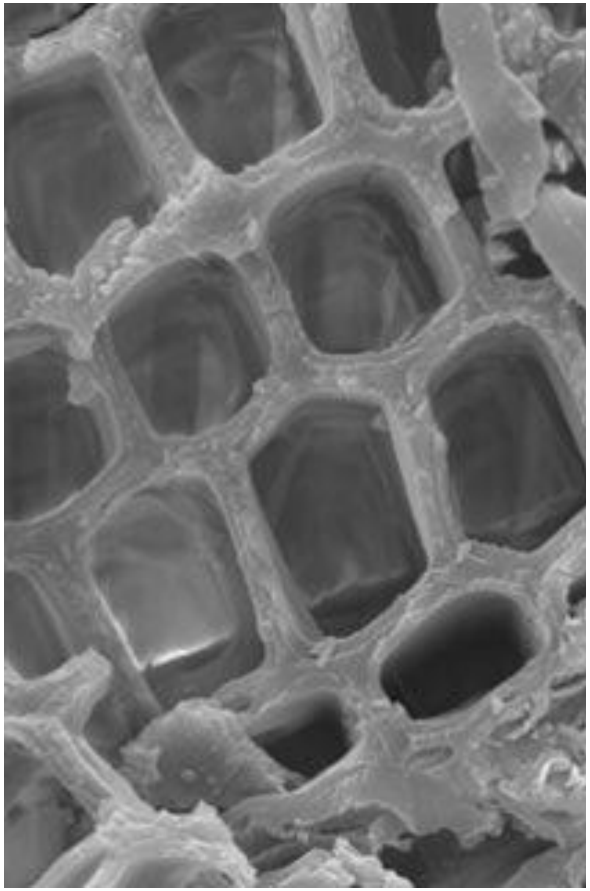
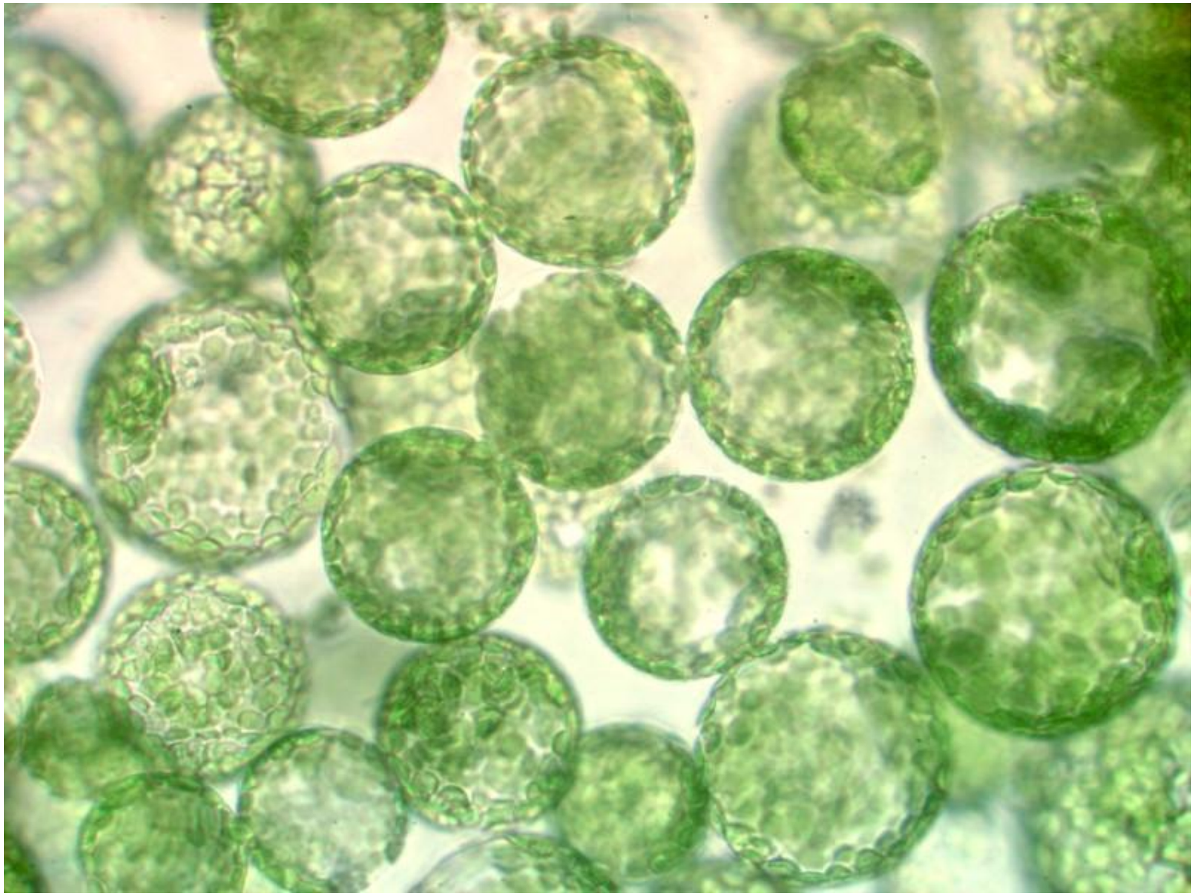
Трансгенные растения

Что нужно, чтобы получить трансгенное растение?

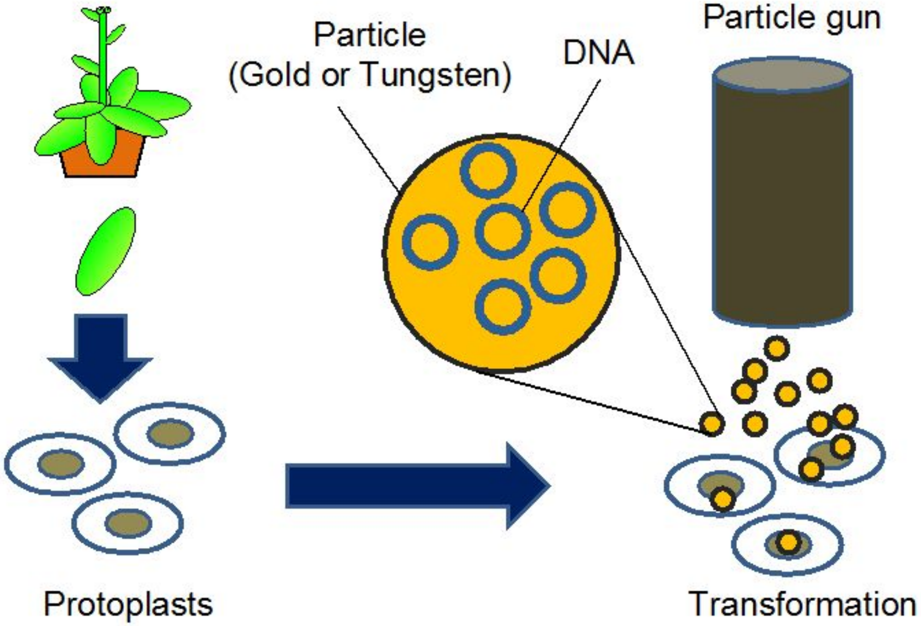
Чужеродная ДНК должна проникнуть в клетку

«Избавление» от клеточной стенки

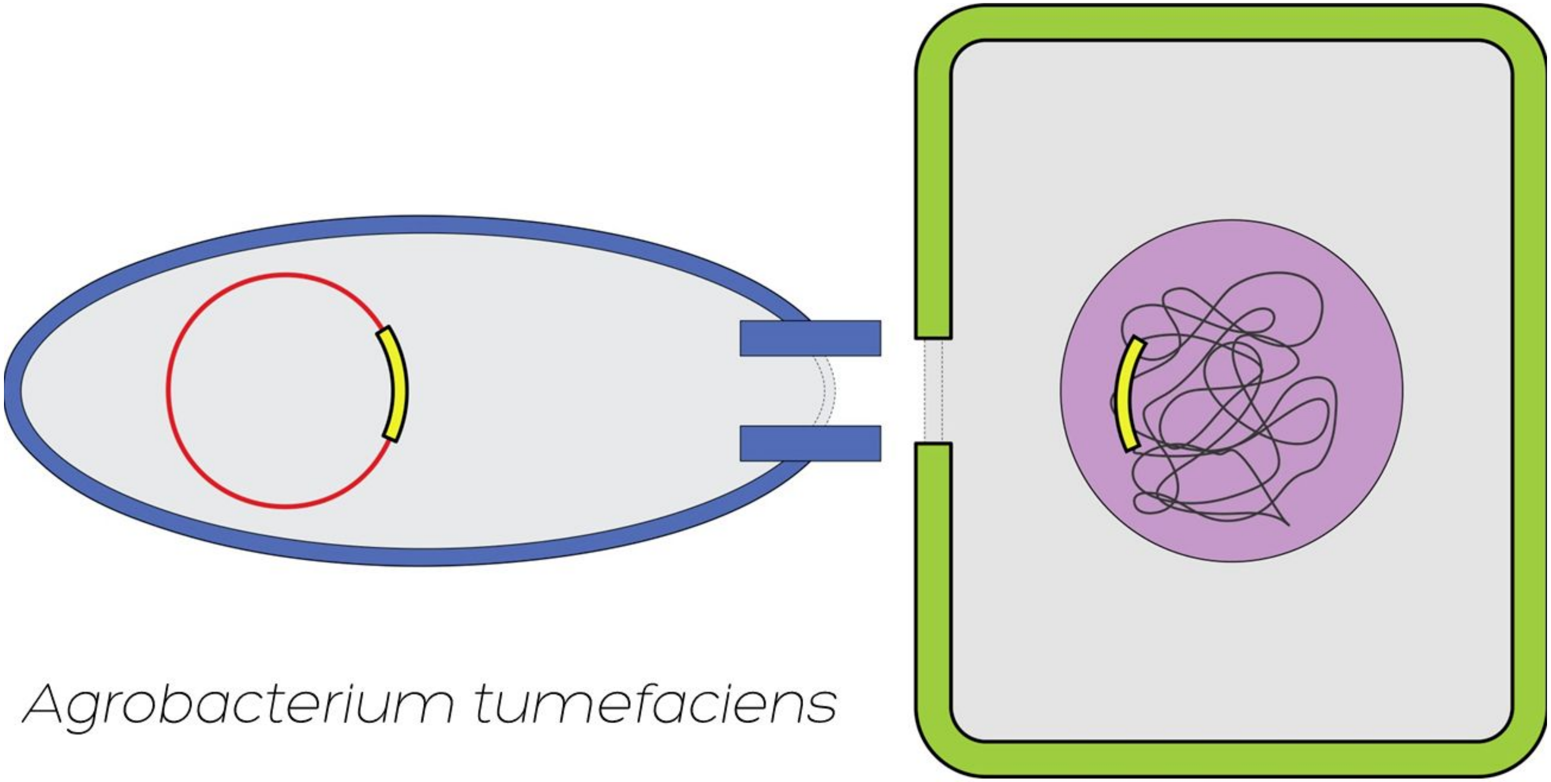




Бомбардировка клеток



Использование потенциала фитопатогенов

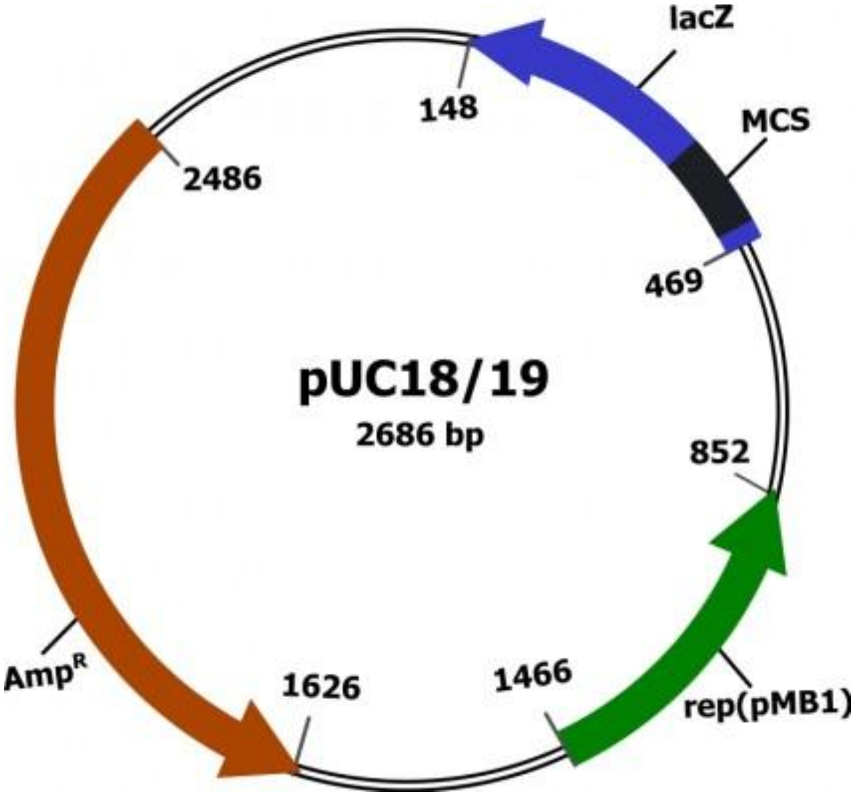


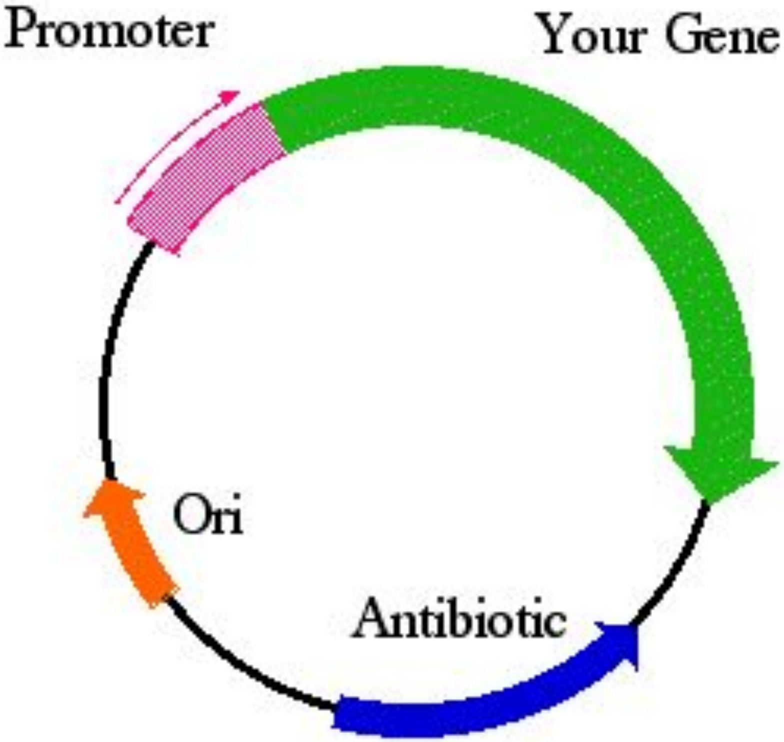
Нужно чтобы целевой участок ДНК:

Встроился в геном

Экспрессировался (чтобы генетическая информация считывалась)

Чужеродные гены находятся в составе векторных плазмид

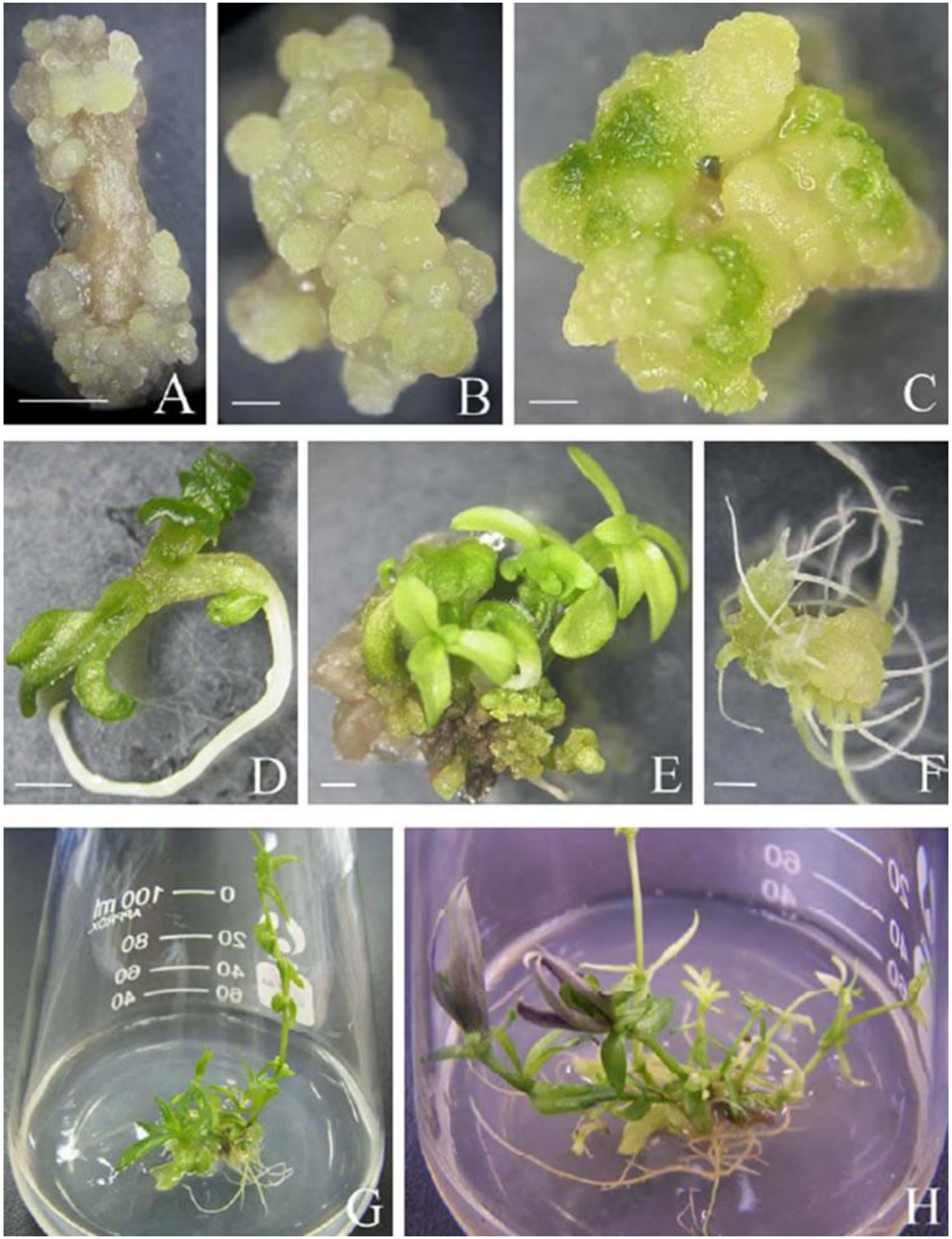




Введение векторных конструкций в клетки растений

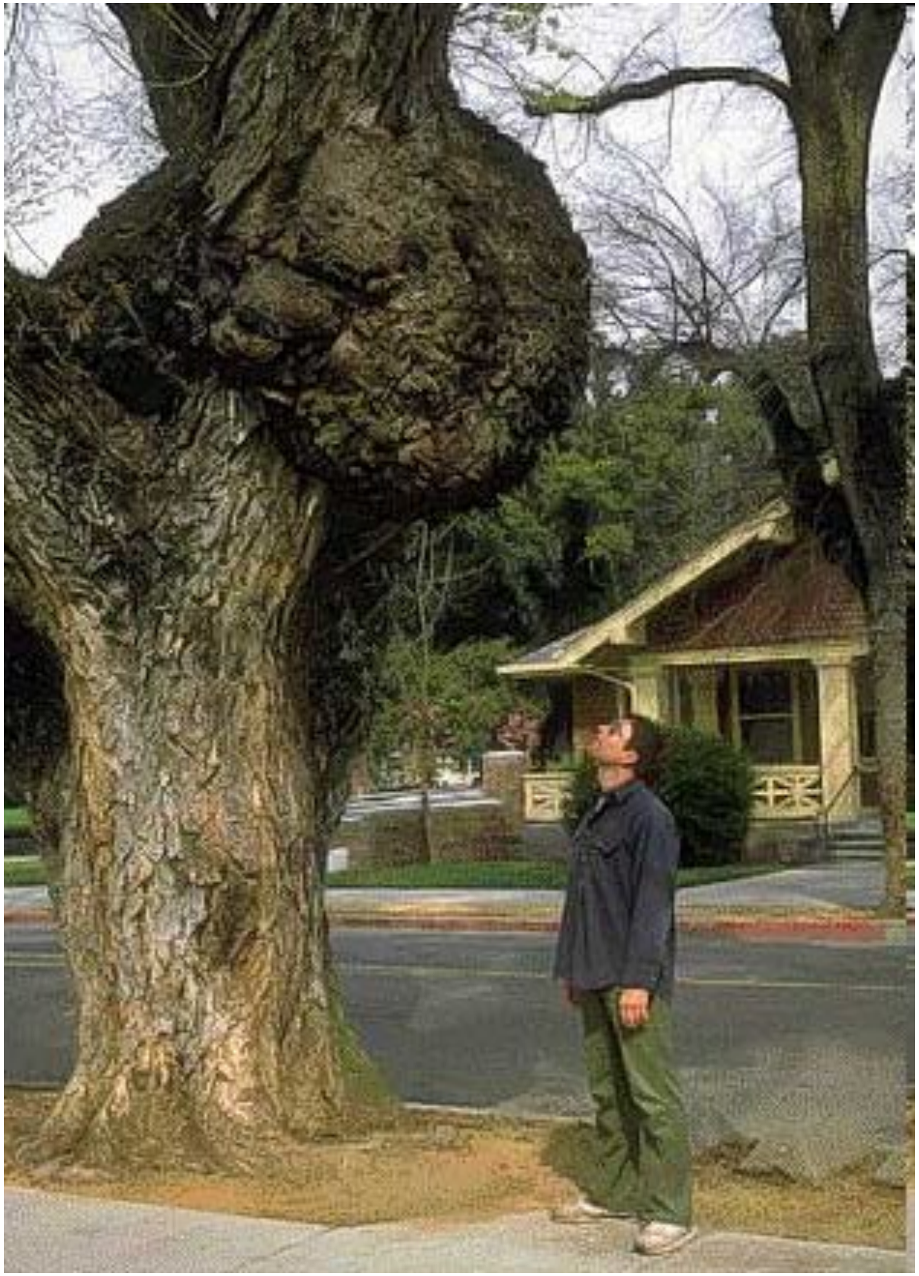
Станет ли после этого растение трансгенным?

