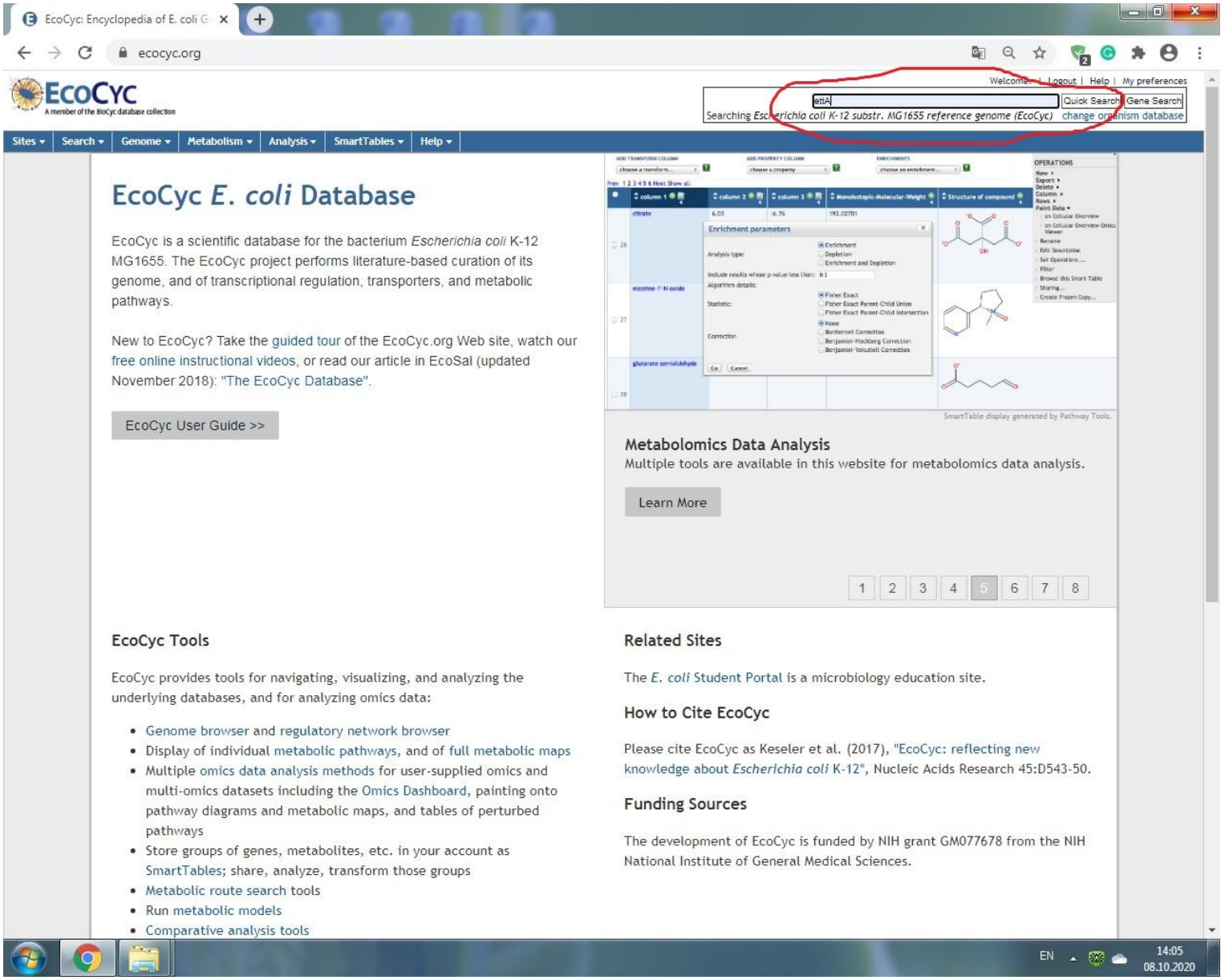
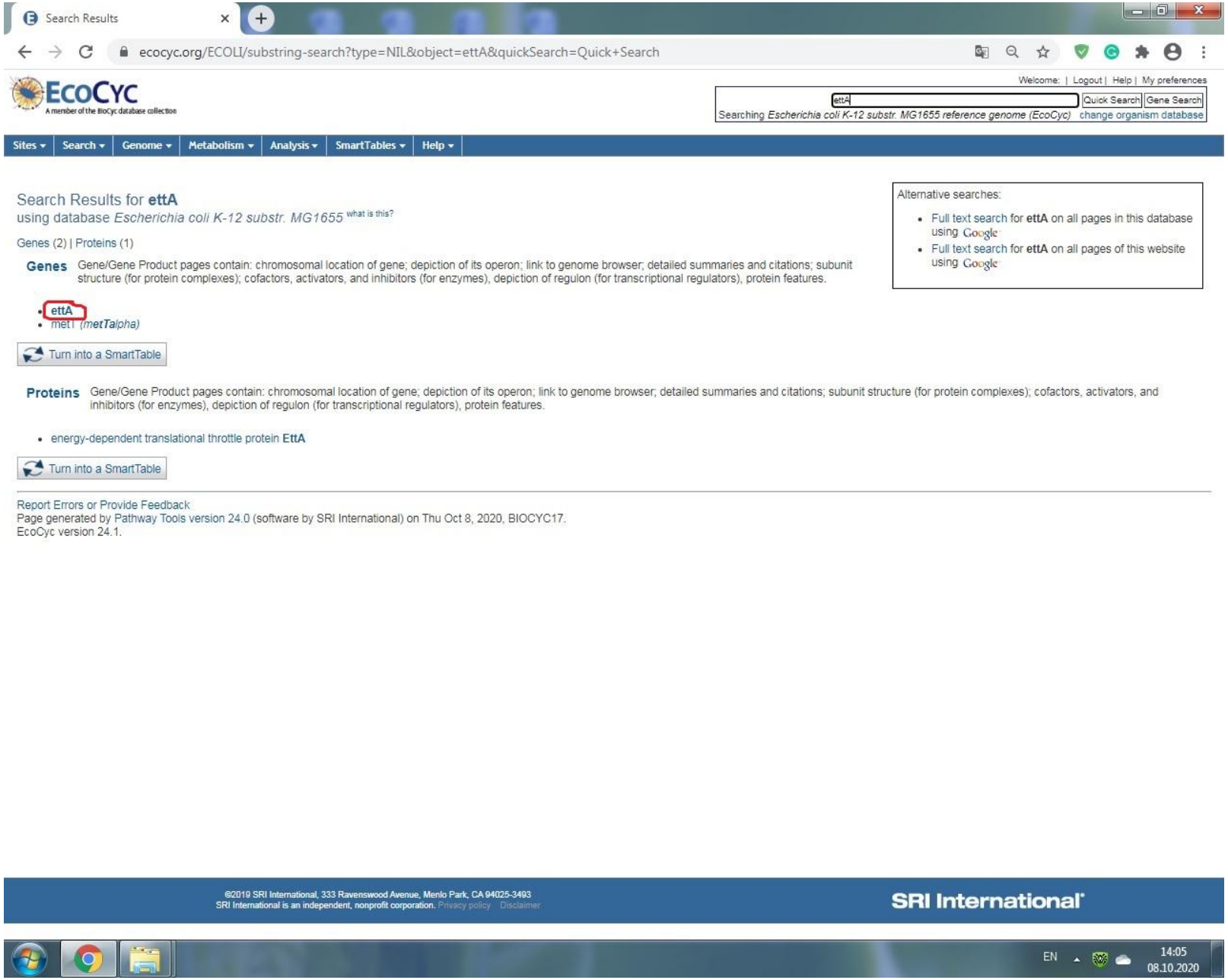
Подбор праймеров

