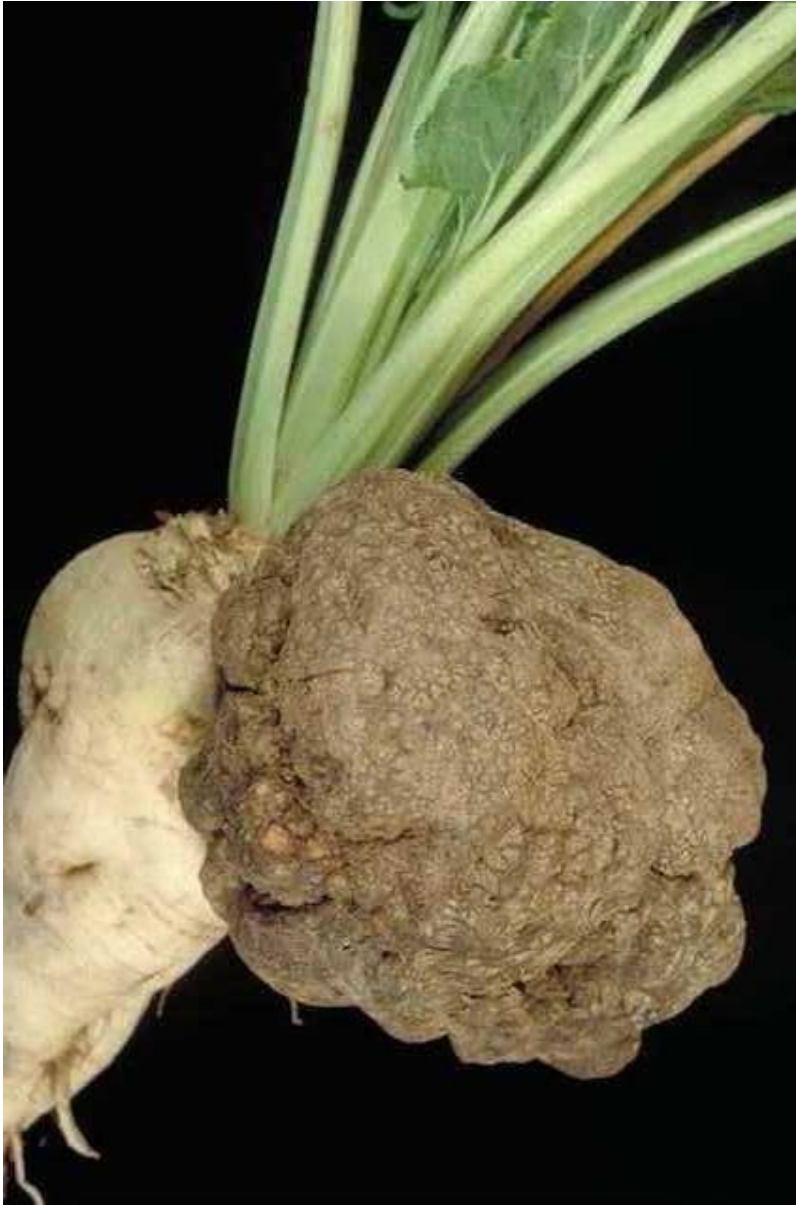


Основной подход для создания
трансгенных растений

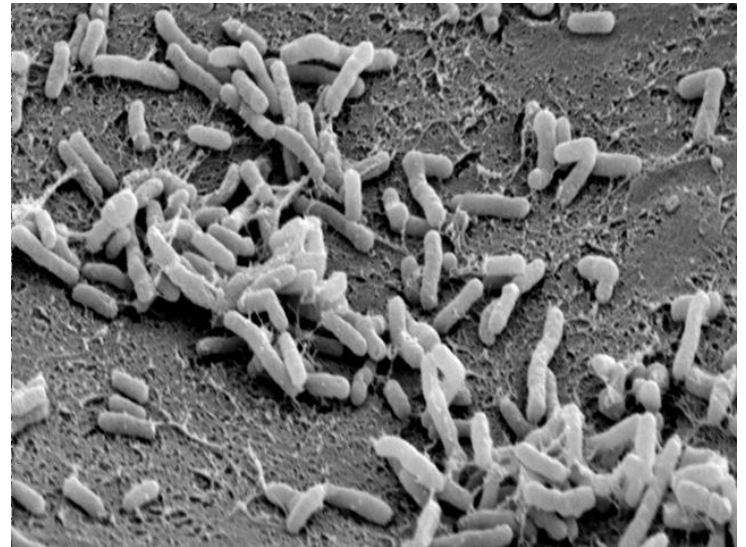
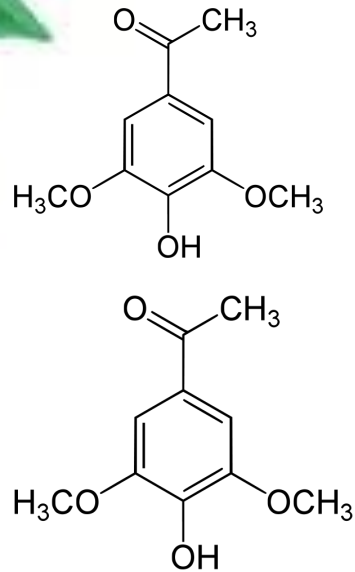
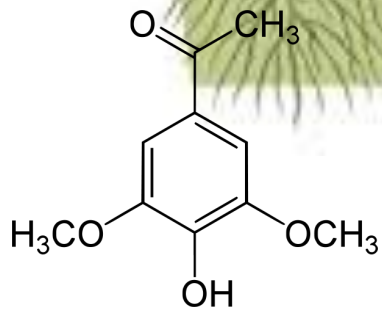
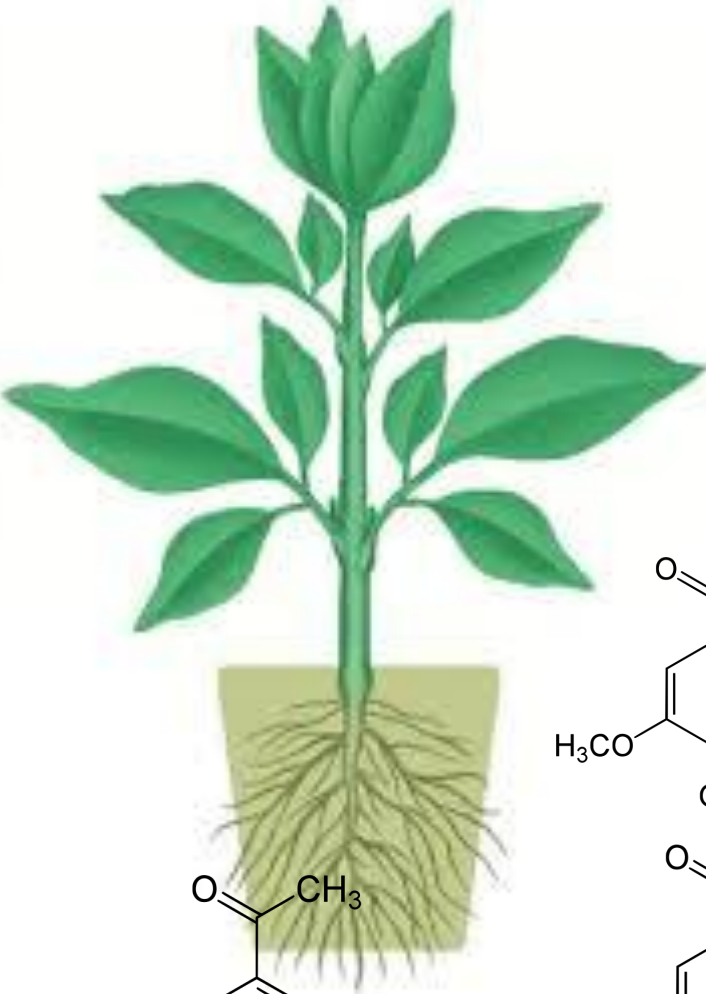
Корончатые галлы



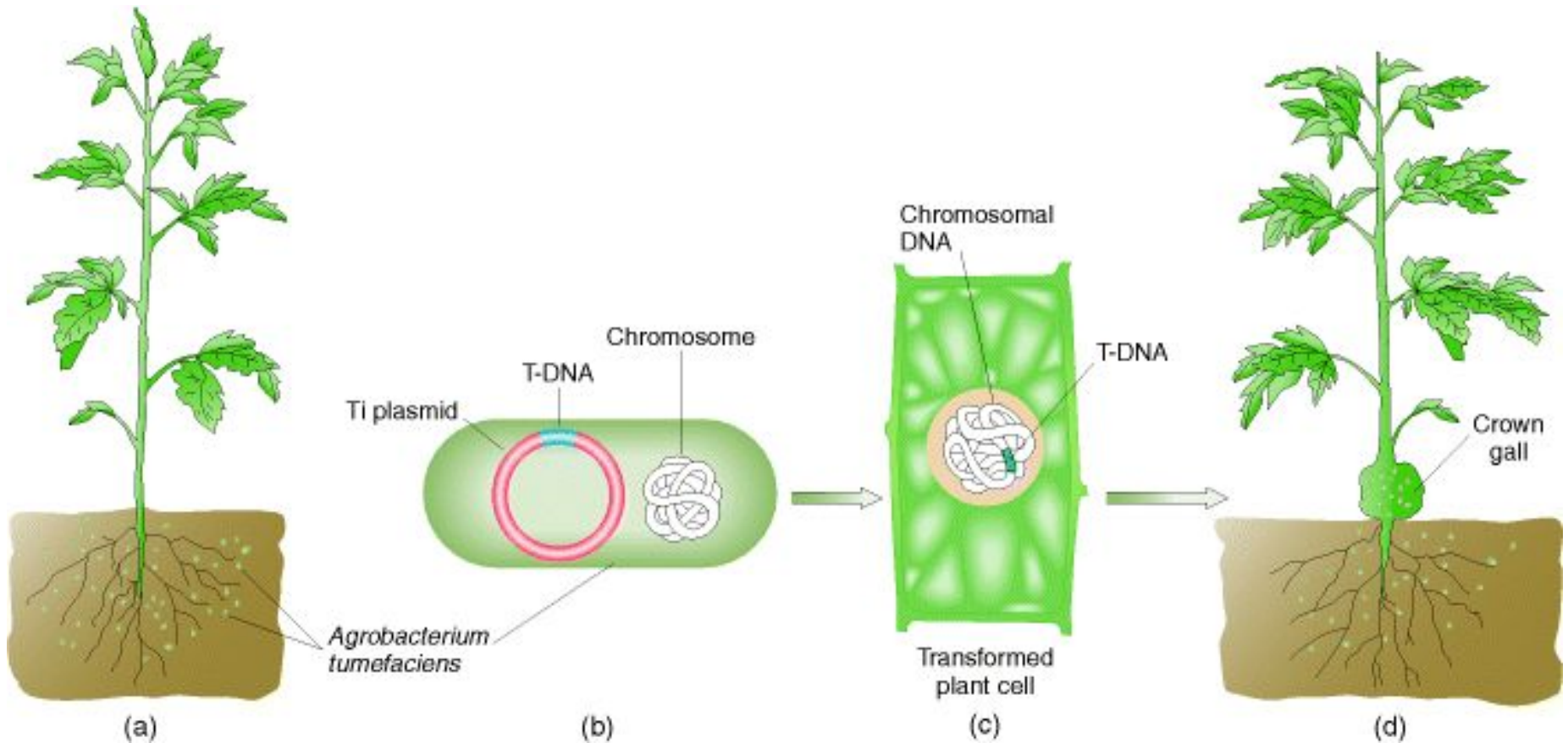
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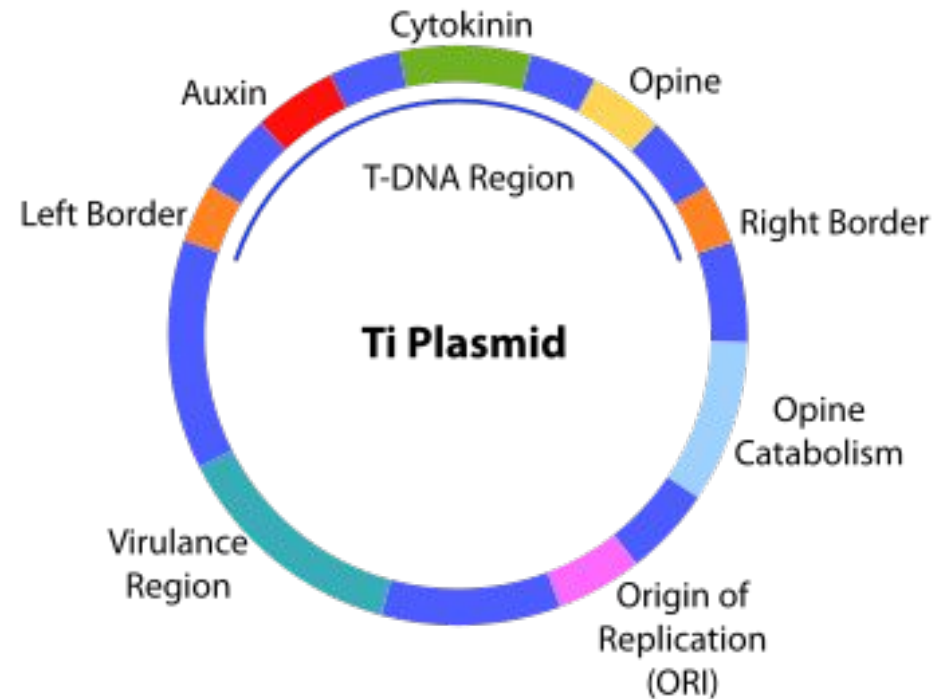
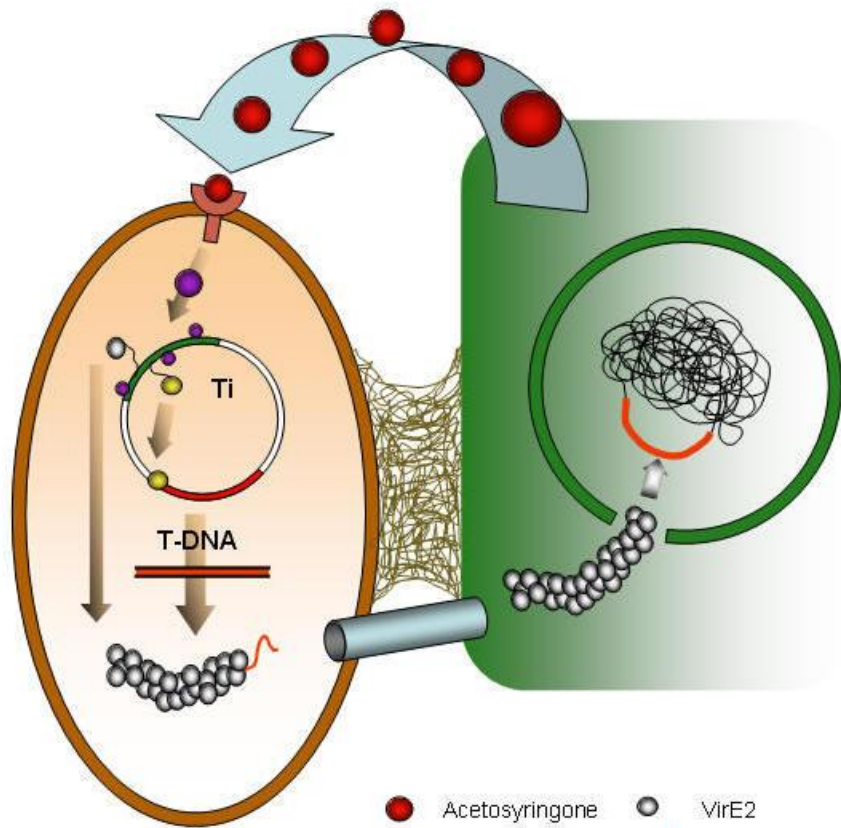
Agrobacterium tumefaciens