Регенерация целых растений из отдельных трансформированных клеток

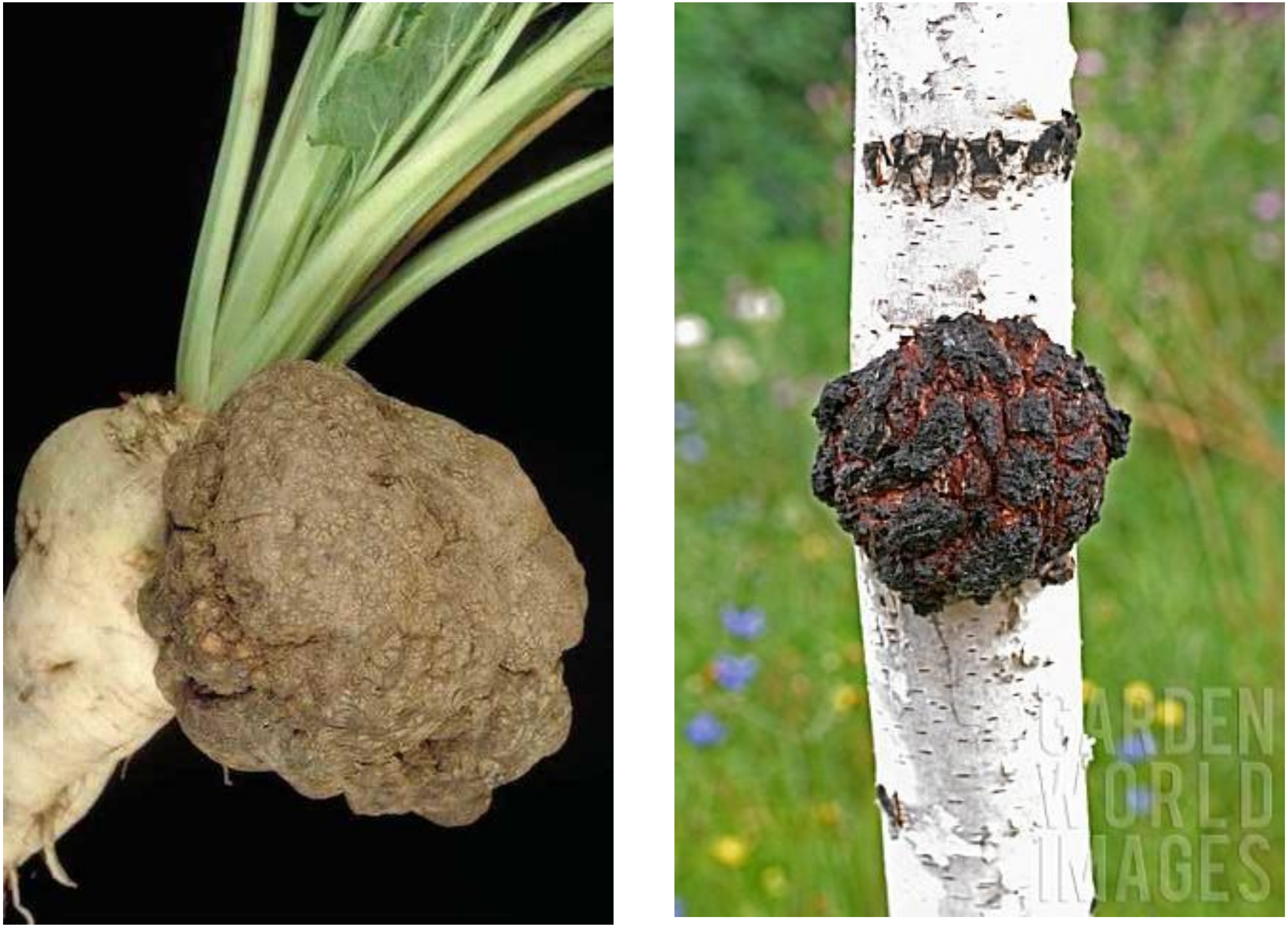


Основной подход для создания трансгенных растений

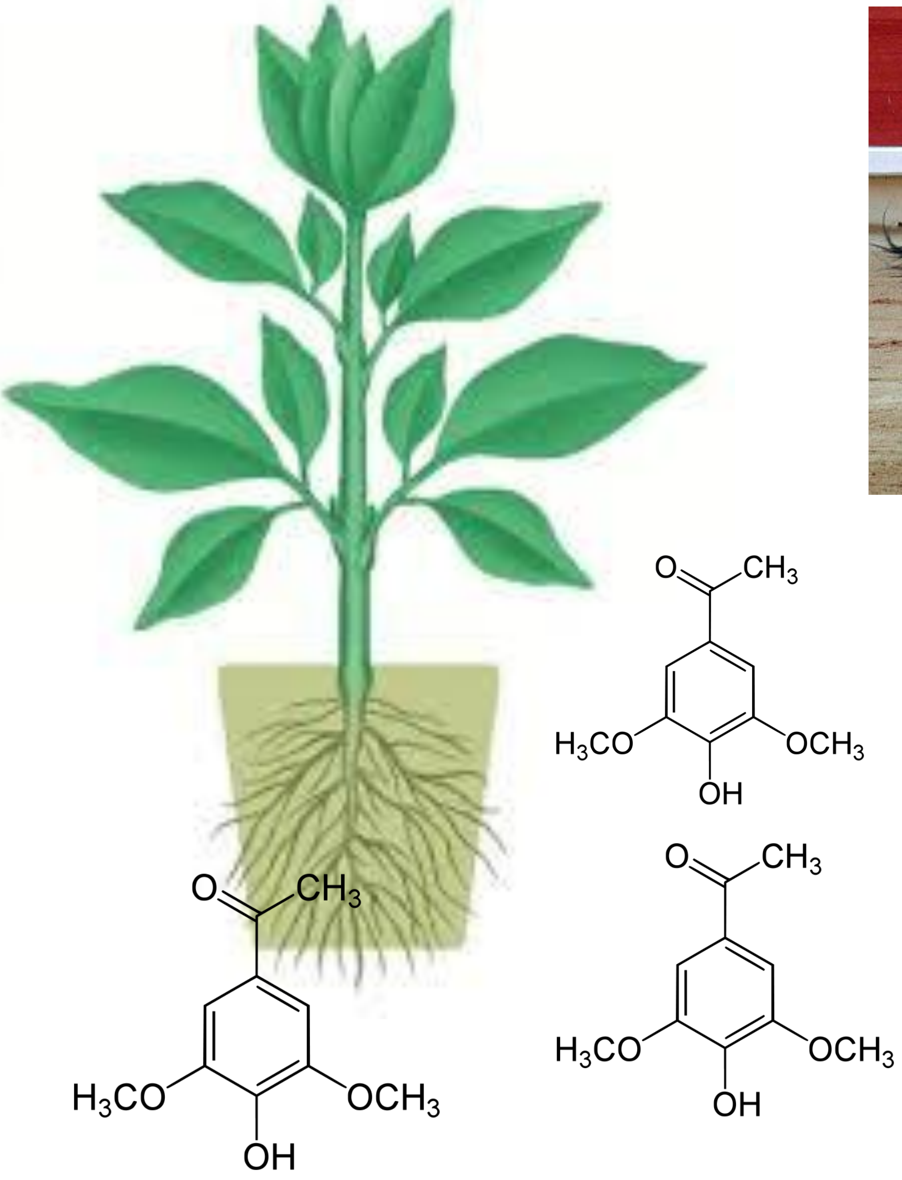
Корончатые галлы

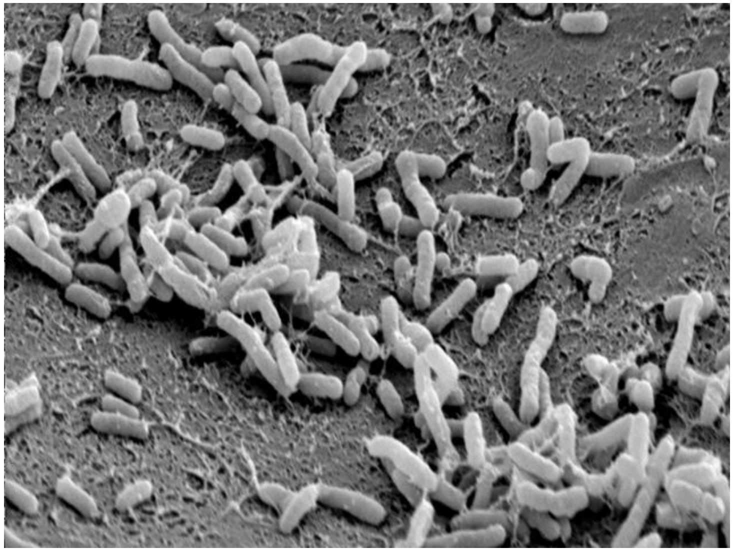


Корончатые галлы

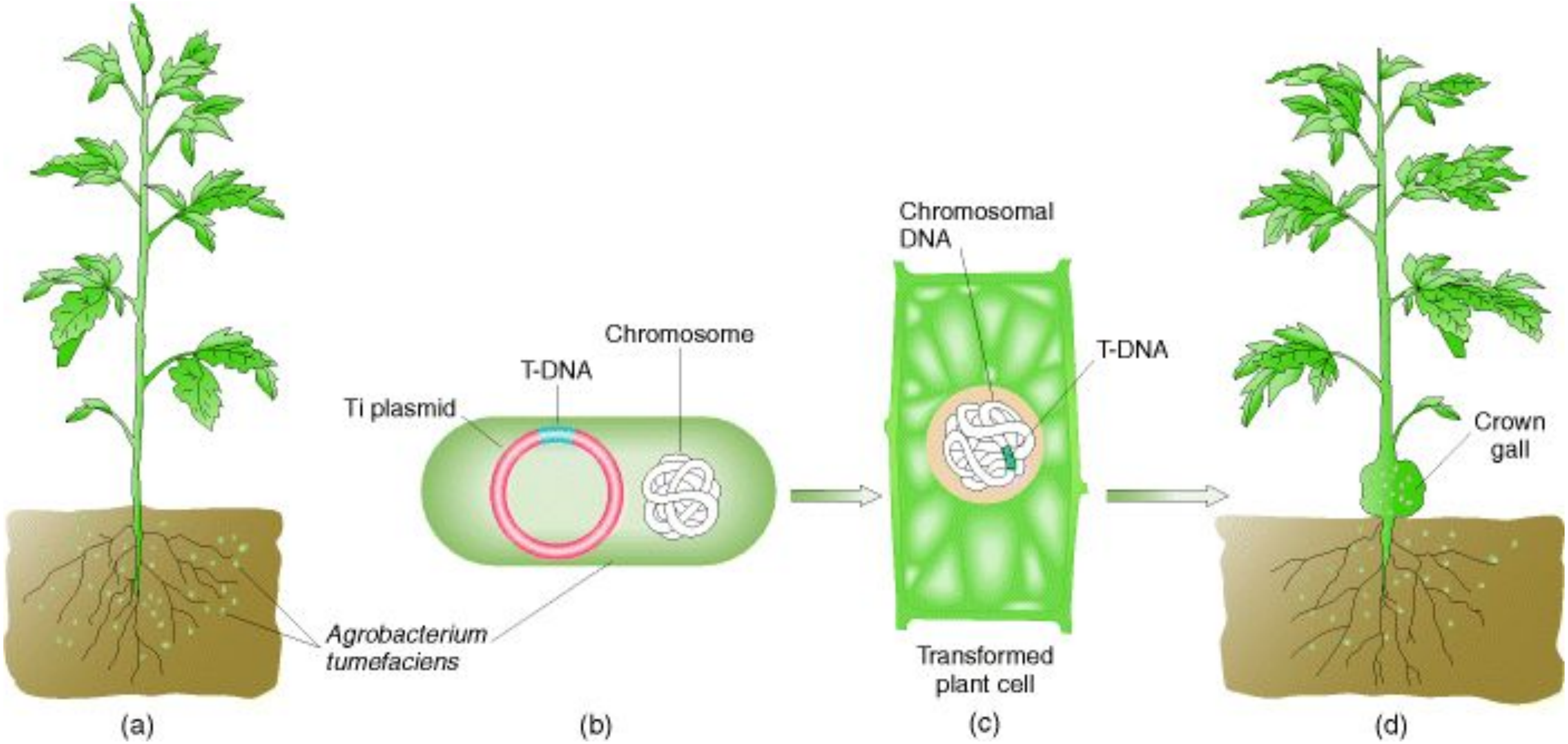


Agrobacterium tumifaciens

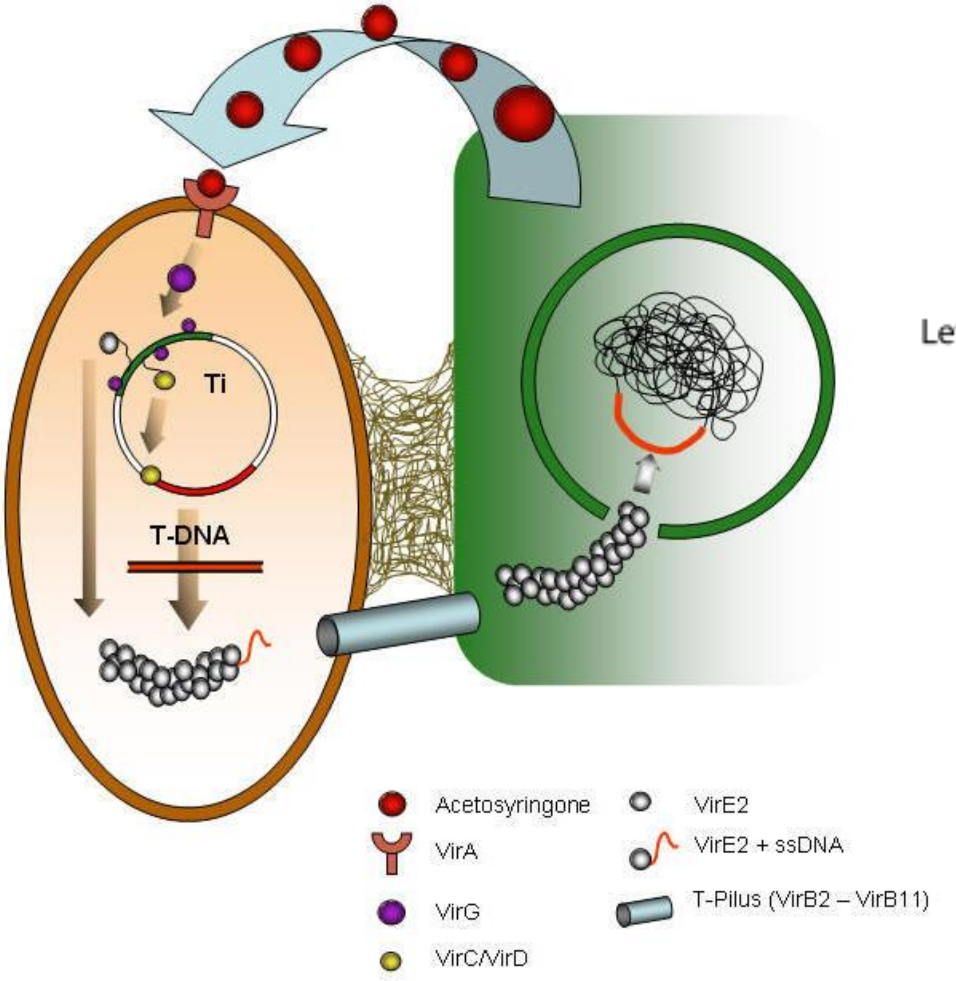


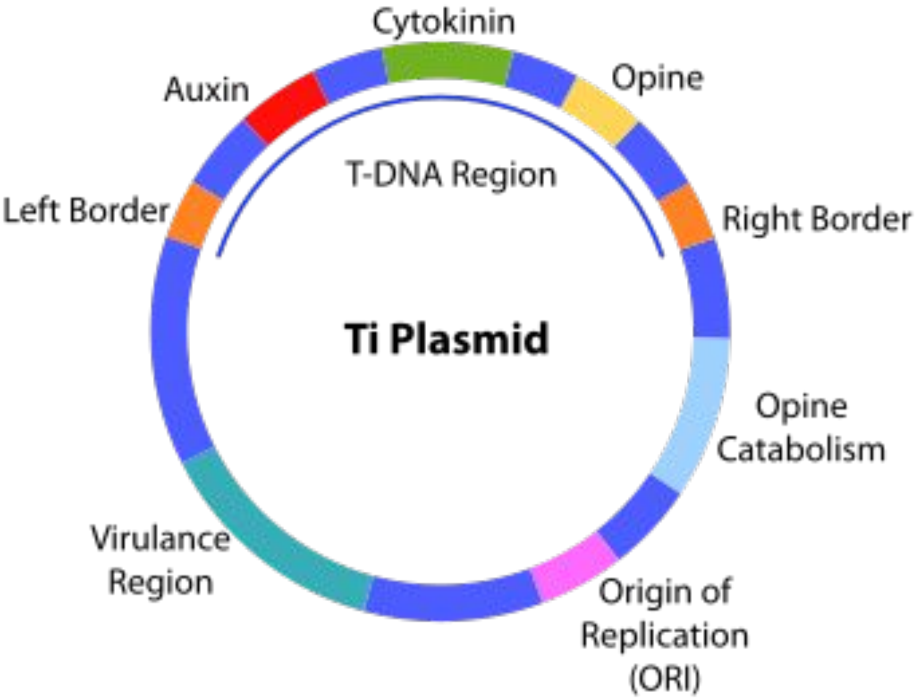


Agrobacterium tumifaciens

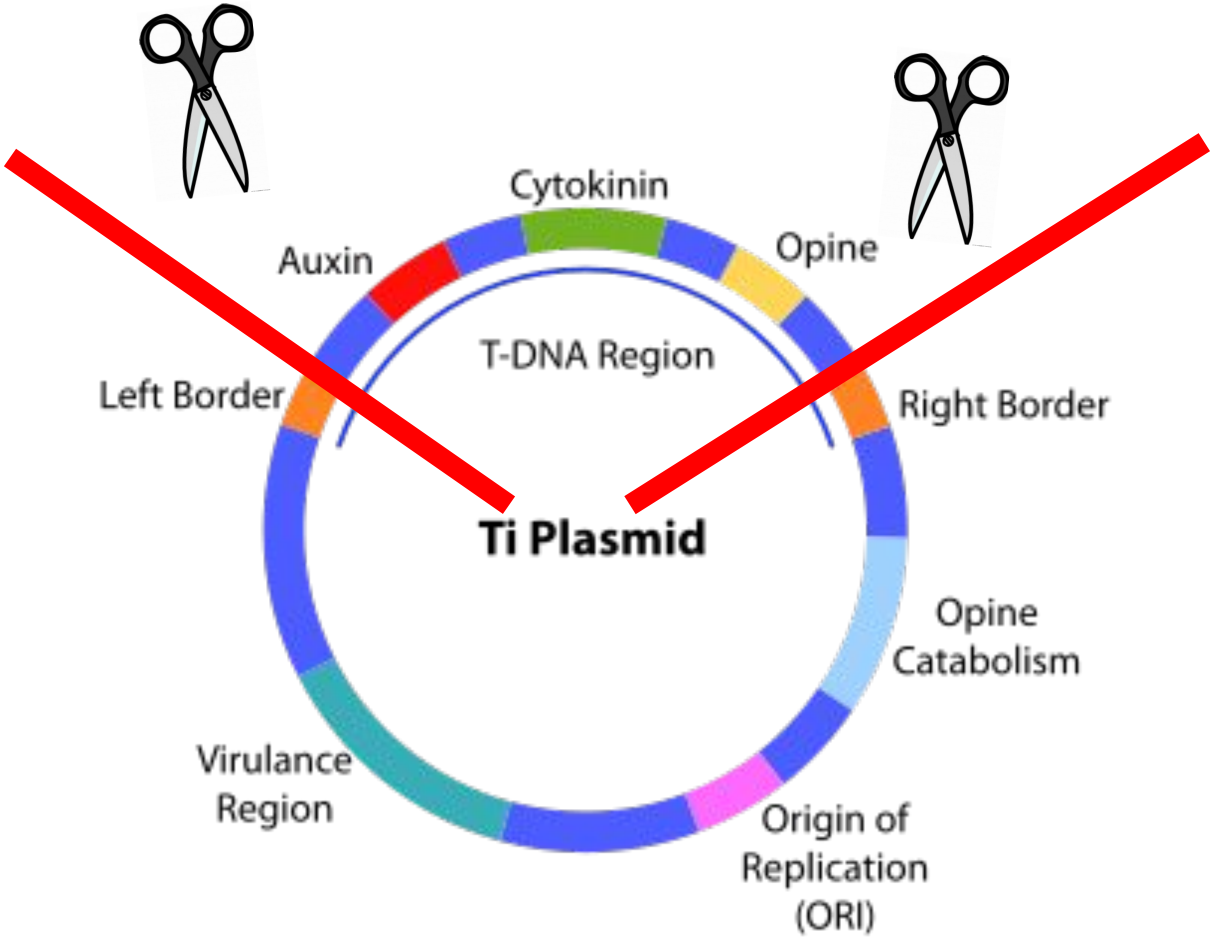


Интеграция тДНК в геном растения

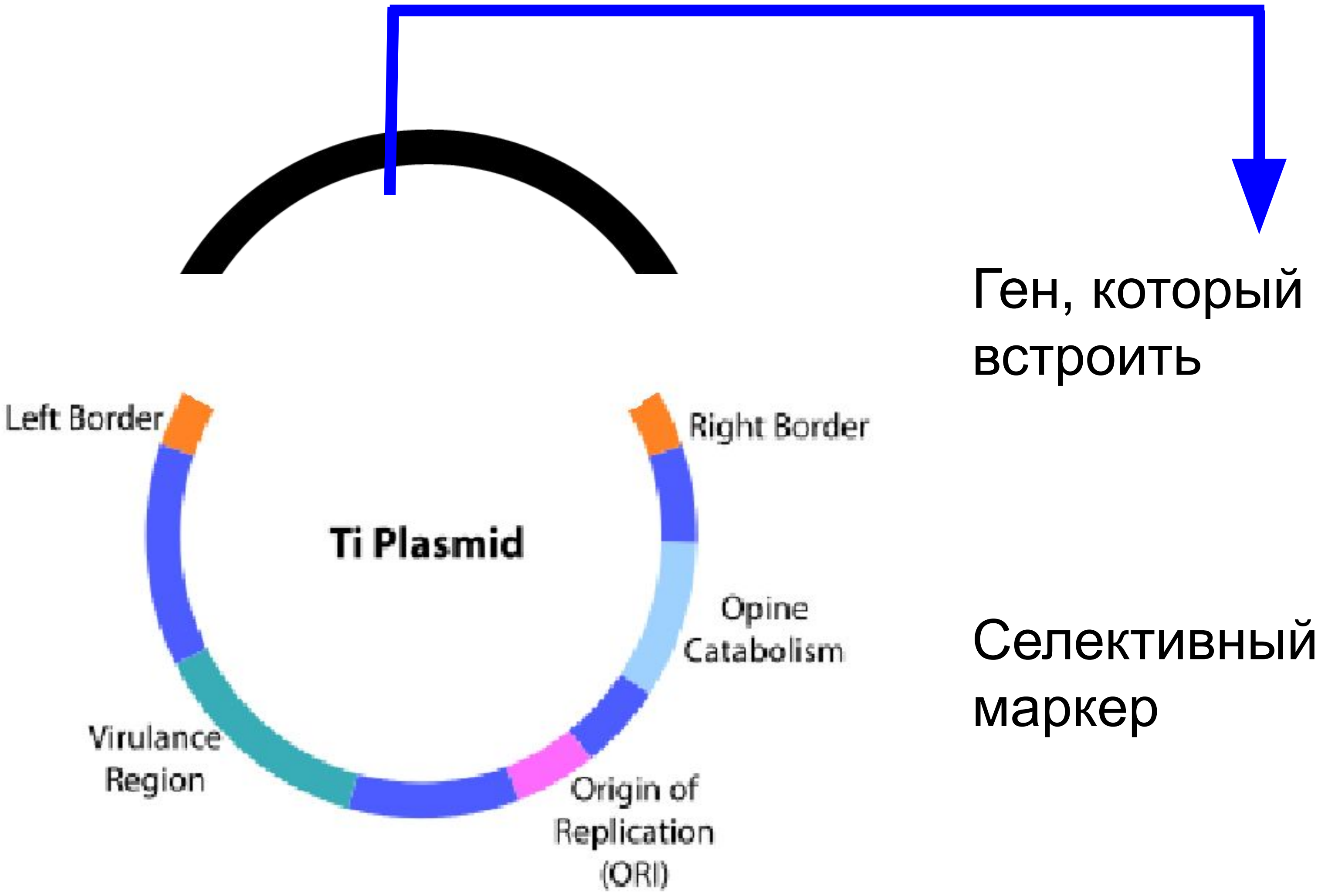




Модификация Ti – плазмиды для создания трансгенных растений



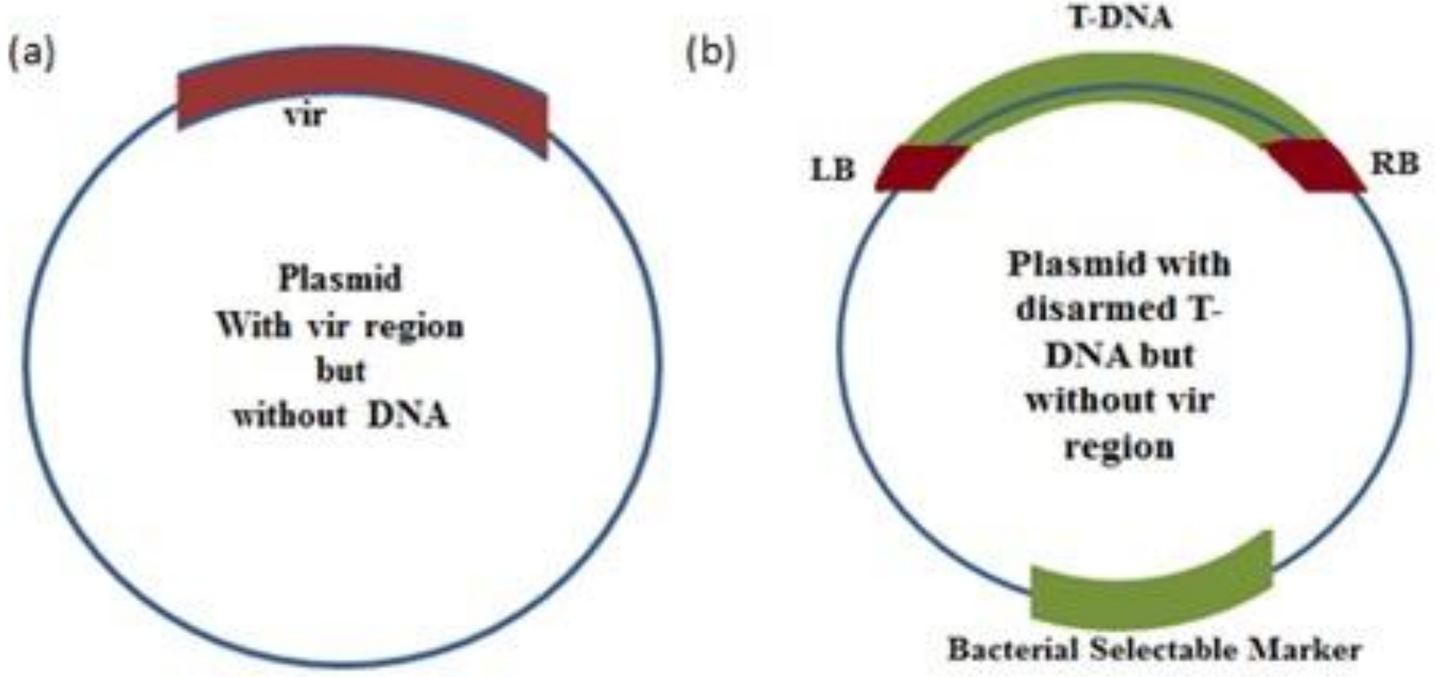
Модификация Ti – плазмиды для создания трансгенных растений

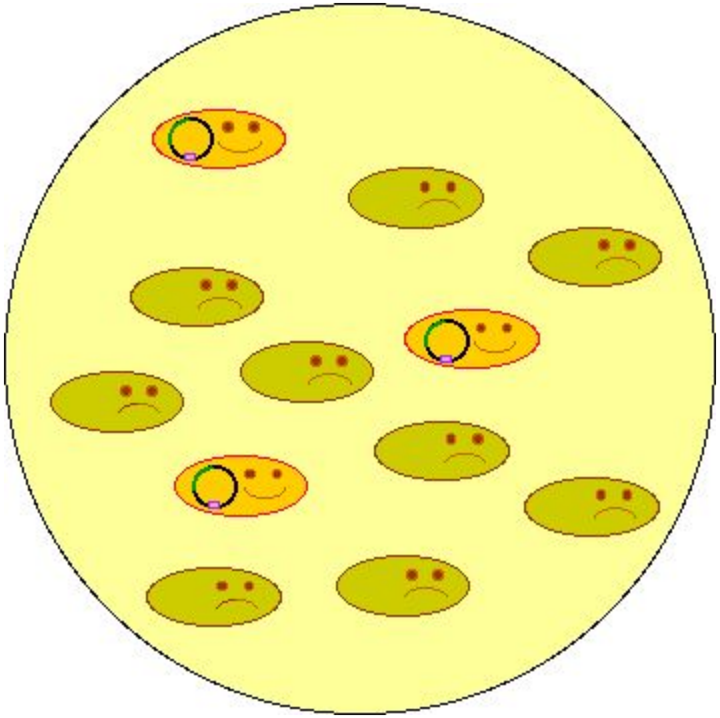


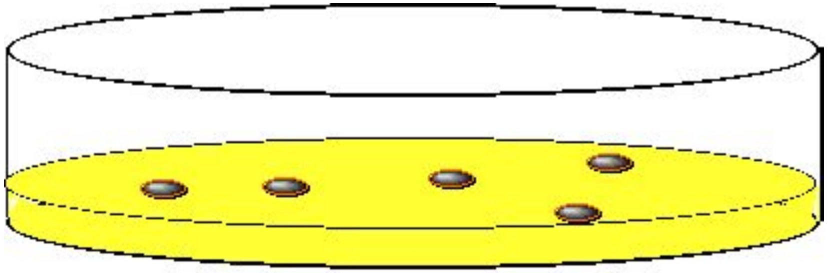
Ген, который хотят встроить

Селективный маркер

Бинарный вектор



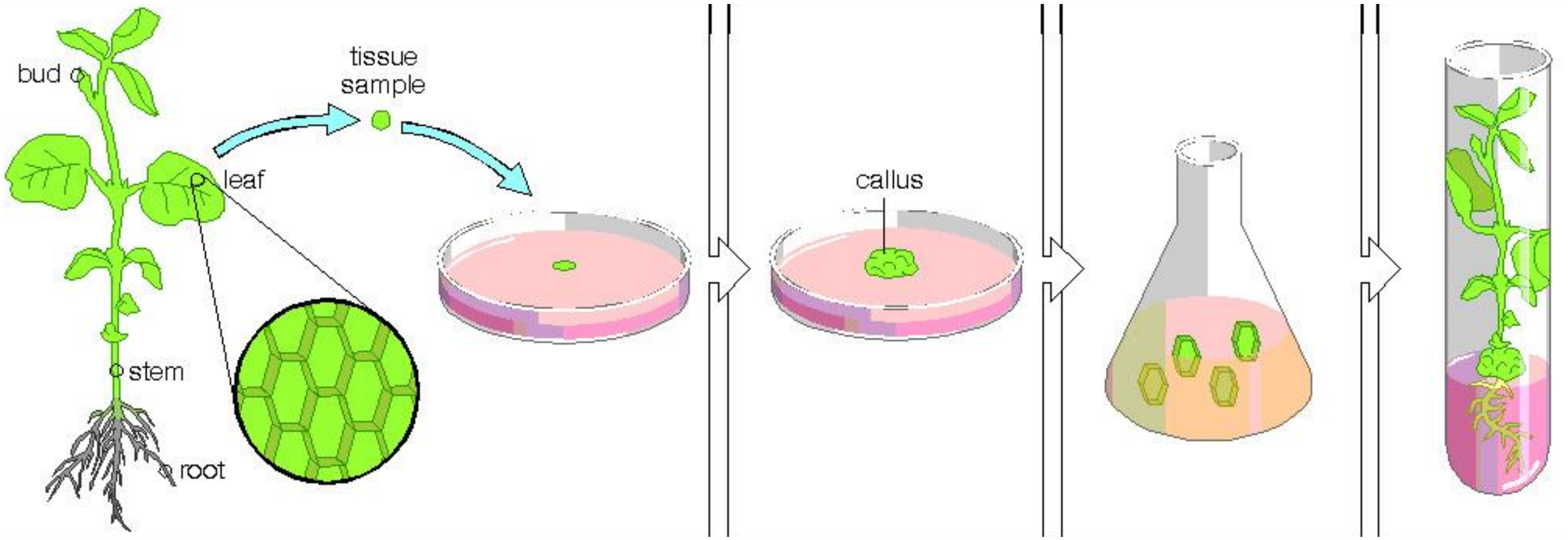




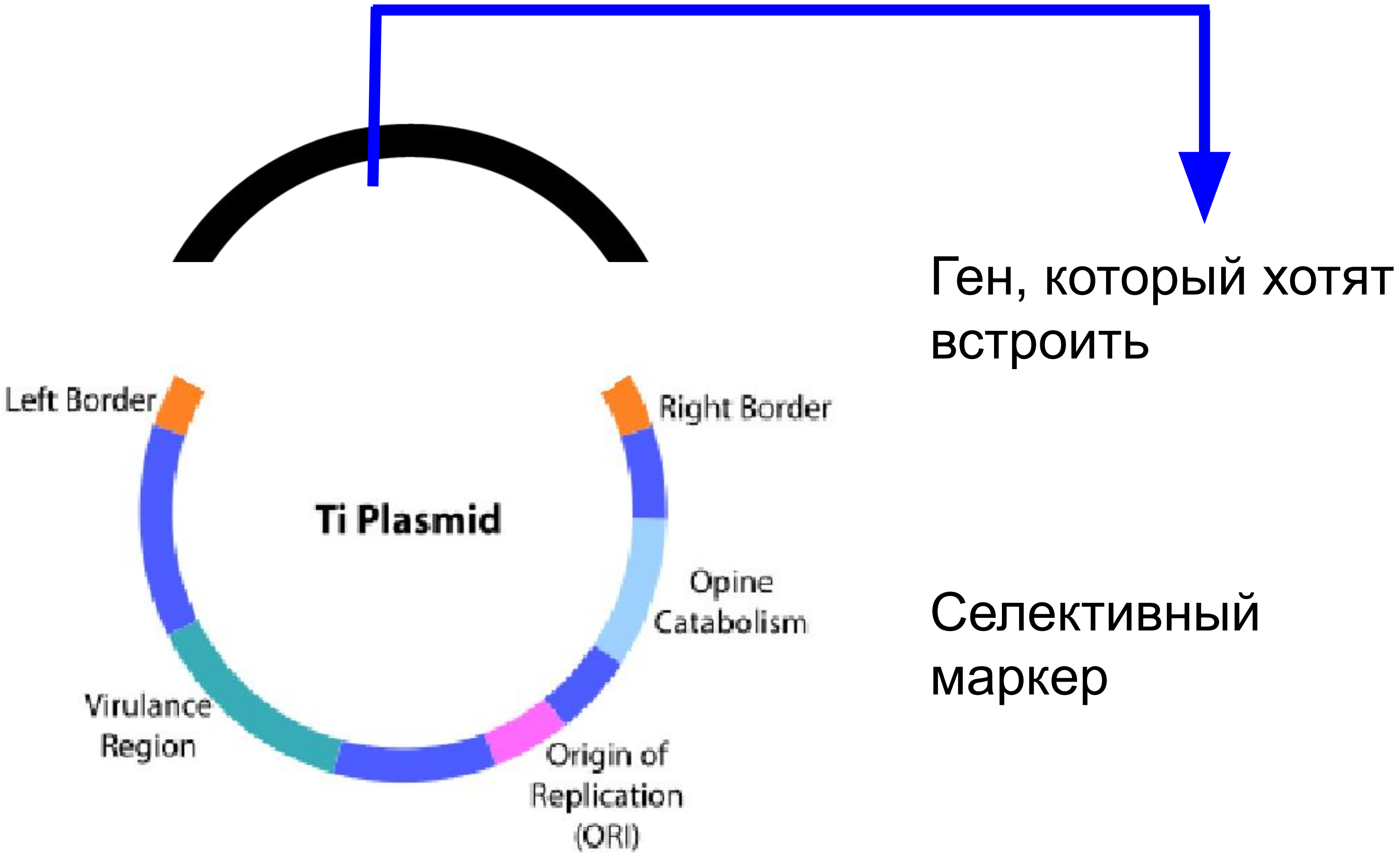
agar medium with antibiotic

Введение векторных конструкций в клетки растений

Регенерация целых растений из отдельных трансформированных клеток



Модификация Ti – плазмиды для создания трансгенных растений



Ген, который хотят встроить

Селективный маркер

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

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

Компоненты реакции:

Вода

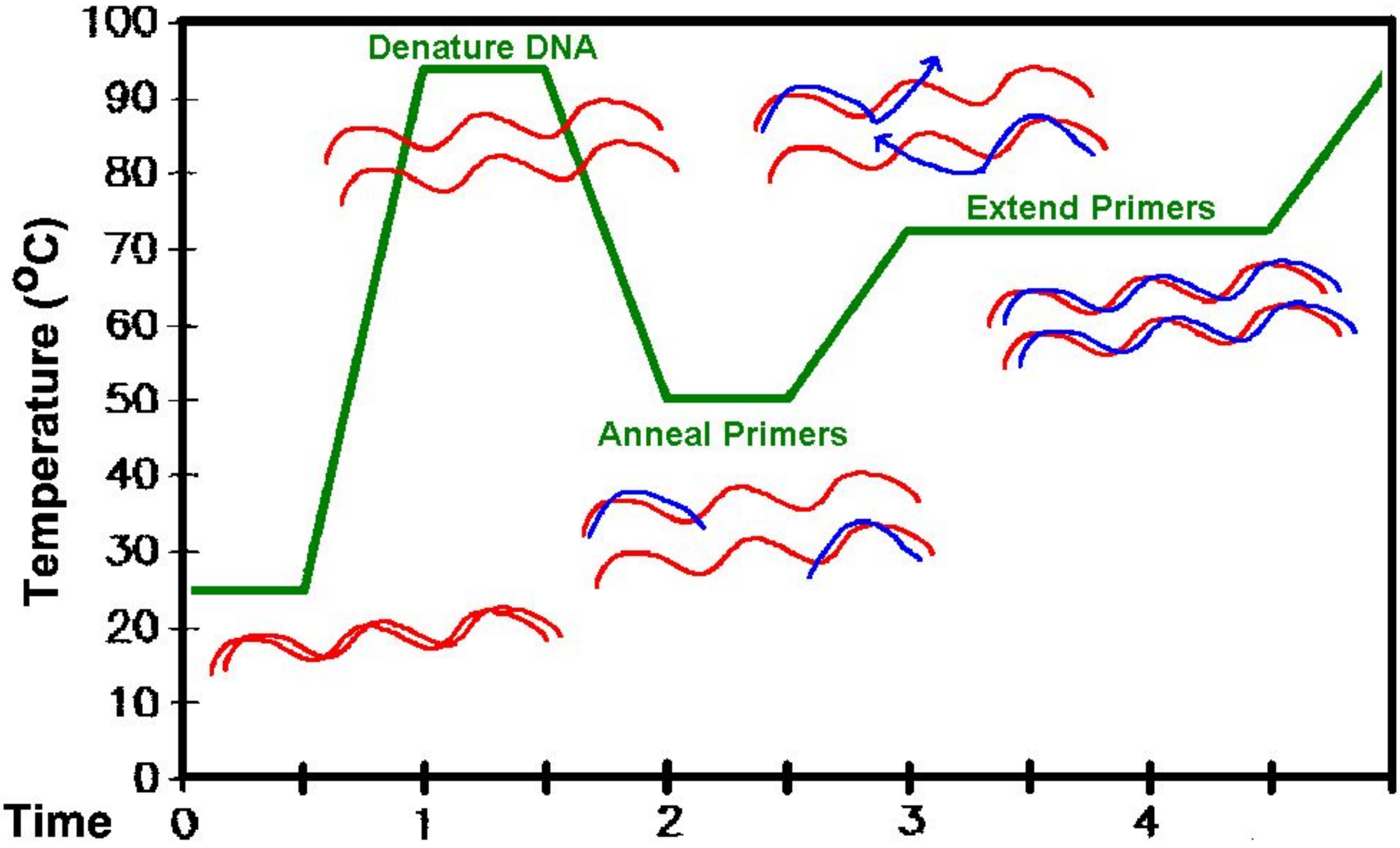
Буфер для фермента (Taq-полимеразы)

Дезоксинуклеотид фосфаты

Праймеры (прямой и обратный)

Фермент (Taq-полимераза)