Основные принципы

- Длина 18-30 нуклеотидов
- Температура отжига 45-65°С, максимально допустимая разница между температурами отжига праймеров 5°С
- Должно быть одно место отжига
- Праймеры могут быть не полностью комплиментарны матрице, но изменения допустимы только на 5’-конце, 3 ’-конец обязательно должен быть полностью комплиментарным
- Желательно, чтобы праймеры заканчивались на 3 ’-конце на A/T
- Недопустима самокомплементарность(димеры и вторичные структуры) праймеров, особенно на 3 ’-конце (минимум 4 нуклеотида)
- Стандартная концентрация праймеров в реакционной смеси 0,2-0,5 мкМ

Поиск последовательности нуклеотидов

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Searching Escherichia coli K-12 substr. MG1655 reference genome (EcoCyc) change organism database
Sites
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using database Escherichia coli K-12 substr. MG1655 what is this?
Genes (2) | Proteins (1)
Genes Gene/Gene Product pages contain: chromosomal location of gene; depiction of its operon; link to genome browser; detailed summaries and citations; subunit structure (for protein complexes); cofactors, activators, and inhibitors (for enzymes), depiction of regulon (for transcriptional regulators), protein features.
ettA
metT (metTalpha)
Turn into a SmartTable
Proteins Gene/Gene Product pages contain: chromosomal location of gene; depiction of its operon; link to genome browser; detailed summaries and citations; subunit structure (for protein complexes); cofactors, activators, and inhibitors (for enzymes), depiction of regulon (for transcriptional regulators), protein features.
energy-dependent translational throttle protein EttA
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gene polypeptide

ettA energy-dependent translational throttle protein EttA

Escherichia coli K-12 substr. MG1655

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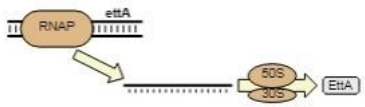
Synonym	yjjK		
Accession IDs	EG12343 (EcoCyc) b4391 ECK4383 P0A9W3 (UniProt)	Length	1668 bp / 555 aa
		Map Position	[4,628,855 <- 4,630,522] (99.72 centisomes, 359°) View in Genome Browser
		Locations	cytosol, inner membrane
Evidence	Inferred from direct assay [Boel14] Inferred from mutant phenotype [Chen14a]		