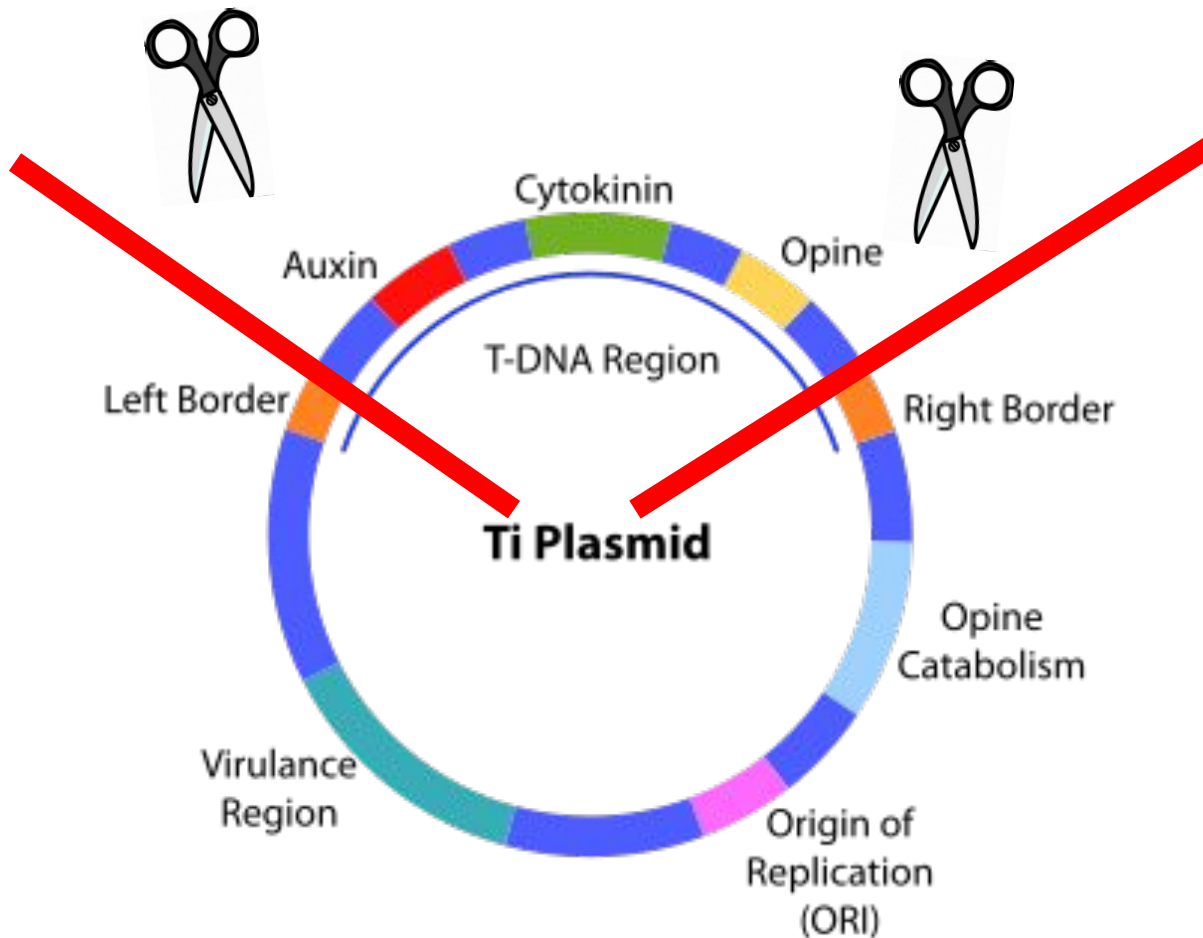
Agrobacterium tumefaciens



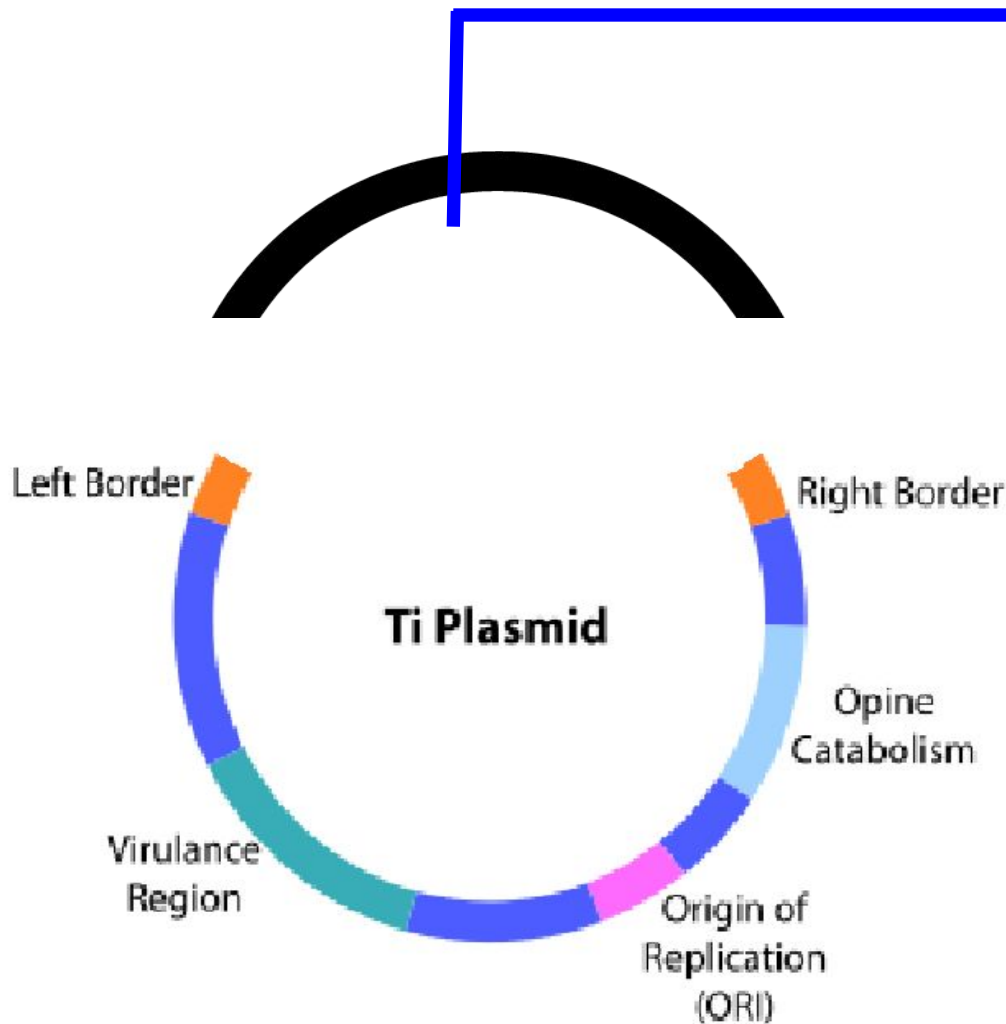
Интеграция тДНК в геном растения



Модификация Ti – плазмиды для создания трансгенных растений



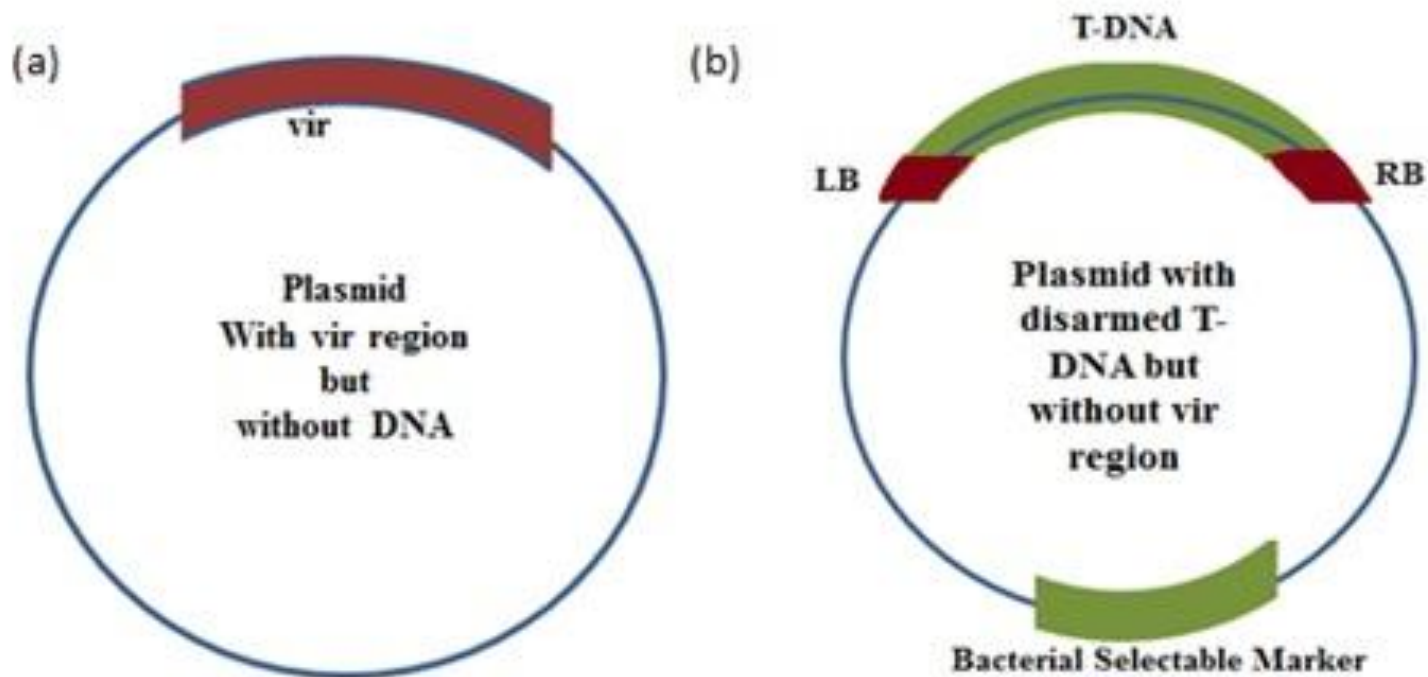
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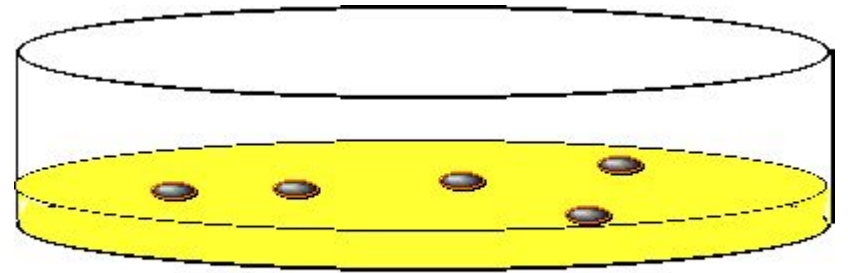
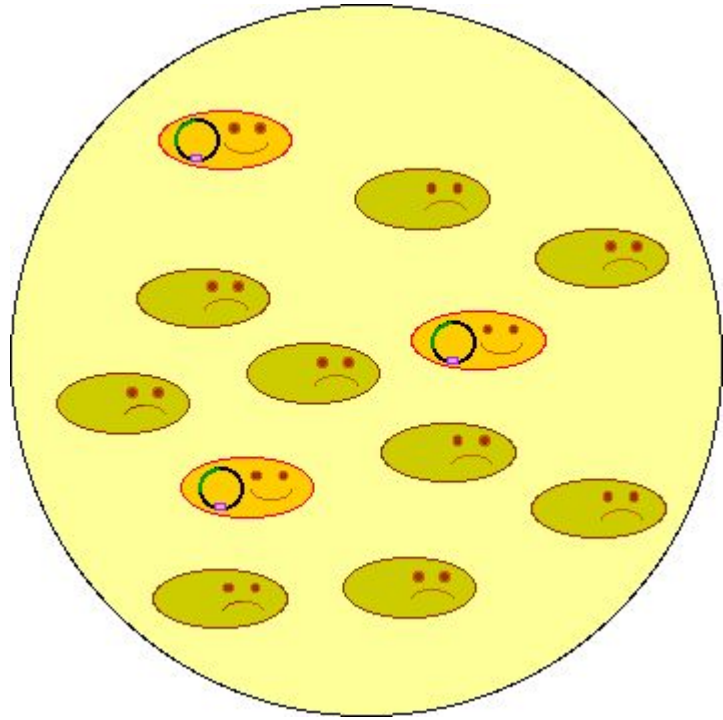


Ген, который хотят встроить

Селективный маркер

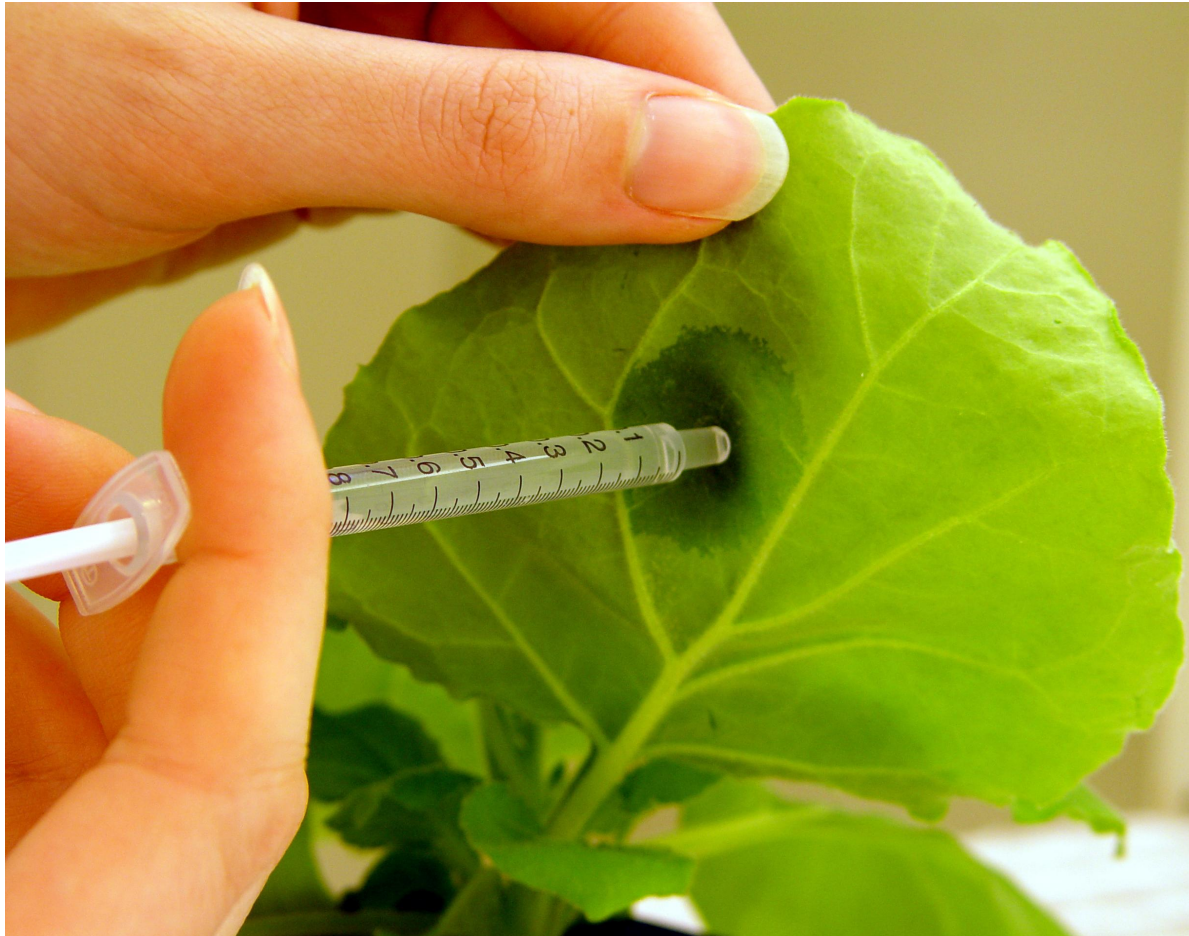
Бинарный вектор



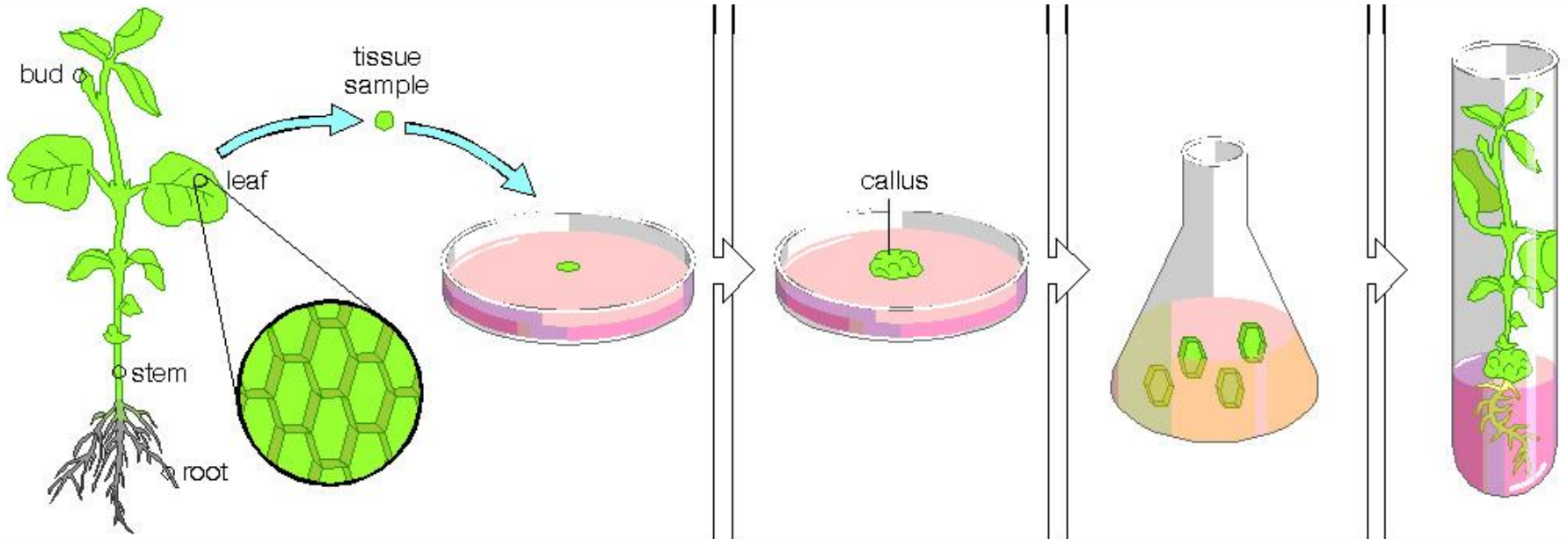


agar medium with antibiotic

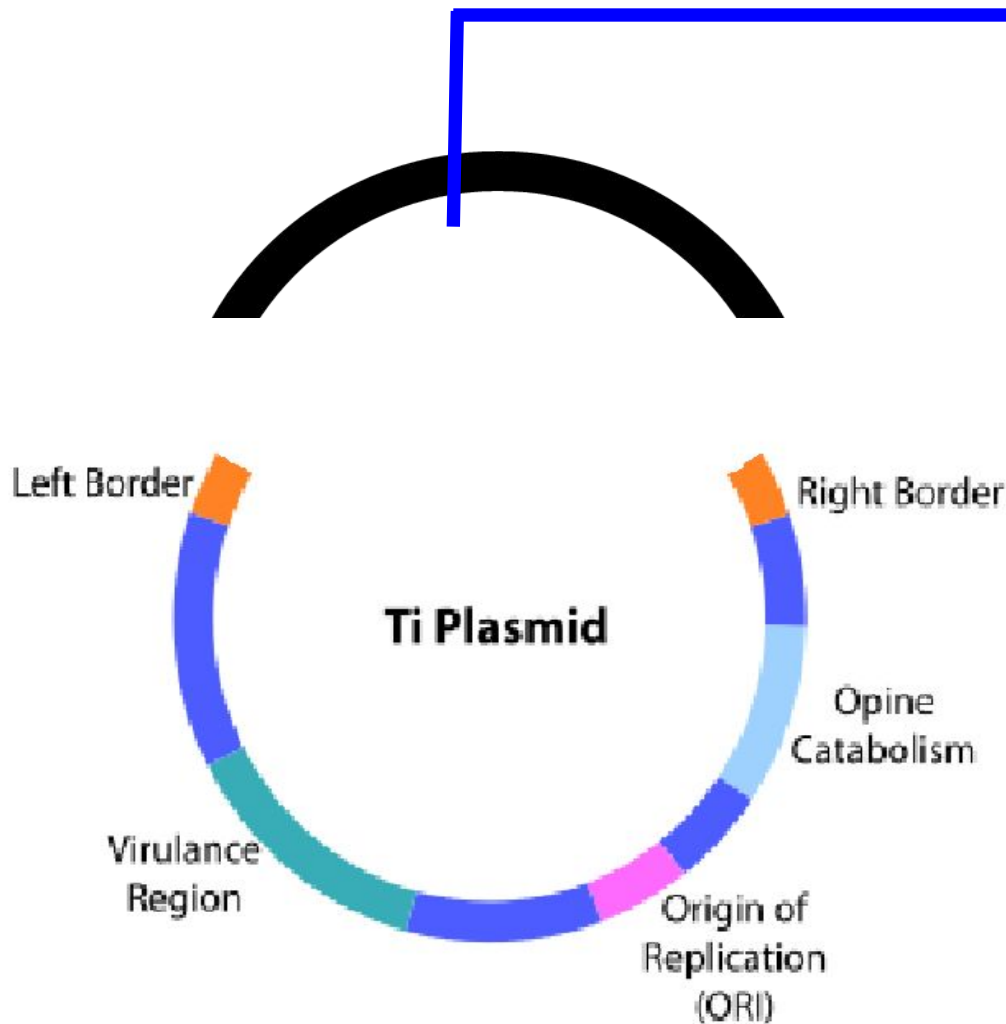
Введение векторных конструкций в клетки растений



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

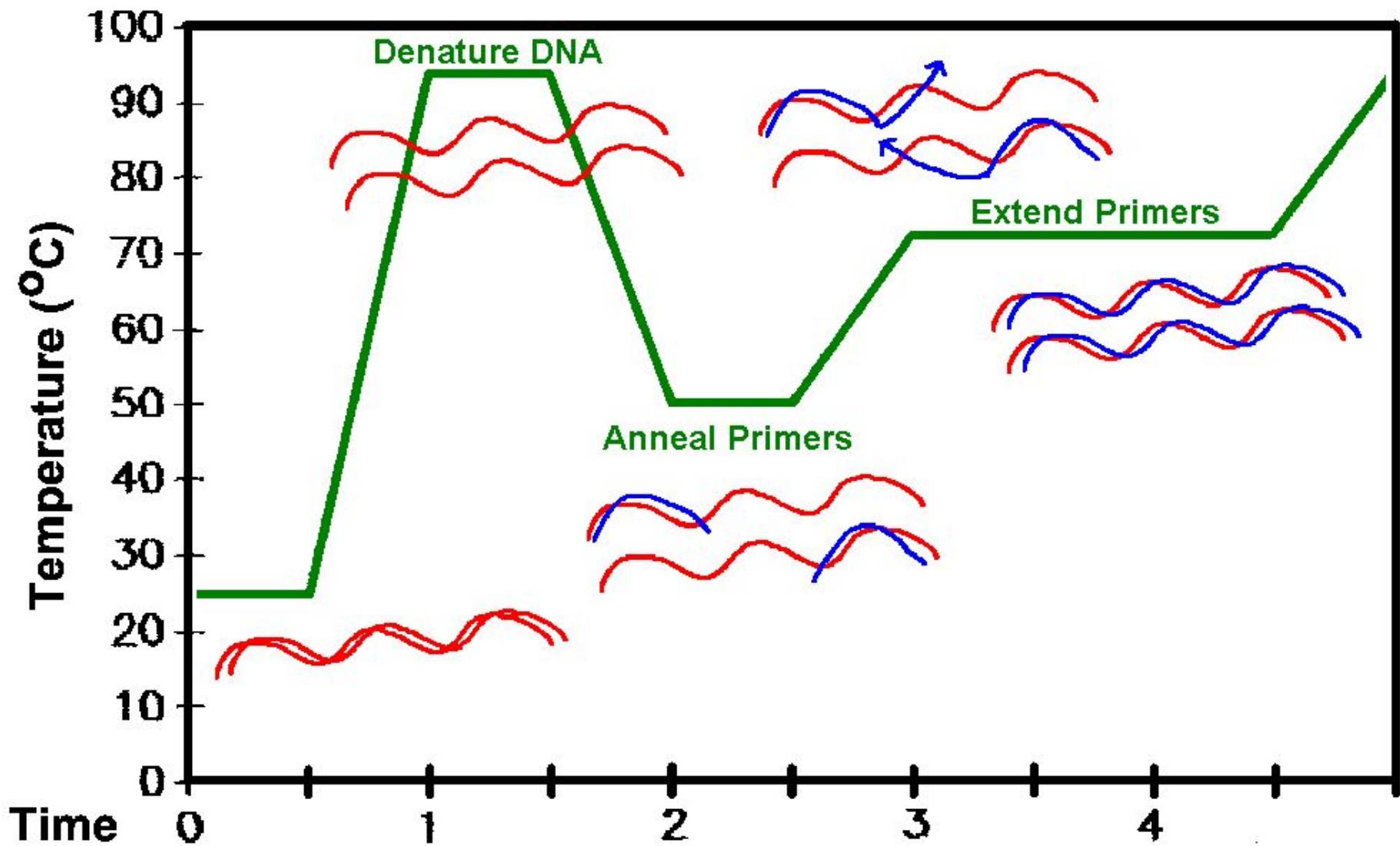
Буфер для фермента (Taq-полимеразы)

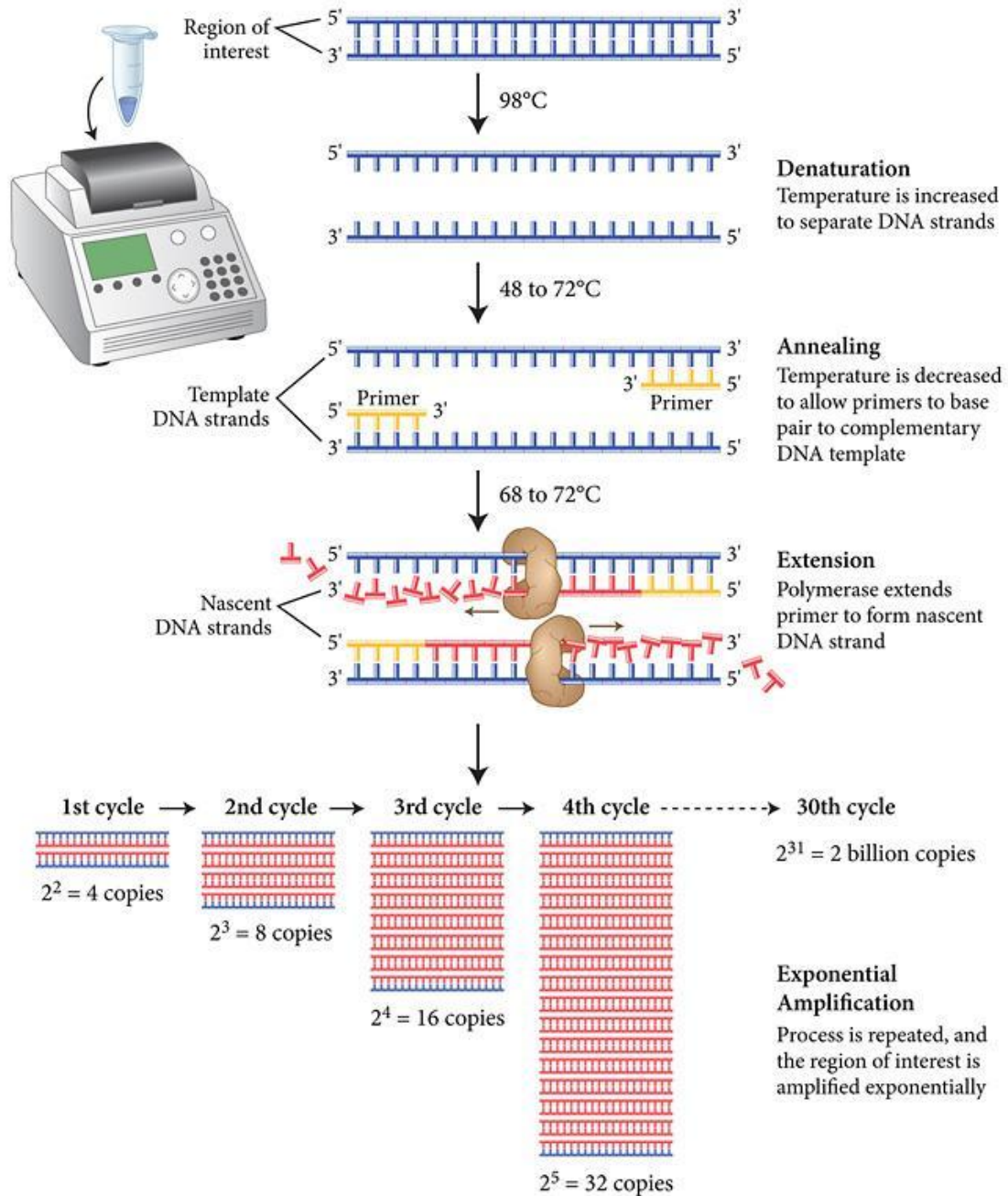
Дезоксинуклеотид фосфаты

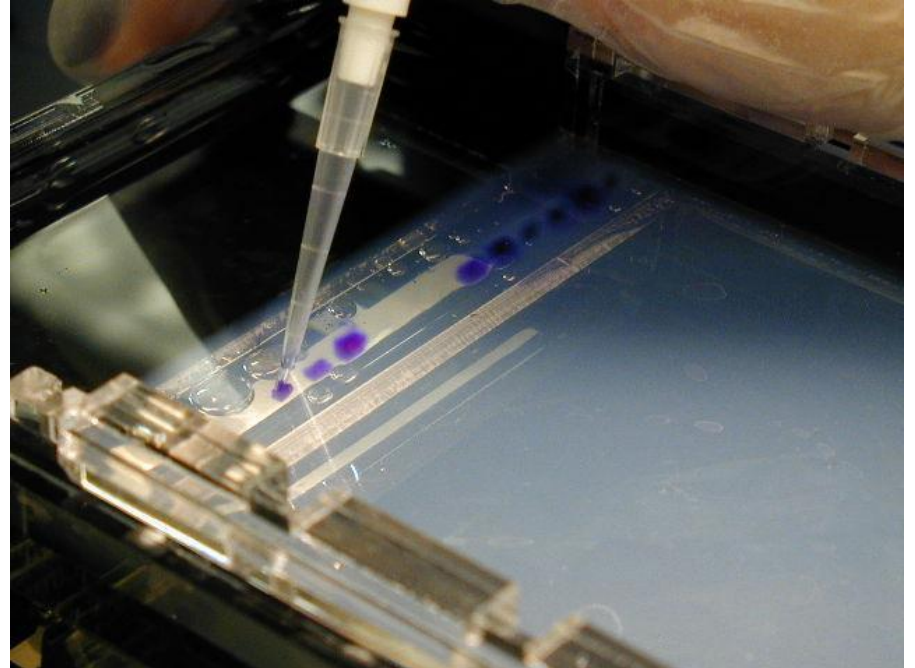
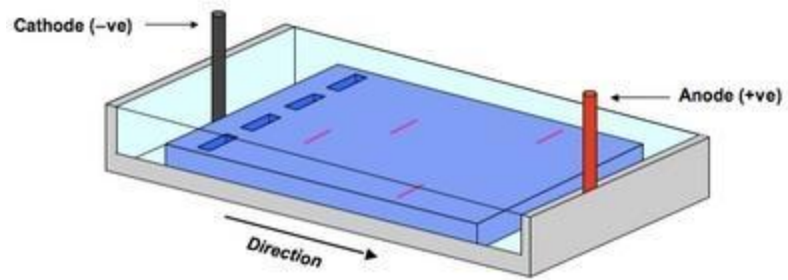
Праймеры (прямой и обратный)