ДНК-матрица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Anneal Primers
Extend Primers
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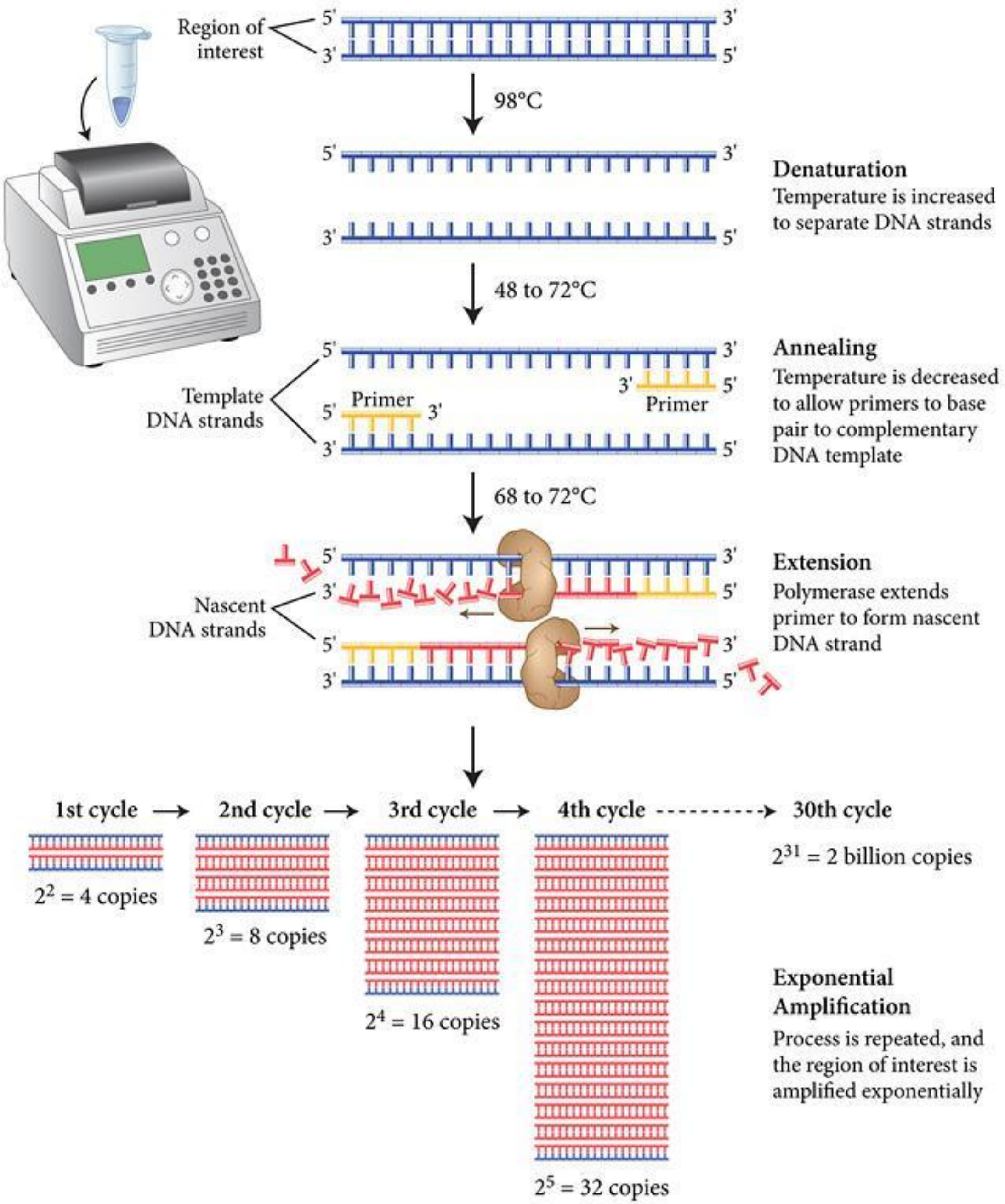
90
80
70
60
50
40
30
20
10
0
Time
0
1
2
3
4

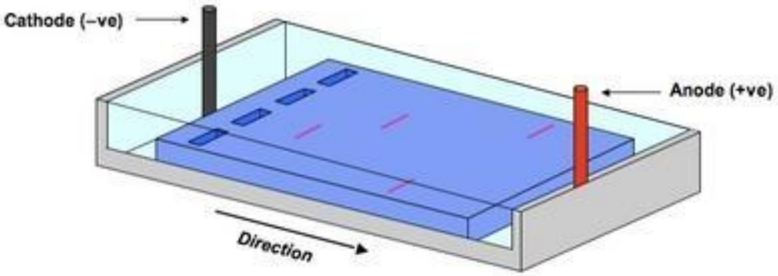


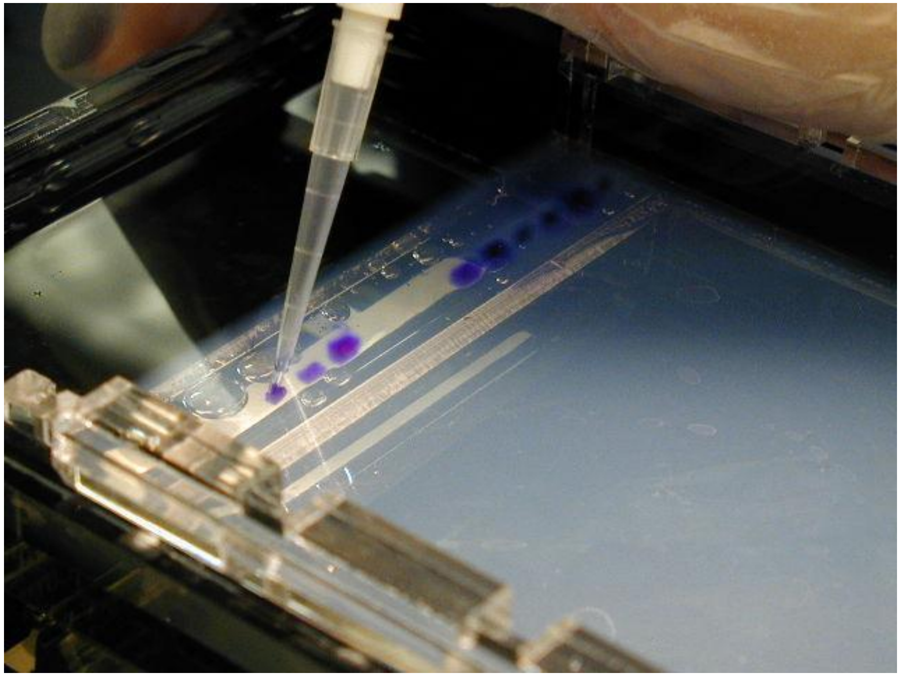
Denaturation
Temperature is increased to separate DNA strands

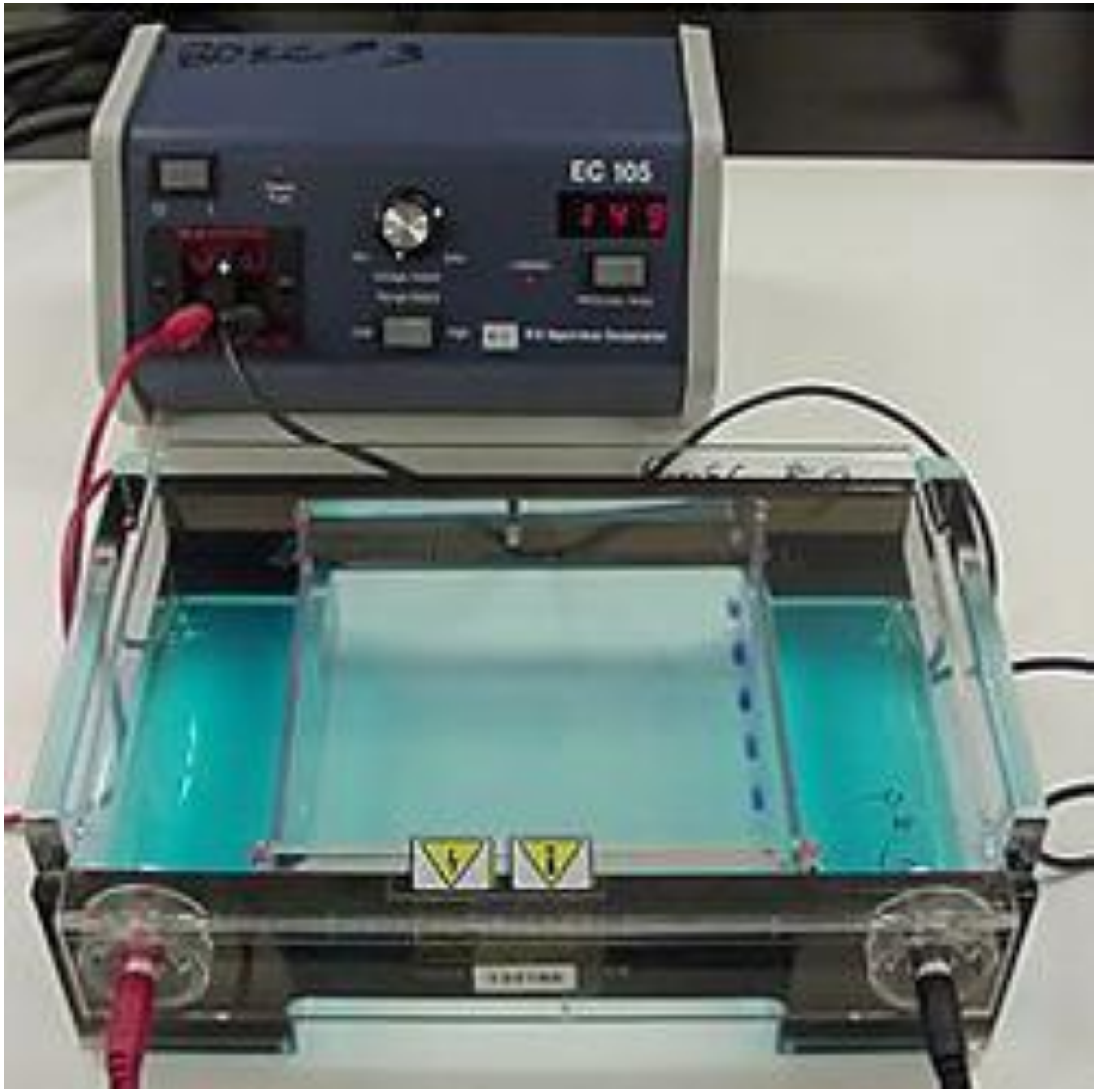
Annealing
Temperature is decreased to allow primers to base pair to complementary DNA template

Extension
Polymerase extends primer to form nascent DNA strand

Exponential Amplification
Process is repeated, and the region of interest is amplified exponentially


Cathode (–ve)
Anode (+ve)
Direction

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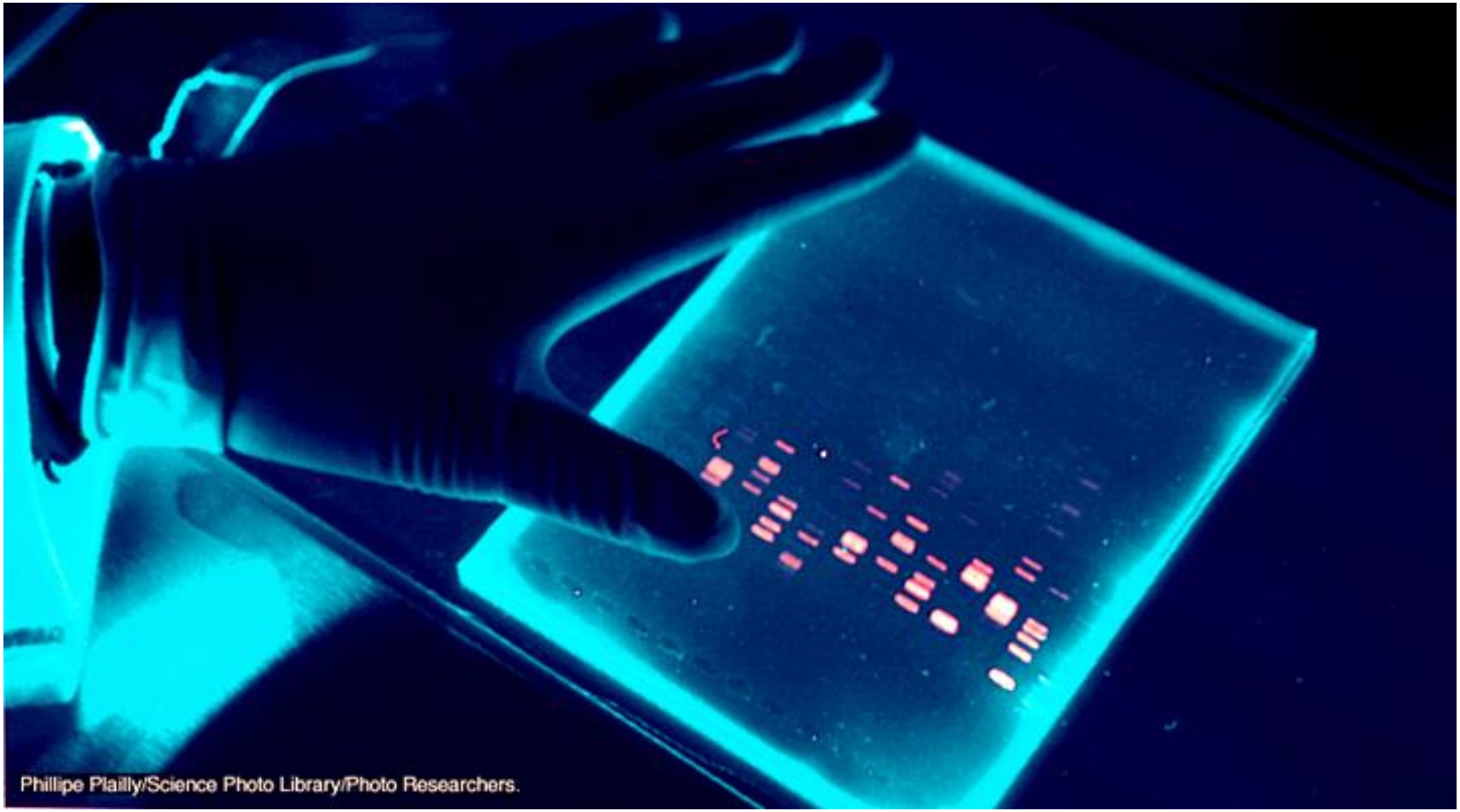
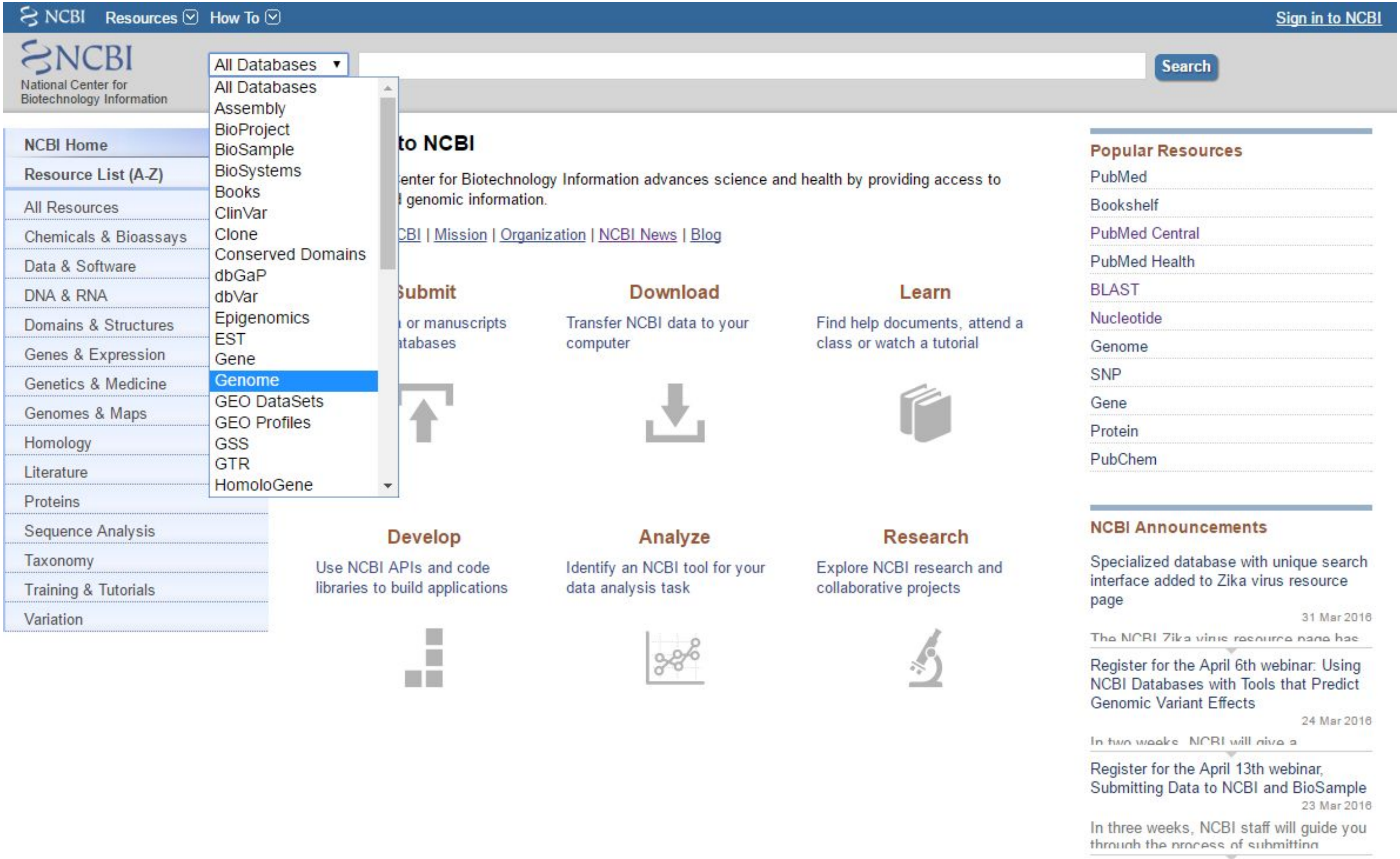


Phillipe Plailly/Science Photo Library/Photo Researchers.

Конструирование праймеров



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enter for Biotechnology Information advances science and health by providing access to
genomic information.

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Explore NCBI research and collaborative projects

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

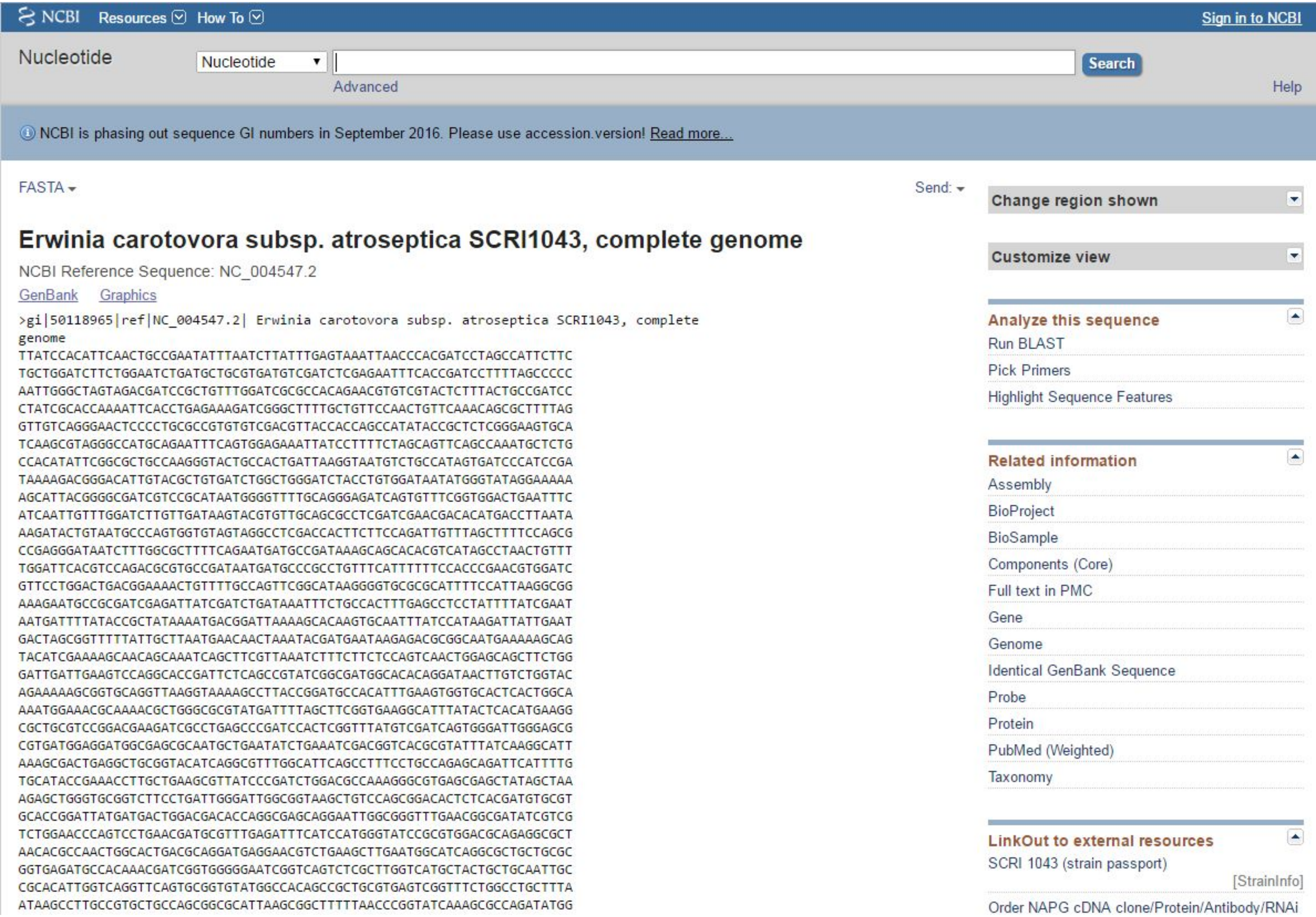
24 Mar 2016

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



NCBI is phasing out sequence GI numbers in September 2016. Please use accession.version! Read more...

Erwinia carotovora subsp. atroseptica SCRI1043, complete genome

NCBI Reference Sequence: NC_004547.2

GenBank Graphics

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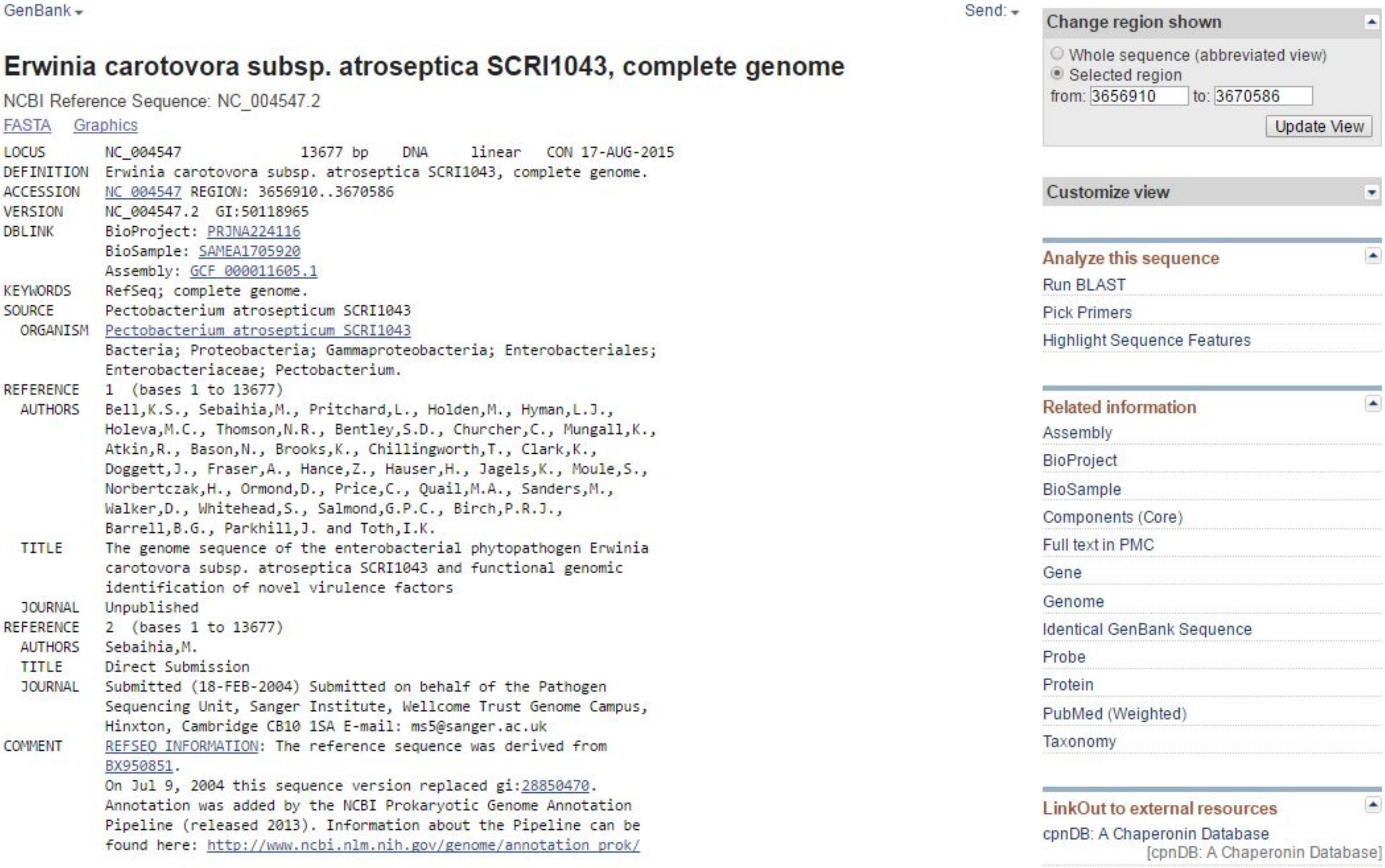

Erwinia carotovora subsp. atroseptica SCRI1043, complete genome

NCBI Reference Sequence: NC_004547.2

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            BioSample: SAMEA1705920
            Assembly: GCF_000011605.1
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SOURCE      Pectobacterium atrosepticum SCRI1043
  ORGANISM  Pectobacterium atrosepticum SCRI1043
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
            Enterobacteriaceae; Pectobacterium.
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  AUTHORS   Bell,K.S., Sebaihia,M., Pritchard,L., Holden,M., Hyman,L.J.,
            Holeva,M.C., Thomson,N.R., Bentley,S.D., Churcher,C., Mungall,K.,
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            Barrell,B.G., Parkhill,J. and Toth,I.K.
  TITLE     The genome sequence of the enterobacterial phytopathogen Erwinia
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  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 13677)
  AUTHORS   Sebaihia,M.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-FEB-2004) Submitted on behalf of the Pathogen
            Sequencing Unit, Sanger Institute, Wellcome Trust Genome Campus,
            Hinxton, Cambridge CB10 1SA E-mail: ms5@sanger.ac.uk
COMMENT     REFSEQ INFORMATION: The reference sequence was derived from
            BX950851.
            On Jul 9, 2004 this sequence version replaced gi:28850470.
            Annotation was added by the NCBI Prokaryotic Genome Annotation
            Pipeline (released 2013). Information about the Pipeline can be
            found here: http://www.ncbi.nlm.nih.gov/genome/annotation_prok/
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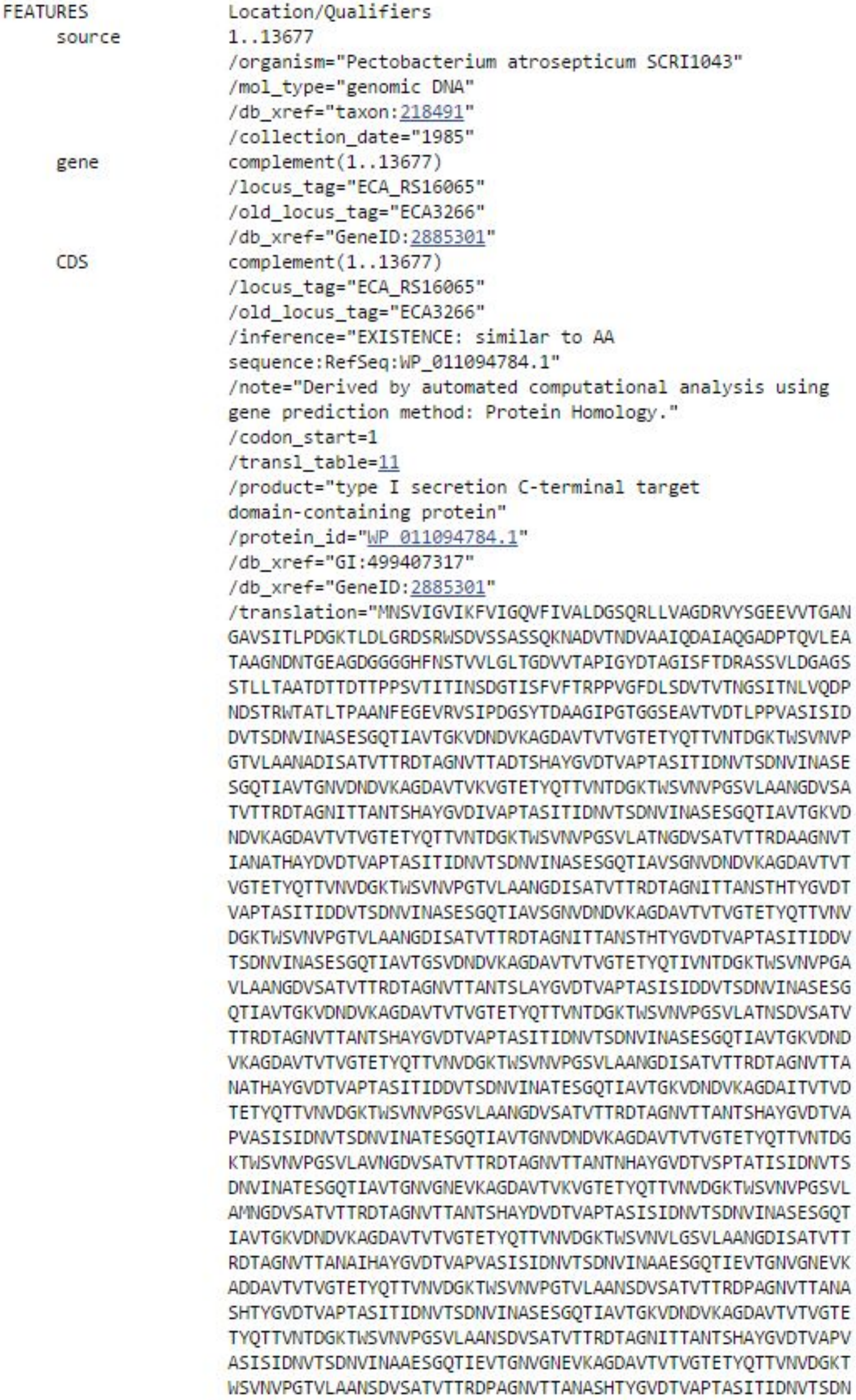
Change region shown

○ Whole sequence (abbreviated view)
● Selected region
from: 3656910 to: 3670586



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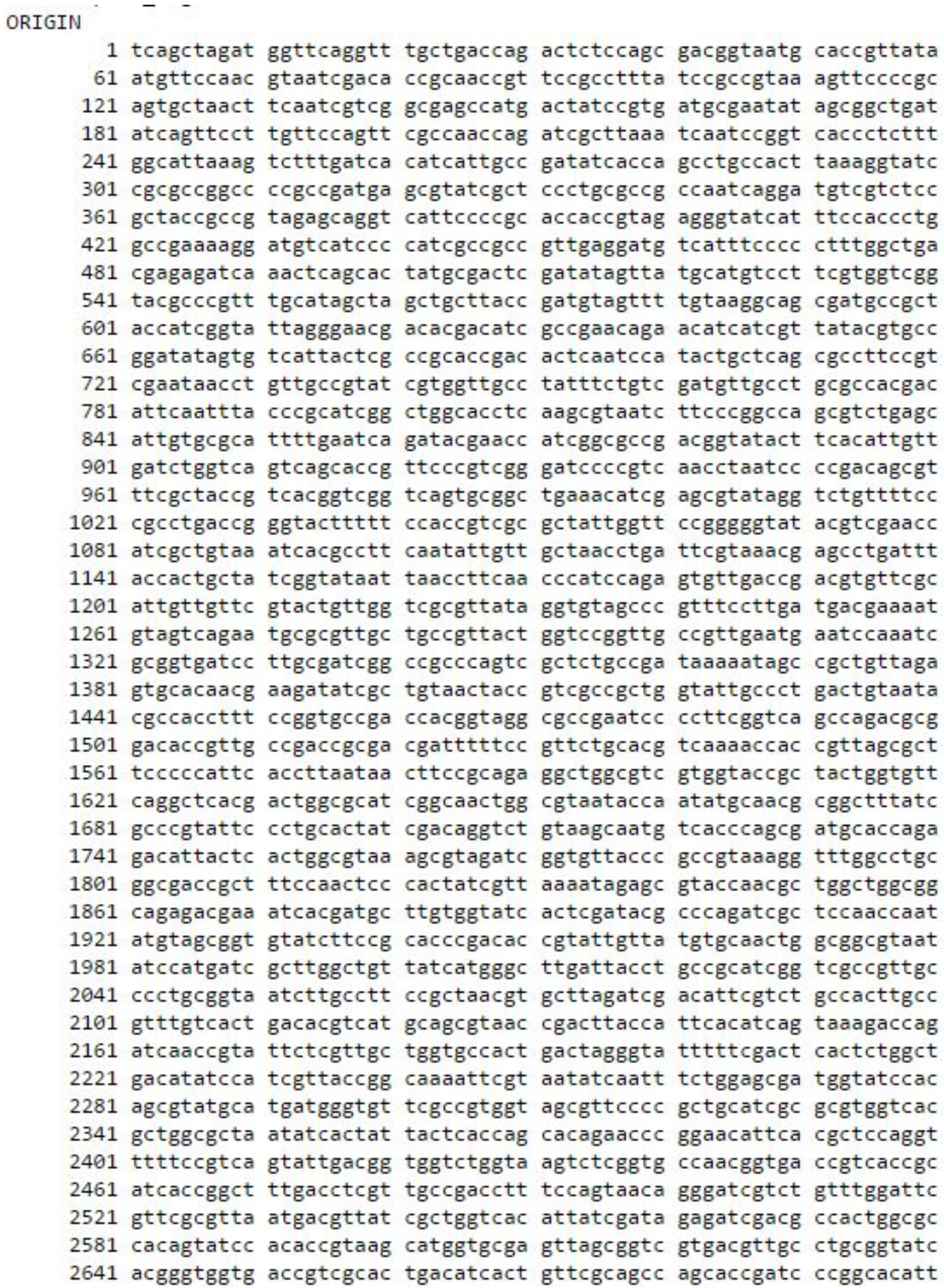