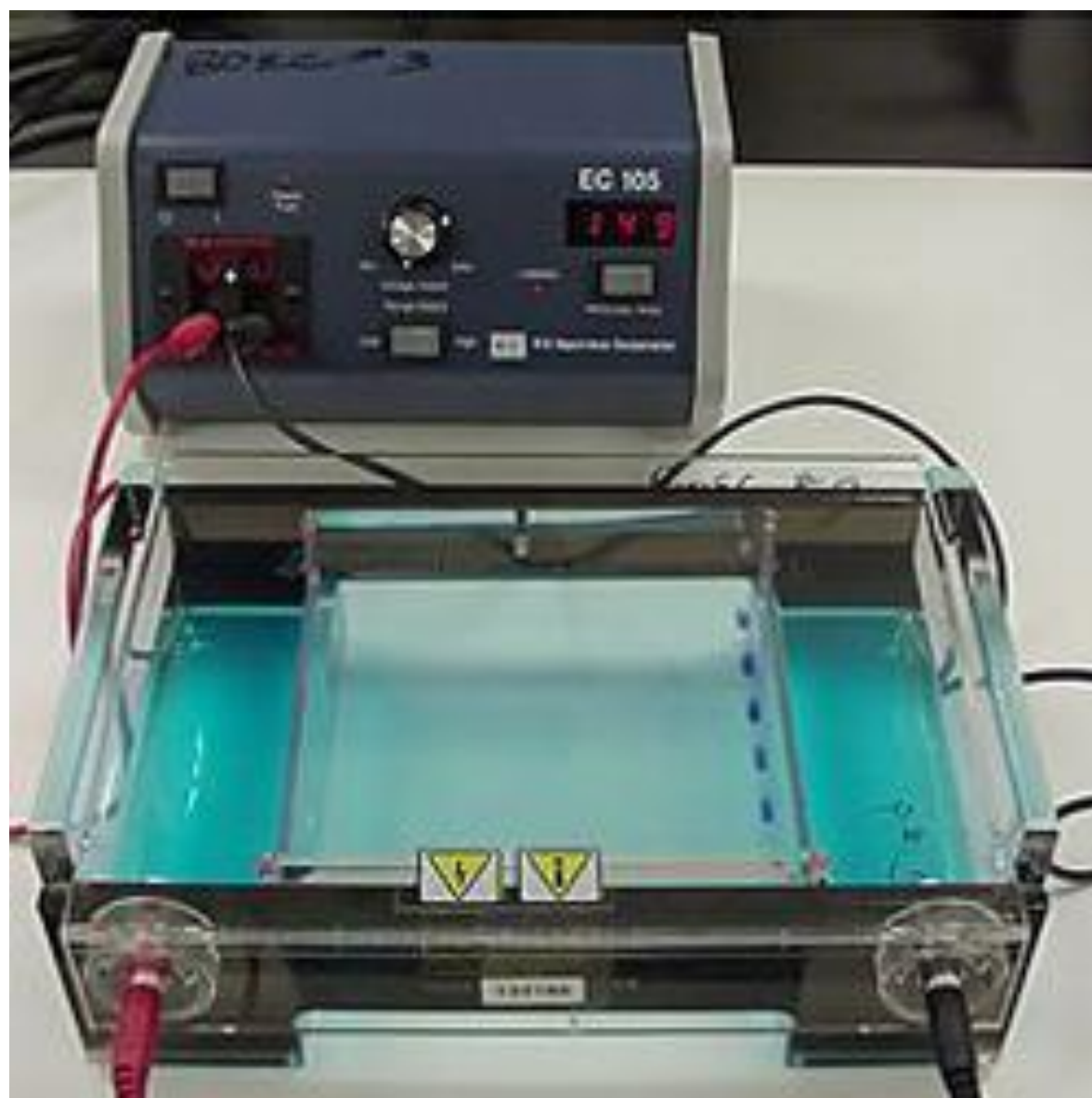
Фермент (Taq-полимераза)

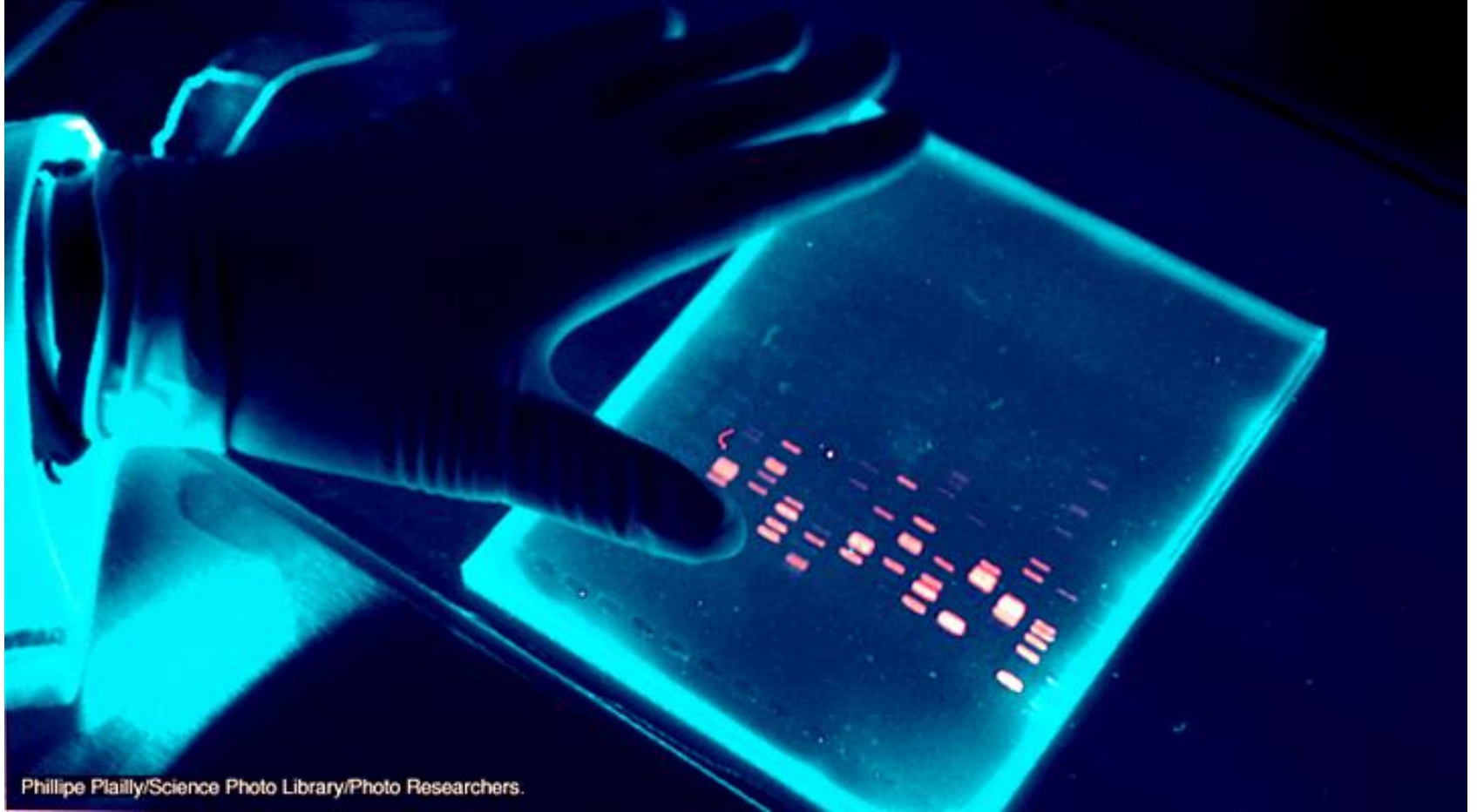
ДНК-матрица











Phillipe Plailly/Science Photo Library/Photo Researchers.

Конструирование праймеров

- All Databases ▾
- All Databases
- Assembly
- BioProject
- BioSample
- BioSystems
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- Conserved Domains
- dbGaP
- dbVar
- Epigenomics
- EST
- Gene
- Genome**
- GEO DataSets
- GEO Profiles
- GSS
- GTR
- HomoloGene

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- DNA & RNA
- Domains & Structures
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- 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

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Nucleotide

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

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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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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](#)[\[cpnDB: A Chaperonin Database\]](#)

FEATURES

Location/Qualifiers

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ORIGIN

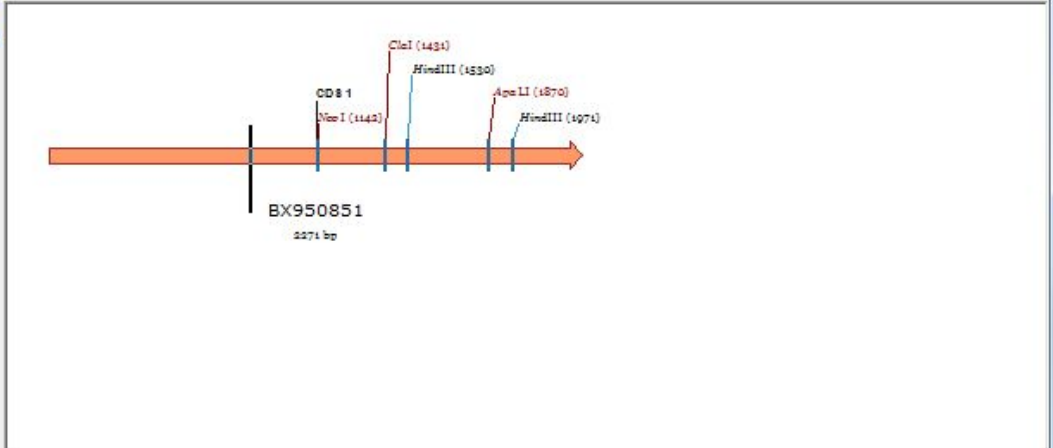
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481 cgagagatca aactcagcac tatgcgactc gatatagtta tgcatgtcct tcgtggctcg
541 tacgcccgtt tgcatagcta gctgcttacc gatgtagttt tgtaaggcag cgatgccgct
601 accatcggtg ttagggaacg acacgacatc gccgaacaga acatcatcgt tatacgtgcc
661 ggatatagtg tcattactcg ccgcaaccgac 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 tcagtgcggc tgaaacatcg agcgtatagg tctgttttcc
1021 cgcctgaccg ggtacttttt ccaccgtcgc gctattgggt ccgggggat acgtcgaacc
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1441 cgccaccttt ccggtgccga ccacggtagg cgccgaatcc ccttcggtca gccagacgcg
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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 atcttgctt ccgctaactg gcttagatcg acattcgtct gccacttggc
2101 gtttgcact gacacgtcat gcagcgtaac cgacttacca ttcacatcag taaagaccg
2161 atcaaccgta ttctcgttgc tgggtgccact gactagggta ttttctgact cactctggct
2221 gacatatcca tcgttacggg caaaattcgt aatatcaatt tctggagcga tggatccac
2281 agcgtatgca tgatgggtgt tcgccgtggg agcgttcccc gctgcatcgc gcgtggtcac
2341 gctggcgcta atatcactat tactcaccag cacagaaccc ggaacattca cgctccagg
2401 ttttccgta gtattgacgg tggctctggta agtctcgggt ccaacgggta ccgtcaccgc
2461 atcaccggtc ttgacctcgt tgccgacctt tccagtaaca gggatcgtct gtttggattc
2521 gttcgcgtta atgacgttat cgctggtcac attatcgata gagatcgacg ccaactggcgc
2581 cacagtatcc acaccgtaag catggtgcga gtttagcggc gtgacgttgc ctgcggatc
2641 acgggtgggt accgtcgac tgacatcact gttcgcagcc agcaccgatc ccggcacatt

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

BX950851

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



1	ATGAGTTTCA	TAGCCATTGA	AAGTAATGCA	GCACGAAAAA	TCGTTTCTGA	AAGAATGCIT	CAGTCAGCAG	ATTATGATAA	AGGCAGATA	CTTGCTGATT
	TACTCAAAGT	ATCGGTAACT	TTCATTACGT	CGTGCTTTTT	AGCAAAGACT	TTCTTACGAA	GTCAGTCGTC	TAATACTATT	TCCAGTCTAT	GAACGACTAA
101	TATTGAAAGA	CAGAAAAATA	CTTGATTTCA	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	GTTTAAAATA	CCCACATAG	AAGAGGAGAA	TGTCACTACA
	TTTTAATCGA	TTAAAGGCGT	TTCGATTCTT	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	ATCGTTGATT	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

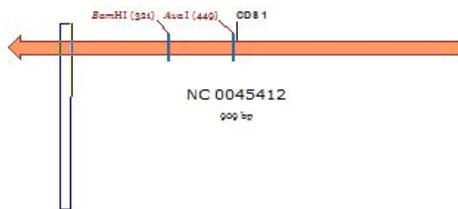
На 3'-конце праймера G или C

Температура отжига 55-60° C

Отсутствие вторичных структур (шпилек и димеров)

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

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



1 TCAGGCGGC TAAAGCAGAG TCTCGTGTG TCTGGCAATG TCCCACAG
AGTCCGCCG CATTTCGTCTC AGAGCACAAC AGACCGTTAC AGGGCTGTC
101 TCAGGCGGC TAAAGCAGAG TCTCGTGTG TCTGGCAATG TCCCACAG
AGTCCGCCG CATTTCGTCTC AGAGCACAAC AGACCGTTAC AGGGCTGTC
201 TACCCGCACC GCCTCCCGCG AGTACCATGT CGGCAATCAC CGAGTAGGT
ATGGCGTGG CGGAGGCGC TCATGTTACA GCCGTTAGTG GTCATCCAT
301 GTGTACCACC TGACCGAGCG GATCCIGATG CTGCATCATC AGCAGGTTA
CACATGGTGG ACTGGCTCGC CTAGGACTAC GACGTAGTAG TCGTCCAAT

Oligo Analysis
Oligonucleotide: From NC_0045412: Start: 102 Length: 25
CGCGGGGCTGATCAGCGTGACTTC
Parameters: dG Temperature (C) 25.0, 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: Mol. Wt 7737.0, %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: 6 of 8
CGCGGGGCTGATCAGCGTGACTTC
CTTCAGTGCGACTAGTCCGGCGCCG
Stem Length = 10
Dimer dG = -13.0 kcal/mol
Hairpin Loops: 1 of 2
CGCGGGGG
CTTCAGTGCGACTAGT
Stem Length = 3 Loop Length = 12
Loop dG = -1.9 kcal/mol

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

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				

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
  
```

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

BLAST

BLAST® » blastn suite » RID-FZ86Z7MR01R

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BLAST Results

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

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

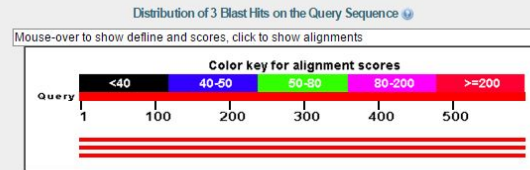
Nucleotide Sequence (599 letters)

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



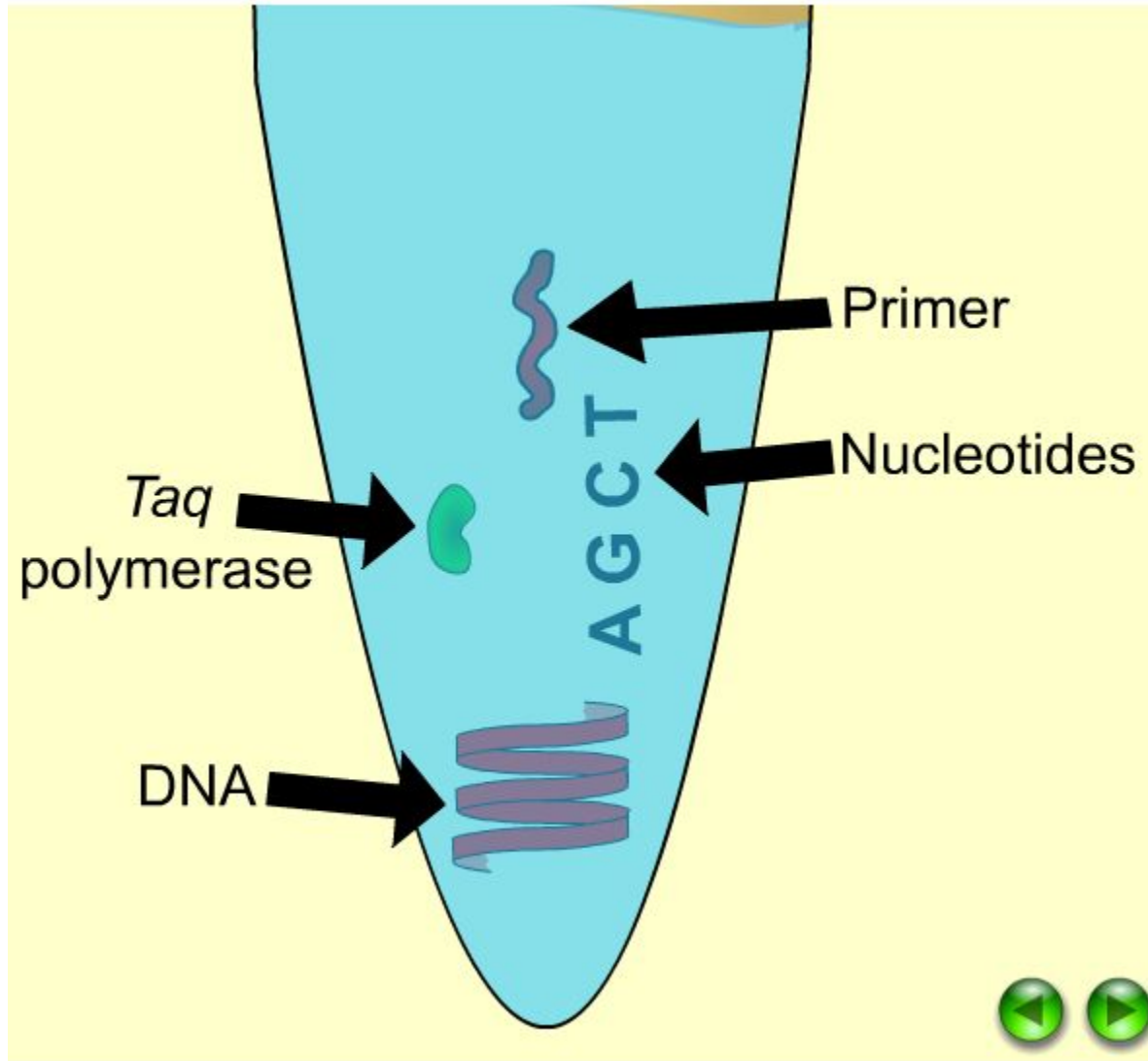
Descriptions

Sequences producing significant alignments:

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

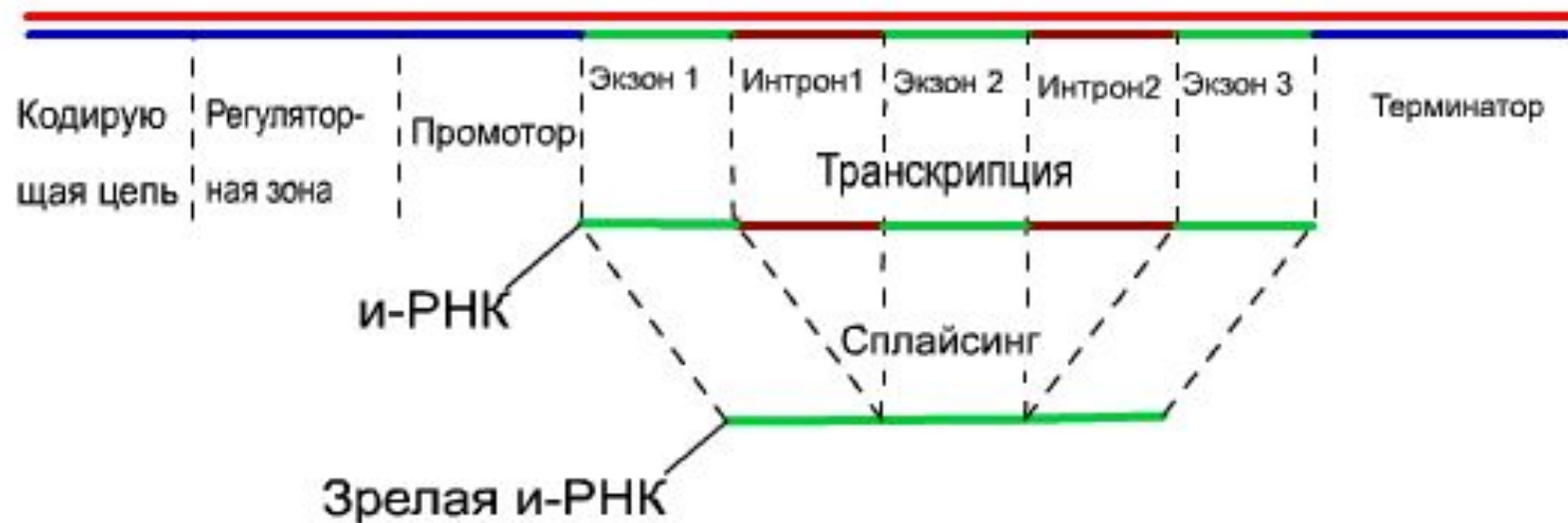
[Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)

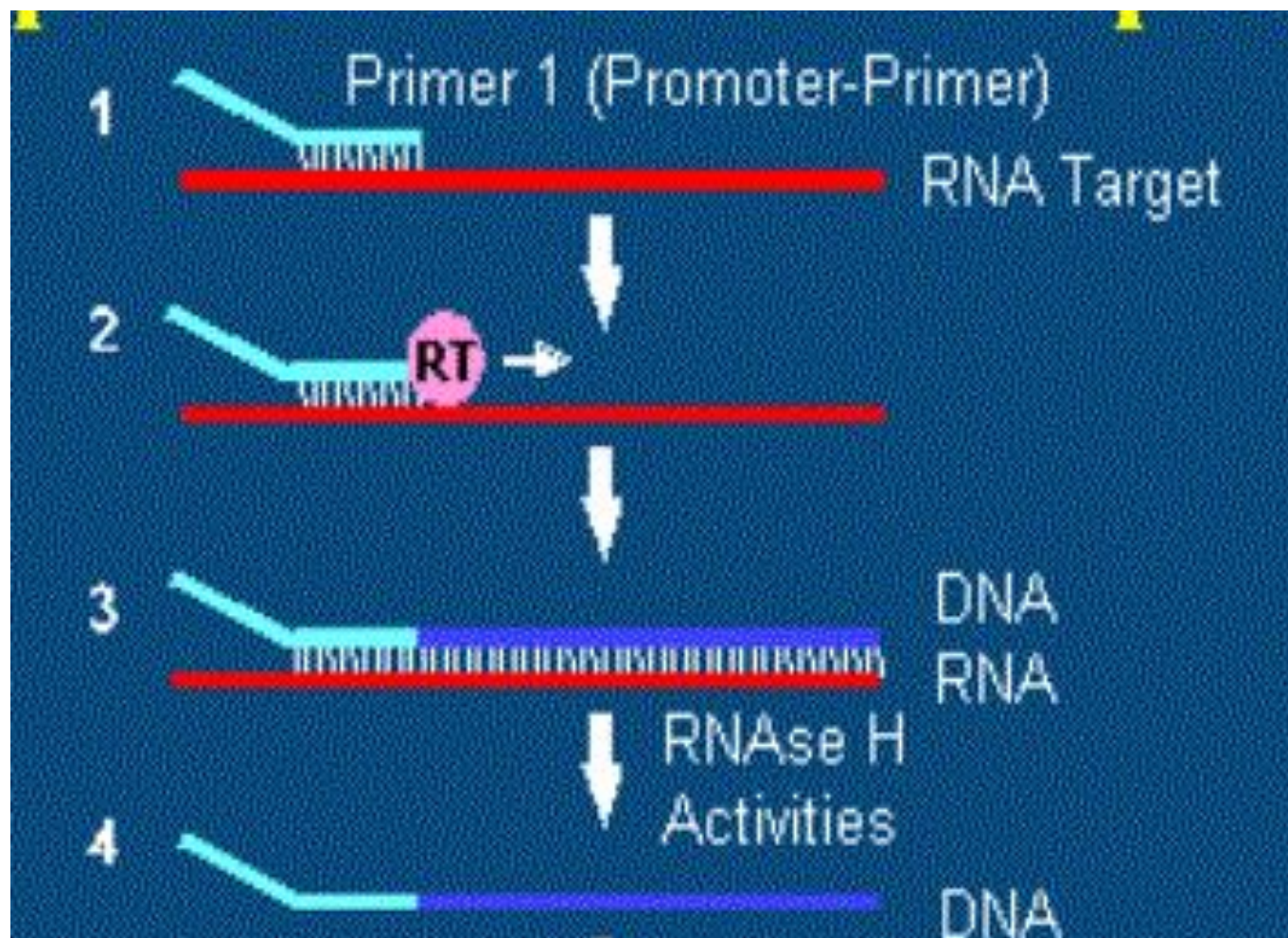
	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Pectobacterium atrosepticum strain 21A, complete genome	1107	1107	100%	0.0	100%	CP009125.1
<input type="checkbox"/>	Pectobacterium atrosepticum strain JG10-08, complete genome	1107	1107	100%	0.0	100%	CP007744.1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

mRNA



B

mRNA



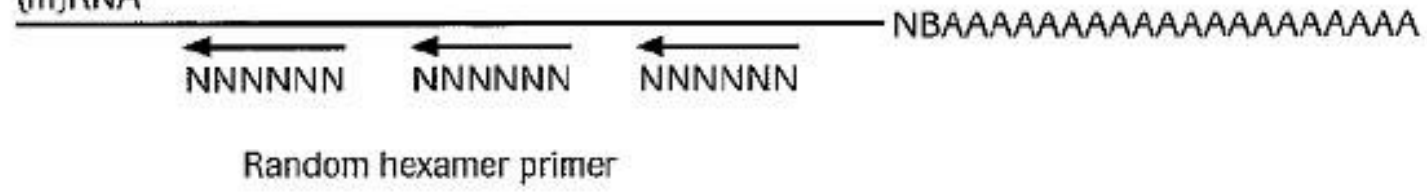
C

(m)RNA



D

(m)RNA



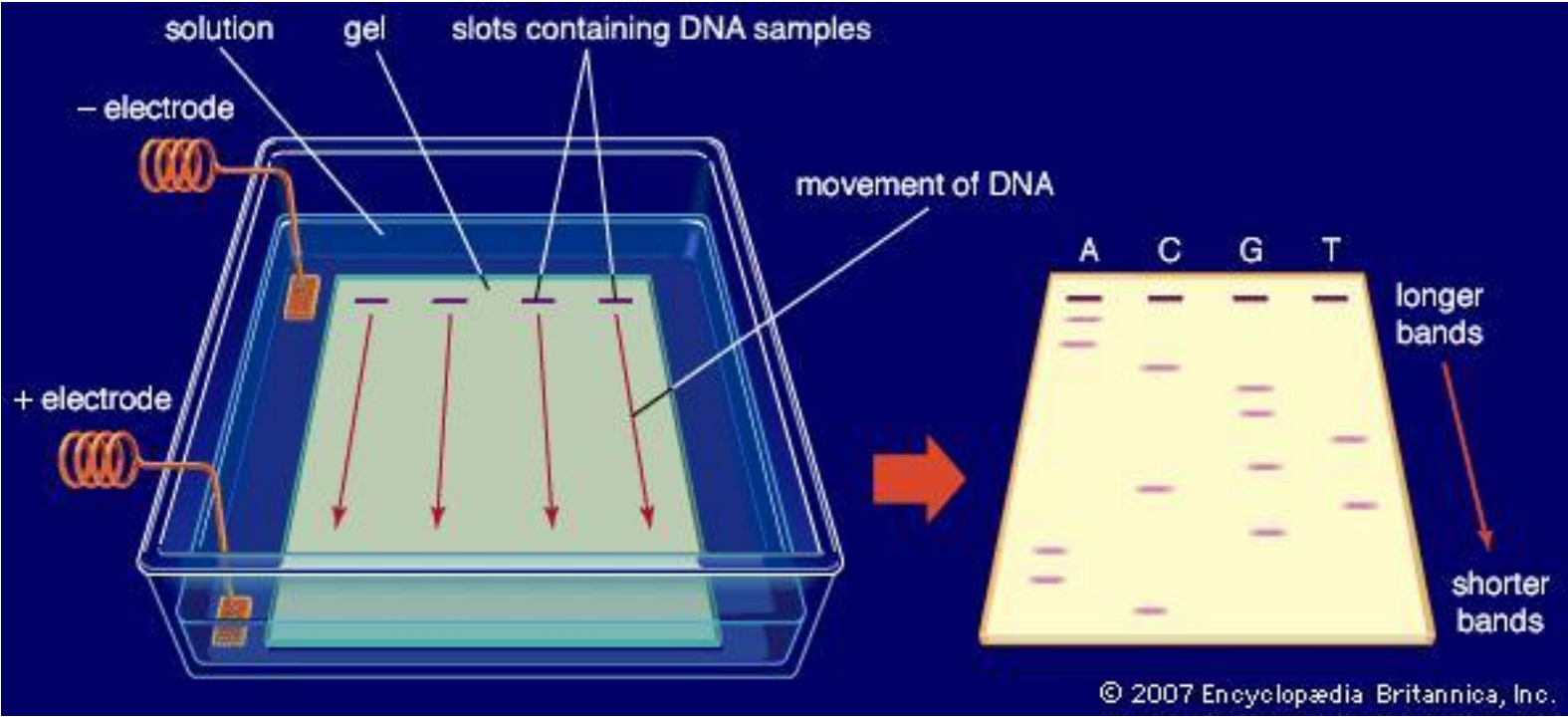
Мы провели ПЦР, чтобы получить целевой фрагмент ДНК

Нужно выяснить получился ли у нас ожидаемый продукт