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      181 atcagttcct tgttccagtt cgccaaccag atcgcttaaa tcaatccggt caccctcttt
      241 ggcattaaag tctttgatca catcattgcc gatatcacca gcctgccact taaaggtatc
      301 cgcgccggcc ccgccgatga gcgtatcgct ccctgcgccg ccaatcagga tgtcgtctcc
      361 gctaccgccg tagagcaggt cattccccgc accaccgtag agggtatcat ttccaccctg
      421 gccgaaaagg atgtcatccc catcgccgcc gttgaggatg tcatttcccc ctttggctga
      481 cgagagatca aactcagcac tatgcgactc gatatagtta tgcatgtcct tcgtggtcgg
      541 tacgcccgtt tgcatagcta gctgcttacc gatgtagttt tgtaaggcag cgatgccgct
      601 accatcggta ttagggaacg acacgacatc gccgaacaga acatcatcgt tatacgtgcc
      661 ggatatagtg tcattactcg ccgcaccgac actcaatcca tactgctcag cgccttccgt
      721 cgaataacct gttgccgtat cgtggttgcc tatttctgtc gatgttgcct gcgccacgac
      781 attcaattta cccgcatcgg ctggcacctc aagcgtaatc ttcccggcca gcgtctgagc
      841 attgtgcgca ttttgaatca gatacgaacc atcggcgccg acggtatact tcacattgtt
      901 gatctggtca gtcagcaccg ttcccgtcgg gatccccgtc aacctaatcc ccgacagcgt
      961 ttcgctaccg tcacggtcgg tcagtgcggc tgaaacatcg agcgtatagg tctgttttcc
     1021 cgcctgaccg ggtacttttt ccaccgtcgc gctattggtt ccgggggtat acgtcgaacc
     1081 atcgctgtaa atcacgcctt caatattgtt gctaacctga ttcgtaaacg agcctgattt
     1141 accactgcta tcggtataat taaccttcaa cccatccaga gtgttgaccg acgtgttcgc
     1201 attgttgttc gtactgttgg tcgcgttata ggtgtagccc gtttccttga tgacgaaaat
     1261 gtagtcagaa tgcgcgttgc tgccgttact ggtccggttg ccgttgaatg aatccaaatc
     1321 gcggtgatcc ttgcgatcgg ccgcccagtc gctctgccga taaaaatagc cgctgttaga
     1381 gtgcacaacg aagatatcgc tgtaactacc gtcgccgctg gtattgccct gactgtaata
     1441 cgccaccttt ccggtgccga ccacggtagg cgccgaatcc ccttcggtca gccagacgcg
     1501 gacaccgttg ccgaccgcga cgatttttcc gttctgcacg tcaaaaccac cgttagcgct
     1561 tcccccattc accttaataa cttccgcaga ggctggcgtc gtggtaccgc tactggtgtt
     1621 caggctcacg actggcgcat cggcaactgg cgtaatacca atatgcaacg cggctttatc
     1681 gcccgtattc cctgcactat cgacaggtct gtaagcaatg tcacccagcg atgcaccaga
     1741 gacattactc actggcgtaa agcgtagatc ggtgttaccc gccgtaaagg tttggcctgc
     1801 ggcgaccgct ttccaactcc cactatcgtt aaaatagagc gtaccaacgc tggctggcgg
     1861 cagagacgaa atcacgatgc ttgtggtatc actcgatacg cccagatcgc tccaaccaat
     1921 atgtagcggt gtatcttccg cacccgacac cgtattgtta tgtgcaactg gcggcgtaat
     1981 atccatgatc gcttggctgt tatcatgggc ttgattacct gccgcatcgg tcgccgttgc
     2041 ccctgcggta atcttgcctt ccgctaacgt gcttagatcg acattcgtct gccacttgcc
     2101 gtttgtcact gacacgtcat gcagcgtaac cgacttacca ttcacatcag taaagaccag
     2161 atcaaccgta ttctcgttgc tggtgccact gactagggta tttttcgact cactctggct
     2221 gacatatcca tcgttaccgg caaaattcgt aatatcaatt tctggagcga tggtatccac
     2281 agcgtatgca tgatgggtgt tcgccgtggt agcgttcccc gctgcatcgc gcgtggtcac
     2341 gctggcgcta atatcactat tactcaccag cacagaaccc ggaacattca cgctccaggt
     2401 ttttccgtca gtattgacgg tggtctggta agtctcggtg ccaacggtga ccgtcaccgc
     2461 atcaccggct ttgacctcgt tgccgacctt tccagtaaca gggatcgtct gtttggattc
     2521 gttcgcgtta atgacgttat cgctggtcac attatcgata gagatcgacg ccactggcgc
     2581 cacagtatcc acaccgtaag catggtgcga gttagcggtc gtgacgttgc ctgcggtatc
     2641 acgggtggtg accgtcgcac tgacatcact gttcgcagcc agcaccgatc ccggcacatt
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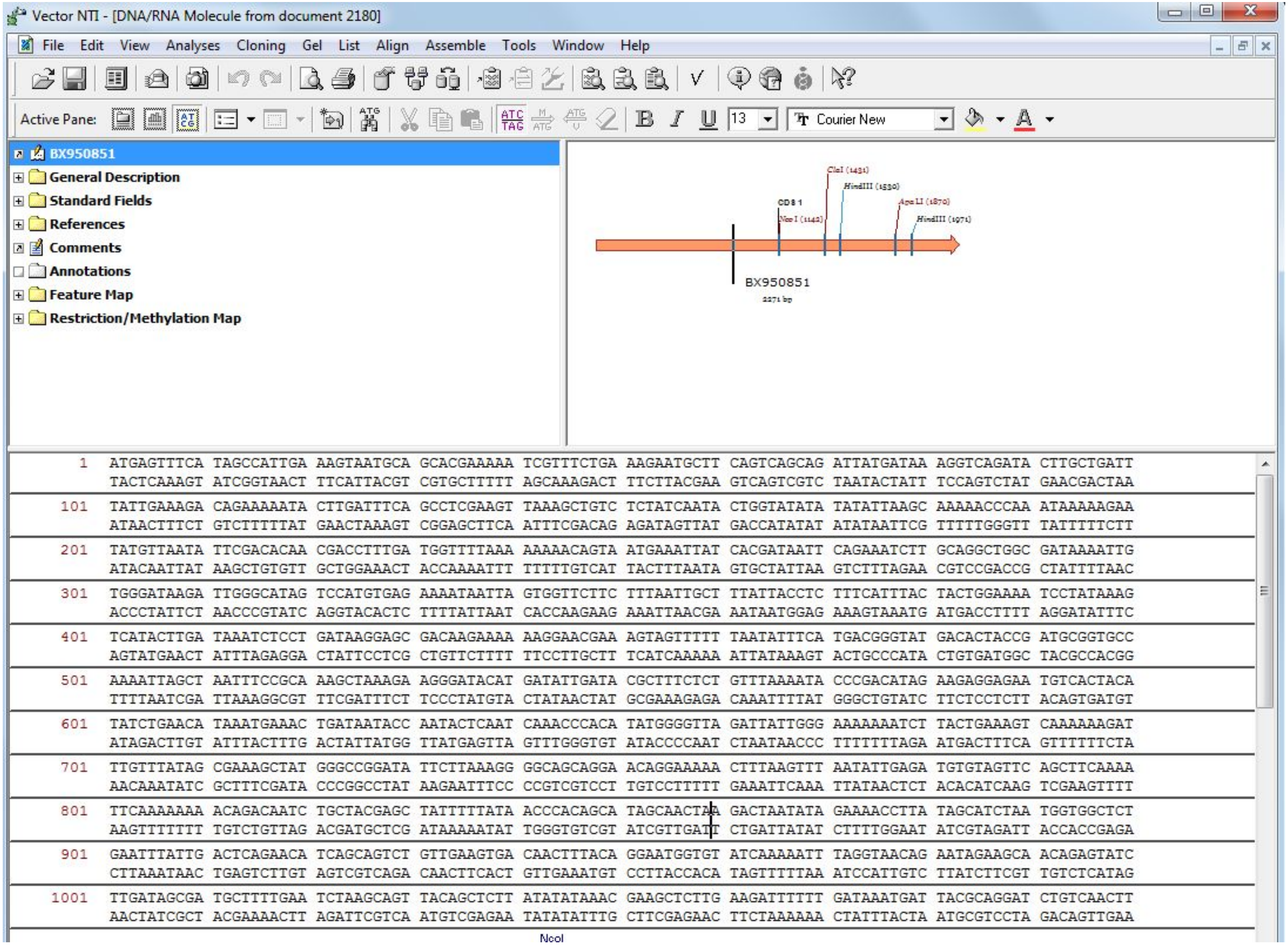


```
   1  ATGAGTTTCA TAGCCATTGA AAGTAATGCA GCACGAAAAA TCGTTTCTGA AAGAATGCTT CAGTCAGCAG ATTATGATAA AGGTCAGATA CTTGCTGATT
      TACTCAAAGT ATCGGTAACT TTCATTACGT CGTGCTTTTT AGCAAAGACT TTCTTACGAA GTCAGTCGTC TAATACTATT TCCAGTCTAT GAACGACTAA
 101  TATTGAAAGA CAGAAAAATA CTTGATTTCA GCCTCGAAGT TAAAGCTGTC TCTATCAATA CTGGTATATA TATATTAAGC AAAAACCCAA ATAAAAAGAA
      ATAACTTTCT GTCTTTTTAT GAACTAAAGT CGGAGCTTCA ATTTCGACAG AGATAGTTAT GACCATATAT ATATAATTCG TTTTTGGGTT TATTTTTCTT
 201  TATGTTAATA TTCGACACAA CGACCTTTGA TGGTTTTAAA AAAAACAGTA ATGAAATTAT CACGATAATT CAGAAATCTT GCAGGCTGGC GATAAAATTG
      ATACAATTAT AAGCTGTGTT GCTGGAAACT ACCAAAATTT TTTTTGTCAT TACTTTAATA GTGCTATTAA GTCTTTAGAA CGTCCGACCG CTATTTTAAC
 301  TGGGATAAGA TTGGGCATAG TCCATGTGAG AAAATAATTA GTGGTTCTTC TTTAATTGCT TTATTACCTC TTTCATTTAC TACTGGAAAA TCCTATAAAG
      ACCCTATTCT AACCCGTATC AGGTACACTC TTTTATTAAT CACCAAGAAG AAATTAACGA AATAATGGAG AAAGTAAATG ATGACCTTTT AGGATATTTC
 401  TCATACTTGA TAAATCTCCT GATAAGGAGC GACAAGAAAA AAGGAACGAA AGTAGTTTTT TAATATTTCA TGACGGGTAT GACACTACCG ATGCGGTGCC
      AGTATGAACT ATTTAGAGGA CTATTCCTCG CTGTTCTTTT TTCCTTGCTT TCATCAAAAA ATTATAAAGT ACTGCCCATA CTGTGATGGC TACGCCACGG
 501  AAAATTAGCT AATTTCCGCA AAGCTAAAGA AGGGATACAT GATATTGATA CGCTTTCTCT GTTTAAAATA CCCGACATAG AAGAGGAGAA TGTCACTACA
      TTTTAATCGA TTAAAGGCGT TTCGATTTCT TCCCTATGTA CTATAACTAT GCGAAAGAGA CAAATTTTAT GGGCTGTATC TTCTCCTCTT 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 GAAAACCTTA TAGCATCTAA TGGTGGCTCT
      AAGTTTTTTT TGTCTGTTAG ACGATGCTCG ATAAAAATAT TGGGTGTCGT ATCGTTGATT CTGATTATAT CTTTTGGAAT 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 GAAGCTCTTG AAGATTTTTT GATAAATGAT TACGCAGGAT CTGTCAACTT
      AACTATCGCT ACGAAAACTT AGATTCGTCA ATGTCGAGAA TATATATTTG CTTCGAGAAC TTCTAAAAAA CTATTTACTA ATGCGTCCTA GACAGTTGAA
                                                        NcoI
```

Характеристики праймеров

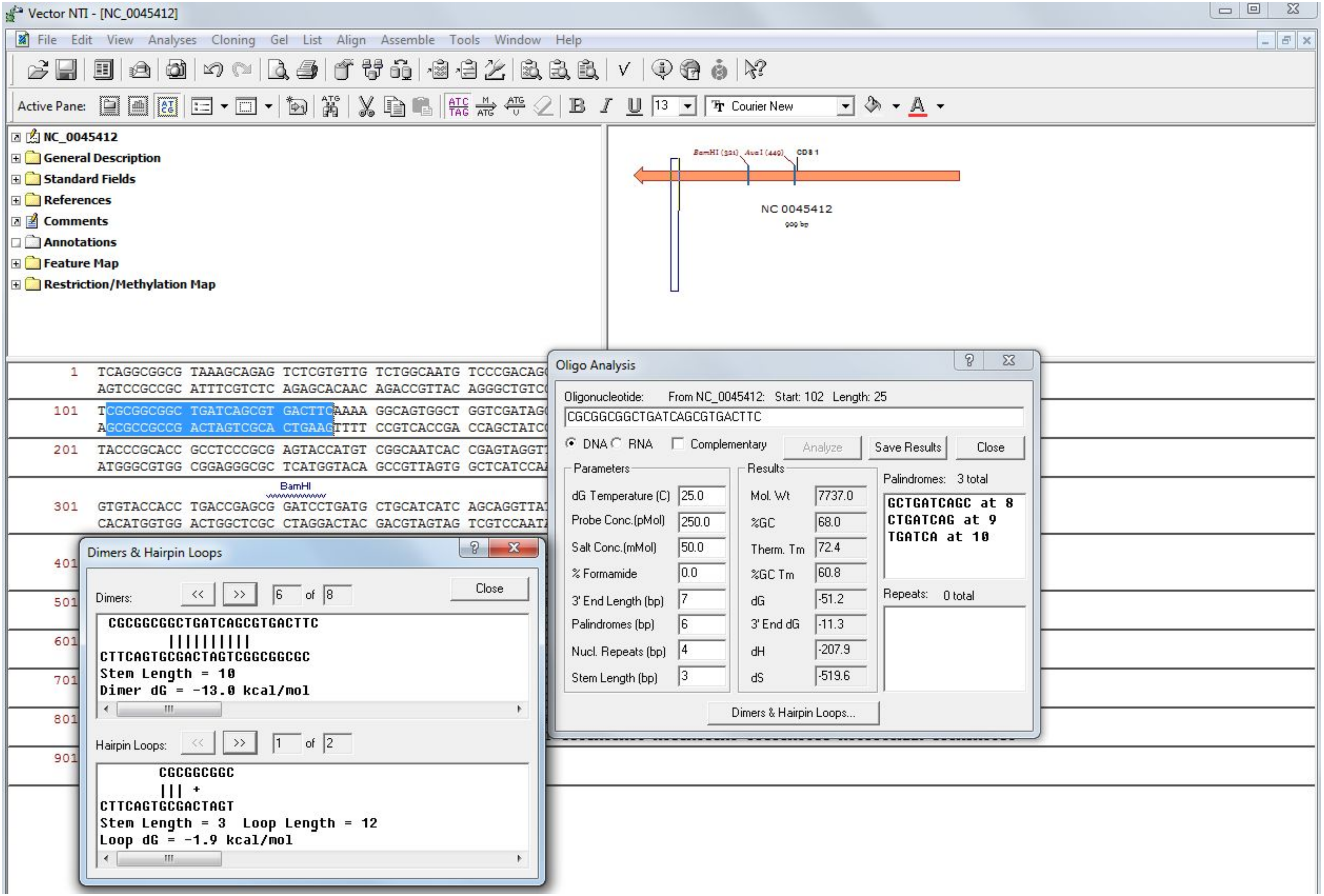
Комплементарность мишени

Высокое GC содержание

На 3’-конце праймера G или C

Температура отжига 55-60° C

Отсутствие вторичных структур (шпилек и димеров)



```
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      AGTCCGCCGC ATTTCGTCTC AGAGCACAAC AGACCGTTAC AGGGCTGTC

101   TCGCGGCGGC TGATCAGCGT GACTTCAAAA GGCAGTGGCT GGTCGATAG
      AGCGCCGCCG ACTAGTCGCA CTGAAGTTTT CCGTCACCGA CCAGCTATC

201   TACCCGCACC GCCTCCCGCG AGTACCATGT CGGCAATCAC CGAGTAGGT
      ATGGGCGTGG CGGAGGGCGC TCATGGTACA GCCGTTAGTG GCTCATCCA

                                 BamHI
301   GTGTACCACC TGACCGAGCG GATCCTGATG CTGCATCATC AGCAGGTTA
      CACATGGTGG ACTGGCTCGC CTAGGACTAC GACGTAGTAG TCGTCCAAT

401
501
601
701
801
901
```

Oligo Analysis

Oligonucleotide: From NC_0045412: Start: 102 Length: 25

CGCGGCGGCTGATCAGCGTGACTTC

● DNA ○ RNA ☐ Complementary

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

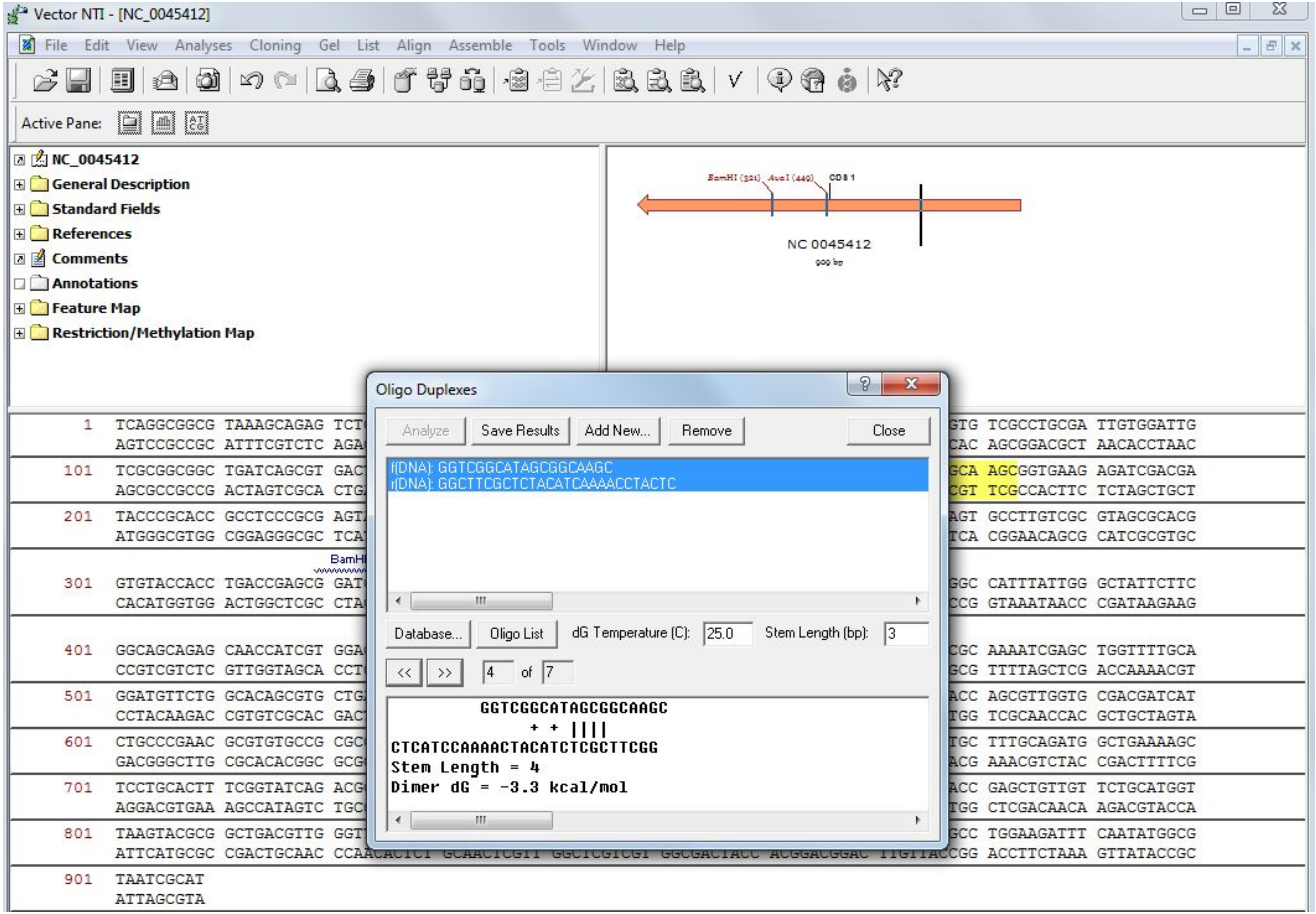
Dimers & Hairpin Loops

Dimers: << >> 6 of 8

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

Hairpin Loops: << >> 1 of 2

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



f(DNA): GGTCGGCATAGCGGCAAGC
r(DNA): GGCTTCGCTCTACATCAAAACCTACTC

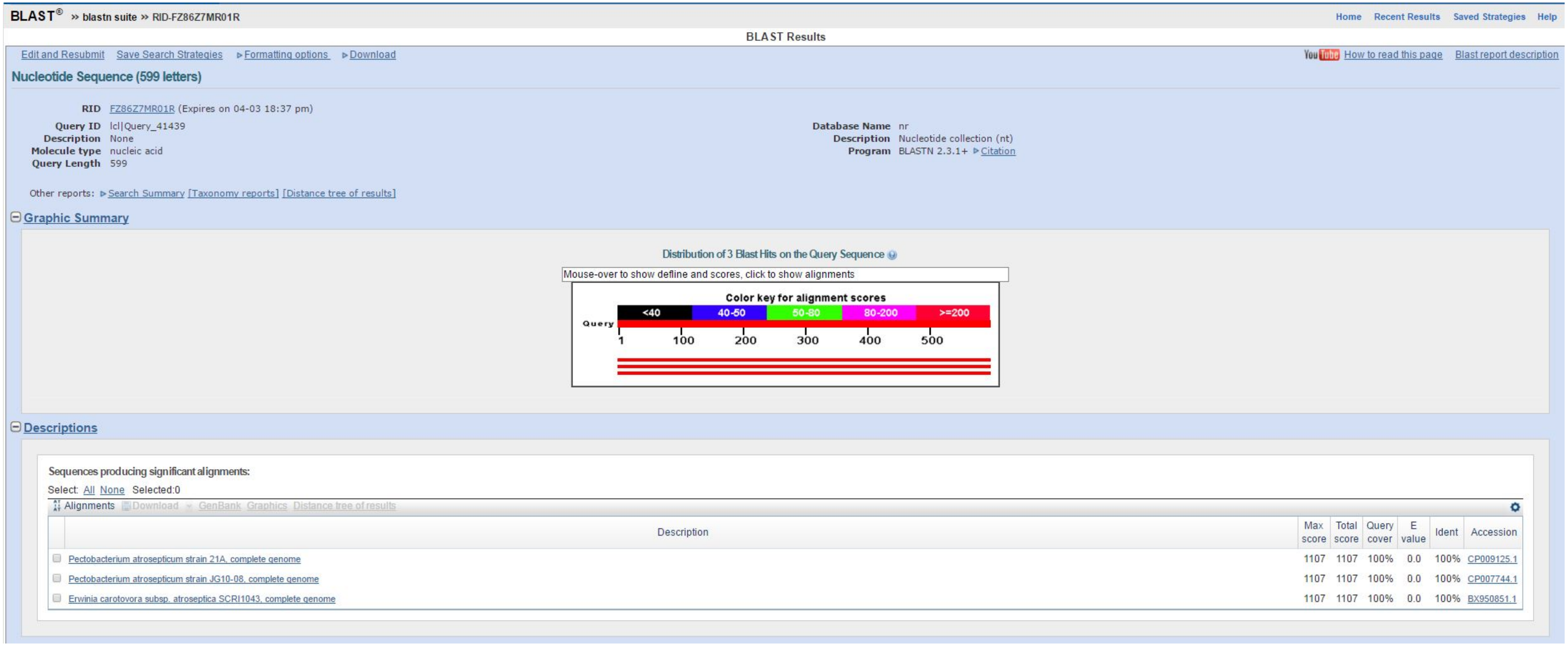
dG Temperature (C): 25.0 Stem Length (bp): 3

4 of 7

```
          GGTCGGCATAGCGGCAAGC
                + + ||||
CTCATCCAAAACTACATCTCGCTTCGG
Stem Length = 4
Dimer dG = -3.3 kcal/mol
```

```
  1  TCAGGCGGCG TAAAGCAGAG TCT                                                  GTG TCGCCTGCGA TTGTGGATTG
     AGTCCGCCGC ATTTCGTCTC AGA                                                  CAC AGCGGACGCT AACACCTAAC
101  TCGCGGCGGC TGATCAGCGT GAC                                                  GCA AGCGGTGAAG AGATCGACGA
     AGCGCCGCCG ACTAGTCGCA CTG                                                  CGT TCGCCACTTC TCTAGCTGCT
201  TACCCGCACC GCCTCCCGCG AGT                                                  AGT GCCTTGTCGC GTAGCGCACG
     ATGGGCGTGG CGGAGGGCGC TCA                                                  TCA CGGAACAGCG CATCGCGTGC
301  GTGTACCACC TGACCGAGCG GAT                                                  GGC CATTTATTGG GCTATTCTTC
     CACATGGTGG ACTGGCTCGC CTA                                                  CCG GTAAATAACC CGATAAGAAG
401  GGCAGCAGAG CAACCATCGT GGA                                                  CGC AAAATCGAGC TGGTTTTGCA
     CCGTCGTCTC GTTGGTAGCA CCT                                                  GCG TTTTAGCTCG ACCAAAACGT
501  GGATGTTCTG GCACAGCGTG CTG                                                  ACC AGCGTTGGTG CGACGATCAT
     CCTACAAGAC CGTGTCGCAC GAC                                                  TGG TCGCAACCAC GCTGCTAGTA
601  CTGCCCGAAC GCGTGTGCCG CGC                                                  TGC TTTGCAGATG GCTGAAAAGC
     GACGGGCTTG CGCACACGGC GCG                                                  ACG AAACGTCTAC CGACTTTTCG
701  TCCTGCACTT TCGGTATCAG ACG                                                  ACC GAGCTGTTGT TCTGCATGGT
     AGGACGTGAA AGCCATAGTC TGC                                                  TGG CTCGACAACA AGACGTACCA
801  TAAGTACGCG GCTGACGTTG GGT                                                  GCC TGGAAGATTT CAATATGGCG
     ATTCATGCGC CGACTGCAAC CCAACACTCT GCAACTCGTT GGCTCGTCGT GGCGACTACC ACGGACGGAC TTGTTACCGG ACCTTCTAAA GTTATACCGC
901  TAATCGCAT
     ATTAGCGTA
```

BLAST



Nucleotide Sequence (599 letters)

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

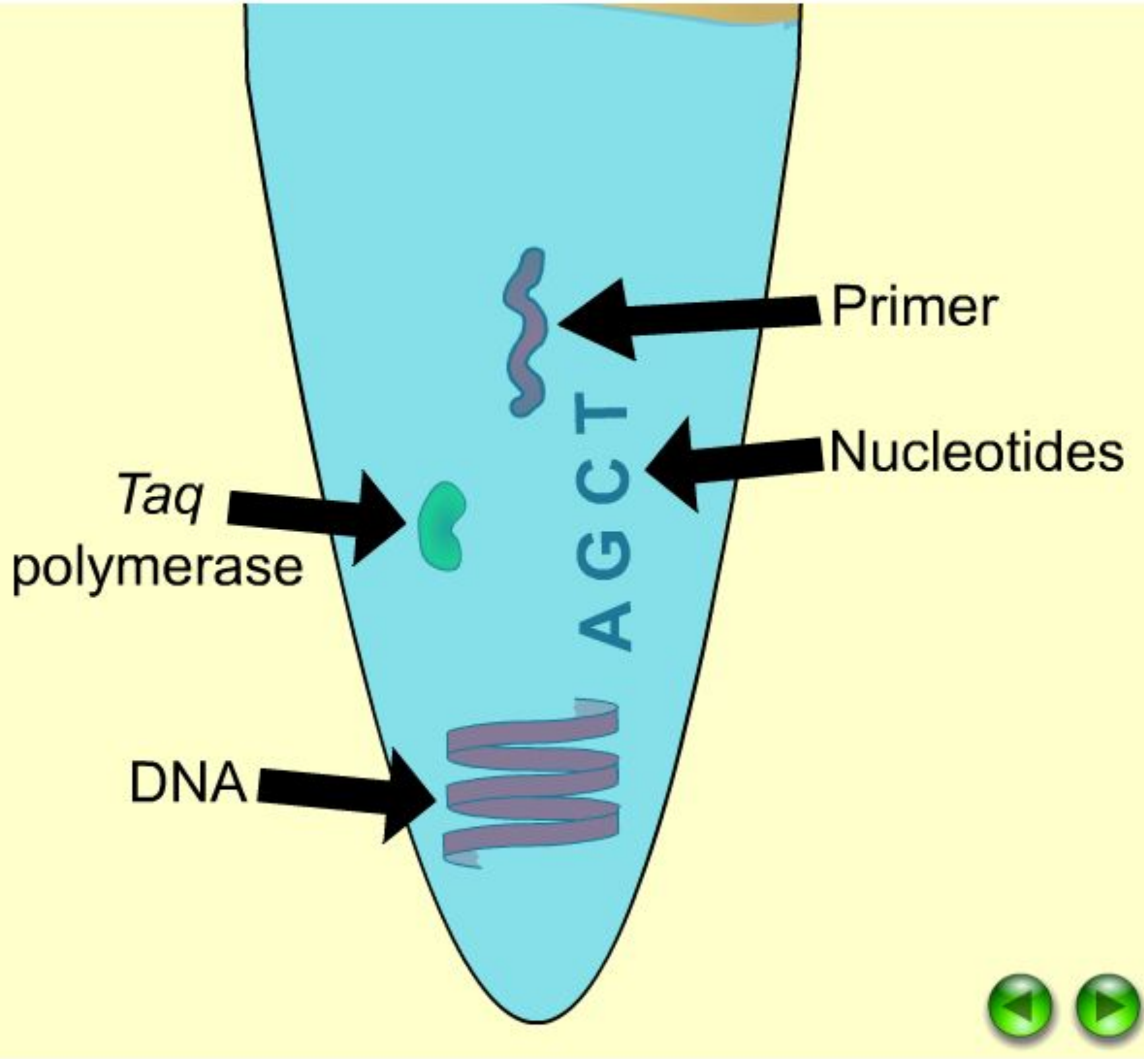
Database Name	nr
Description	Nucleotide collection (nt)
Program	BLASTN 2.3.1+ Citation