Электрофорез

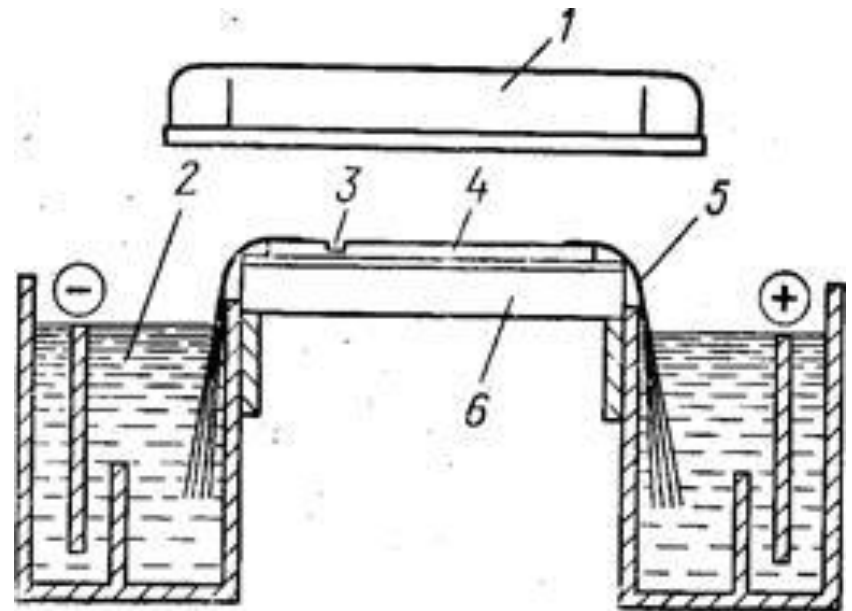
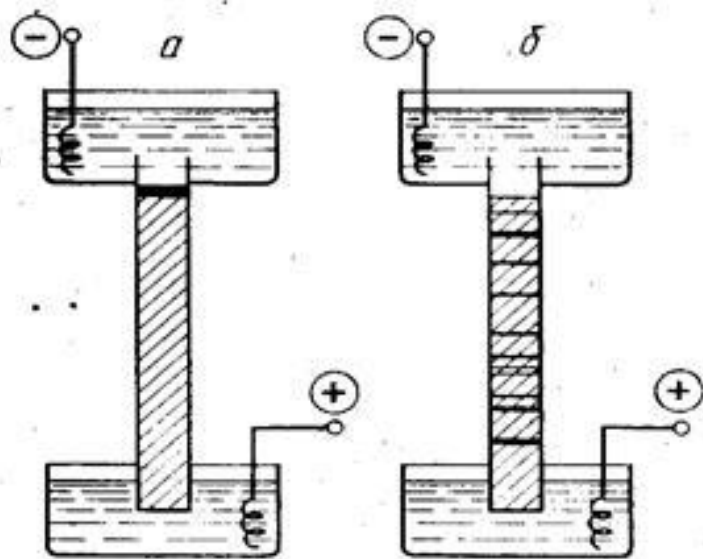
Электрофоретическое разделение нуклеиновых кислот

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

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

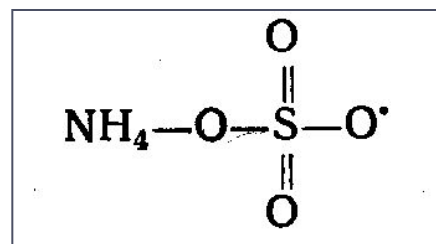
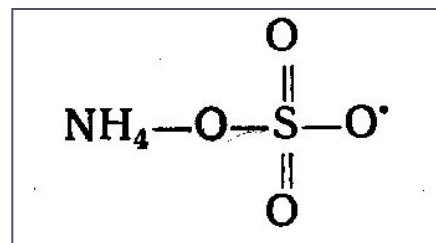
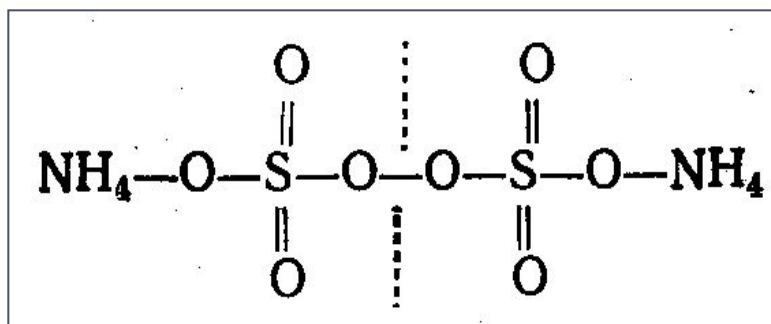
NN'-Метиленабисакриламид

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

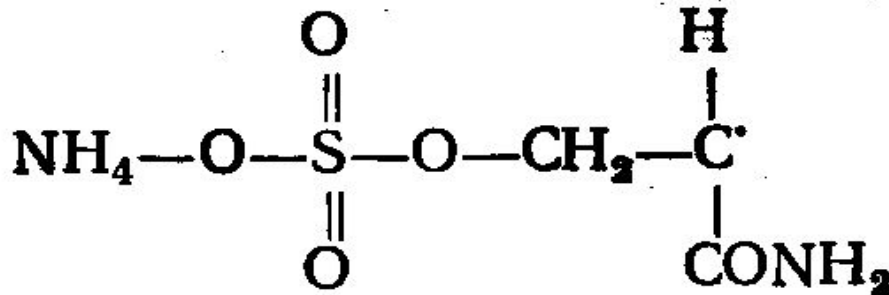
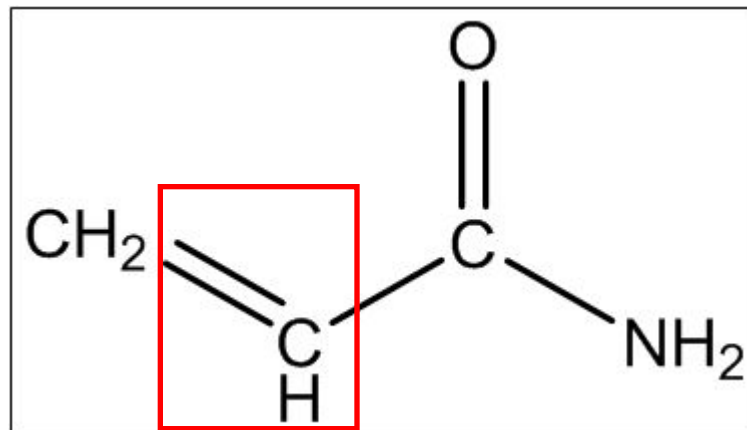
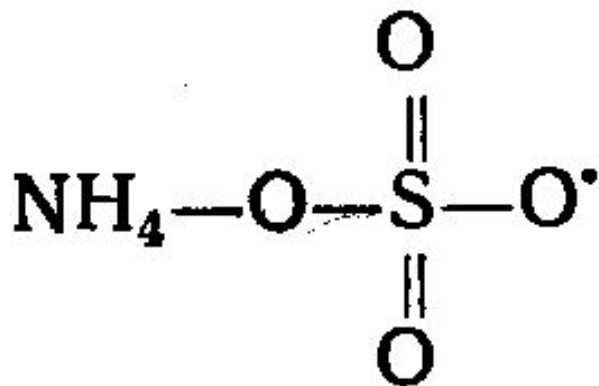
Буферные растворы

Индукция полимеризации акриламида

Персульфат аммония



Полимеризация акриламида

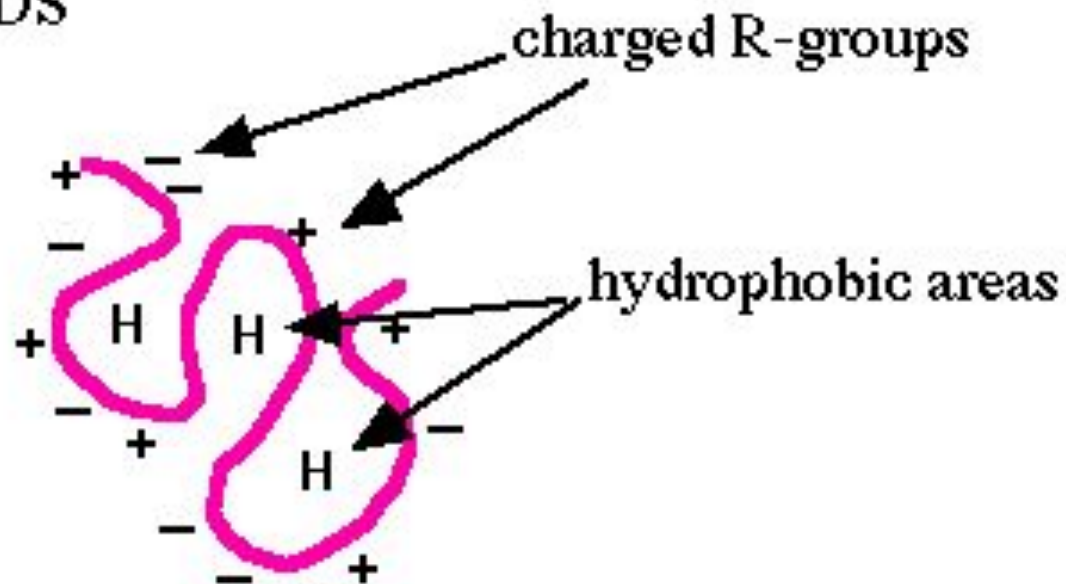


Катализатор процесса полимеризации

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

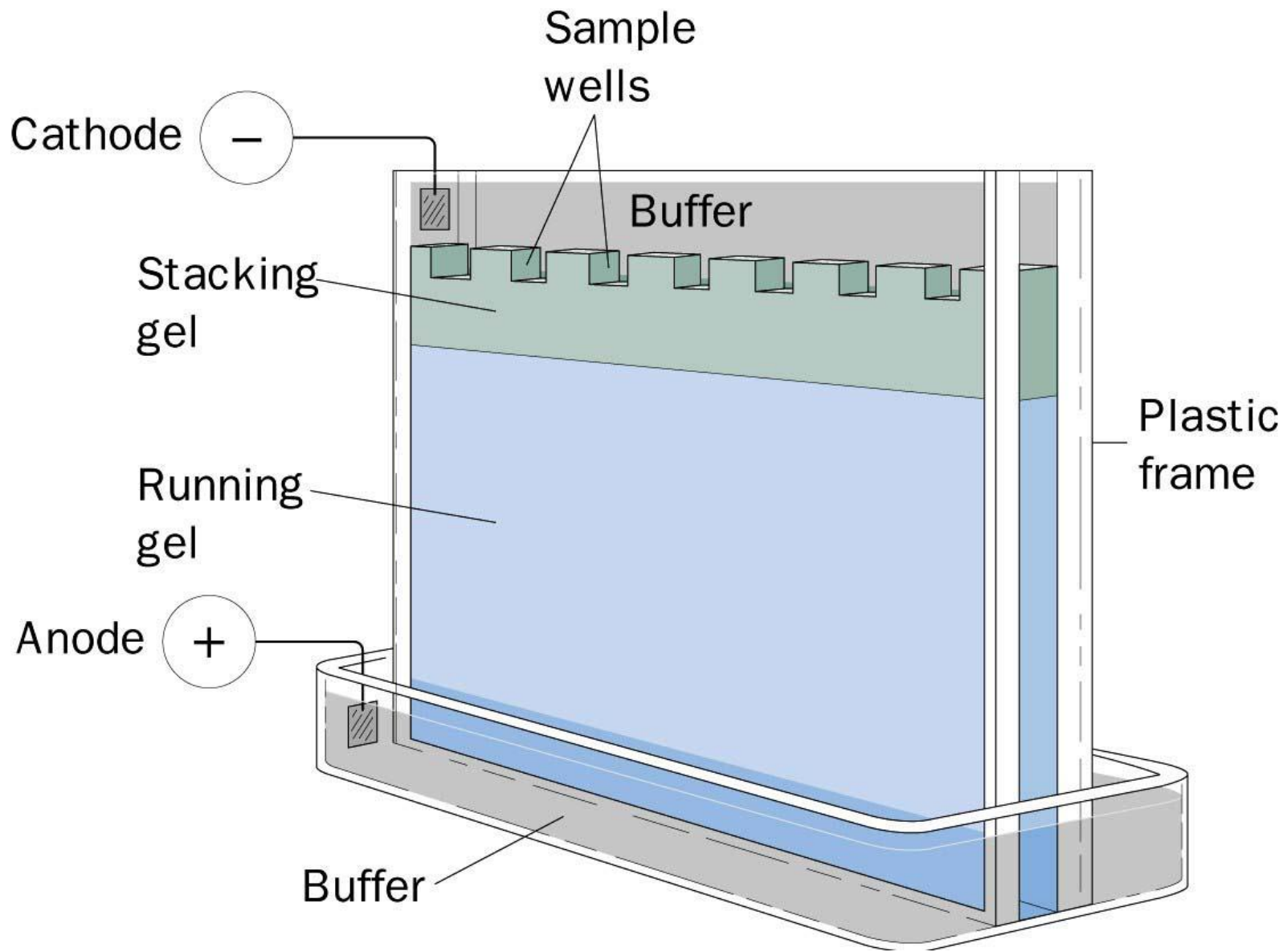
Денатурация белка с помощью ДДС

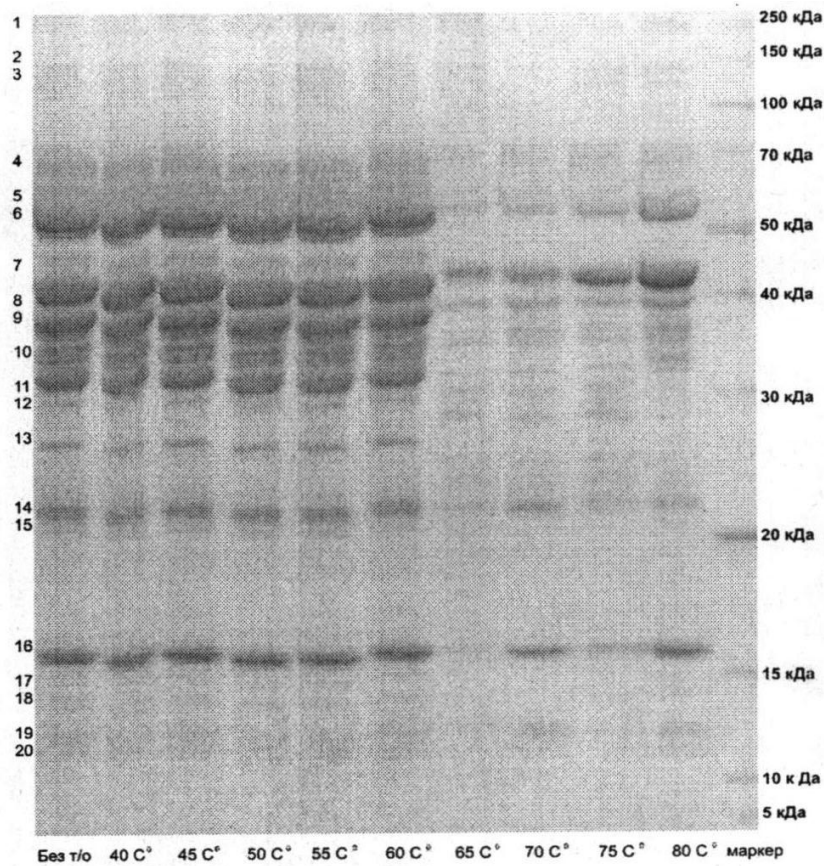
BEFORE SDS



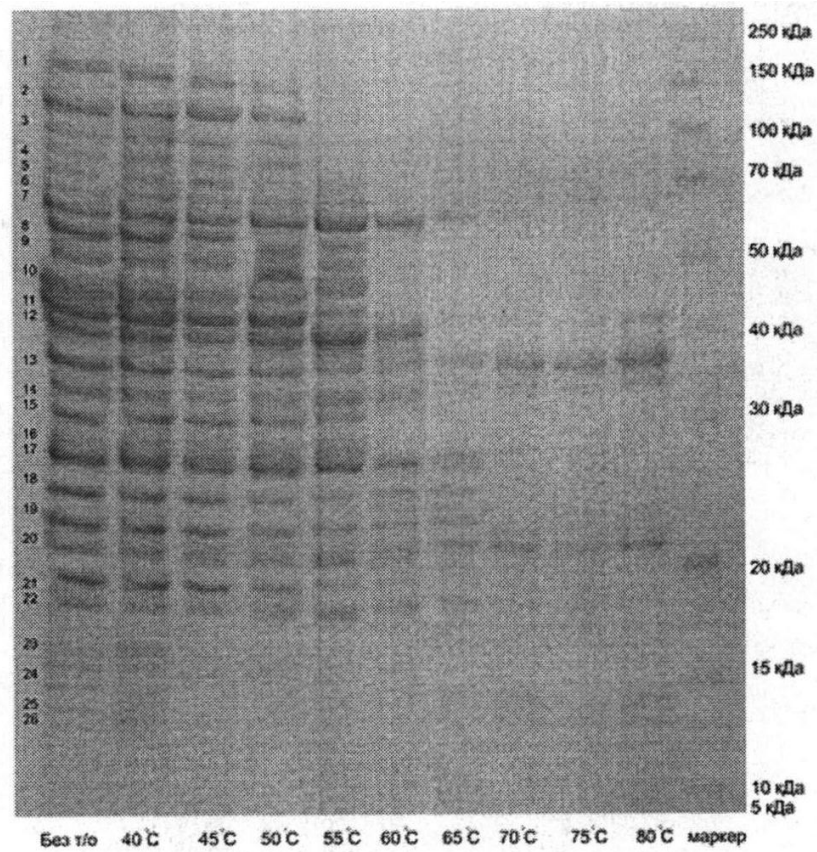
AFTER SDS







а)

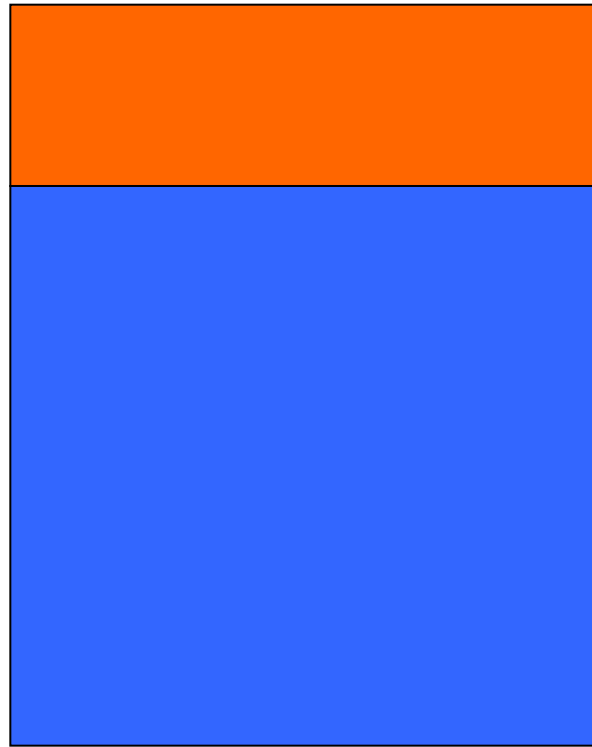


б)

Фиг. 2



Постоянная сила тока



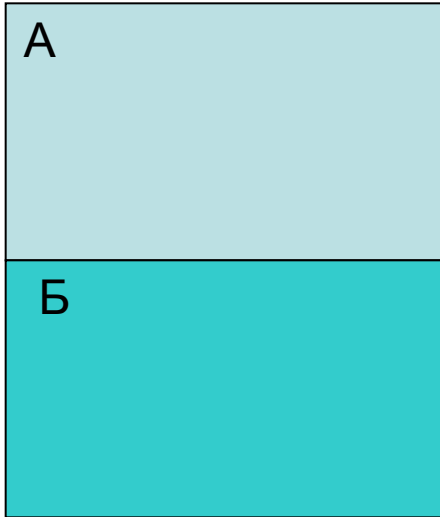
Высокая напряженность

Низкая напряженность

Подвижность ионов

Заряд ионов

Изоэлектрическая точка

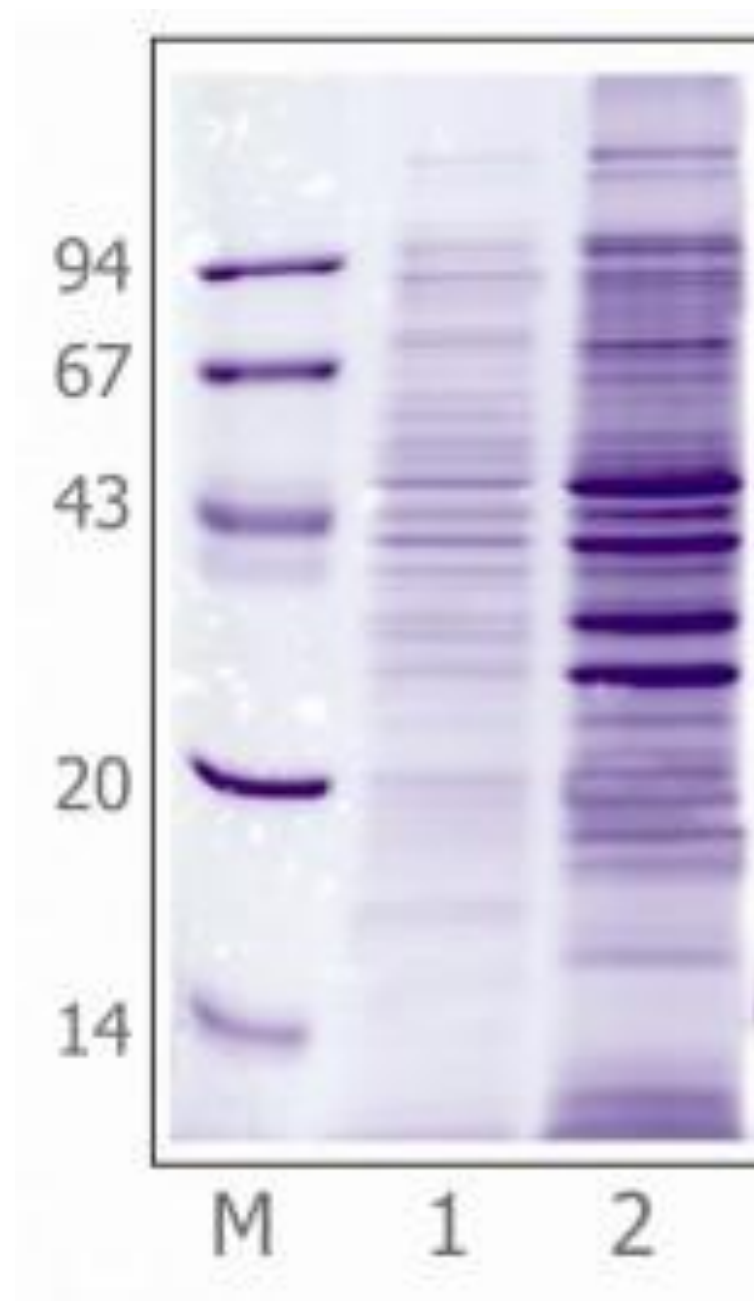
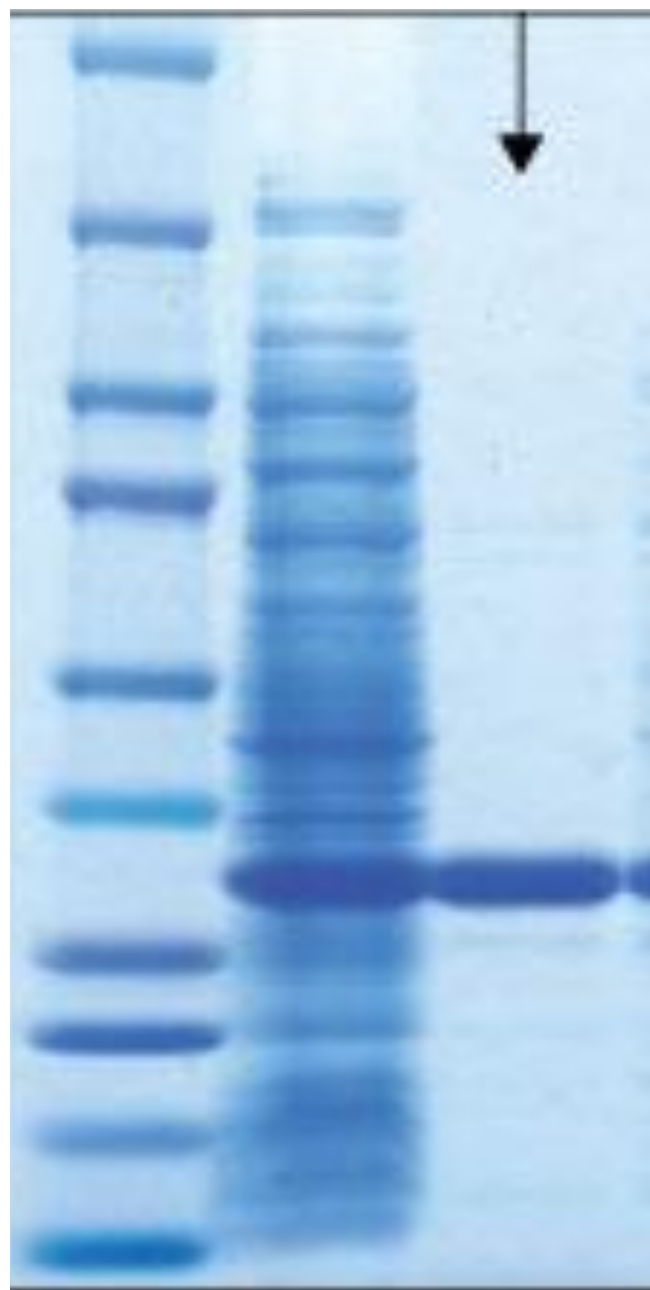


Глицин – малоподвижный ион. Высокое сопротивление. Высокая напряженность