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

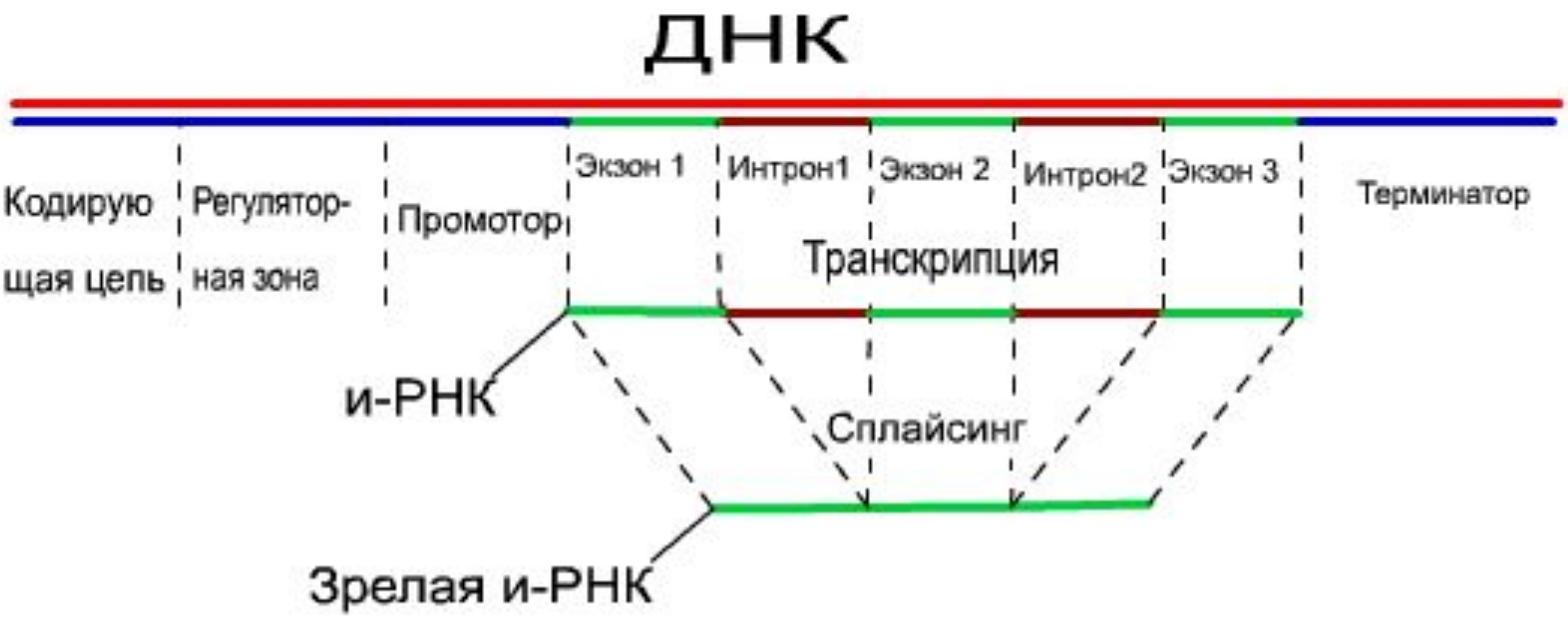
Sequences producing significant alignments:

Select: All None Selected:0

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Pectobacterium atrosepticum strain 21A, complete genome	1107	1107	100%	0.0	100%	CP009125.1
☐	Pectobacterium atrosepticum strain JG10-08, complete genome	1107	1107	100%	0.0	100%	CP007744.1
☐	Erwinia carotovora subsp. atroseptica SCRI1043, complete genome	1107	1107	100%	0.0	100%	BX950851.1


Primer
Nucleotides
Taq polymerase
A G C T
DNA

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



ДНК

Кодирующая цепь

Регуляторная зона

Промотор

Экзон 1

Интрон1

Экзон 2

Интрон2

Экзон 3

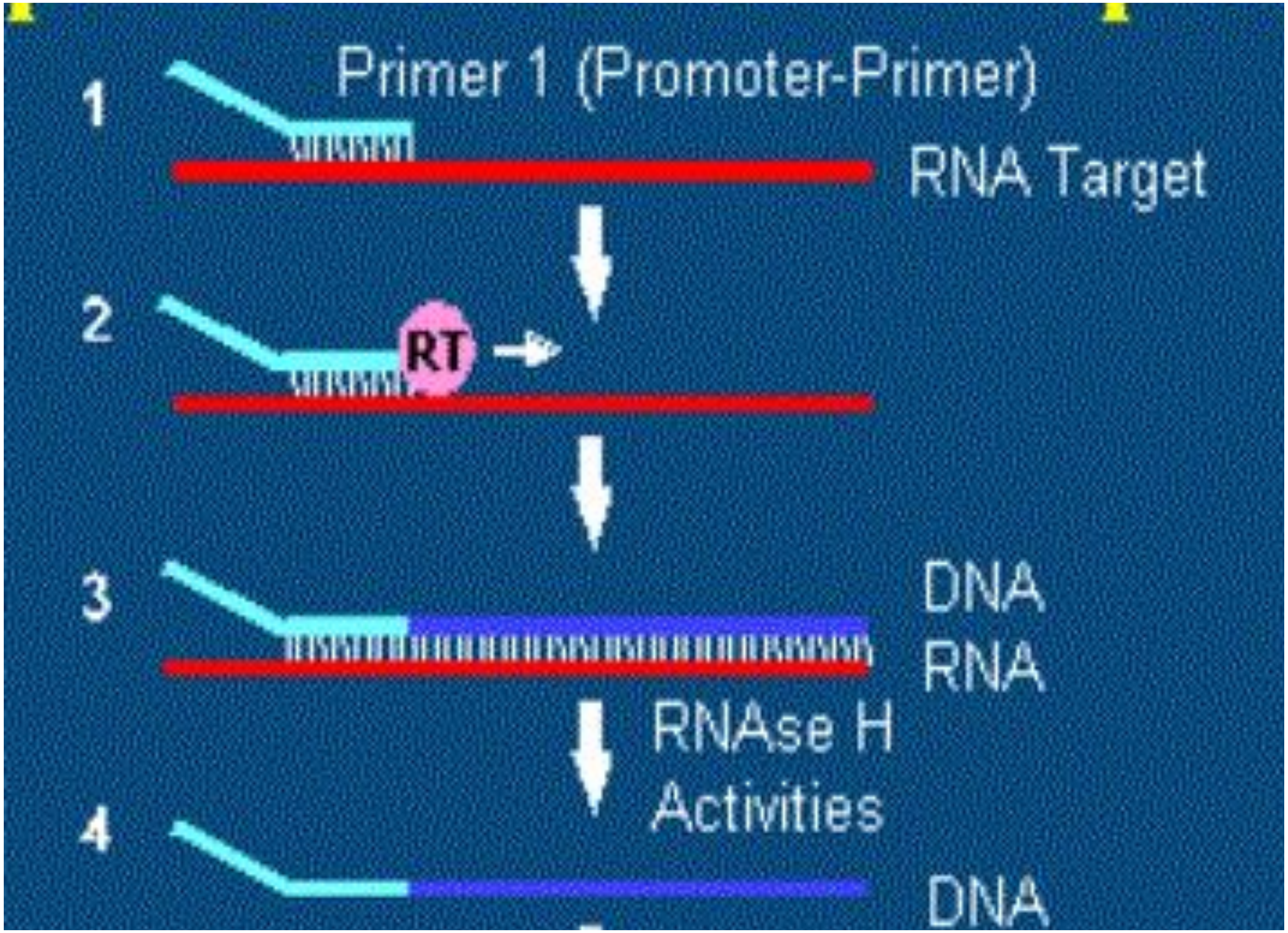
Терминатор

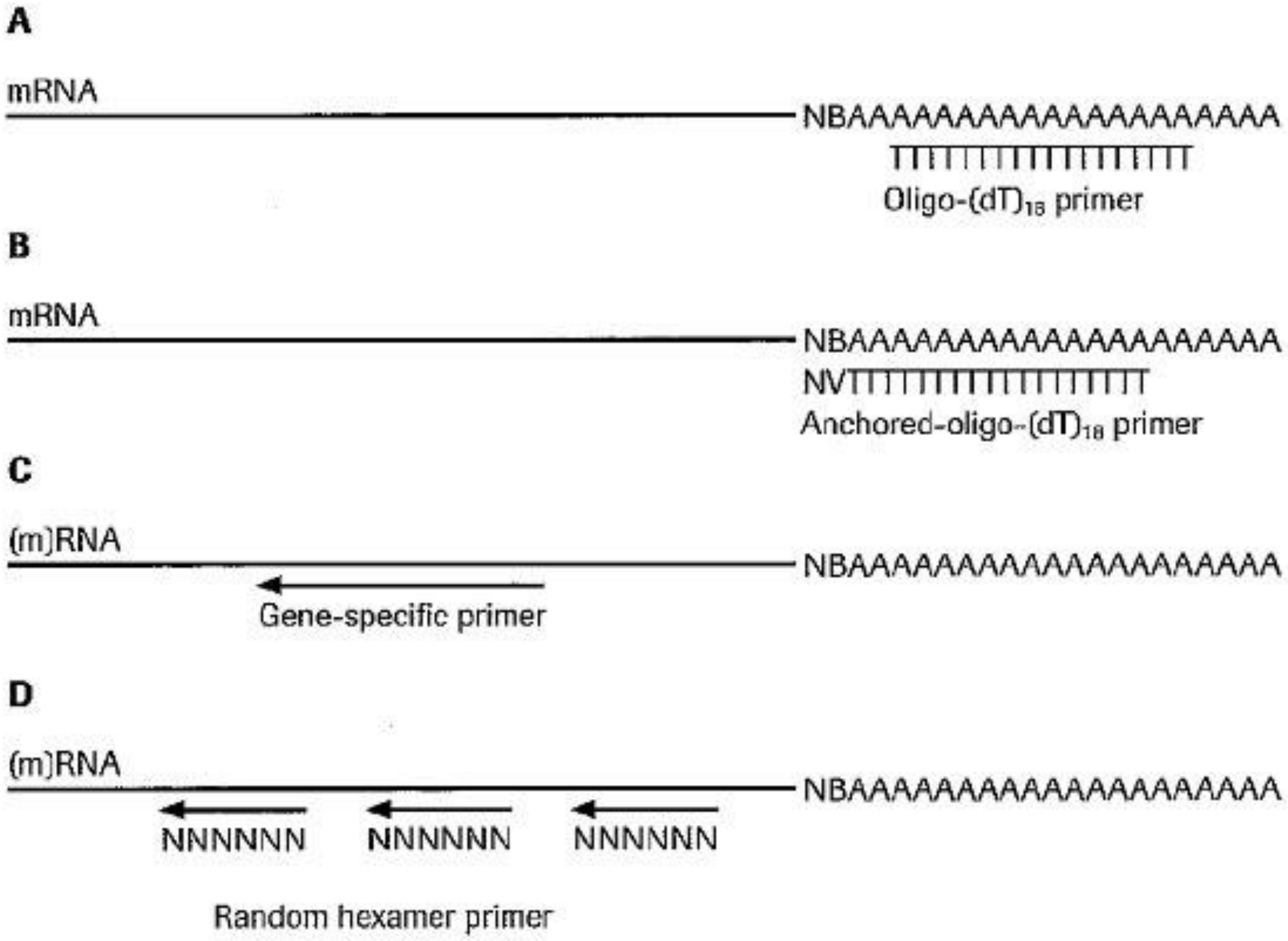
Транскрипция

и-РНК

Сплайсинг

Зрелая и-РНК


1
Primer 1 (Promoter-Primer)
RNA Target
2
RT
3
DNA
RNA
RNAse H
Activities
4
DNA


A
mRNA
NBAAAAAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTTTTT
Oligo-(dT)18 primer
B
mRNA
NBAAAAAAAAAAAAAAAAAAAA
NVTTTTTTTTTTTTTTTTTT
Anchored-oligo-(dT)18 primer
C
(m)RNA
NBAAAAAAAAAAAAAAAAAAAA
Gene-specific primer
D
(m)RNA
NBAAAAAAAAAAAAAAAAAAAA
NNNNNN
NNNNNN
NNNNNN
Random hexamer primer

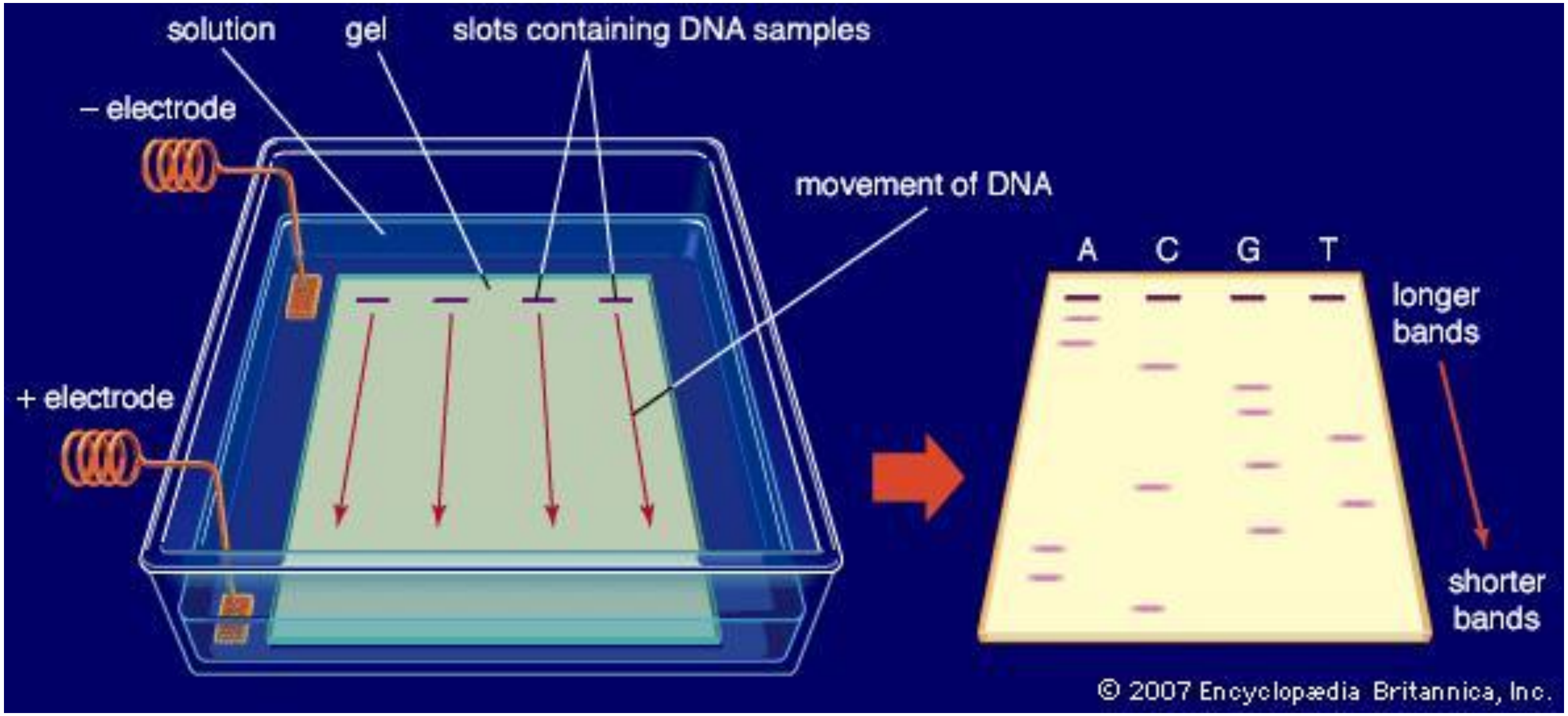
Мы провели ПЦР, чтобы получить целевой фрагмент ДНК

Нужно выяснить получился ли у нас ожидаемый продукт

Электрофорез

Электрофоретическое разделение нуклеиновых кислот

Электрофорез ДНК в агарозном геле



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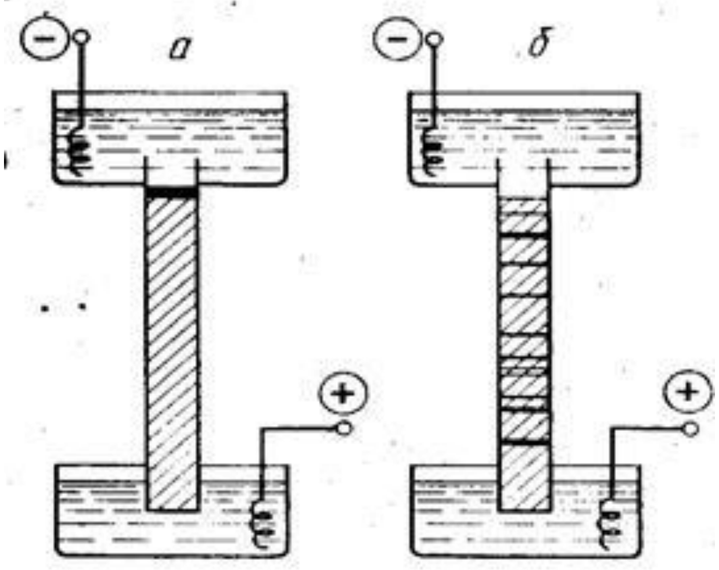
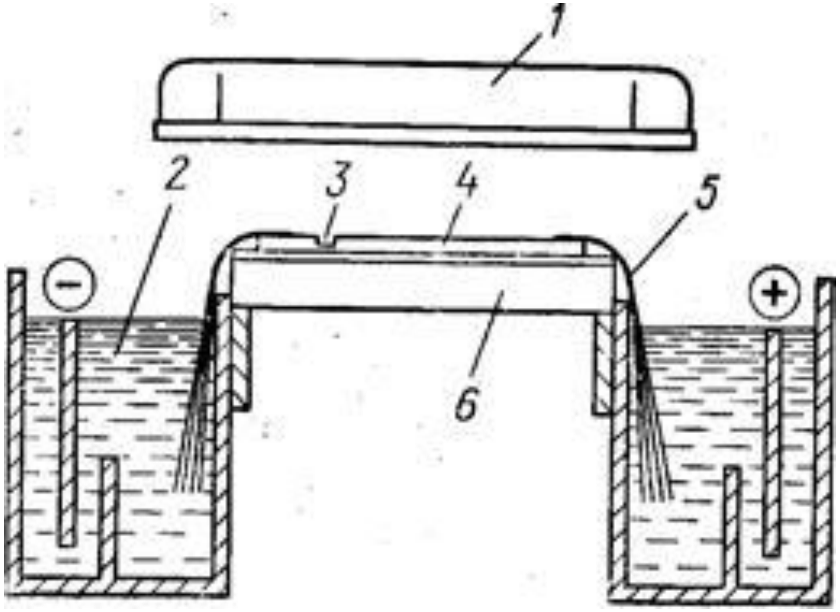
Электрофорез белков

Разделение молекул в электрическом поле





Разделение макромолекул в зависимости от:

размера,

пространственной конфигурации,

электрического заряда

Разделение проводят в полиакриламидном геле

Акриламид

(CH2 = CH — CONH2)

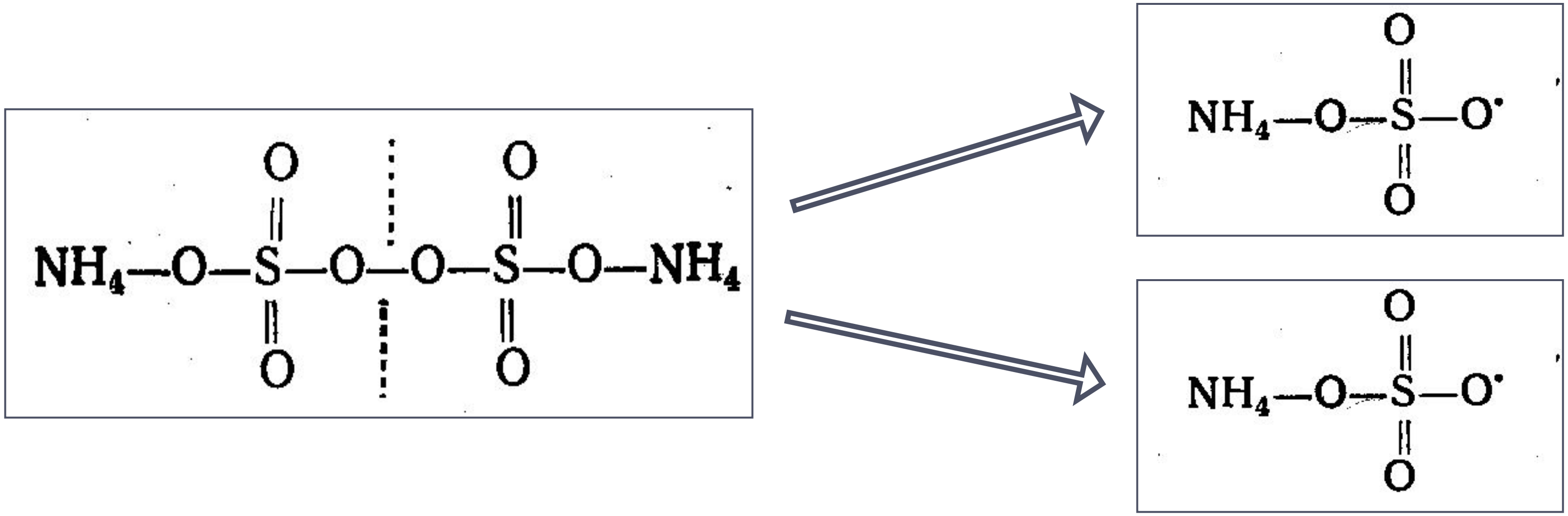
NN'-Метиленбисакриламид

(CH2 = CH — CONH)2 – CH2

Буферные растворы

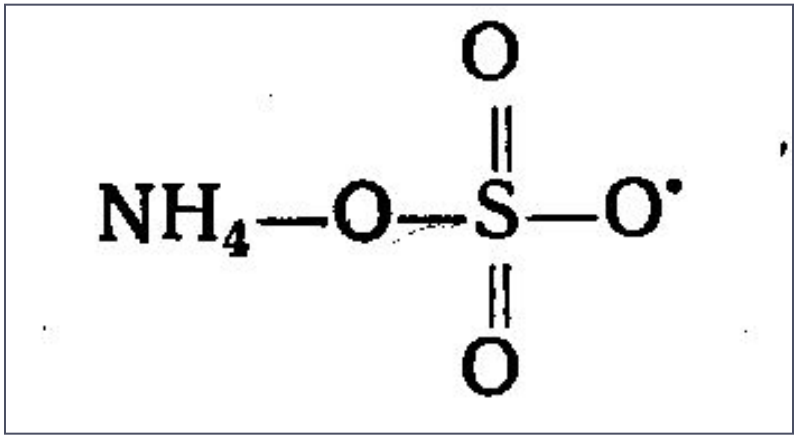
Индукция полимеризации акриламида

Персульфат аммония

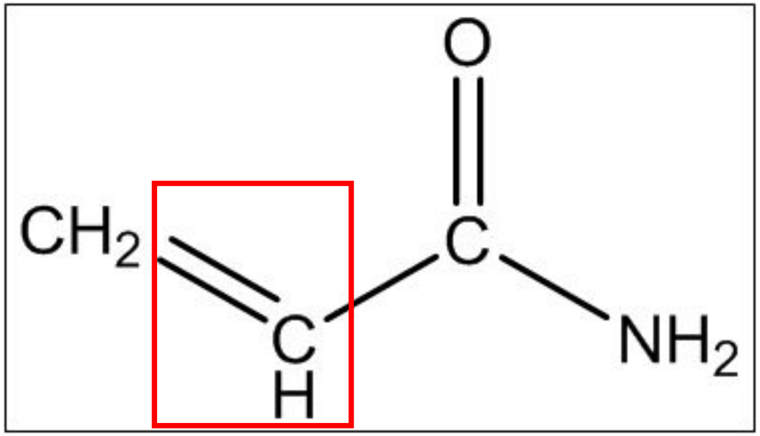


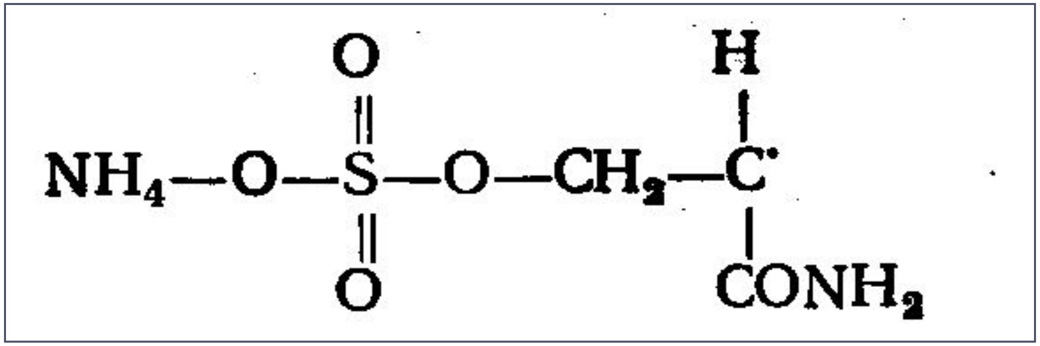
$$NH_4-O-\overset{O}{\underset{O}{\overset{\|}{\underset{\|}{S}}}}-O-O-\overset{O}{\underset{O}{\overset{\|}{\underset{\|}{S}}}}-O-NH_4 \Rightarrow NH_4-O-\overset{O}{\underset{O}{\overset{\|}{\underset{\|}{S}}}}-O^{\bullet} \quad NH_4-O-\overset{O}{\underset{O}{\overset{\|}{\underset{\|}{S}}}}-O^{\bullet}$$

Полимеризация акриламида



$$NH_4-O-\overset{O}{\overset{\|}{\underset{\underset{O}{\|}}{S}}}-O^{\bullet}$$



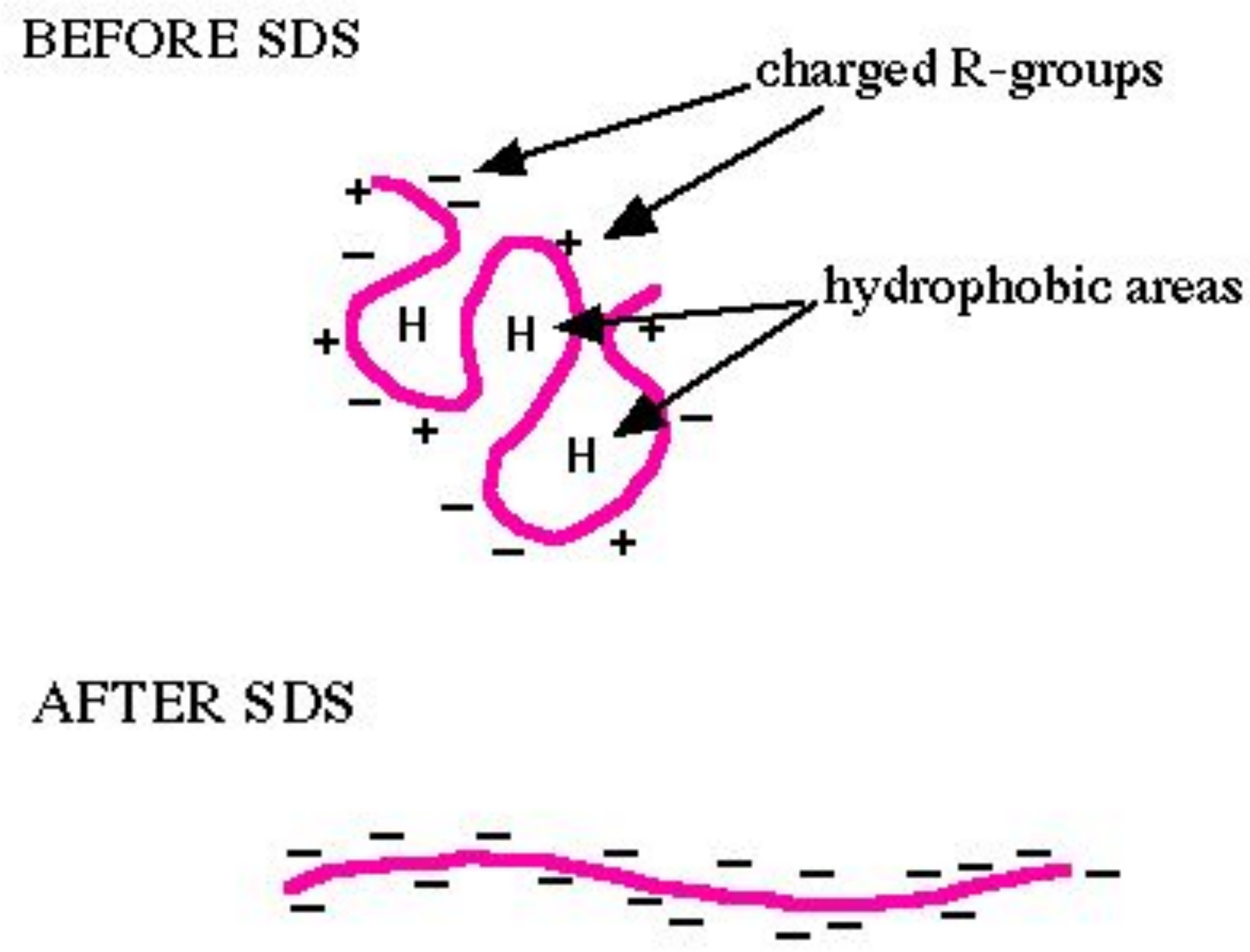


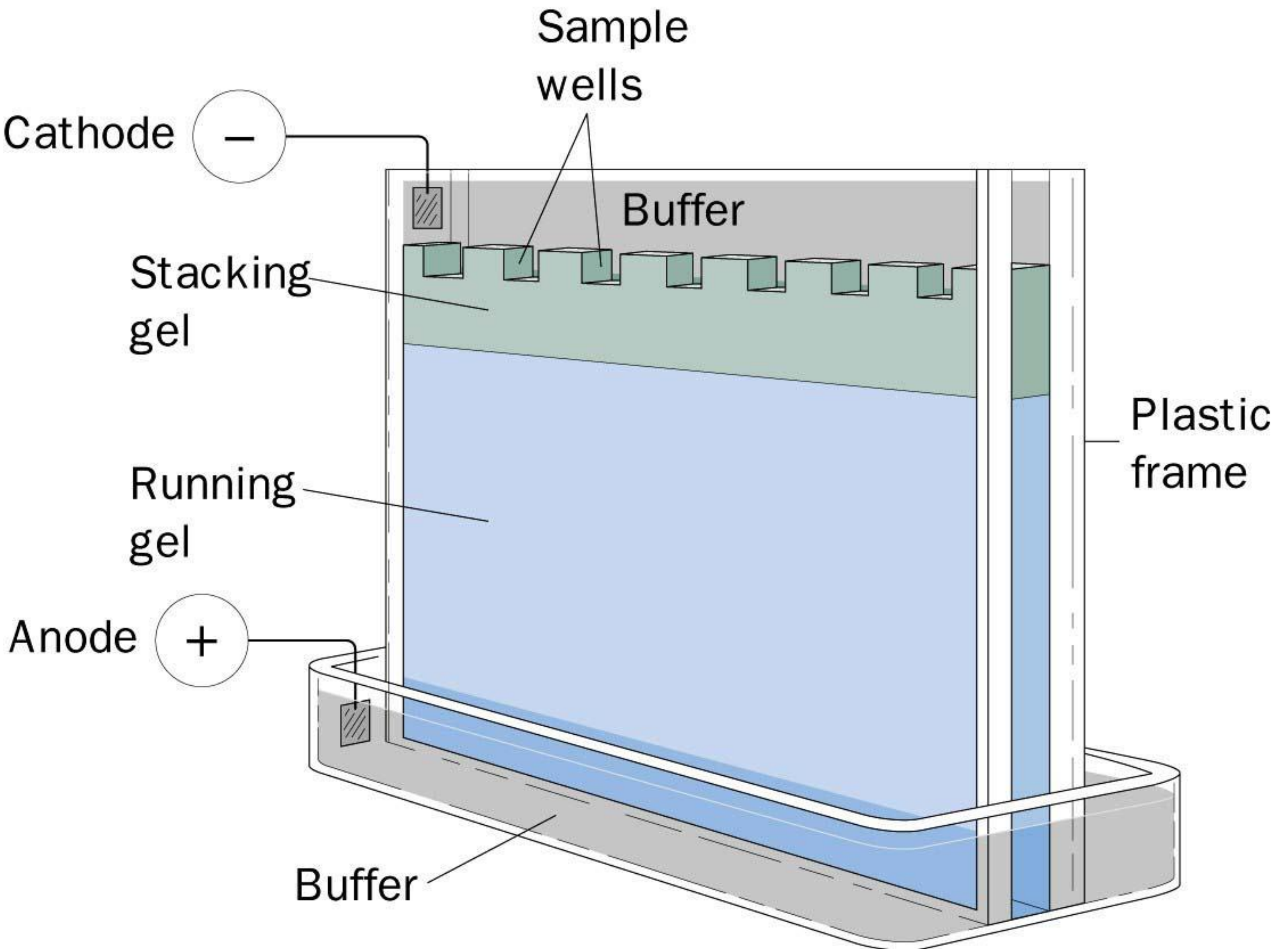
$$NH_4-O-\overset{O}{\overset{\|}{\underset{\underset{O}{\|}}{S}}}-O-CH_2-\overset{H}{\overset{|}{\underset{\underset{CONH_2}{|}}{C^{\bullet}}}}$$

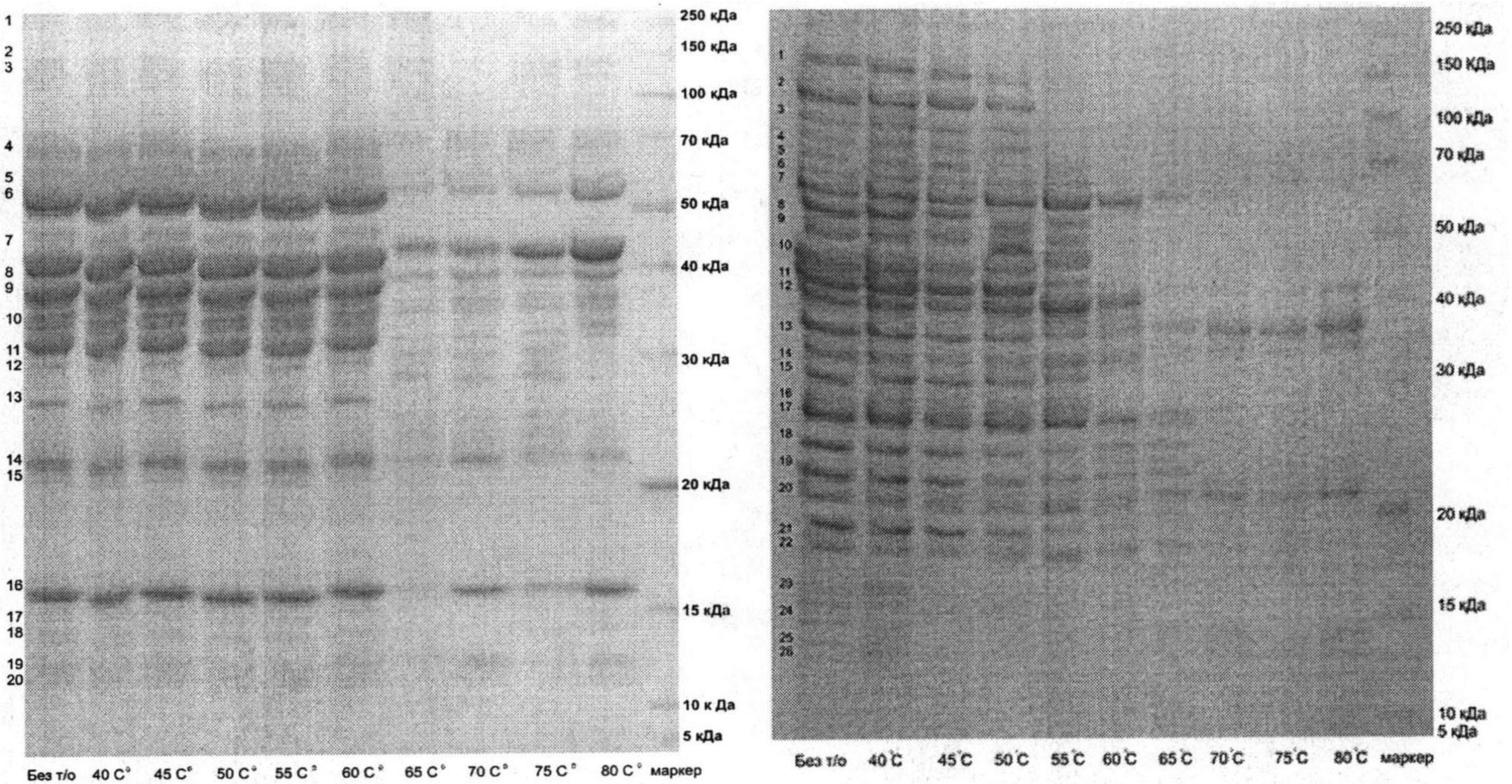
Катализатор процесса полимеризации

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

Денатурация белка с помощью ДДС



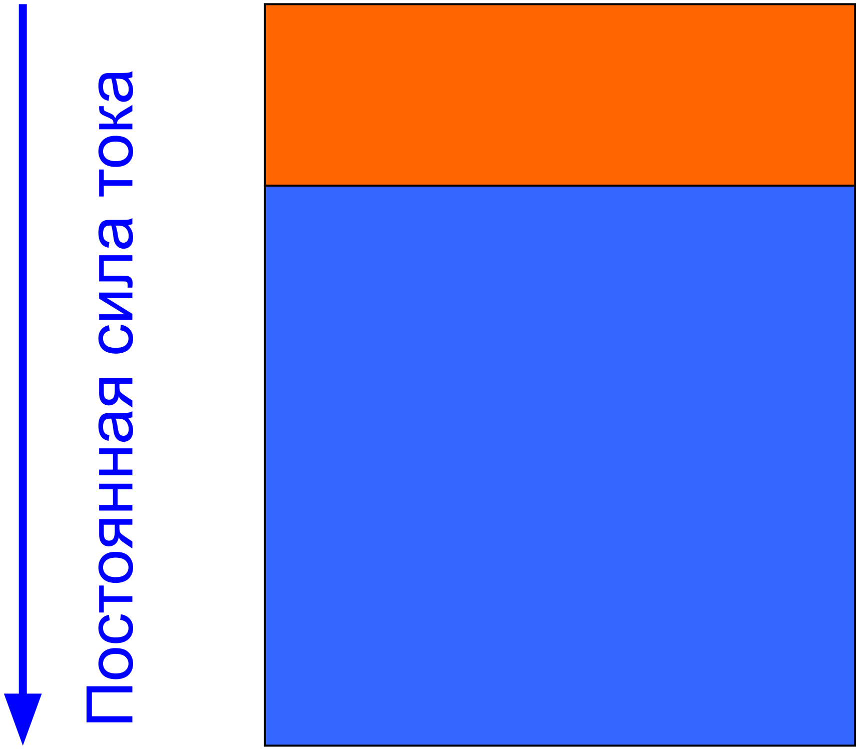

Sample wells
Cathode –
Buffer
Stacking gel
Plastic frame
Running gel
Anode +
Buffer


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
250 кДа
150 кДа
100 кДа
70 кДа
50 кДа
40 кДа
30 кДа
20 кДа
15 кДа
10 к Да
5 кДа
Без т/о
40 С°
45 С°
50 С°
55 С°
60 С°
65 С°
70 С°
75 С°
80 С°
маркер
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
250 кДа
150 КДа
100 кДа
70 кДа
50 кДа
40 кДа
30 кДа
20 кДа
15 кДа
10 кДа
5 кДа
Без т/о
40°С
45°С
50°С
55°С
60°С
65°С
70°С
75°С
80°С
маркер

а)

б)

Фиг. 2



Постоянная сила тока

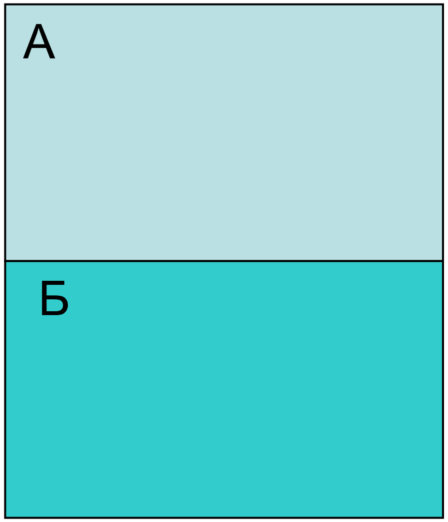
Высокая напряженность

Низкая напряженность

Подвижность ионов

Заряд ионов

Изоэлектрическая точка

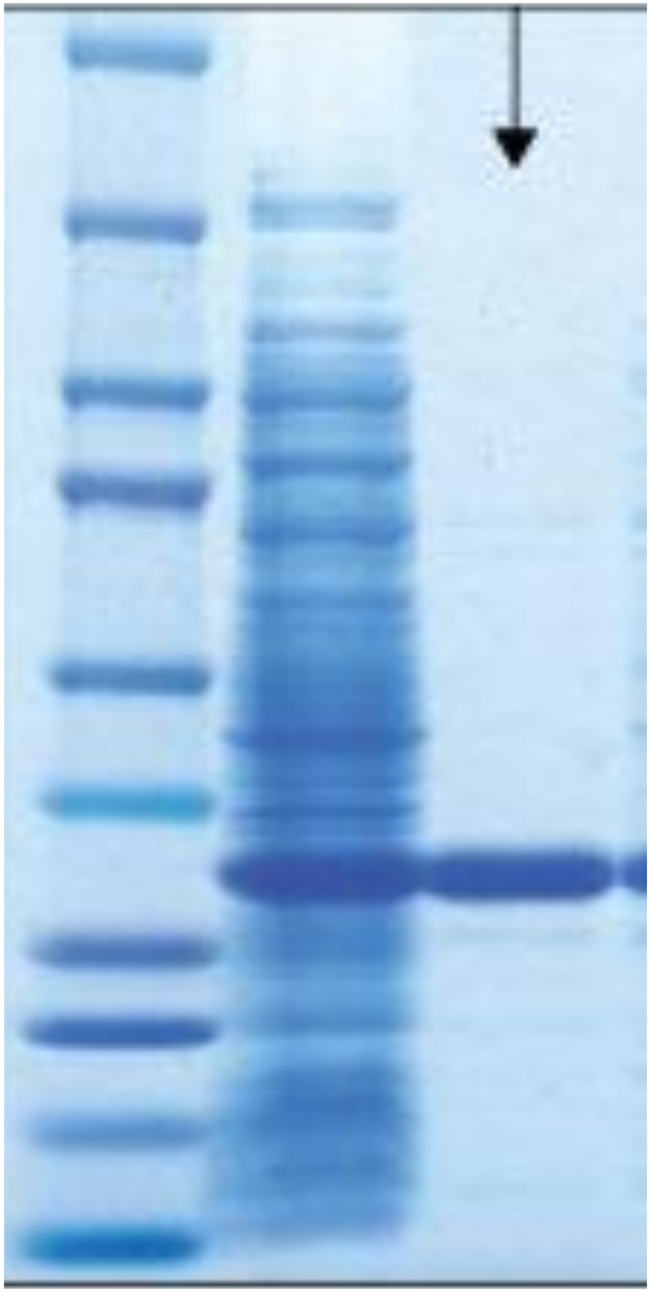


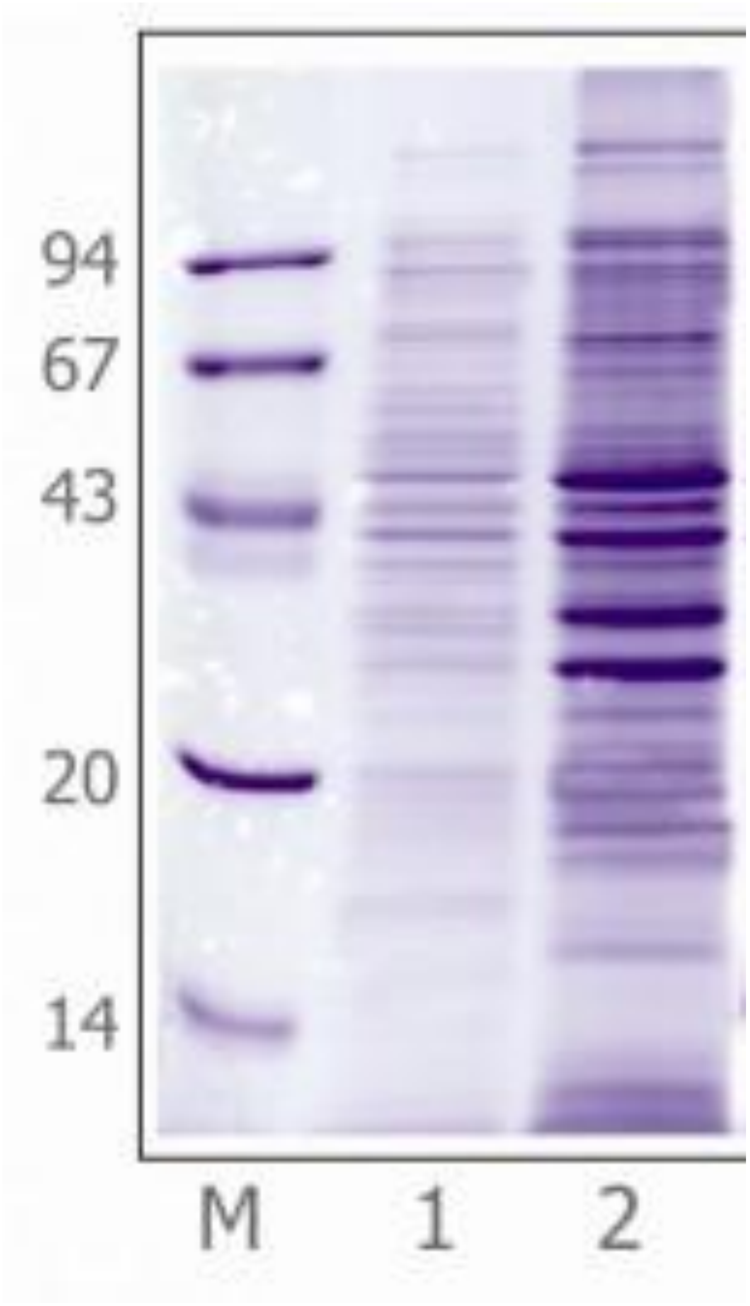
Глицин – малоподвижный ион. Высокое сопротивление. Высокая напряженность

Хлор – подвижный ион. Низкое сопротивление. Низкая напряженность

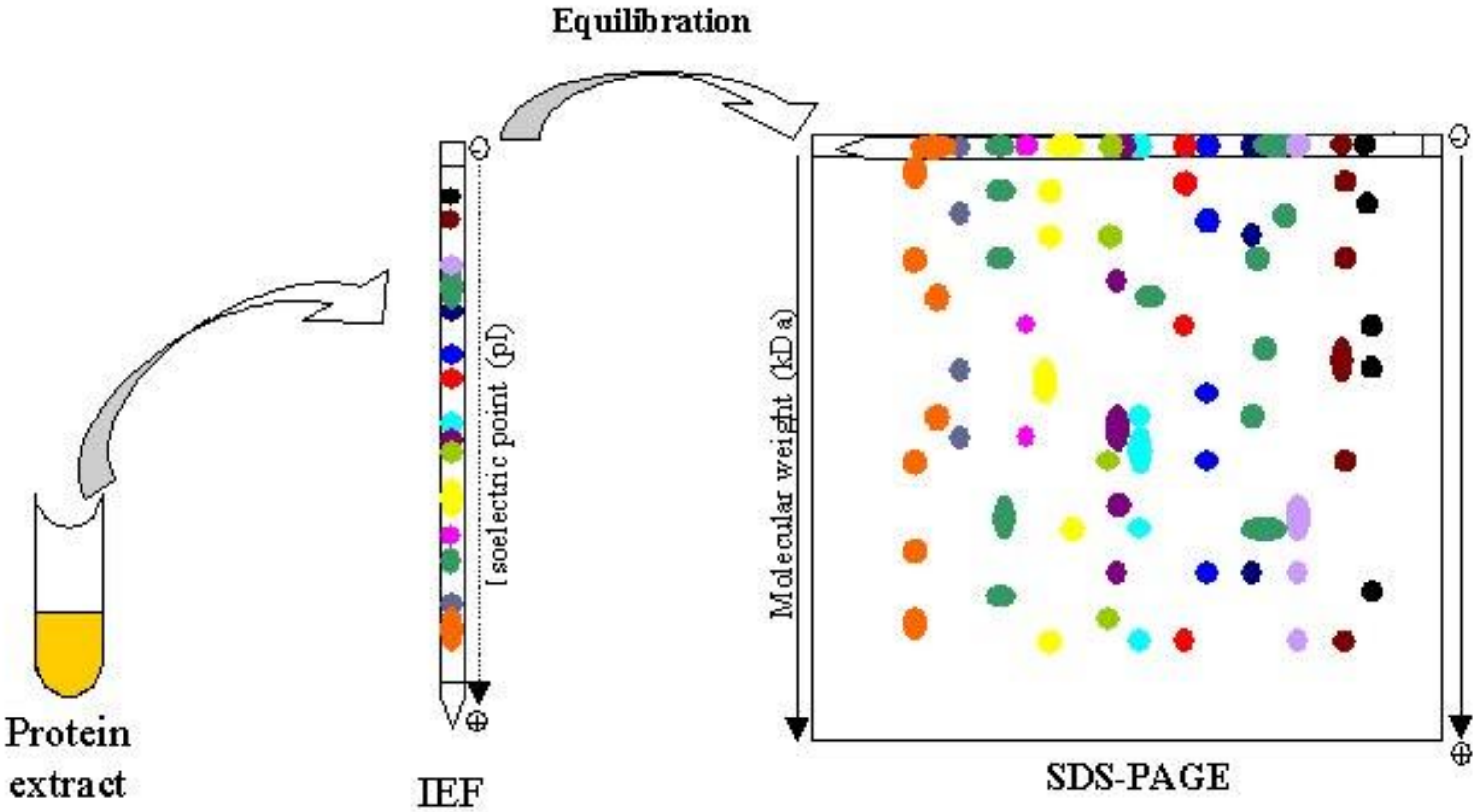
Одинаковая сила тока

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).

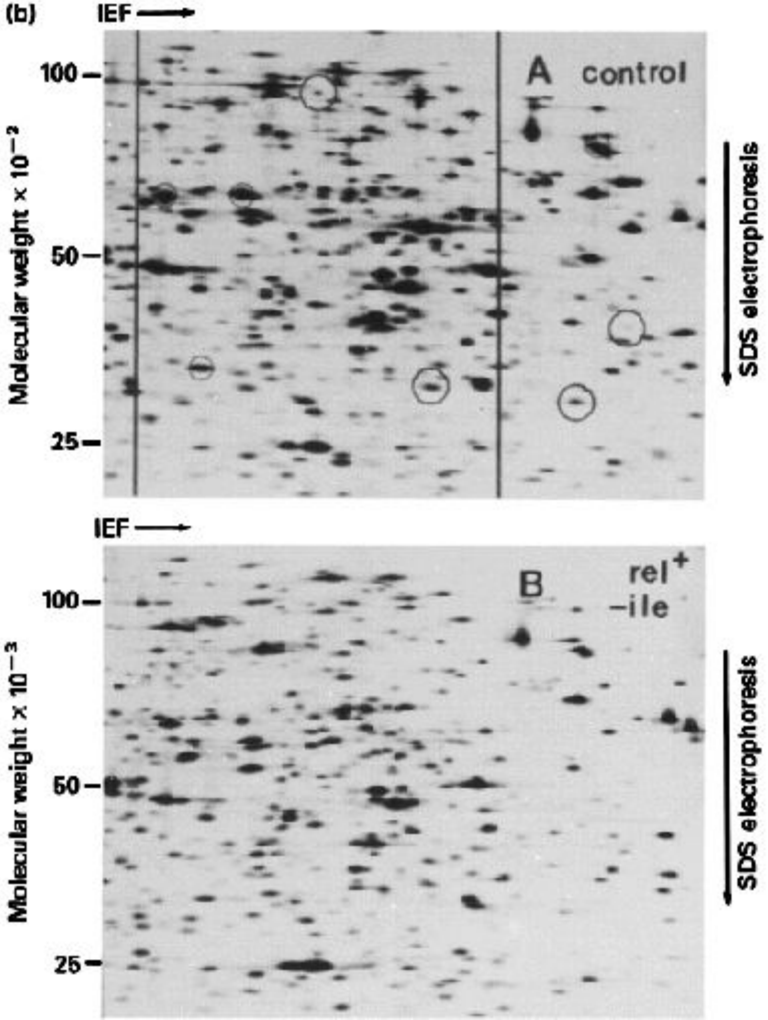


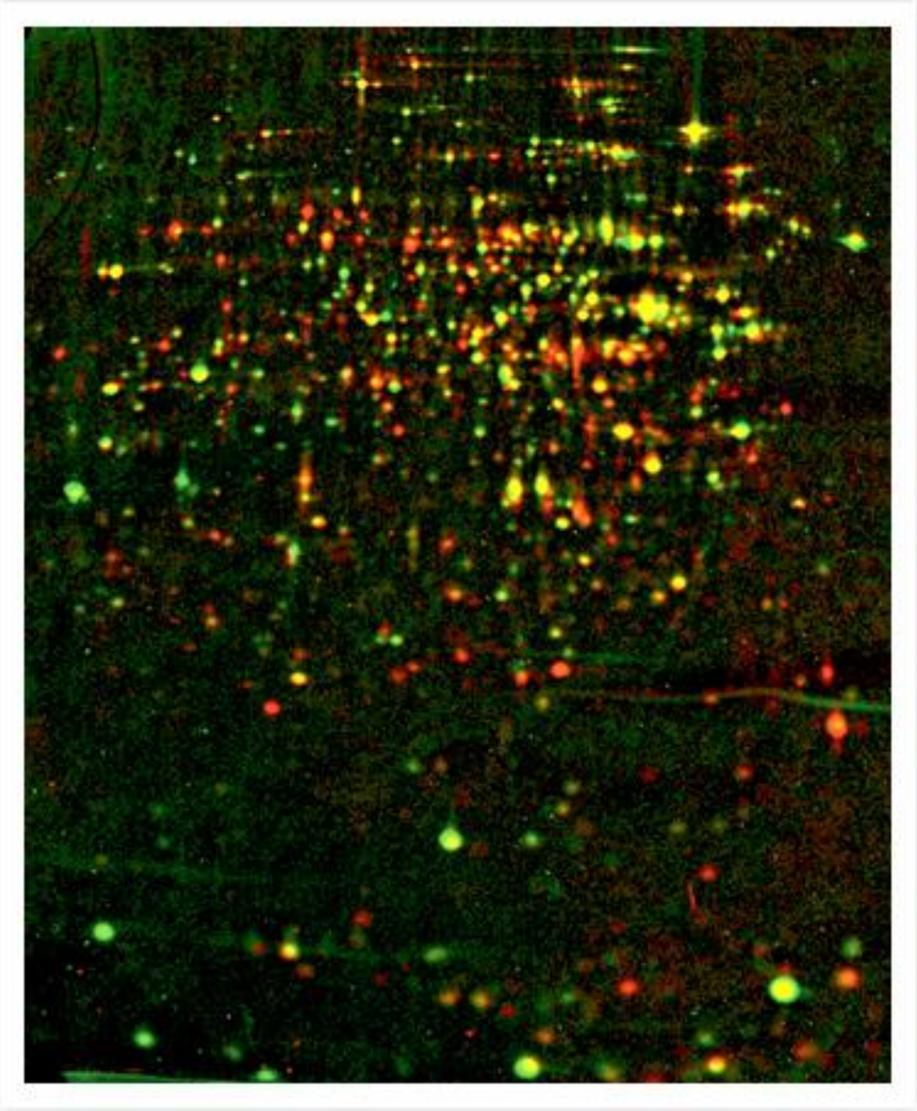

94
67
43
20
14
M
1
2

Двумерный электрофорез белков

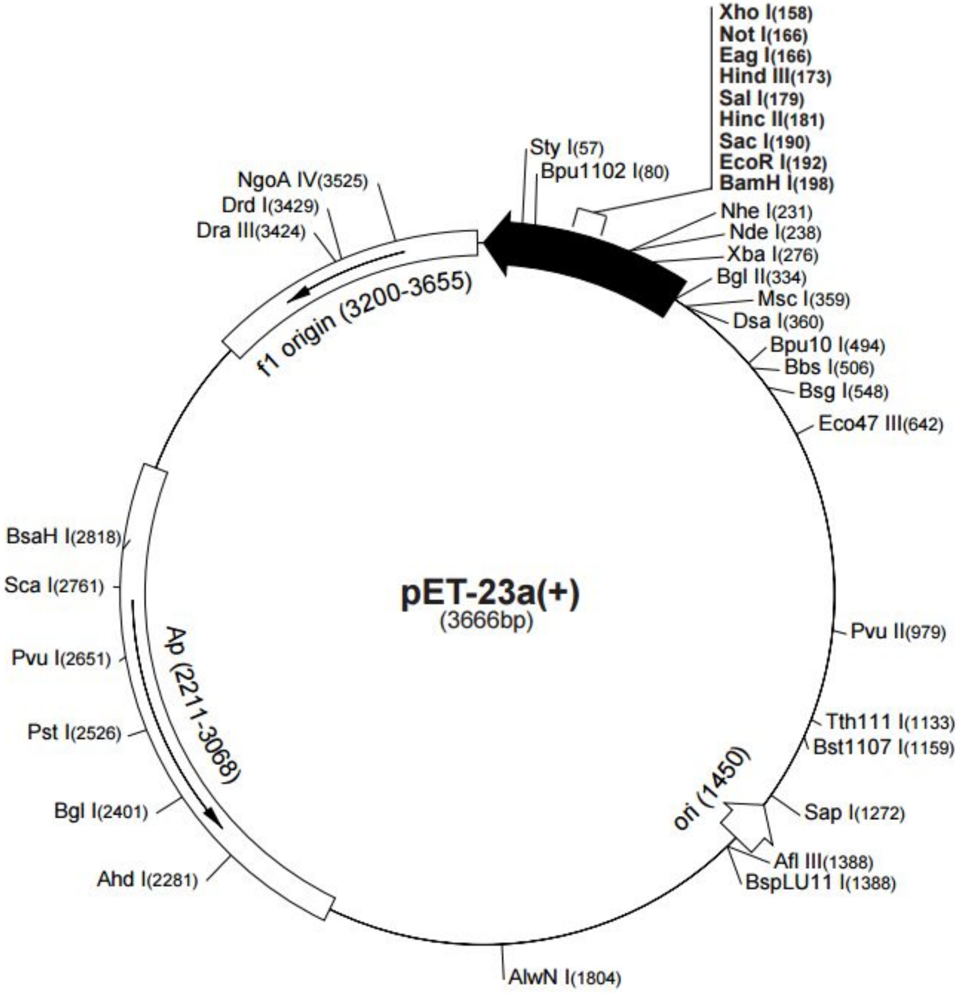


(b)

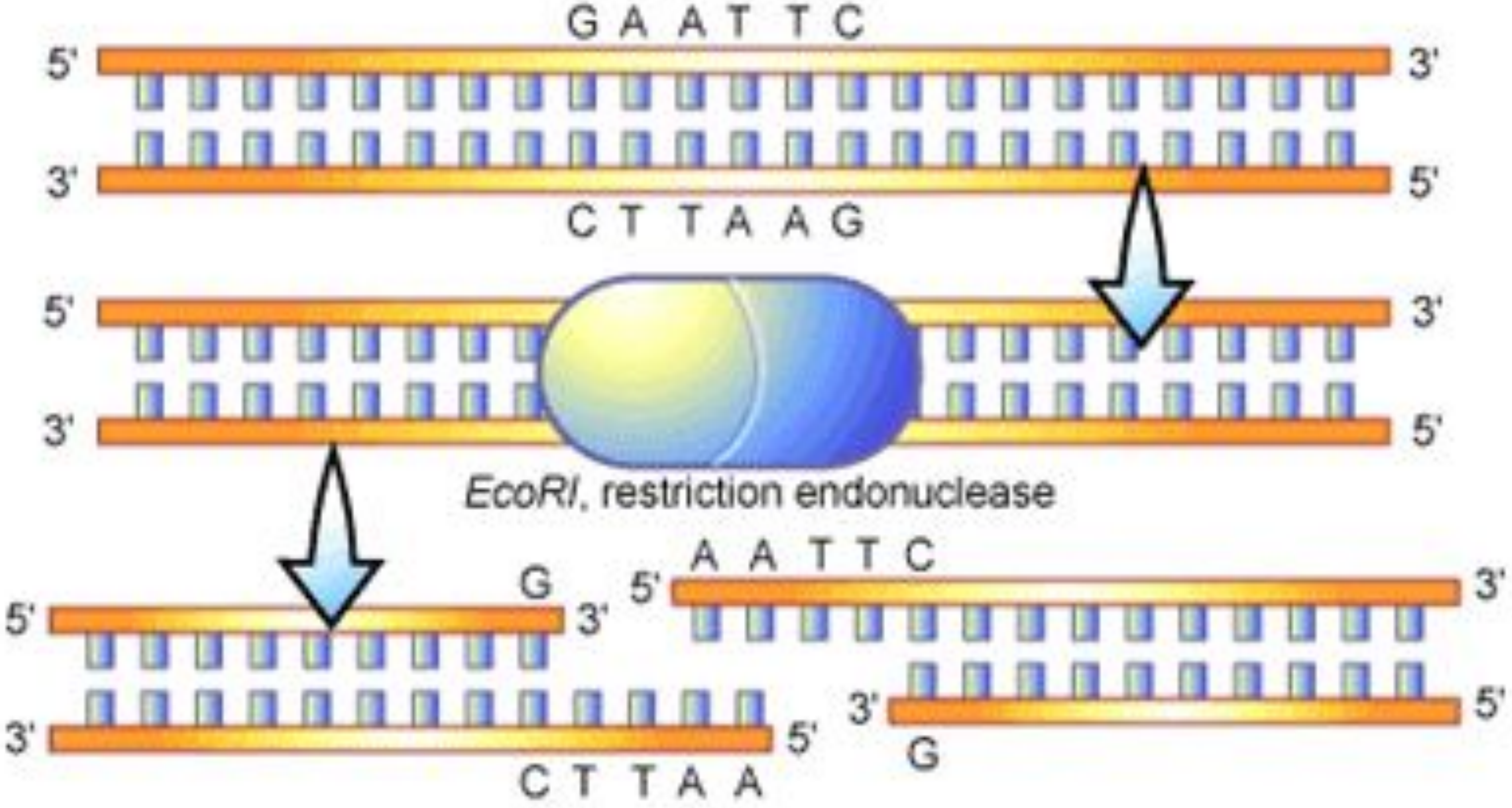

IEF
100
50
25
Molecular weight × 10^{-3}
A control
SDS electrophoresis
IEF
100
50
25
Molecular weight × 10^{-3}
B rel$^+$
−ile
SDS electrophoresis