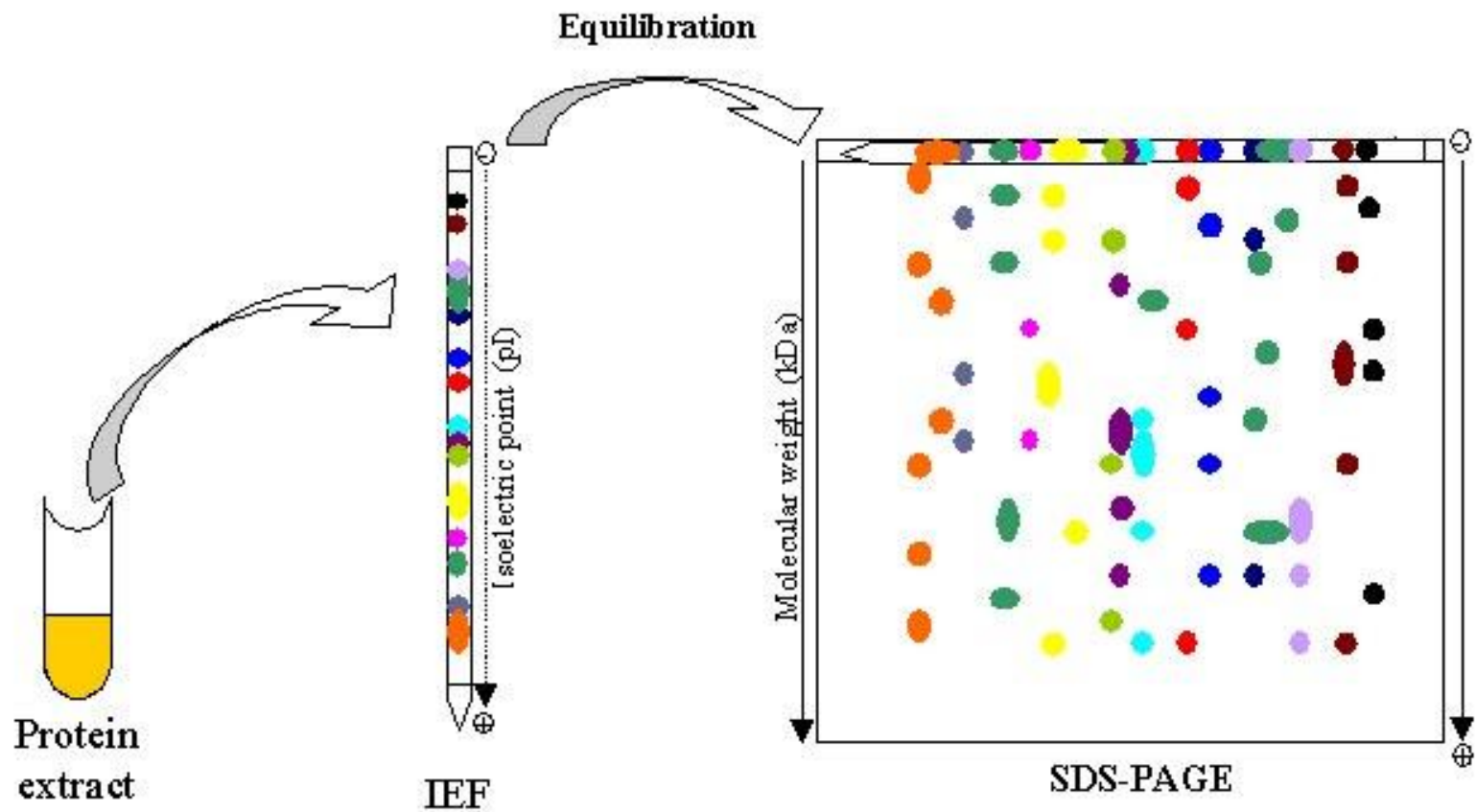
Хлор – подвижный ион. Низкое сопротивление. Низкая напряженность

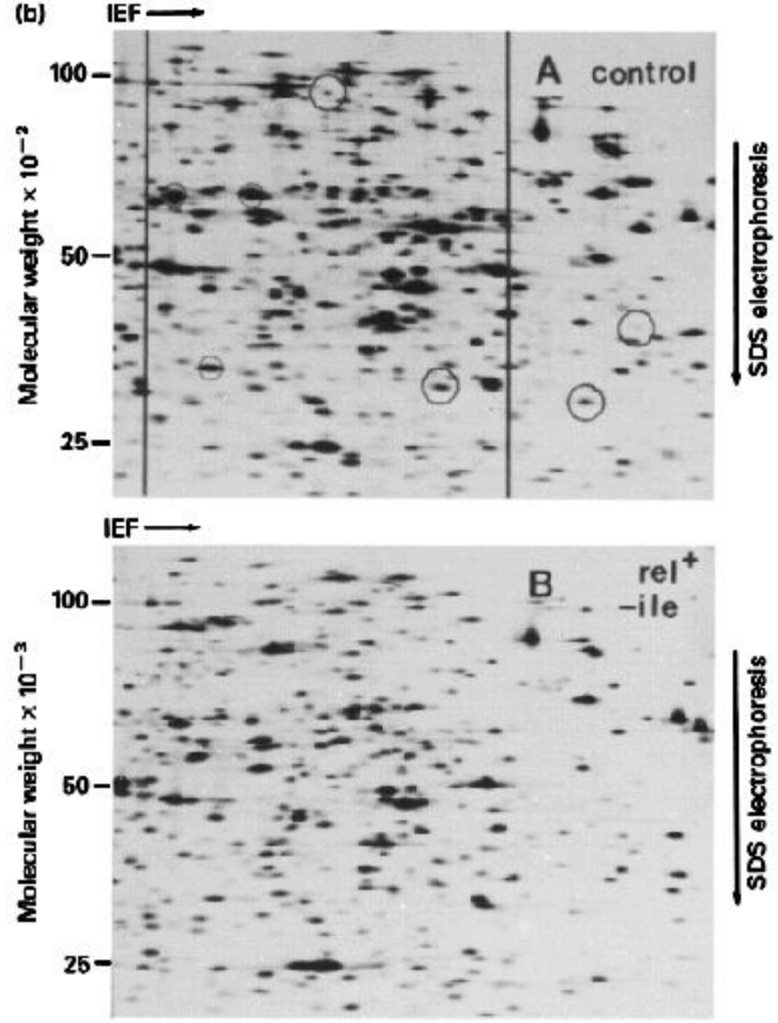
Одинаковая сила тока

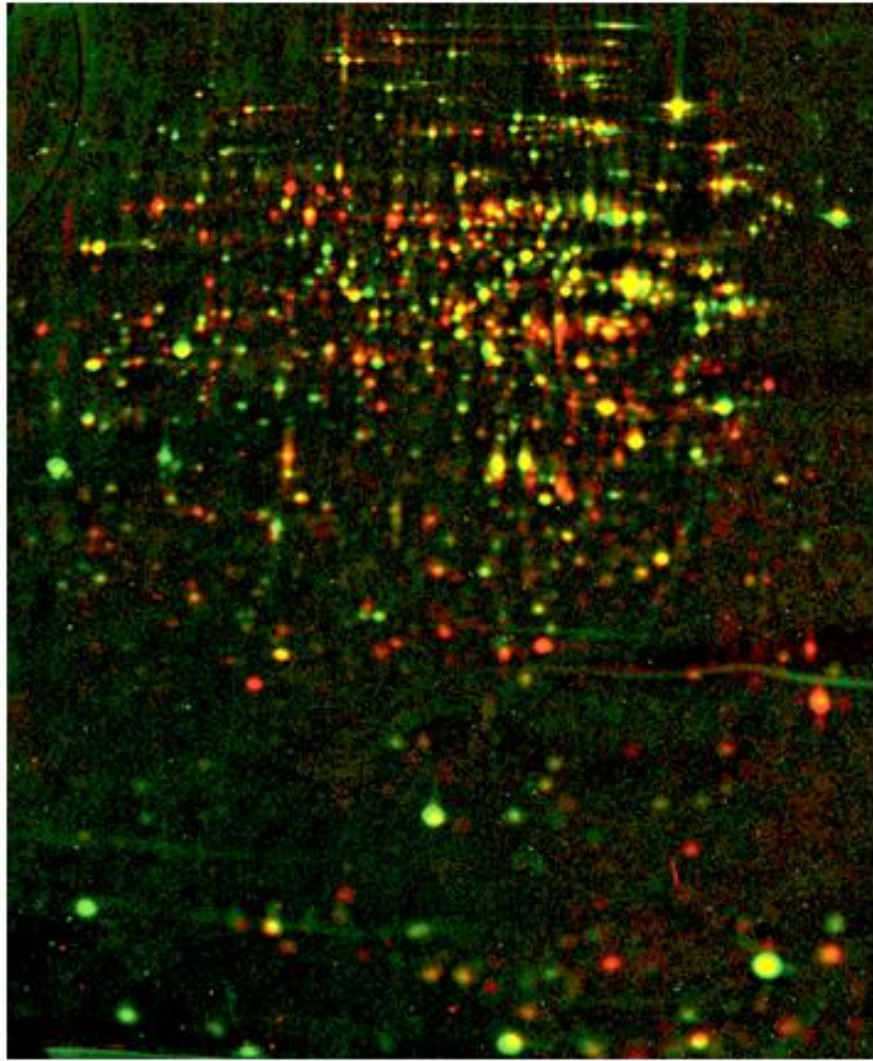
Суммарное напряжение распределится между участками А и Б так, что напряженность поля А будет выше, причем настолько, чтобы скорость миграции ионов глицина стала точно такой же как у ионов хлора. Этого требует условие неизменности величины тока вдоль всего геля (постоянство тока во всем геле).



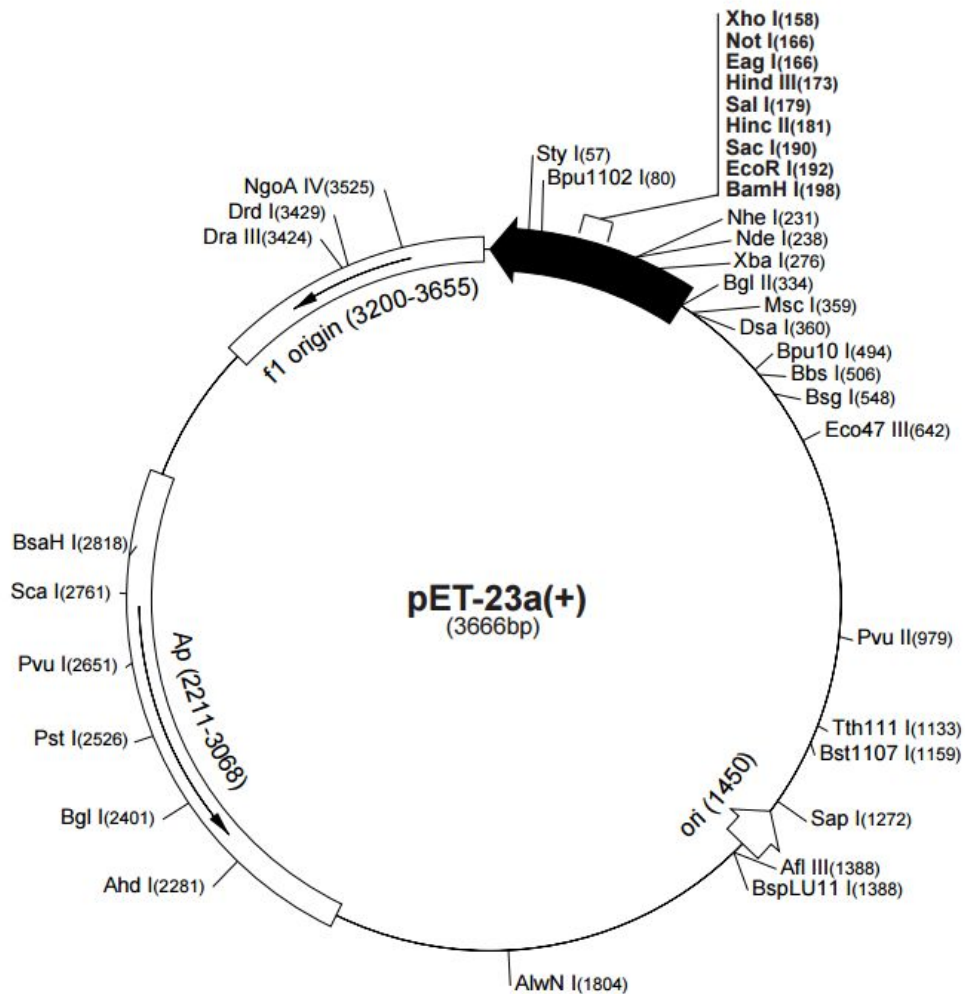
Двумерный электрофорез белков



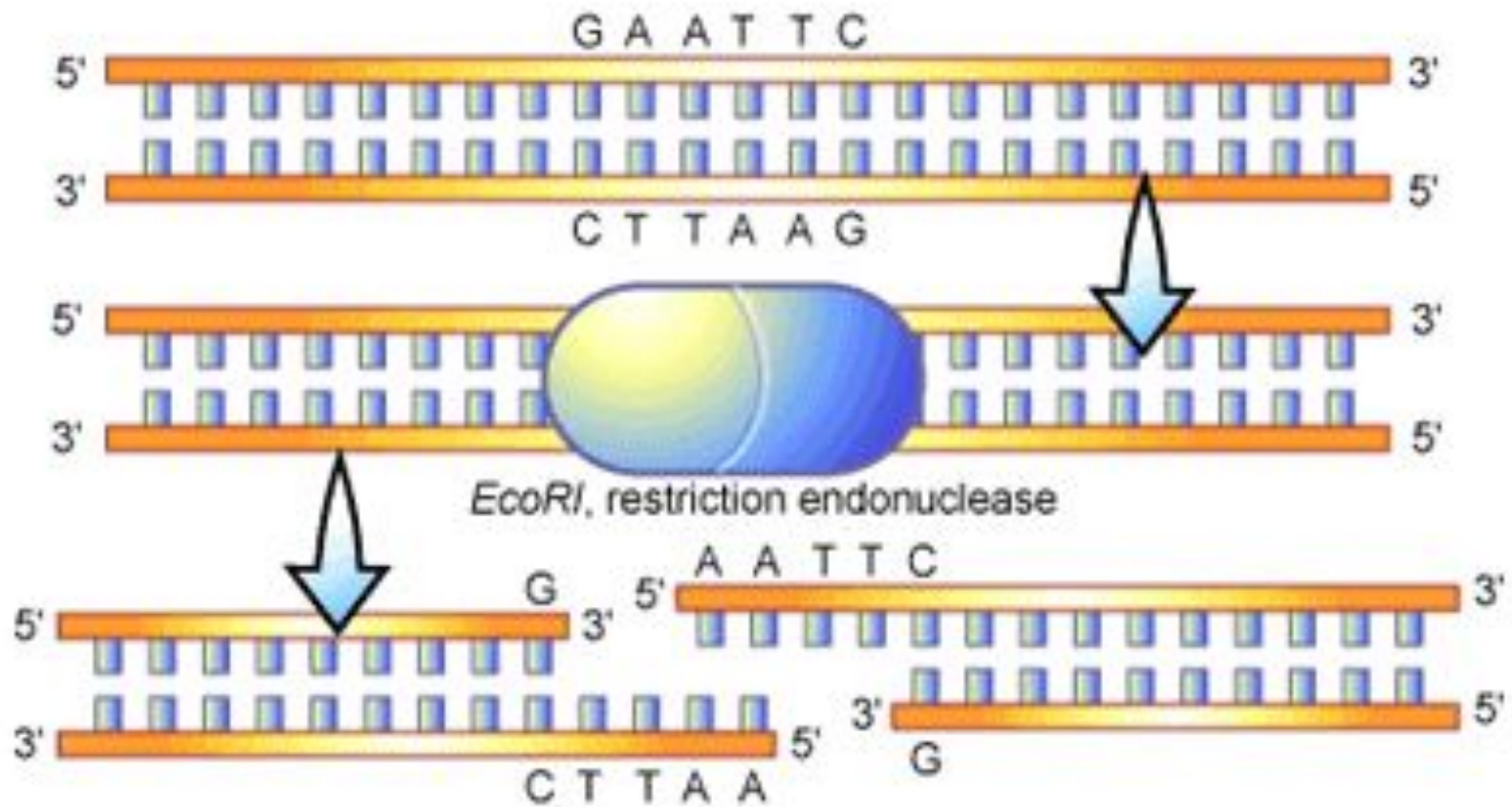




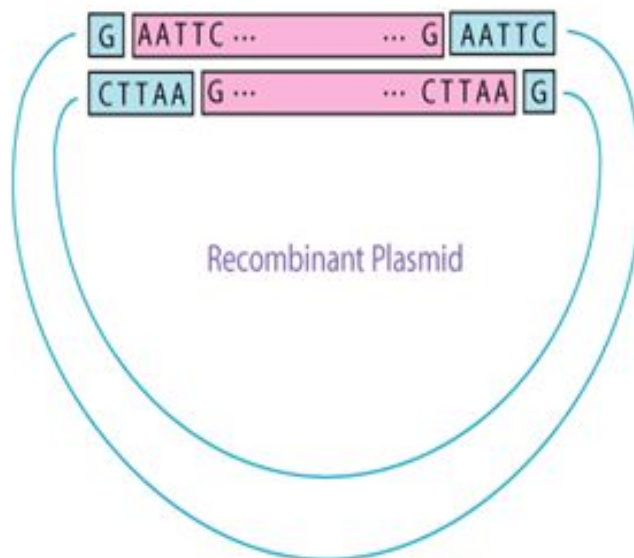
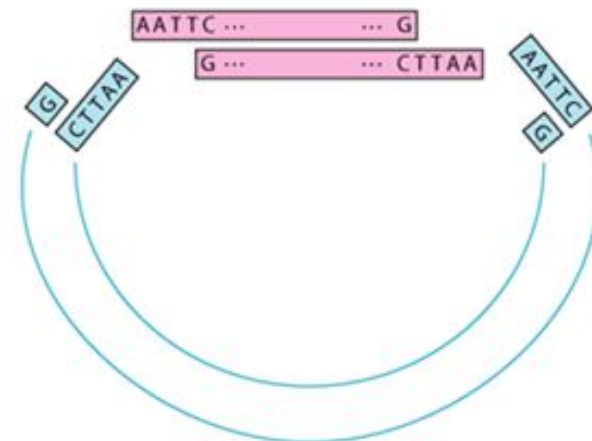
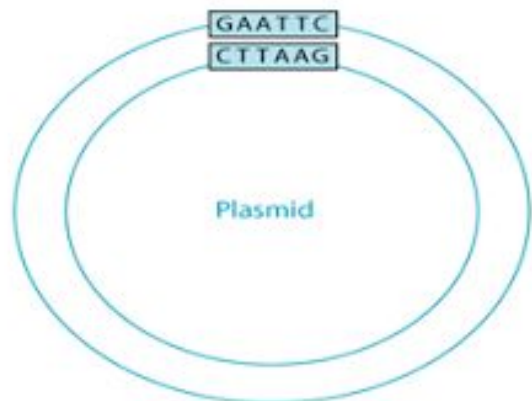
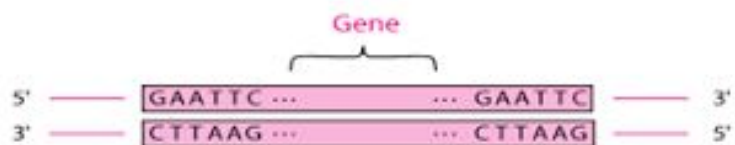
Векторные конструкции

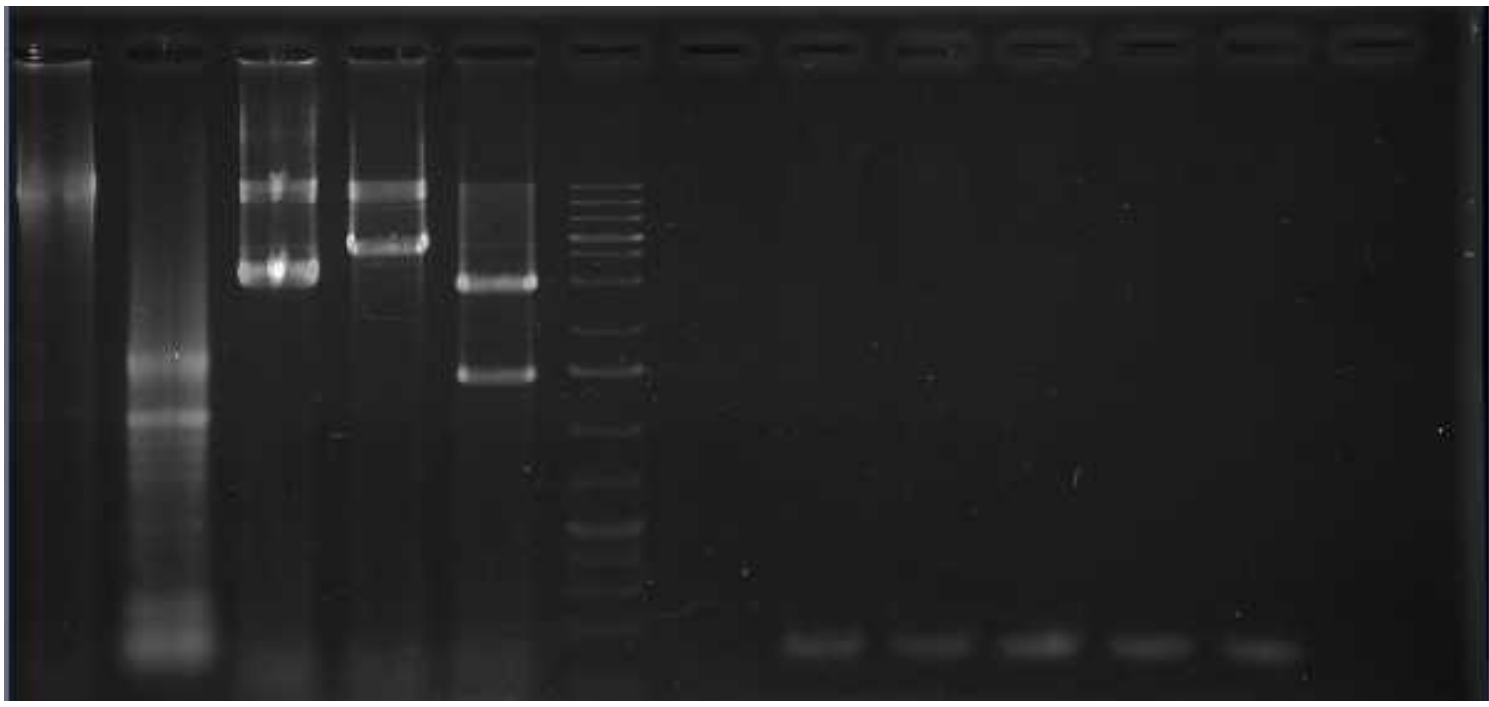


Рестриктазы



Сборка плазмиды. Рестрикция-лигирование.

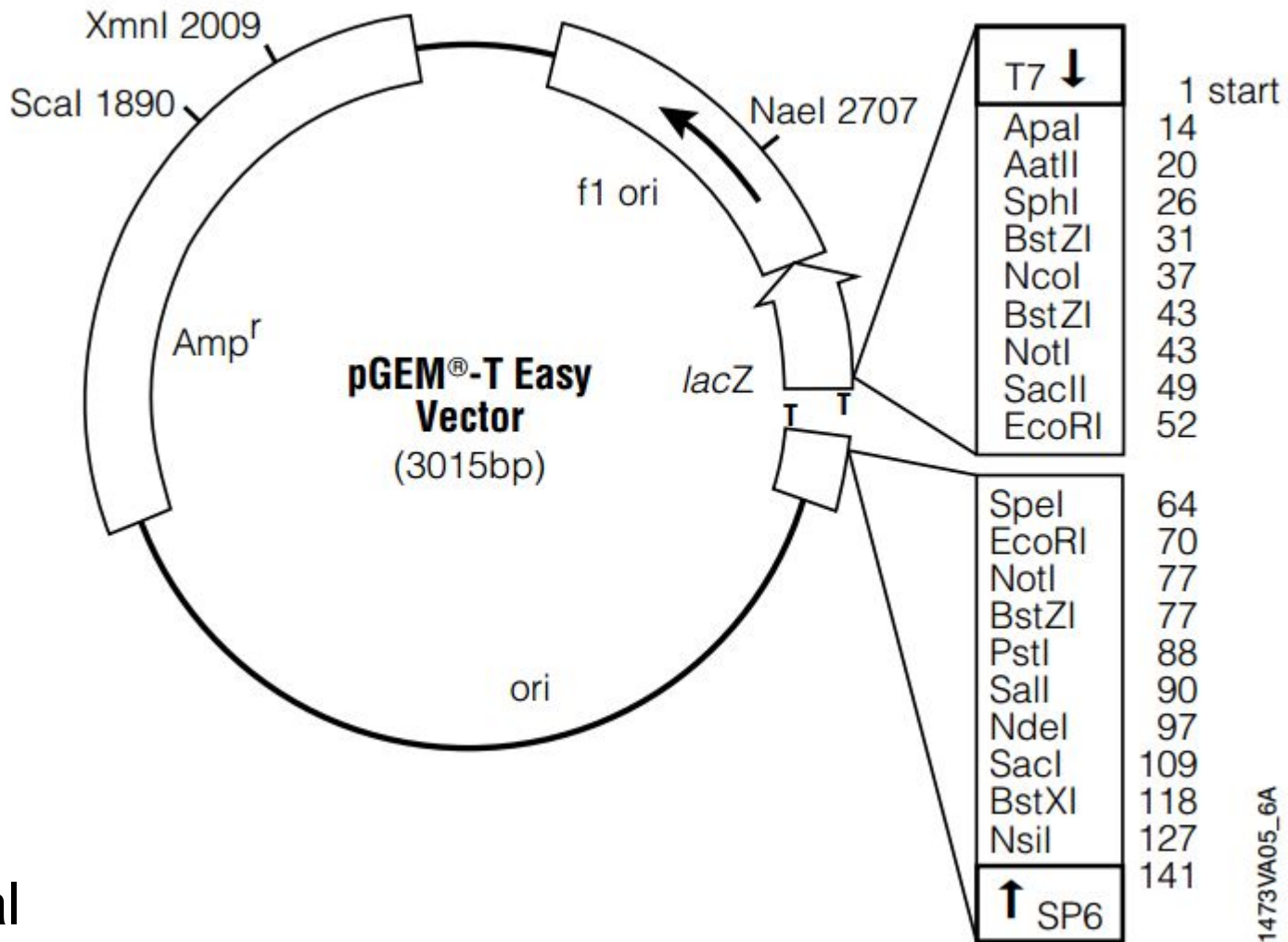




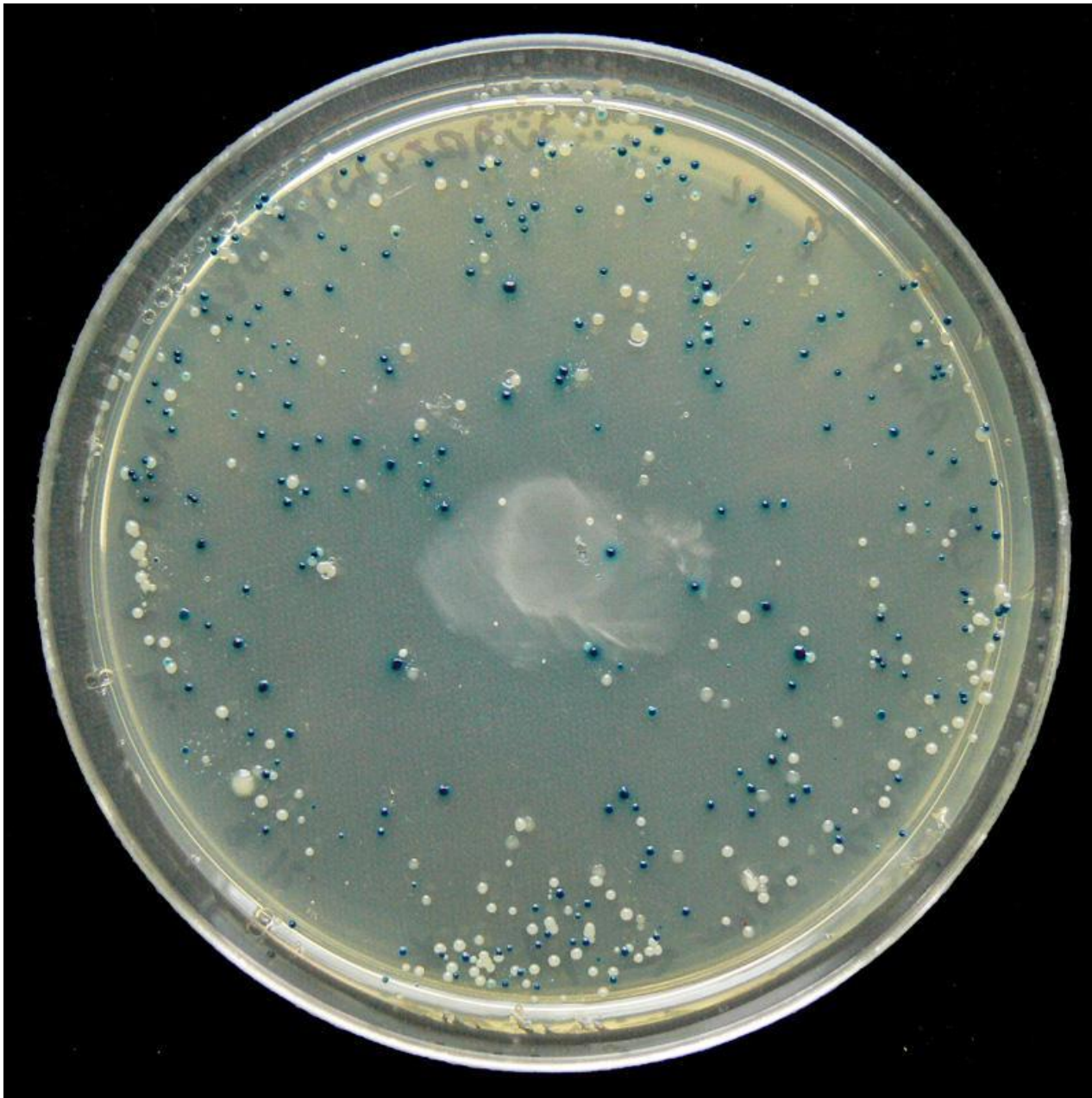
bp	ng/0.5 µg	%
20000	20	4
10000	20	4
7000	20	4
5000	75	15
4000	20	4
3000	20	4
2000	20	4
1500	80	16
1000	25	5
700	25	5
500	75	15
400	25	5
300	25	5
200	25	5
75	25	5

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

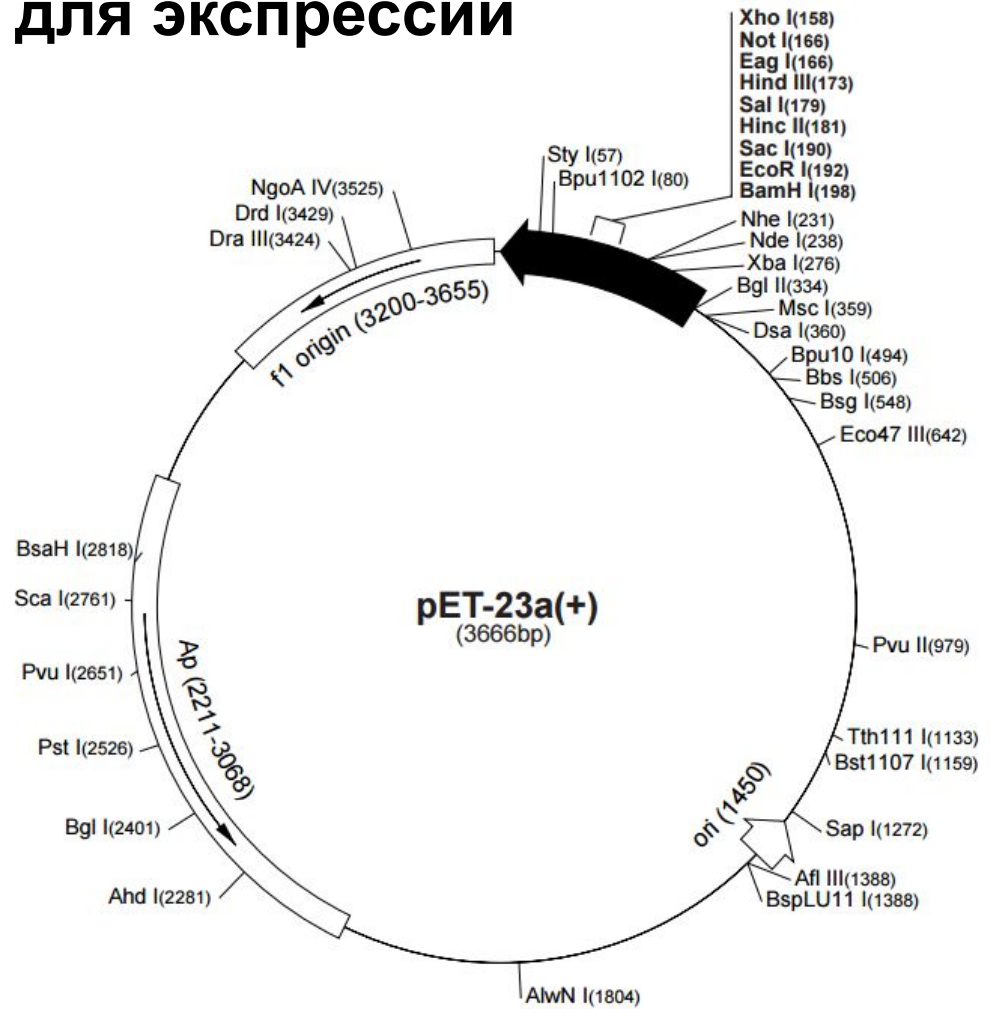
Векторы для клонирования



X-Gal



Векторы для экспрессии



T7 promoter primer #69348-3

T7 promoter

Bgl II

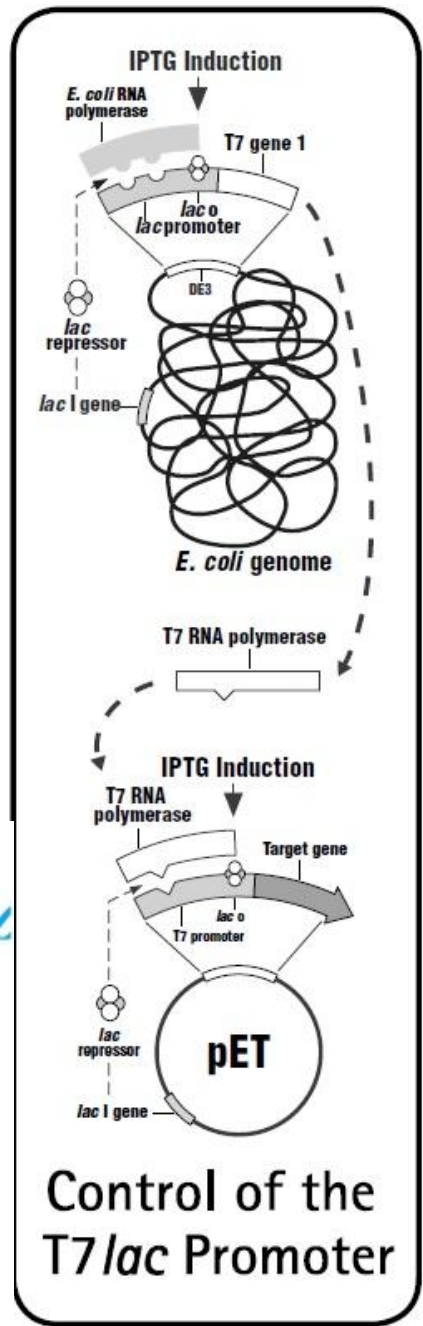
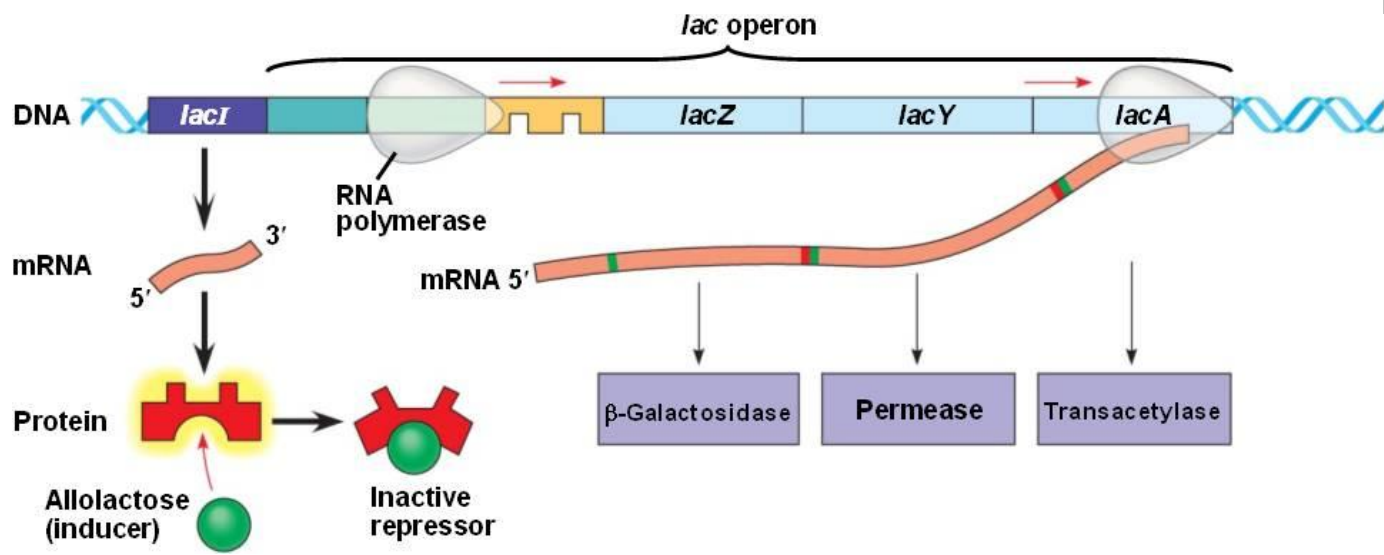