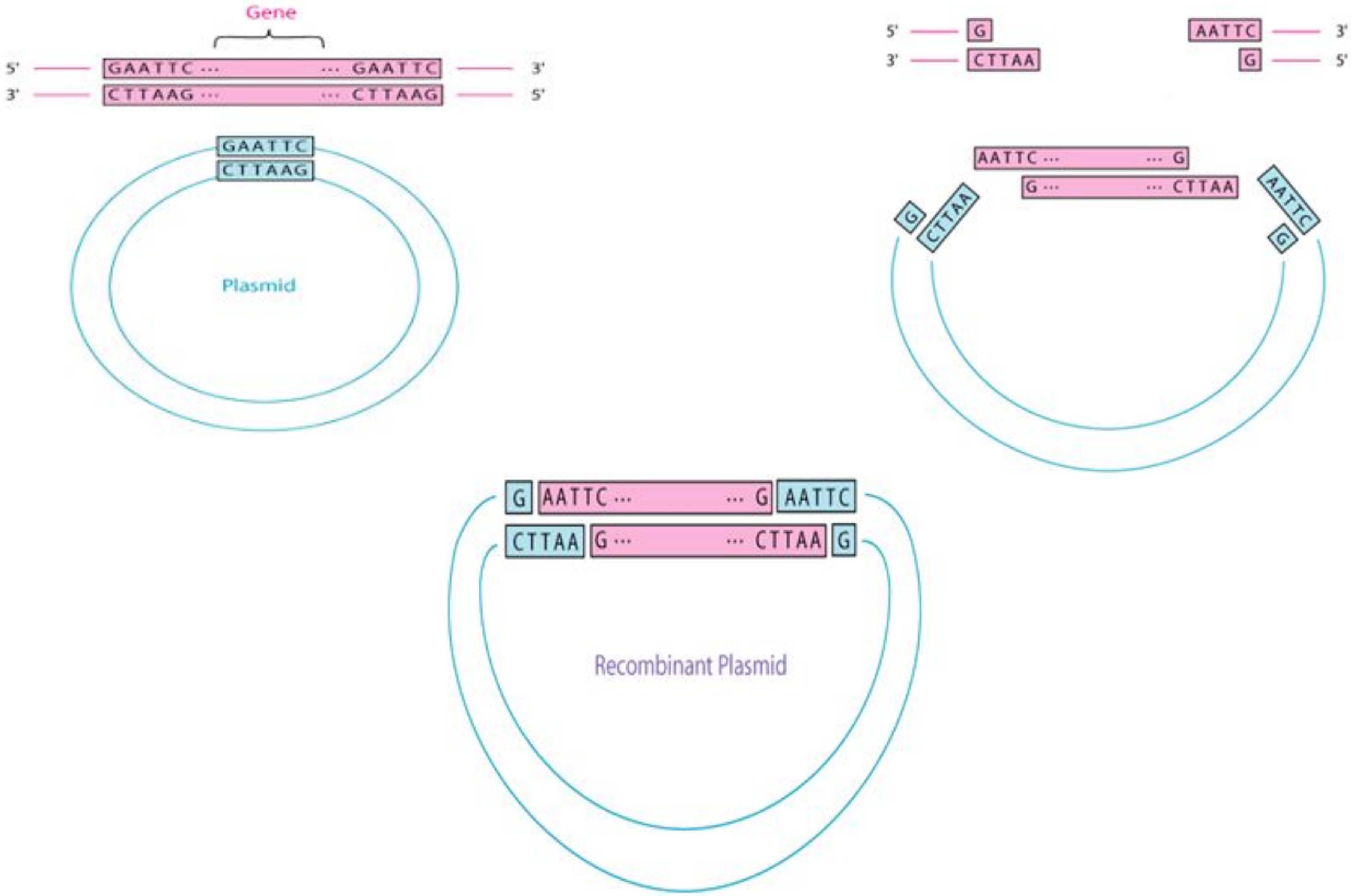
Векторные конструкции

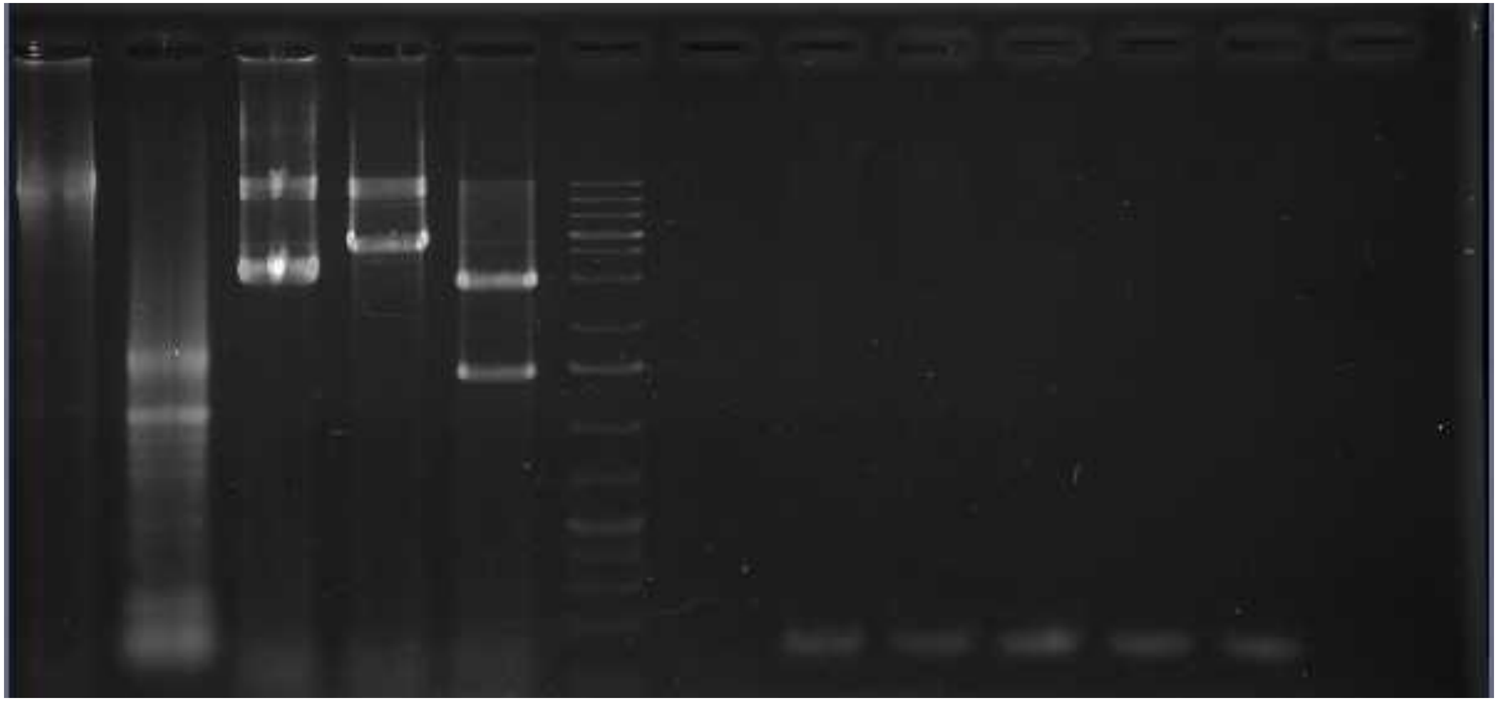


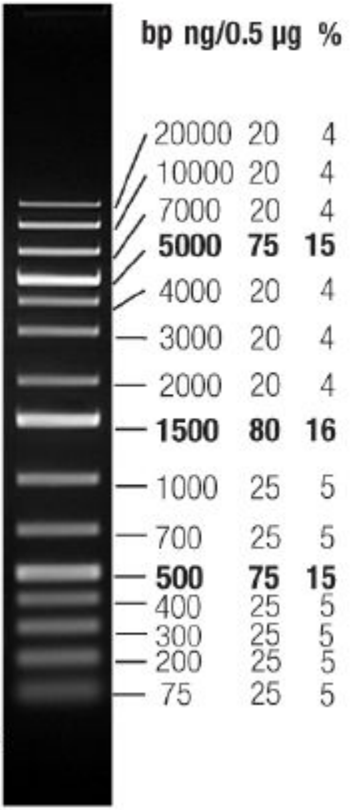
Рестриктазы



Сборка плазмиды. Рестрикция-лигирование.

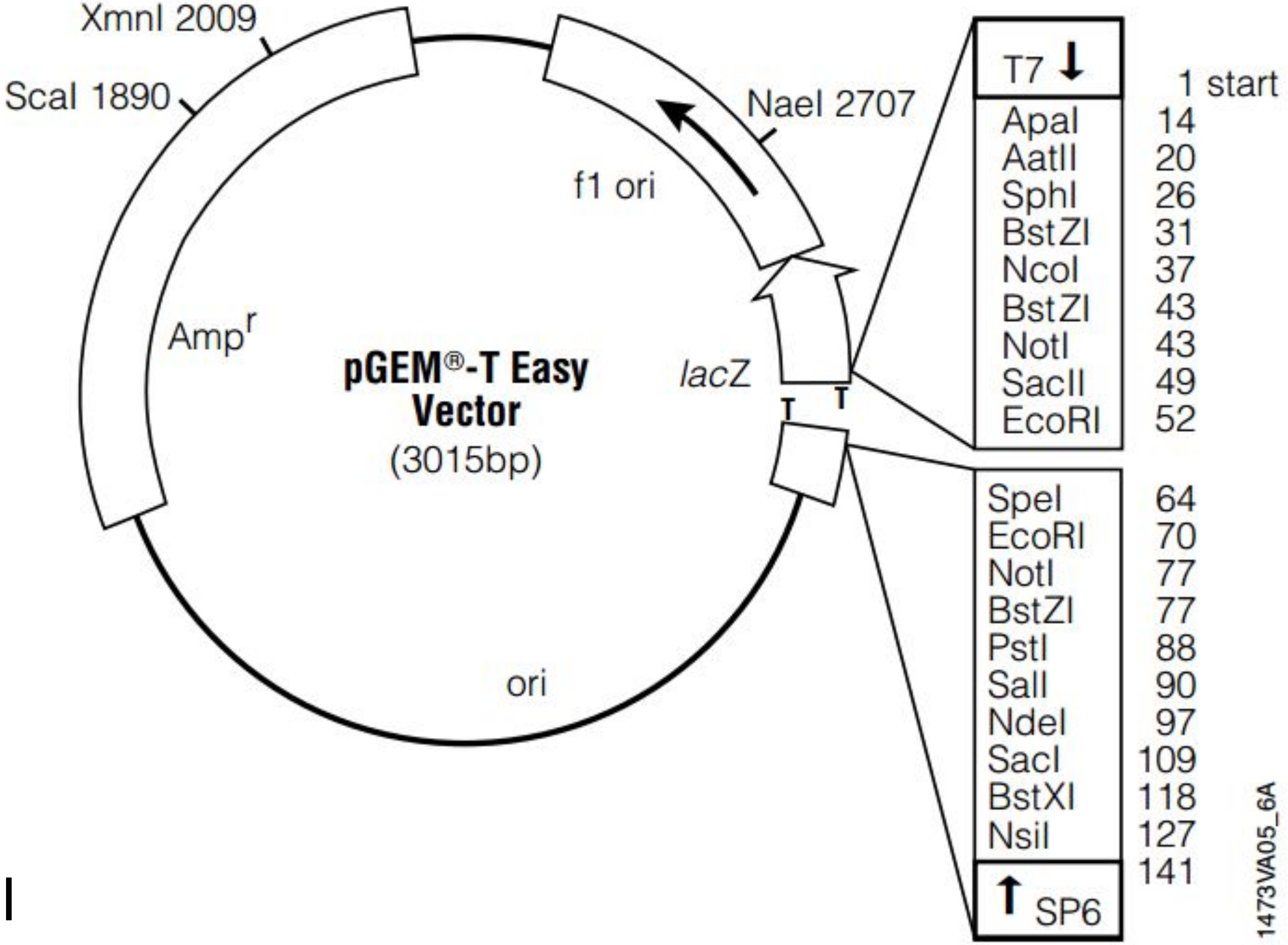






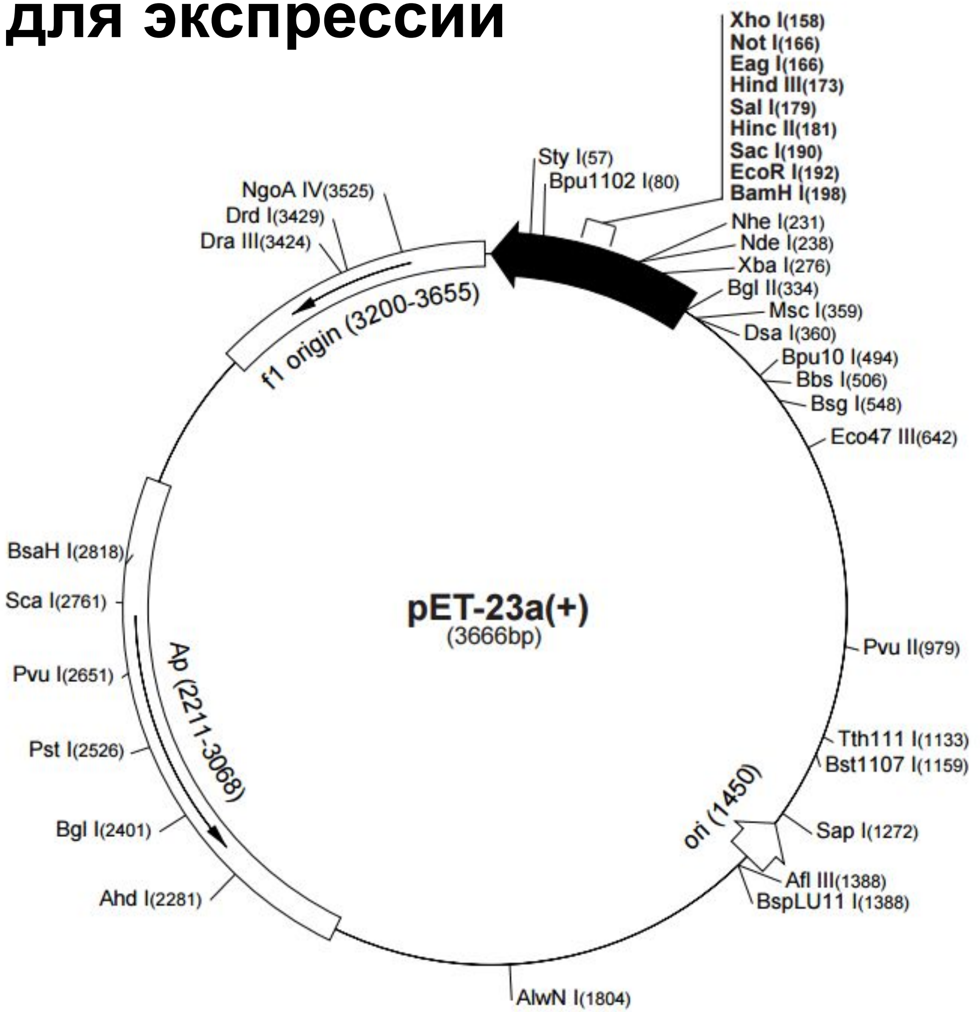
0.5 µg/lane, 8 cm length gel,
1X TAE, 7 V/cm, 45 min

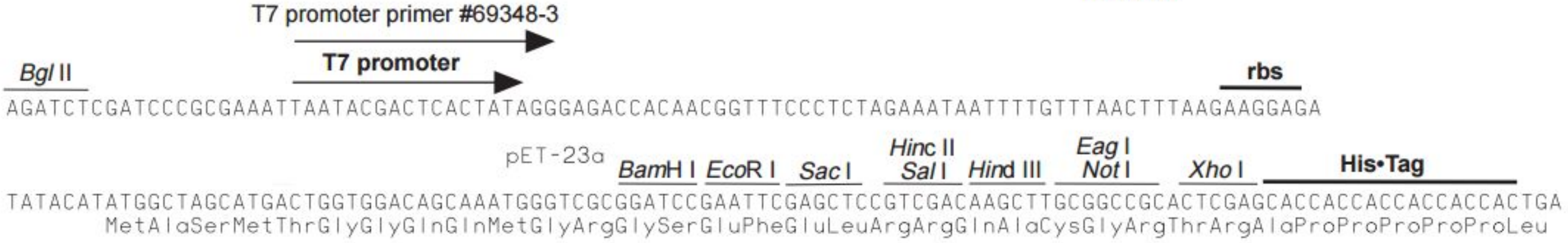
Векторы для клонирования



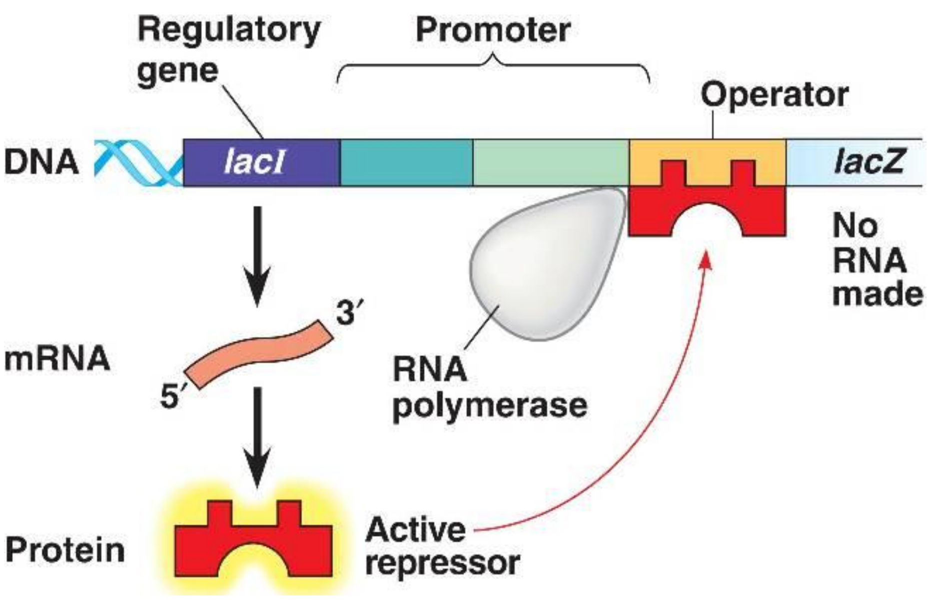
X-Gal

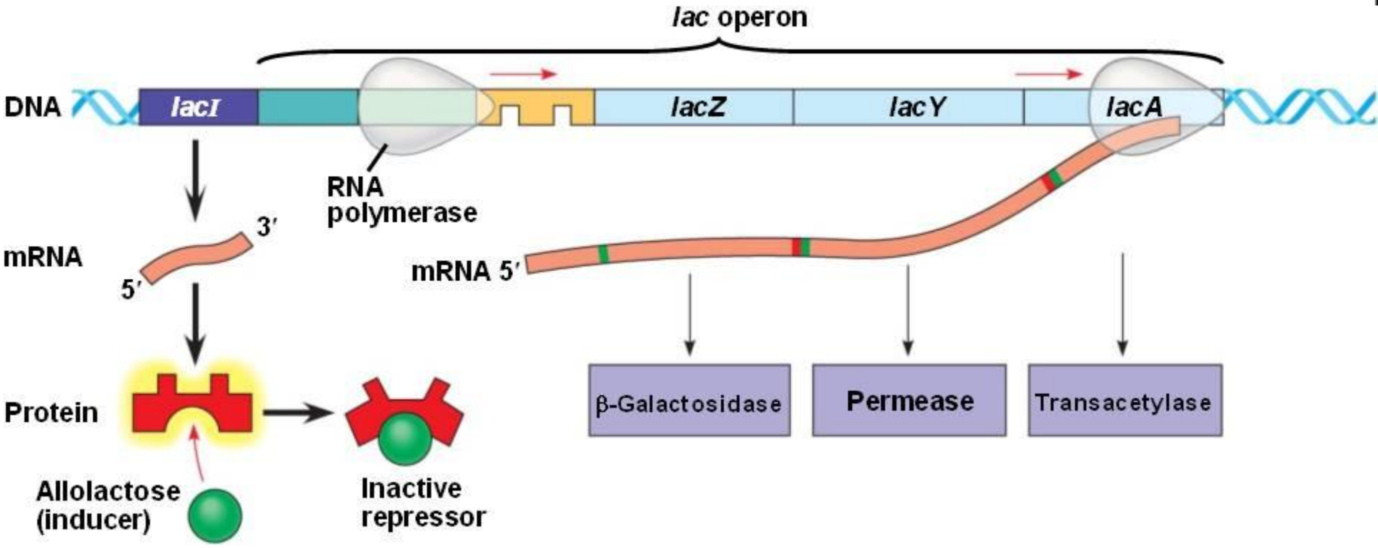
Векторы для экспрессии

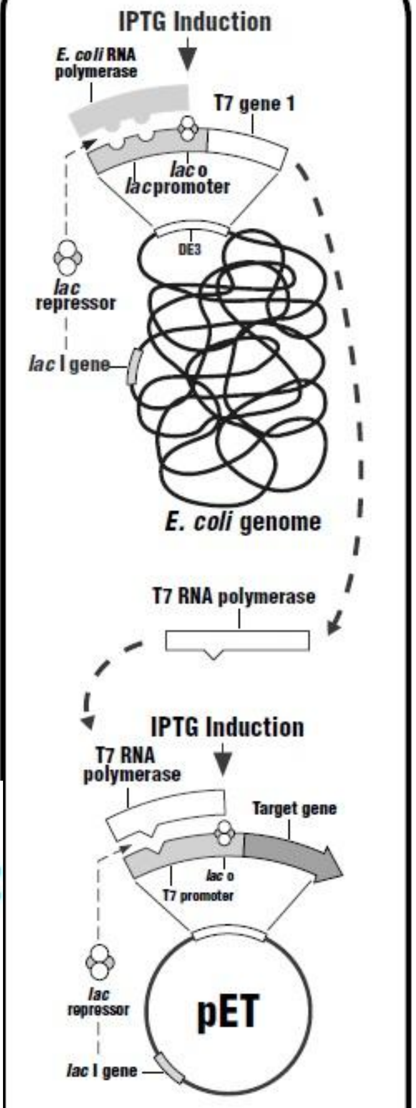




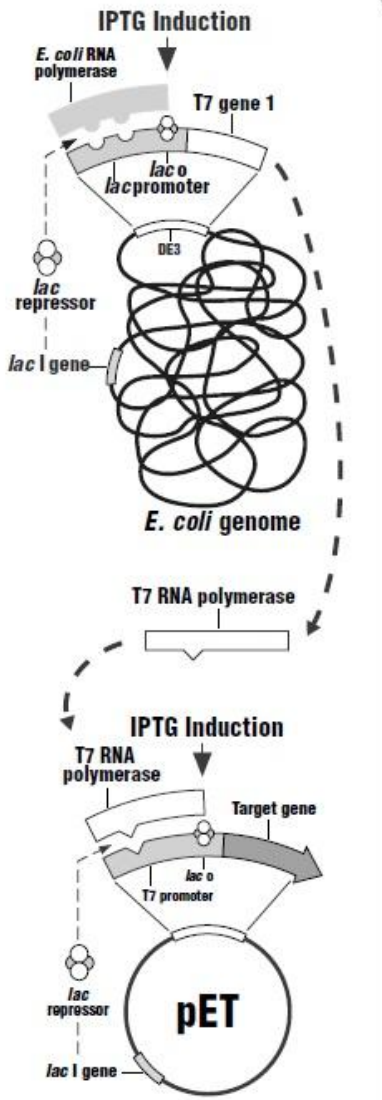
```
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA

TATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGA
      MetAlaSerMetThrGlyGlyGlnGlnMetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProProLeu
```



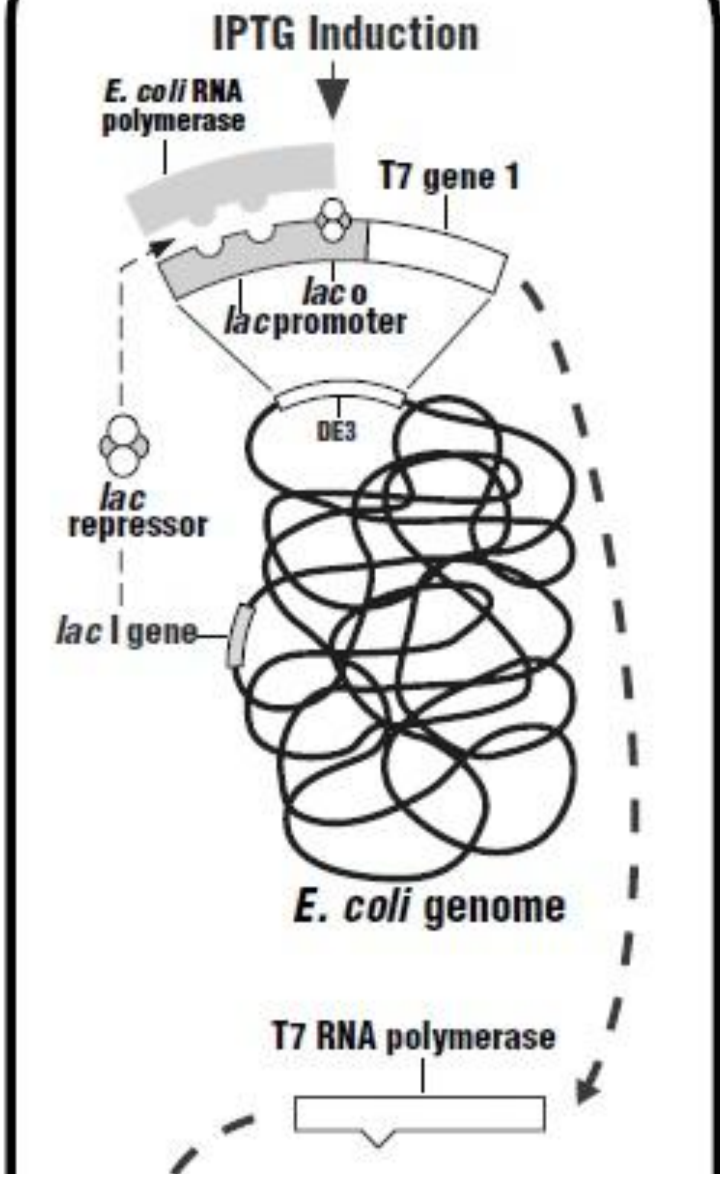


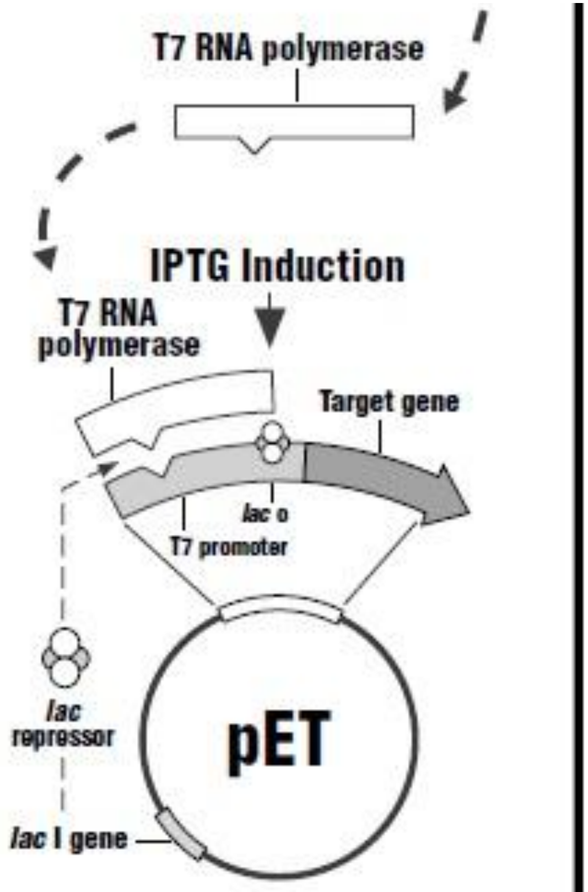


Control of the T7 *lac* Promoter



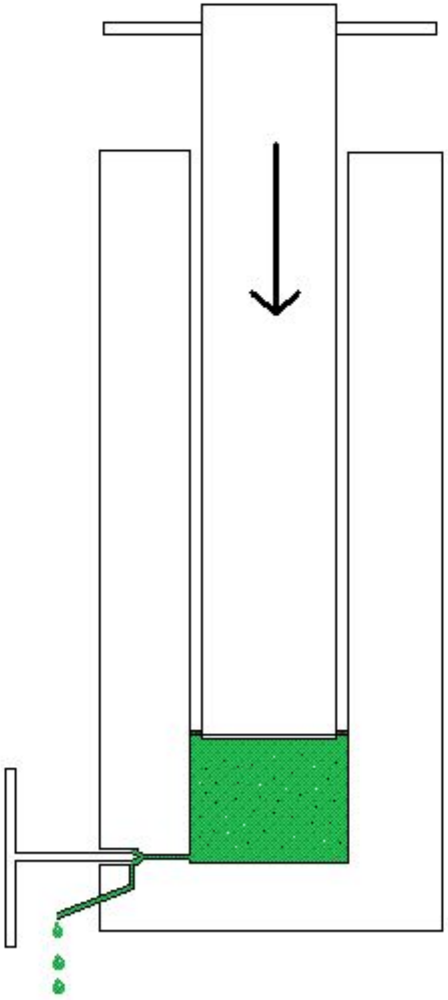
Control of the T7*lac* Promoter





Control of the T7*lac* Promoter

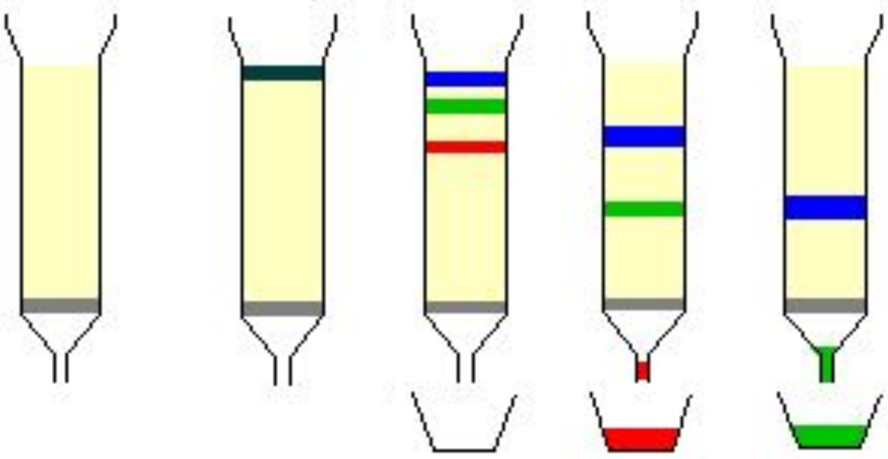
Лизис клеток



Очистка целевого белка

Хроматография

column containing stationary phase

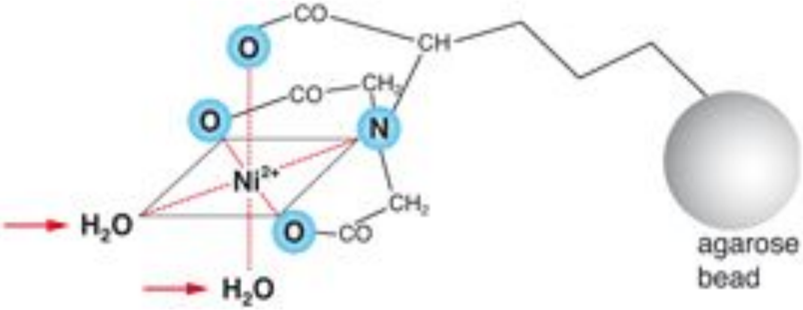


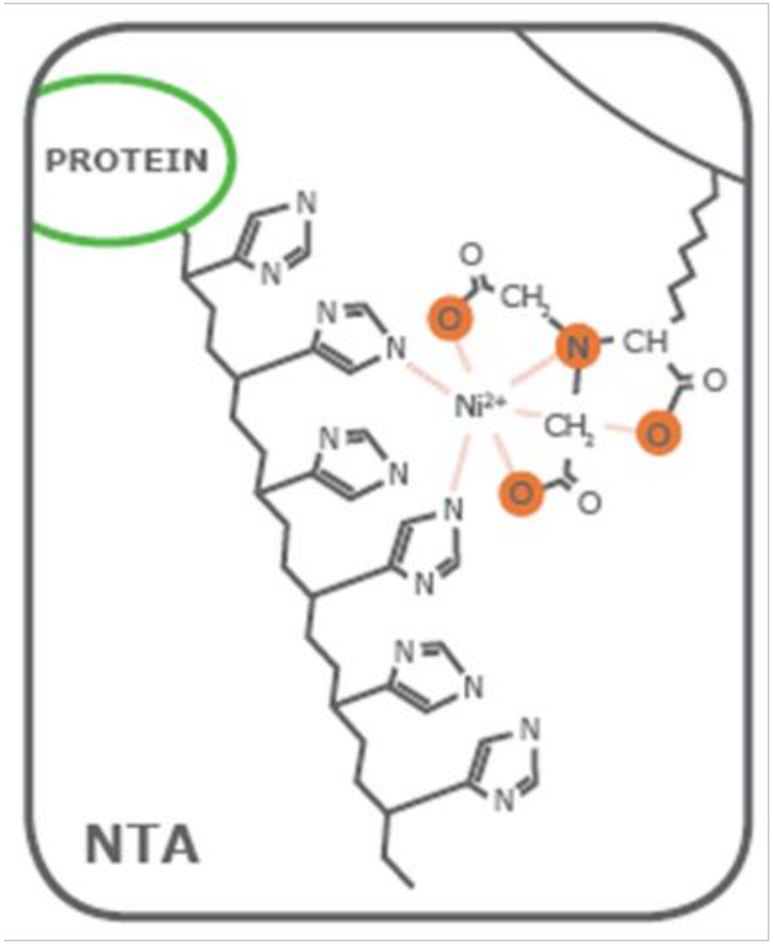
load sample

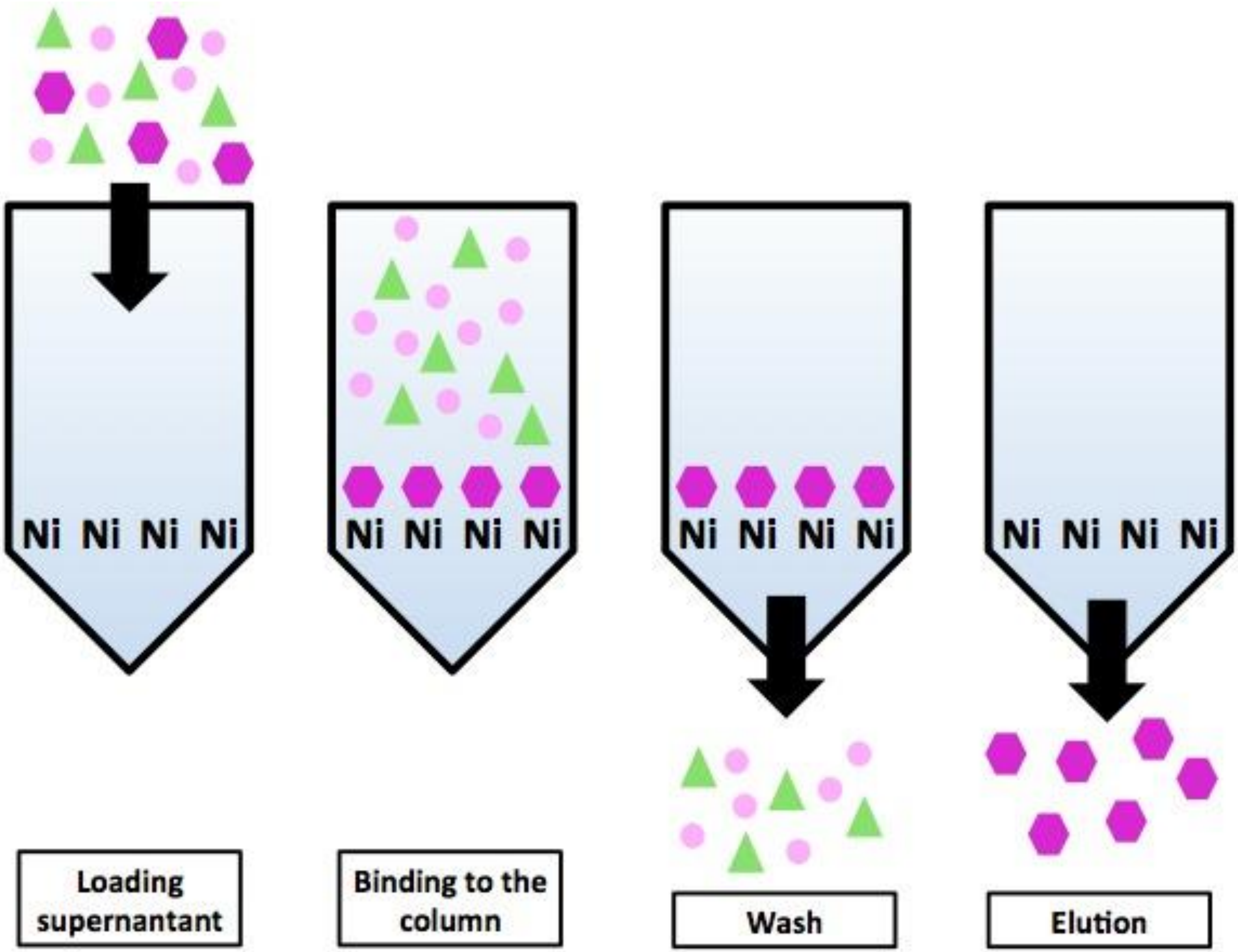
add solvent

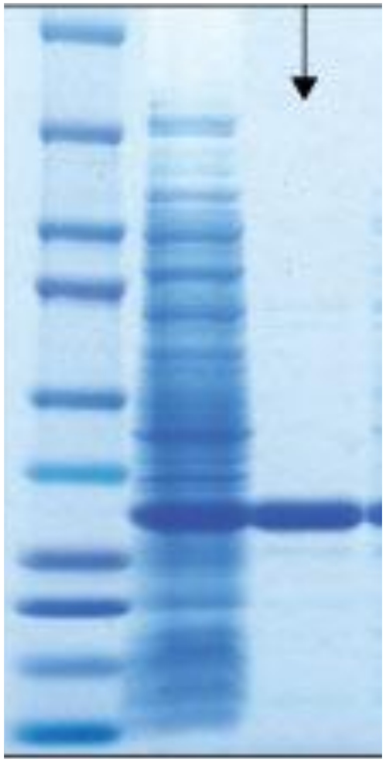
collect components

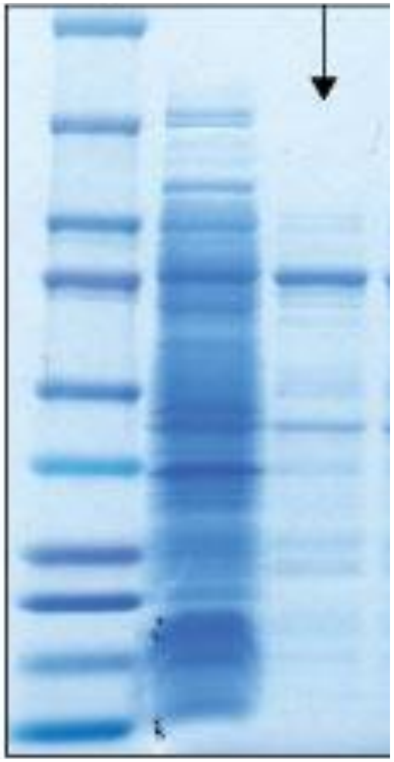

HisTrap™ HP
5 ml
HisTrap™ HP
1 ml


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

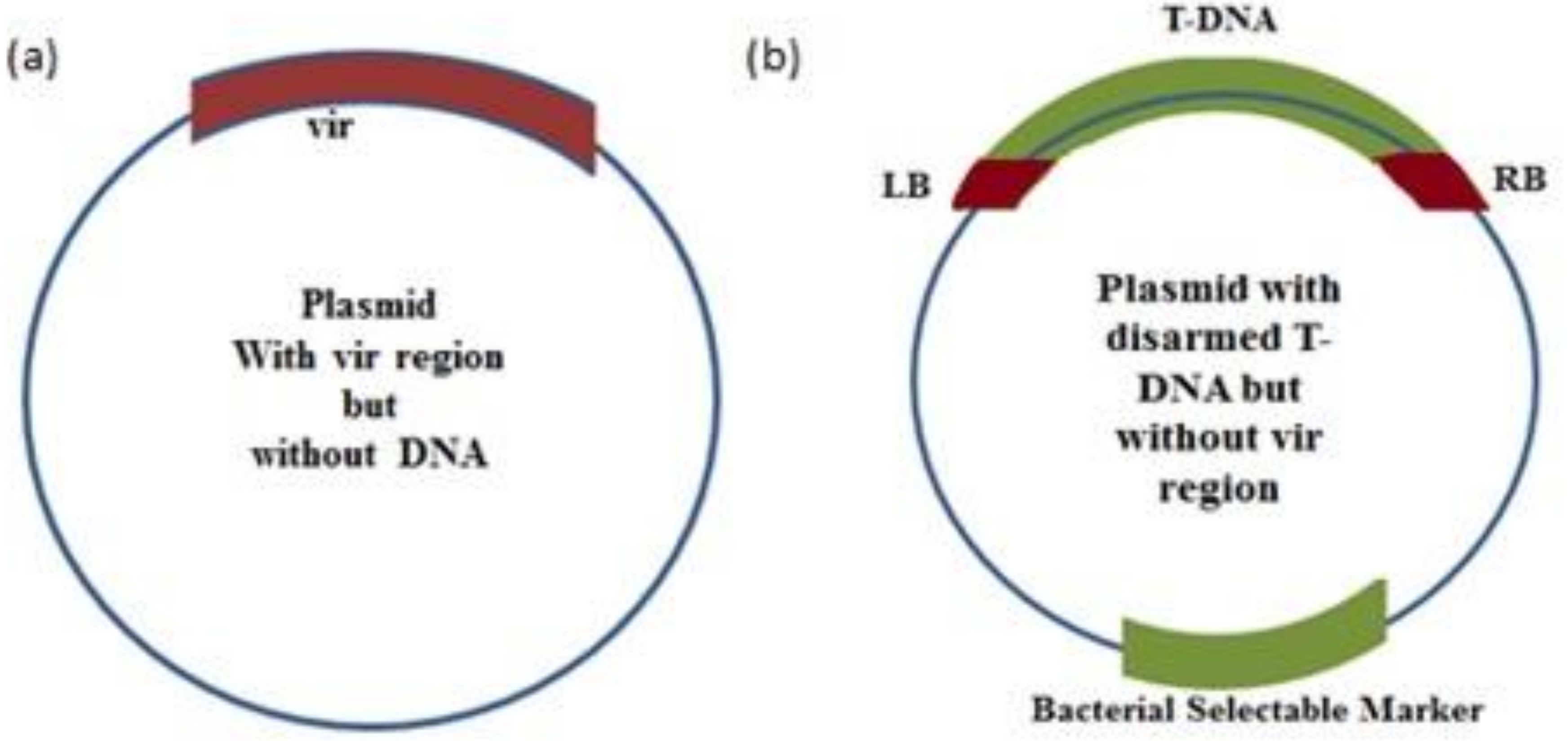

PROTEIN
N
N
O
CH2
O
N
N
N
CH
O
Ni2+
CH2
O
N
N
O
O
N
N
N
N
N
N
N
NTA

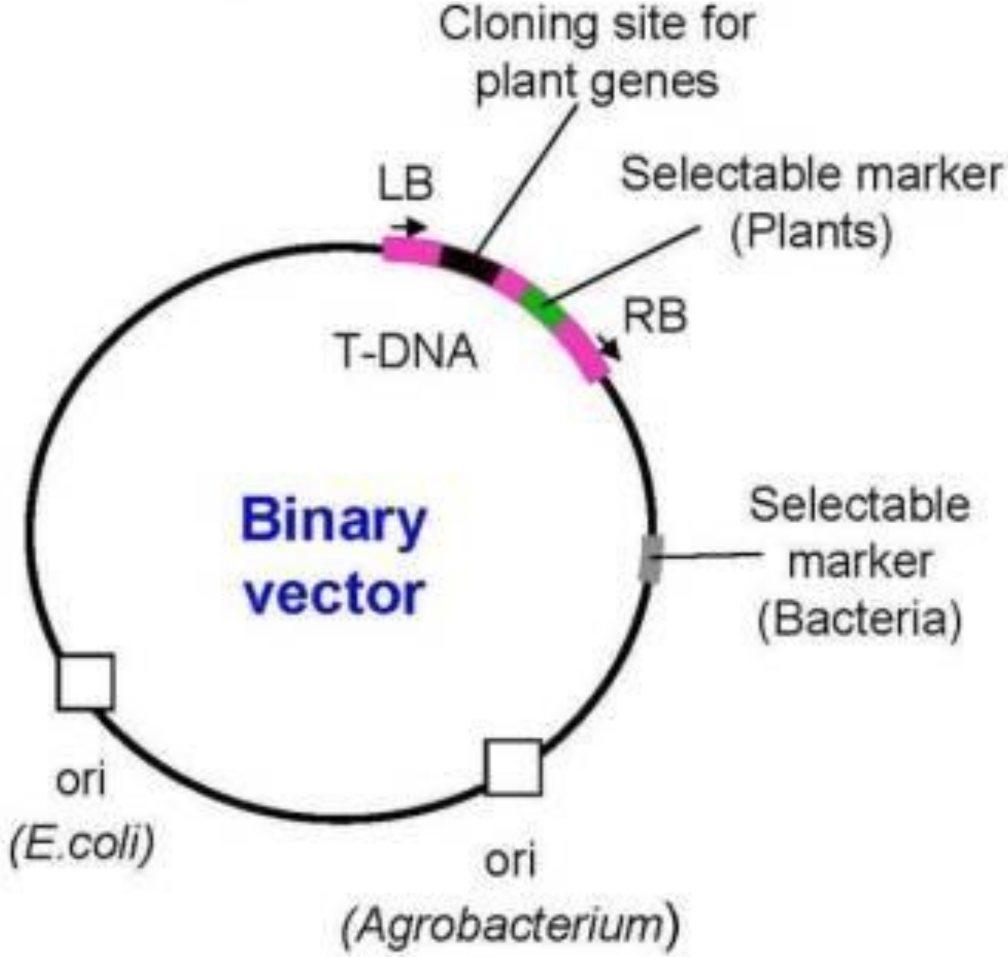

Ni Ni Ni Ni
Ni Ni Ni Ni
Ni Ni Ni Ni
Ni Ni Ni Ni
Loading supernantant
Binding to the column
Wash
Elution



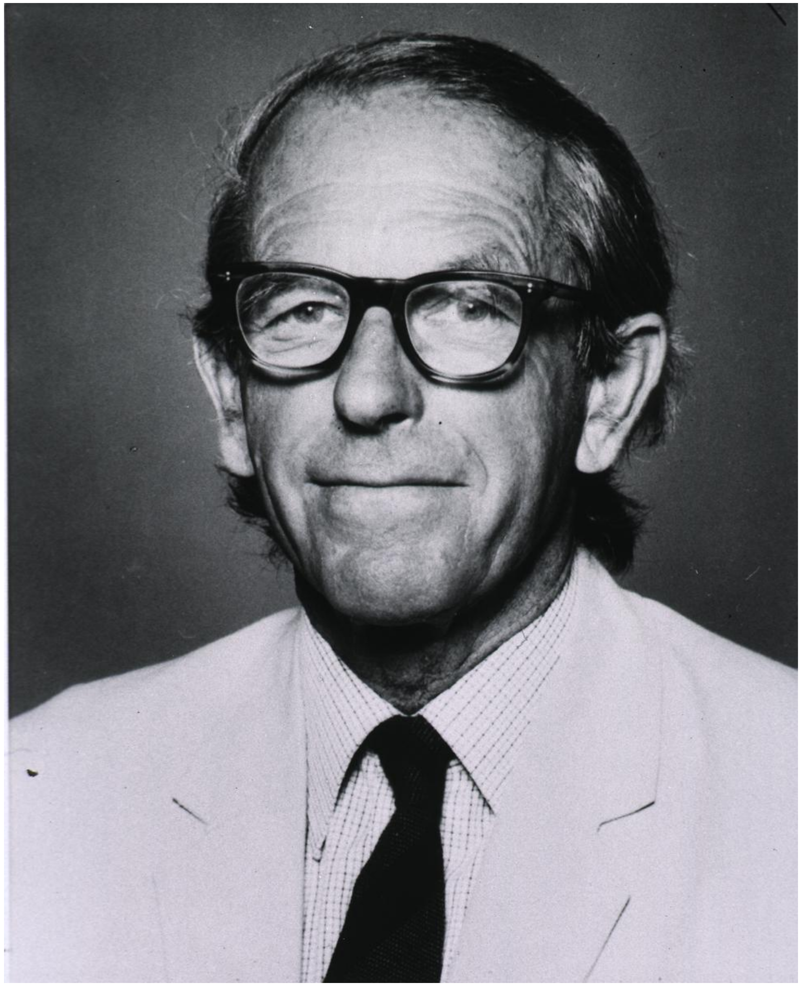


Векторы для трансформации растений

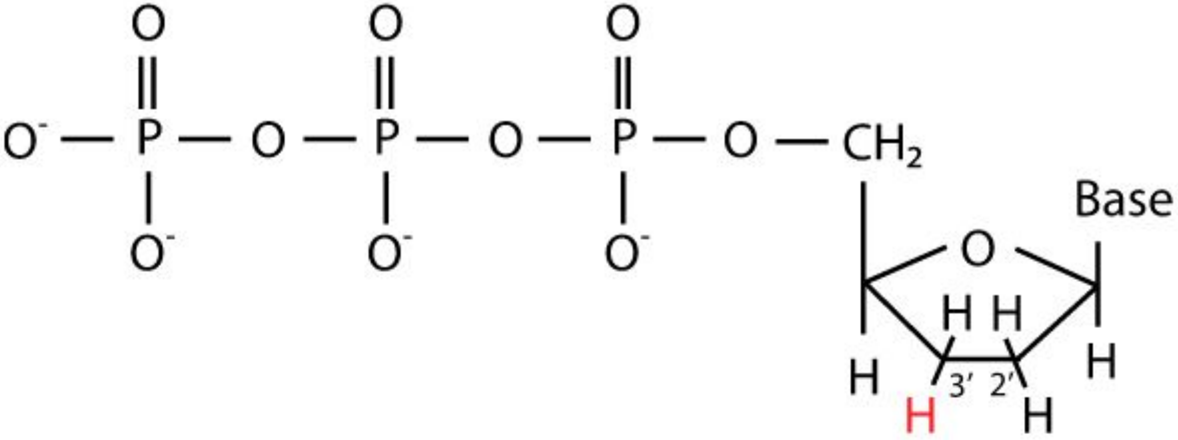



Cloning site for plant genes
LB
Selectable marker (Plants)
RB
T-DNA
Binary vector
Selectable marker (Bacteria)
ori (*E.coli*)
ori (*Agrobacterium*)

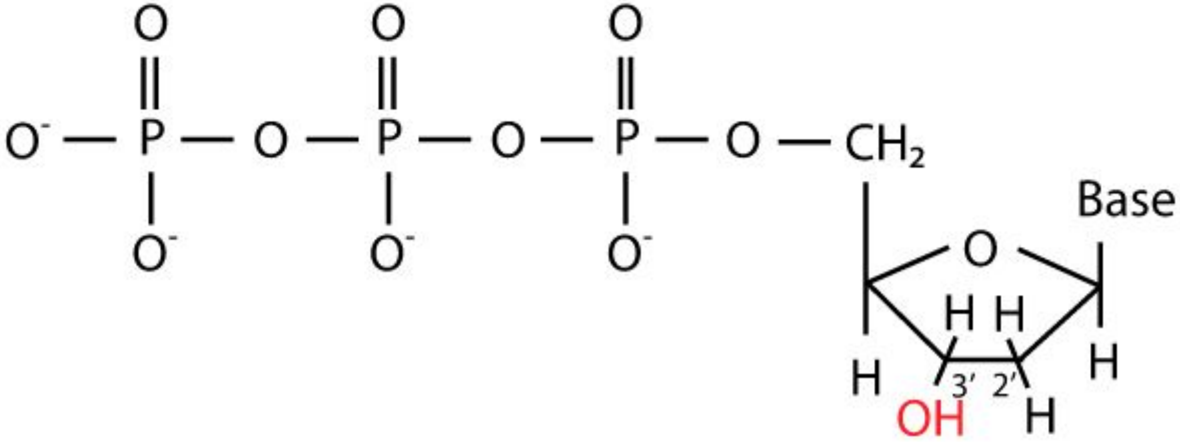
Определение первичной структуры ДНК

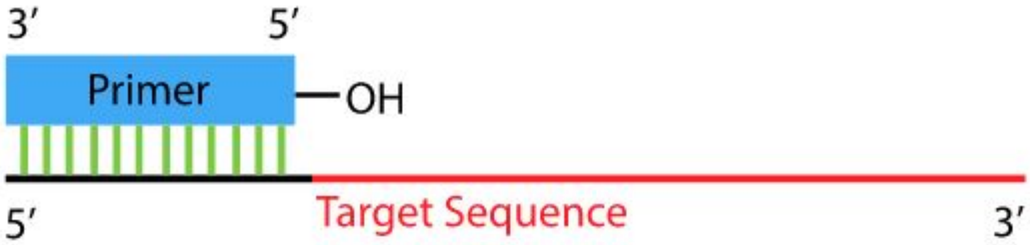


Dideoxynucleotide (ddNTP)

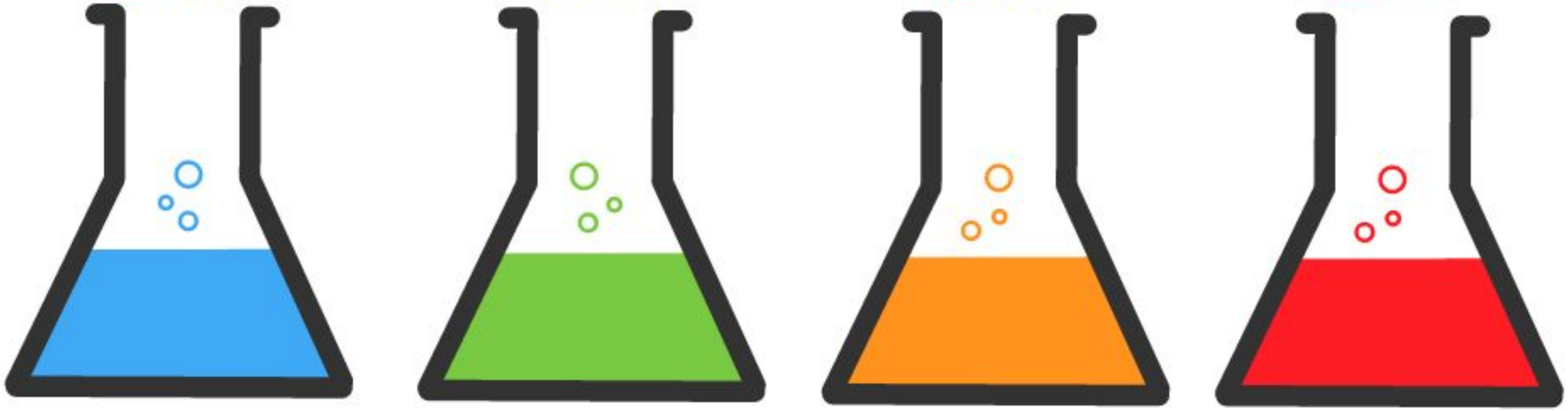


Deoxynucleotide (dNTP)

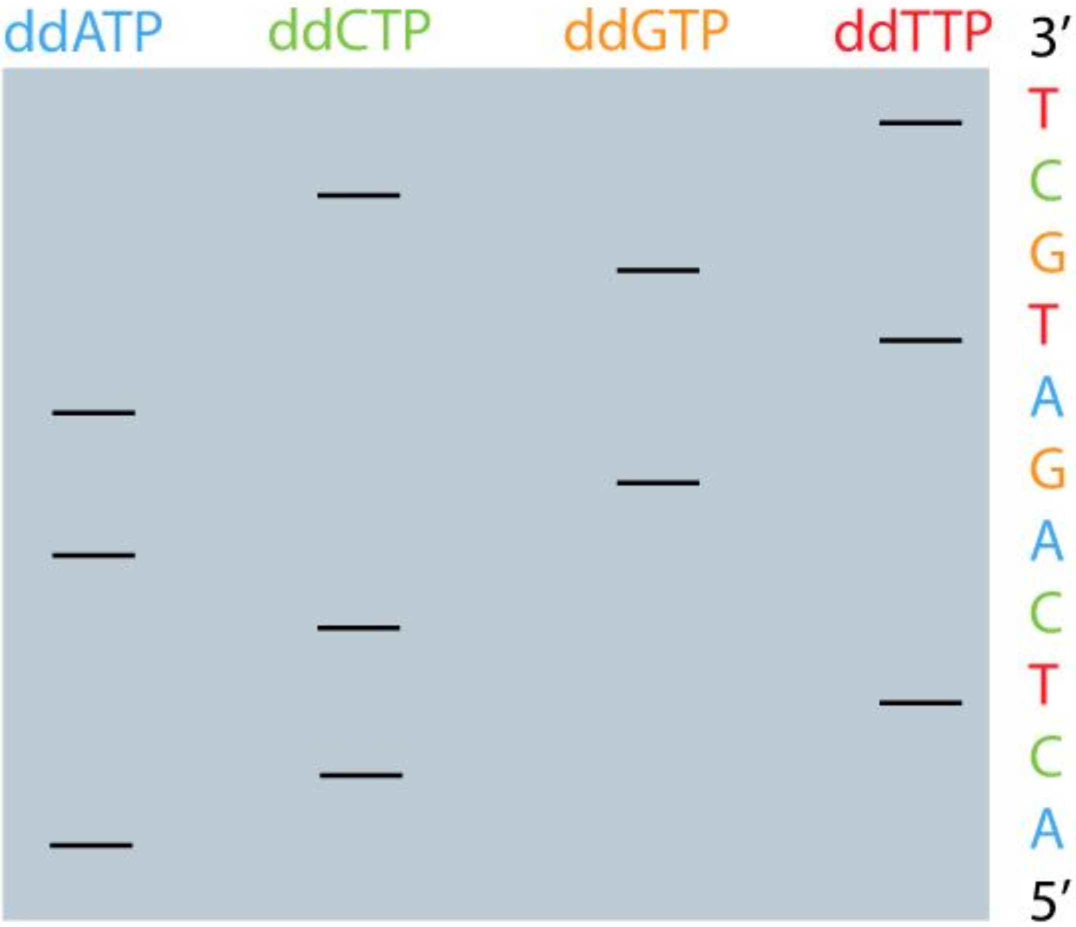


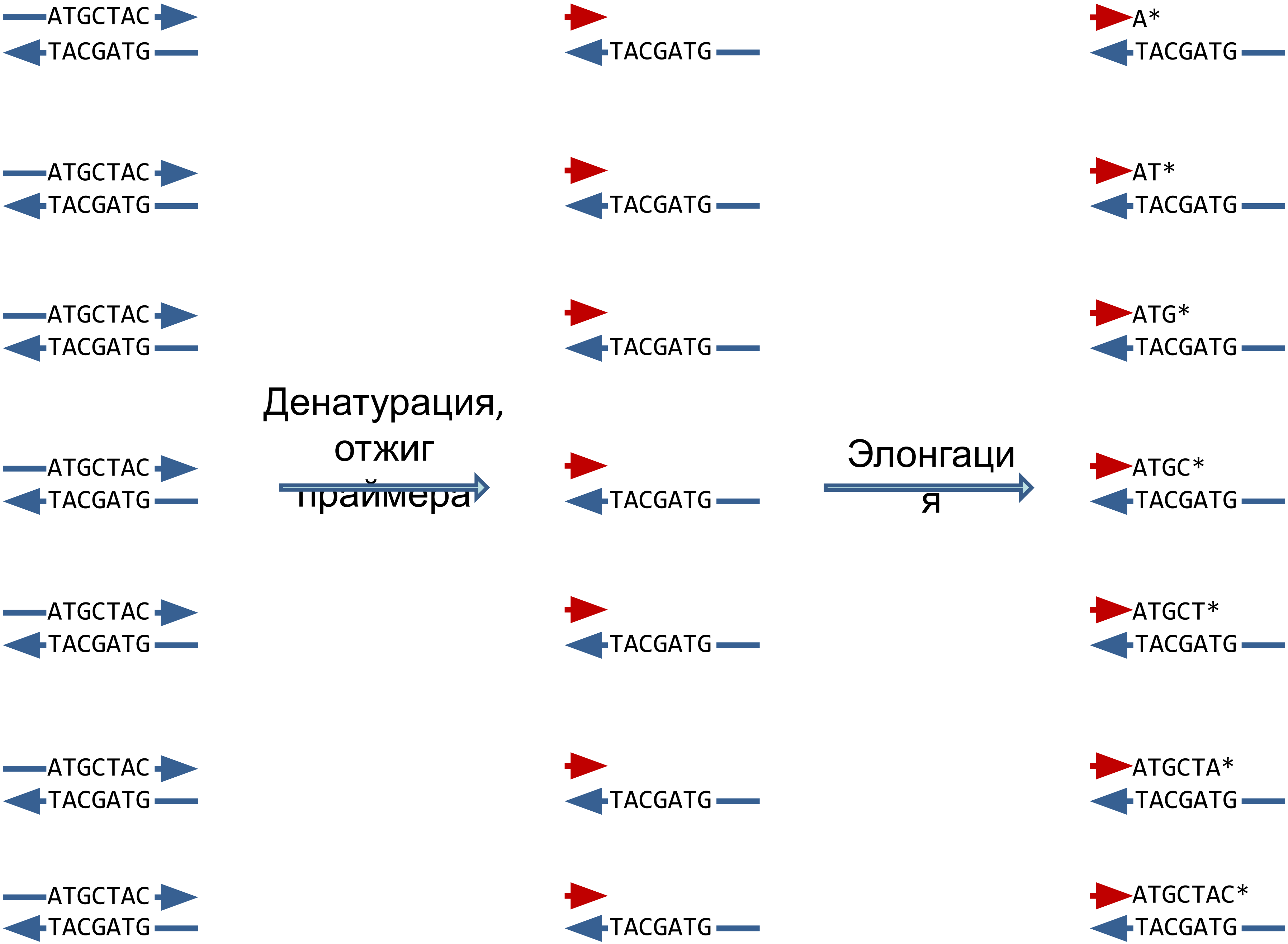

3′
5′
Primer
OH
5′
Target Sequence
3′

dATP + dCTP + dGTP + dTTP
DNA Polymerase
Template DNA
Primer



ddATP	ddCTP	ddGTP	ddTTP
ACTCAGATGCT	ACTCAGATGCT	ACTCAGATGCT	ACTCAGATGCT
ACTCAGA	ACTCAGATGC	ACTCAGATG	ACTCAGAT
ACTCA	ACTC	ACTCAG	ACT
A	AC		


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



ATGCTAC
TACGATG

ATGCTAC
TACGATG

ATGCTAC
TACGATG

ATGCTAC
TACGATG

ATGCTAC
TACGATG

ATGCTAC
TACGATG

ATGCTAC
TACGATG

Денатурация, отжиг праймера

TACGATG

TACGATG

TACGATG

TACGATG

TACGATG

TACGATG

TACGATG

Элонгация

A*
TACGATG

AT*
TACGATG

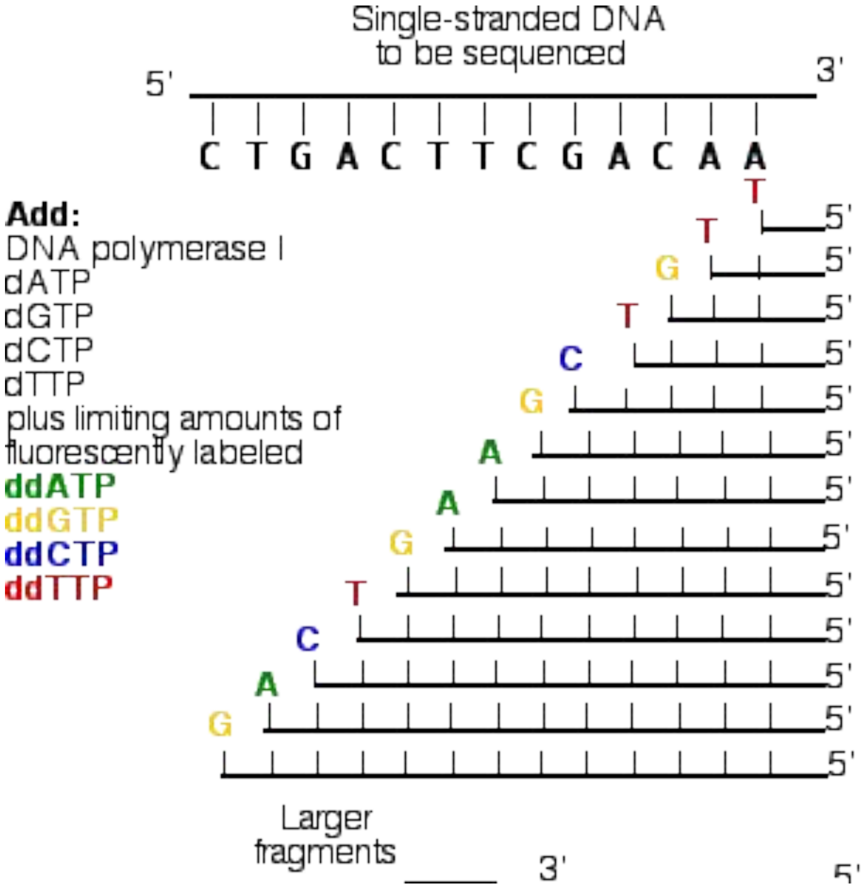
ATG*
TACGATG

ATGC*
TACGATG

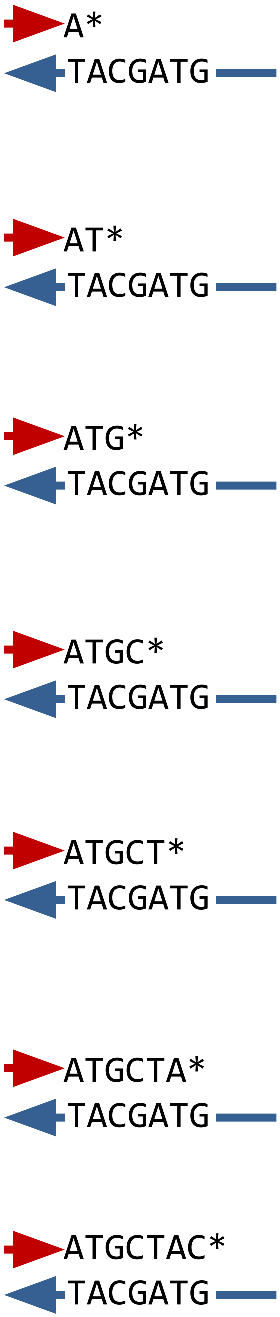
ATGCT*
TACGATG

ATGCTA*
TACGATG

ATGCTAC*
TACGATG



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



A*
TACGATG

AT*
TACGATG

ATG*
TACGATG

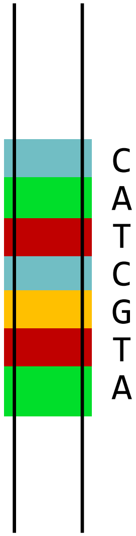
ATGC*
TACGATG

ATGCT*
TACGATG

ATGCTA*
TACGATG

ATGCTAC*
TACGATG

Капиллярный электрофорез



Анализ