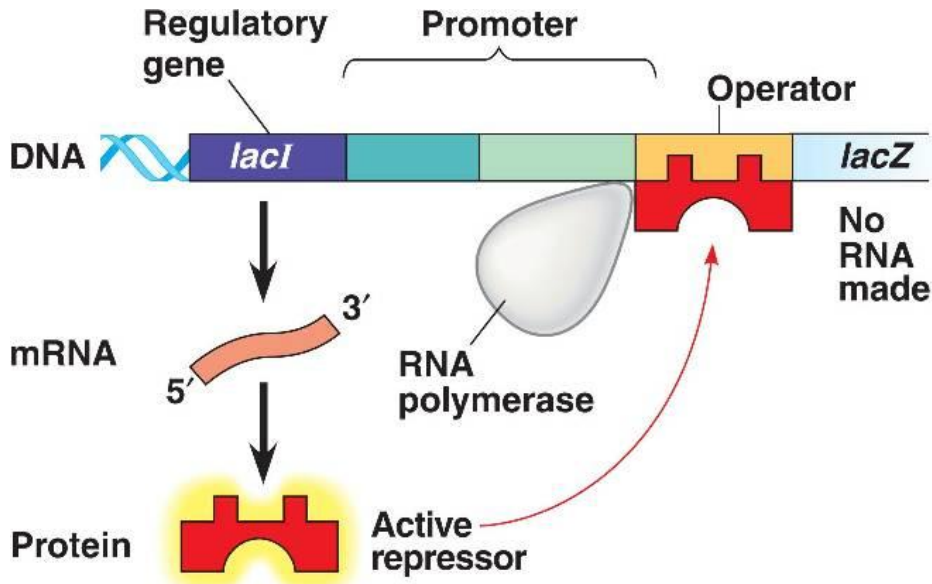
rbs

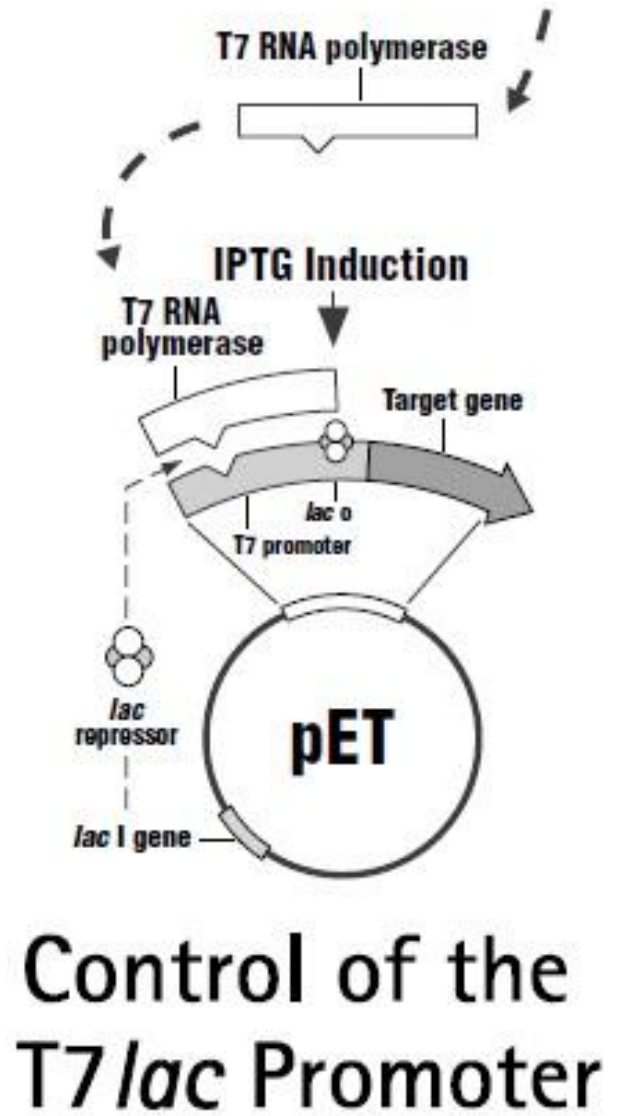
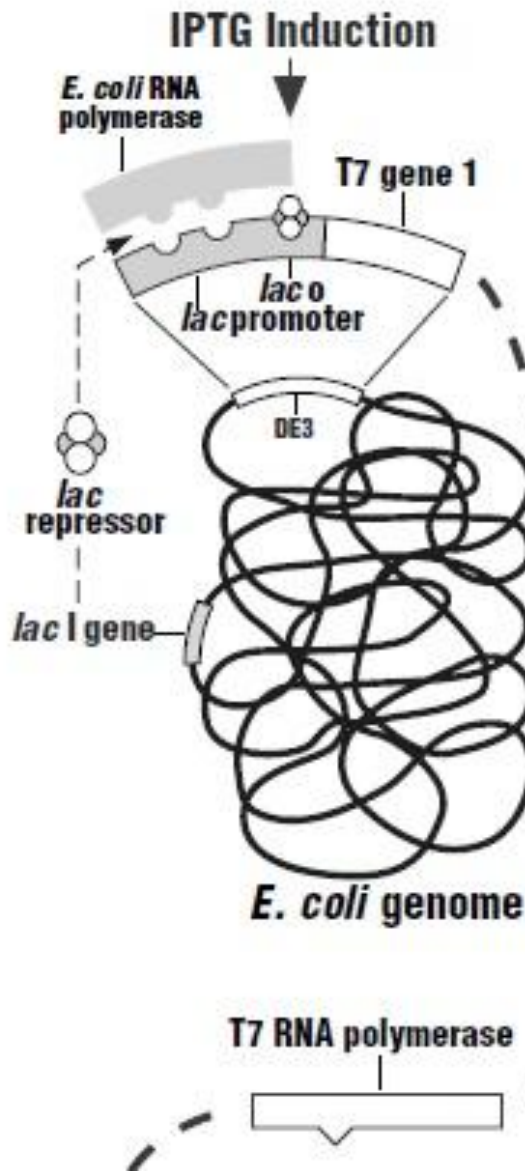
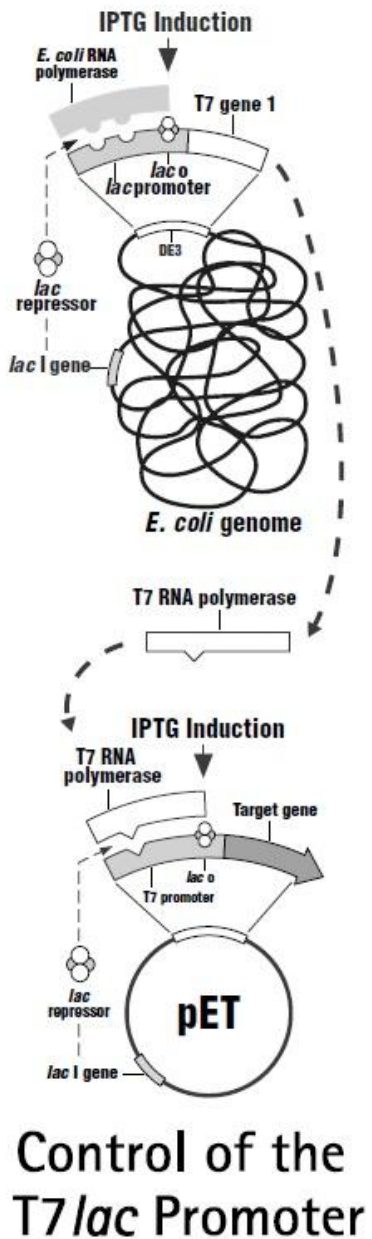
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA

pET-23a

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

TATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGC GGCCGCACTCGAGCACCACCACCACCACCCTGA
MetAlaSerMetThrGlyGlyGlnGlnMetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProLeu

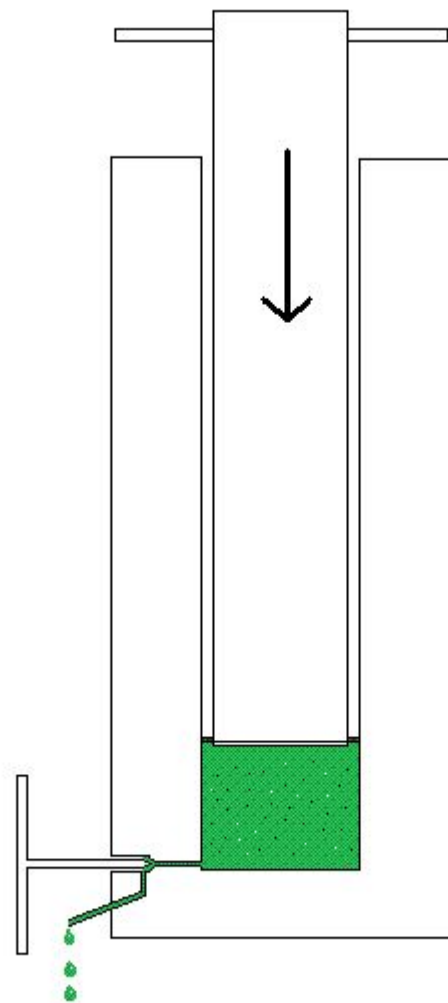




Лизис клеток



Pressure Cells



Очистка целевого белка

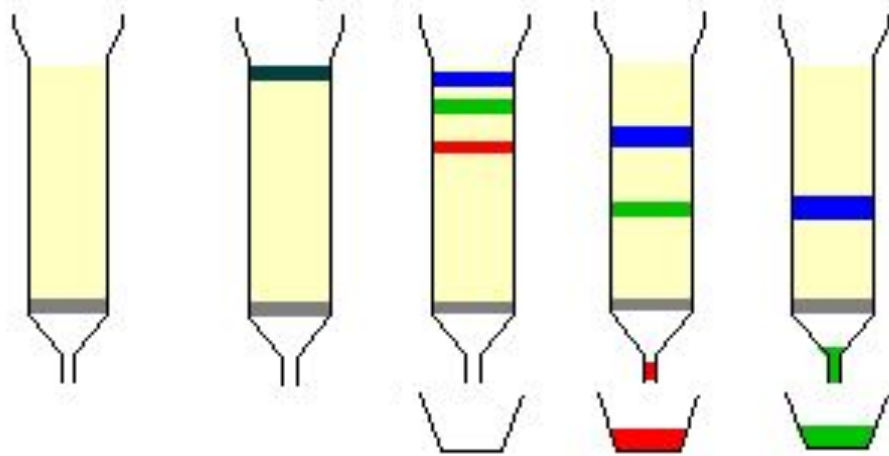
Хроматография



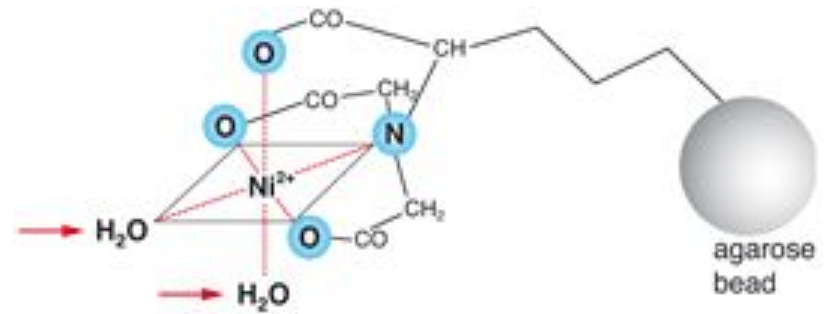
column containing stationary phase

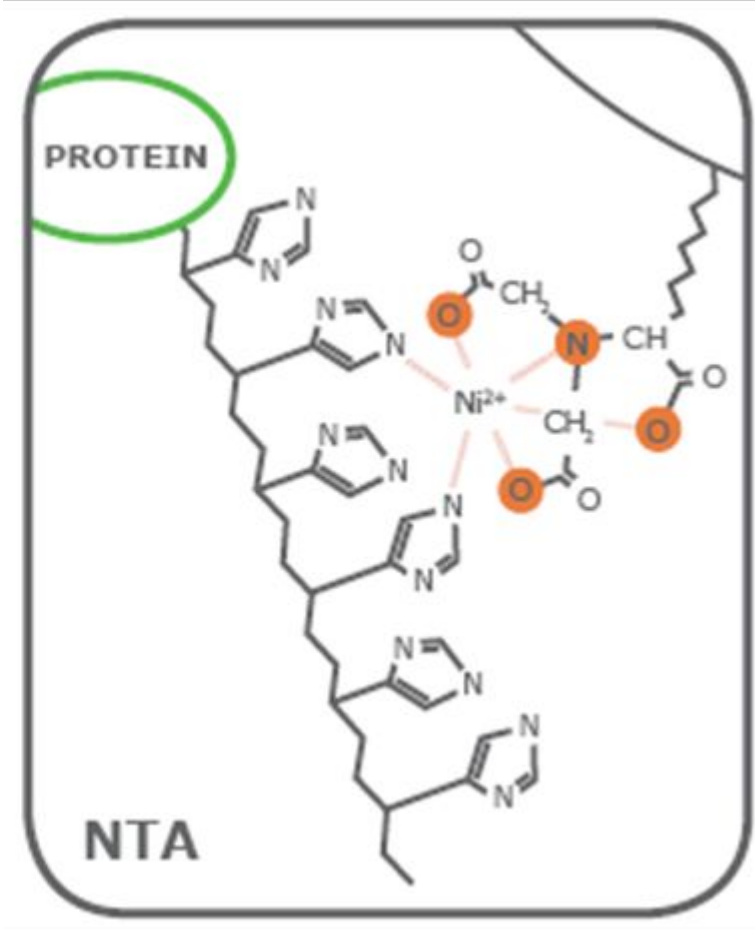
load sample

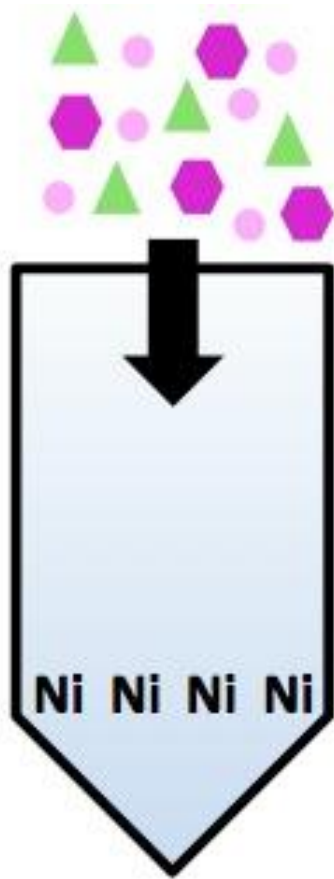
add solvent



collect components







Loading
supernatant



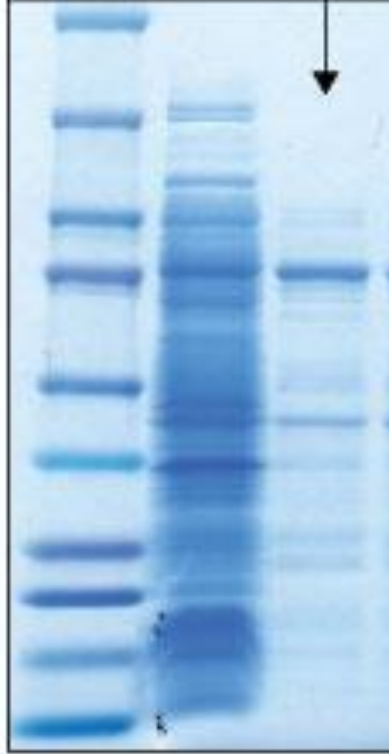
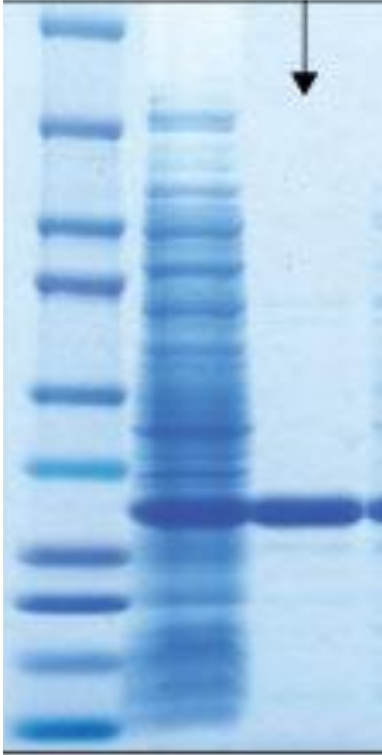
Binding to the
column



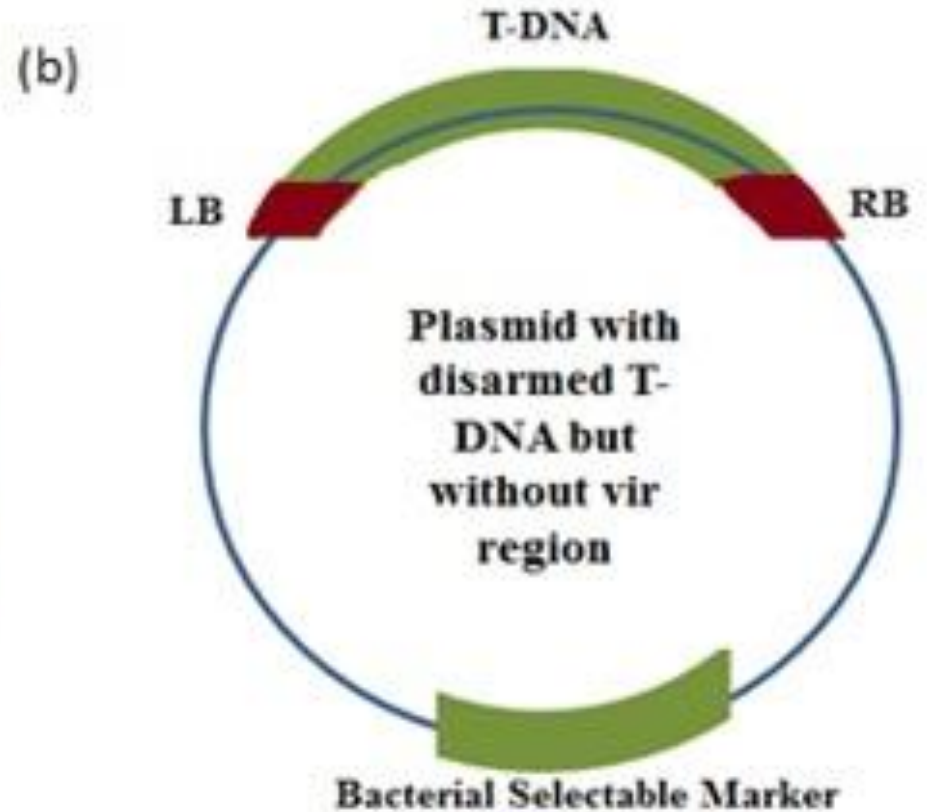
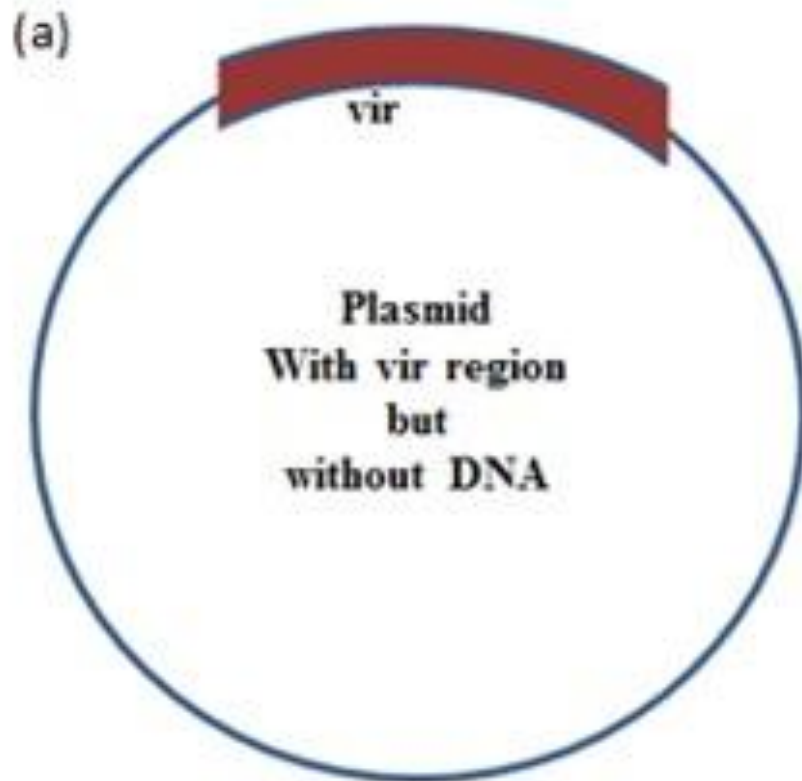
Wash

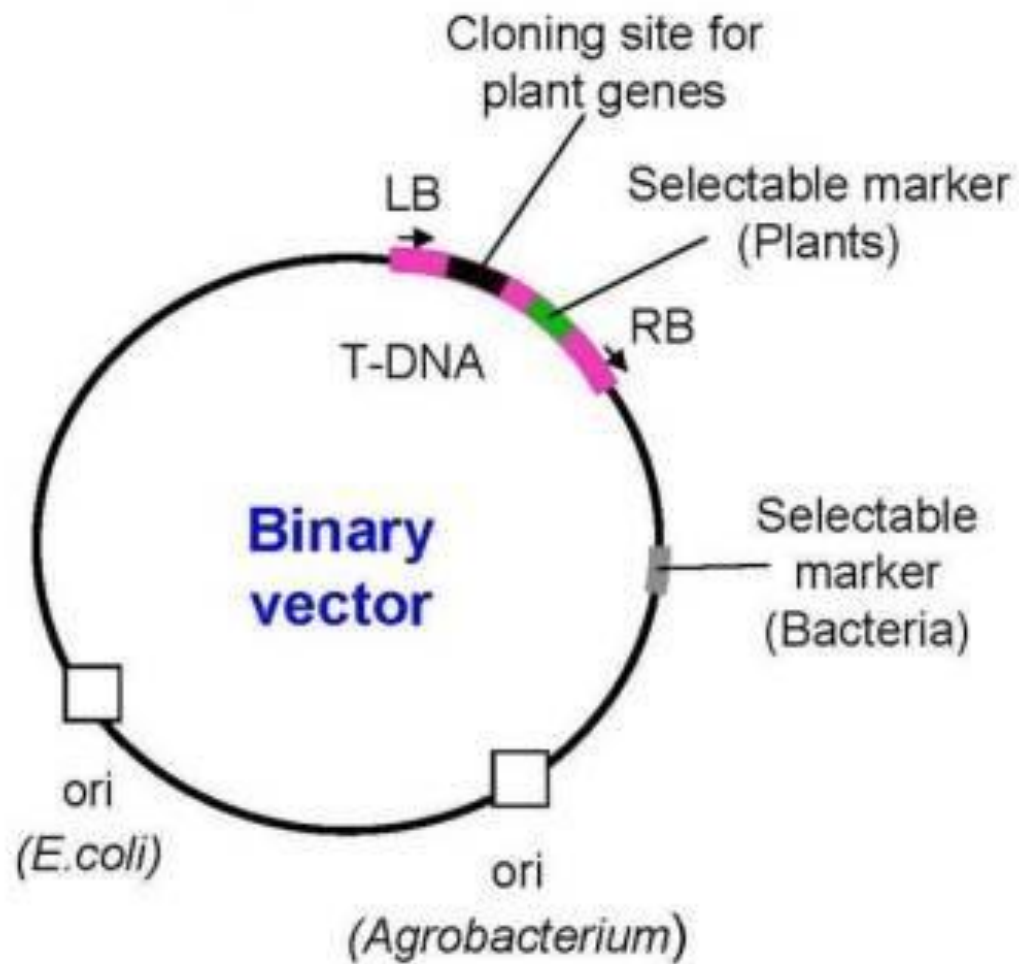


Elution

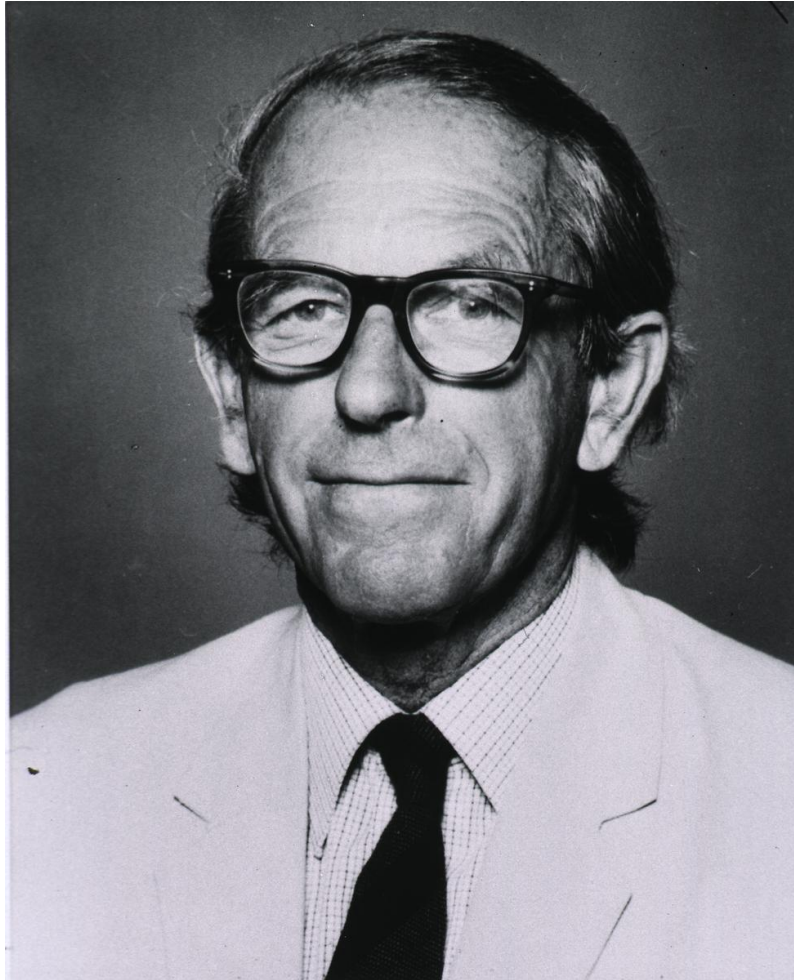


Векторы для трансформации растений

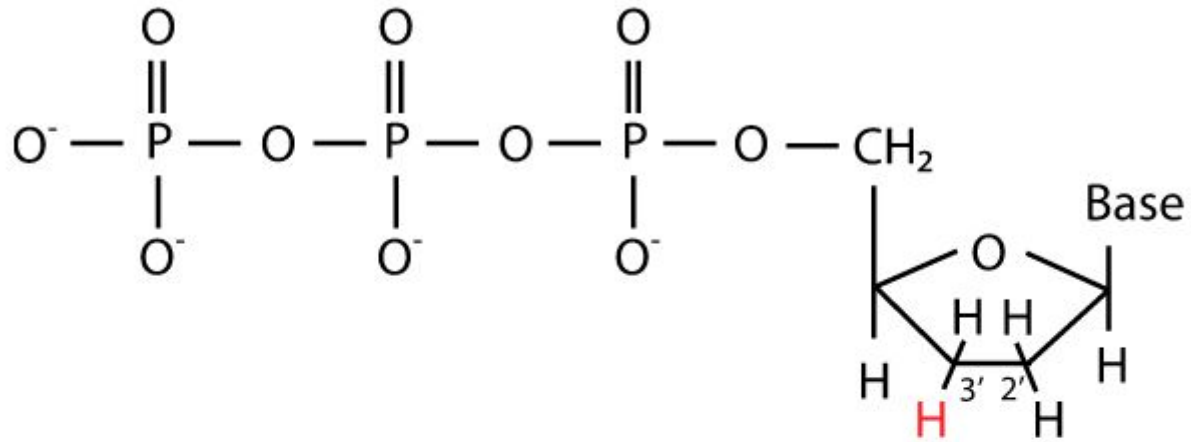




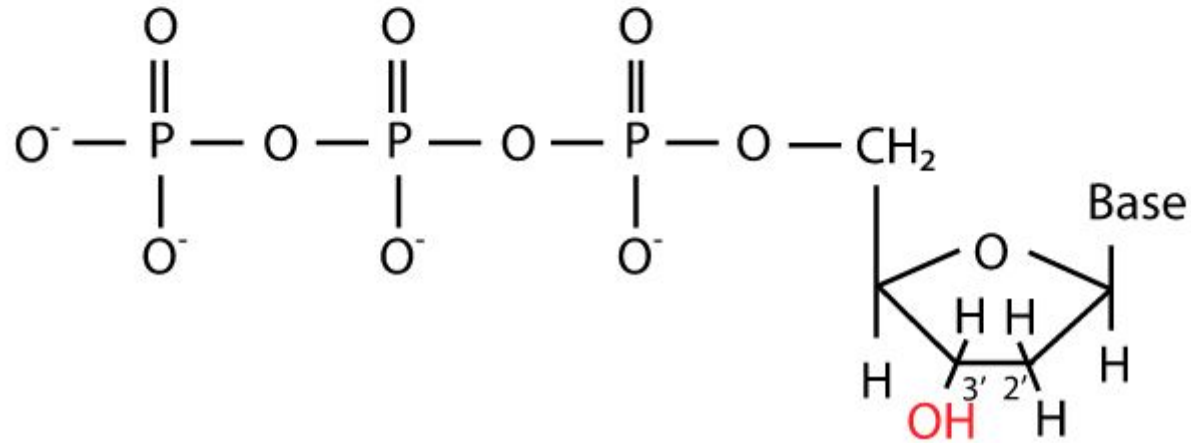
Определение первичной структуры ДНК



Dideoxynucleotide (ddNTP)



Deoxynucleotide (dNTP)





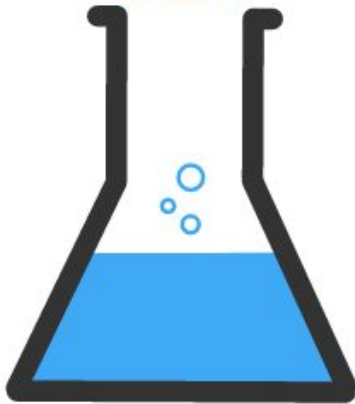
dATP + dCTP + dGTP + dTTP

DNA Polymerase

Template DNA

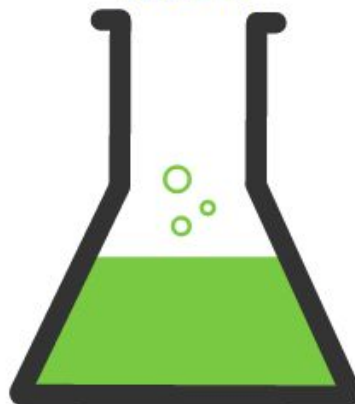
Primer

ddATP



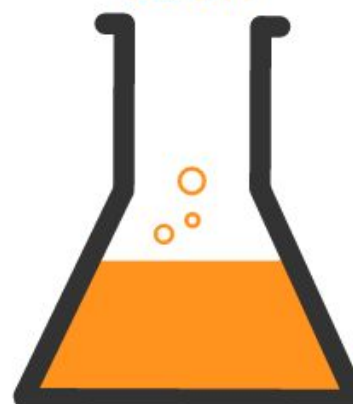
ACTCAGATGCT
ACTCAGA
ACTCA
A

ddCTP



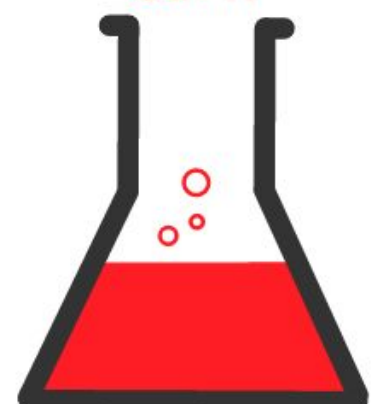
ACTCAGATGCT
ACTCAGATGC
ACTC
AC

ddGTP

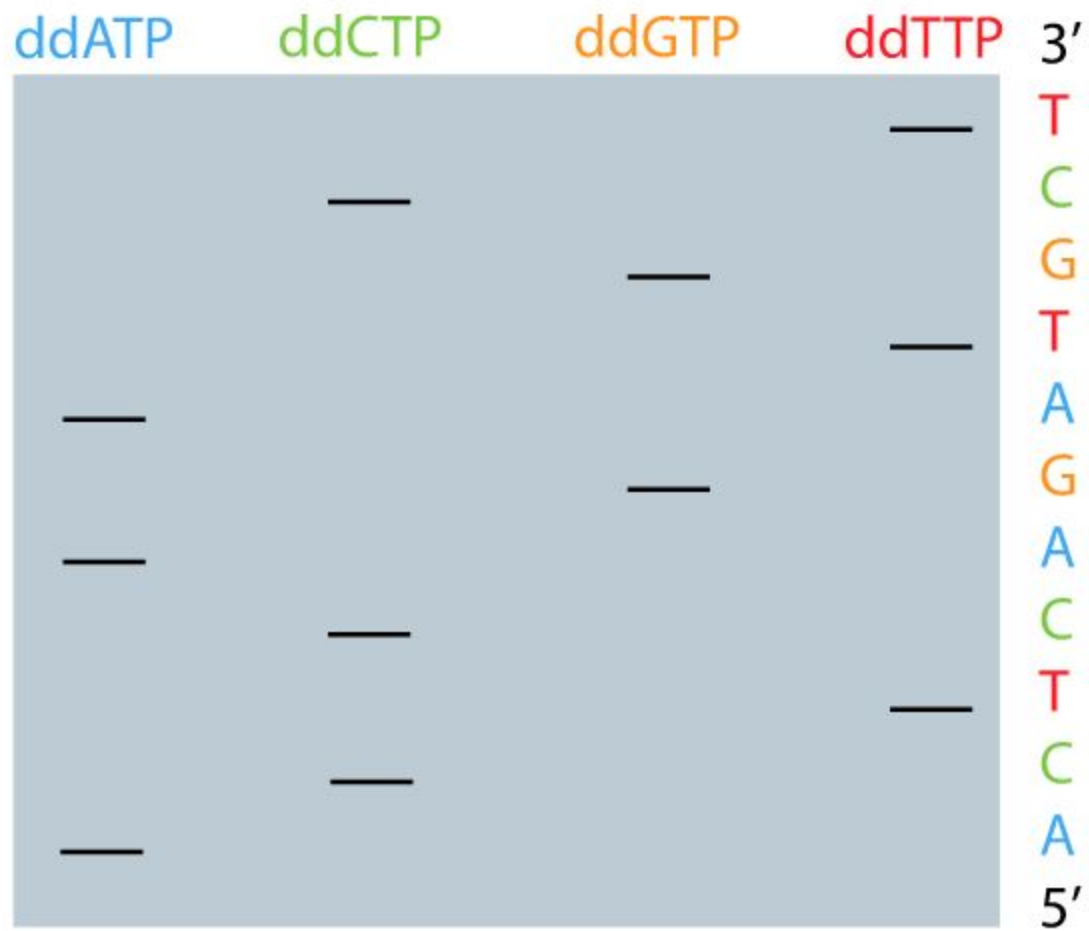


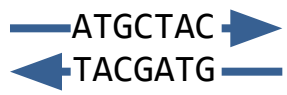
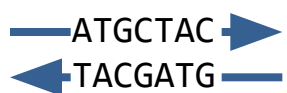
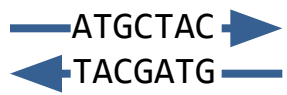
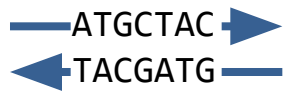
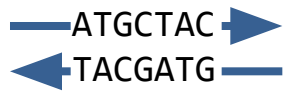
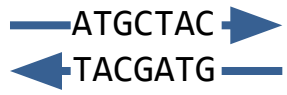
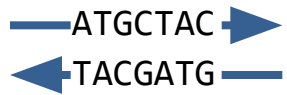
ACTCAGATGCT
ACTCAGATG
ACTCAG

ddTTP



ACTCAGATGCT
ACTCAGAT
ACT

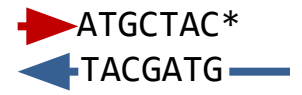
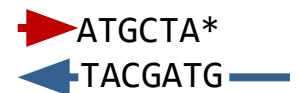
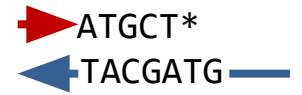
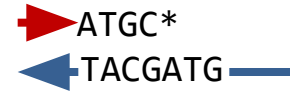
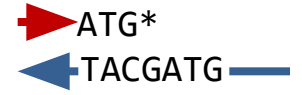


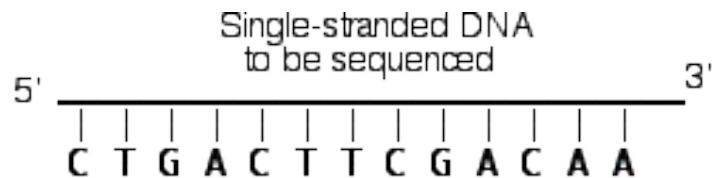


Денатурация,
отжиг
праймера

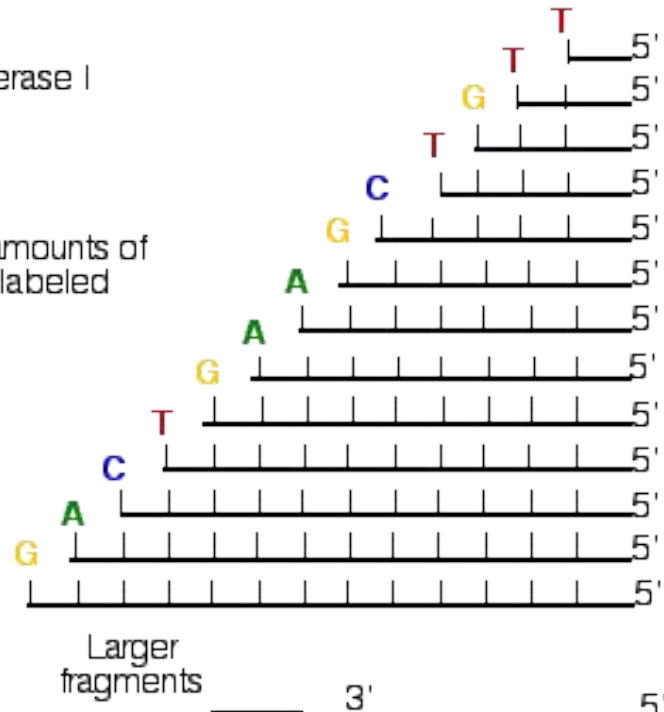


Элонгаци
я

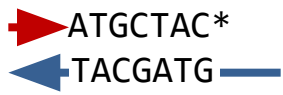
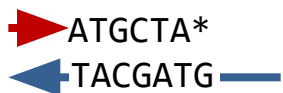
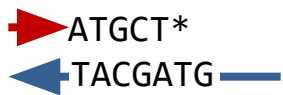
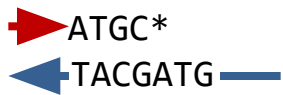
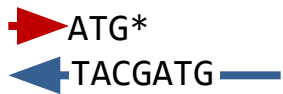




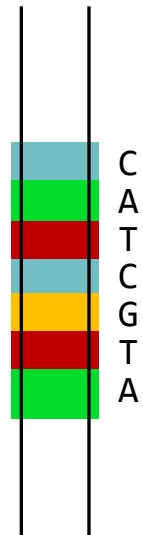
Add:
 DNA polymerase I
 dATP
 dGTP
 dCTP
 dTTP
 plus limiting amounts of
 fluorescently labeled
ddATP
ddGTP
ddCTP
ddTTP







Капиллярный
электрофорез



Анали
з