Summary | GO Terms (13) | Essentiality | Protein Features | Operons | References | Show All

Regulation Summary Diagram



Summary

EttA is a translation factor that gates ribosome entry into the translation elongation cycle through a nucleotide-dependent interaction that is sensitive to the ATP/ADP ratio. Higher amounts of ATP relieve ADP-dependent inhibition of protein synthesis by EttA, suggesting that the elevated ADP/ATP ratio found in energy-depleted cells leads to stabilization of 70S translation initiation complexes in a hibernating conformation [Boel14]. Mechanistically, EttA modulates the movements of the ribosome and tRNAs that are required for polypeptide elongation. A model for regulation of translation by EttA has been proposed [Chen14a].

EttA is a member of the ATP-binding cassette F (ABC-F) protein family with two ABC domains, each containing an insertion in the loop after the first of the three α helices in the ABCα subdomain, and separated by an 81 residue linker domain [Boel14]. A cryo-EM structure of EttA bound to the ribosome showed that the linker domain is a P-site tRNA-interaction domain [Chen14a]. A crystal structure of EttA has been solved at 2.4 Å resolution, showing a domain-swapped dimer. Soluble EttA exists in a slowly reversible monomer-dimer equilibrium that favors the monomeric form at concentrations found *in vivo* [Boel14].

The ATPase activity of wild type EttA is stimulated by the presence of ribosomes. The EttA-EQ_2 mutant, which is expected to prevent ATP hydrolysis and trap EttA in its ATP-bound conformation, has a dominant negative effect on growth by inhibiting protein synthesis after formation of the first peptide bond [Boel14]. ATP-bound EttA-EQ_2 binds to the ribosomal E site and kinetically traps the ribosomal PRE complex in the MS-I state [Chen14a]. An *ettA* deletion strain shows decreased survival of long-term stationary phase [Boel14].

EttA: energy-dependent translational throttle **A** [Boel14]

Review: [Prossliner18]

Comment: [Fredrick14]

Additional Citations: [Linton98, ParadisBleau14, Xu17, Cochrane15, Murina19]

Unification Links

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DIP	DIP-48138N
EchoBASE	EB2247
EcoliWiki	b4391
Mint	MINT-1222078
OU-Microarray	b4391
PortEco	yjjK
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RefSeq	NP_418808
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Relationship Links

InterPro In-Family	IPR003439, IPR003593, IPR017871, IPR022374, IPR027417, IPR032781
Panther In-Family	PTHR43858
PDB Structure	3J5S, 4FIN
Pfam In-Family	PF00005, PF12848

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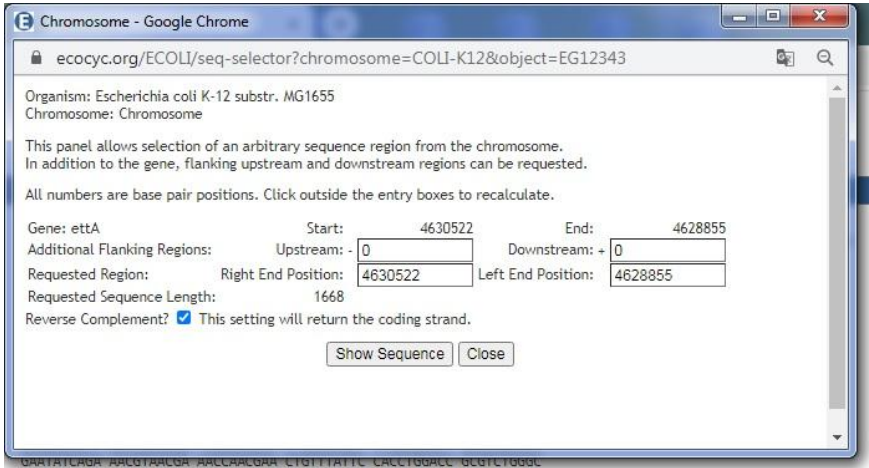
OPERATIONS
Sequences
- Get Protein Sequence
- Get Nucleotide Sequence
- BLAST the Nucleotide Sequence
- BLAST the Protein Sequence
- Save Nucleotide Sequence to File
- Save Protein Sequence to File

Comparison Operations
- Change Organisms/Databases for Comparison Operations
- Show This Gene in Another Database
- Search for This Gene in Multiple Databases
- Show Orthologs (with Operon Diagrams) in Multiple Databases
- Align in Multi-Genome Browser
- Align Gene Nucleotide Sequence with Orthologs
- Align Gene Product Amino Acid Sequence with Orthologs

Functional Linkage
- Genome Context Analysis

Other
- Get Email Notification of Updates to This Gene

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Chromosome: **Chromosome**
Region: 4628855 - 4630522 (reverse complement)

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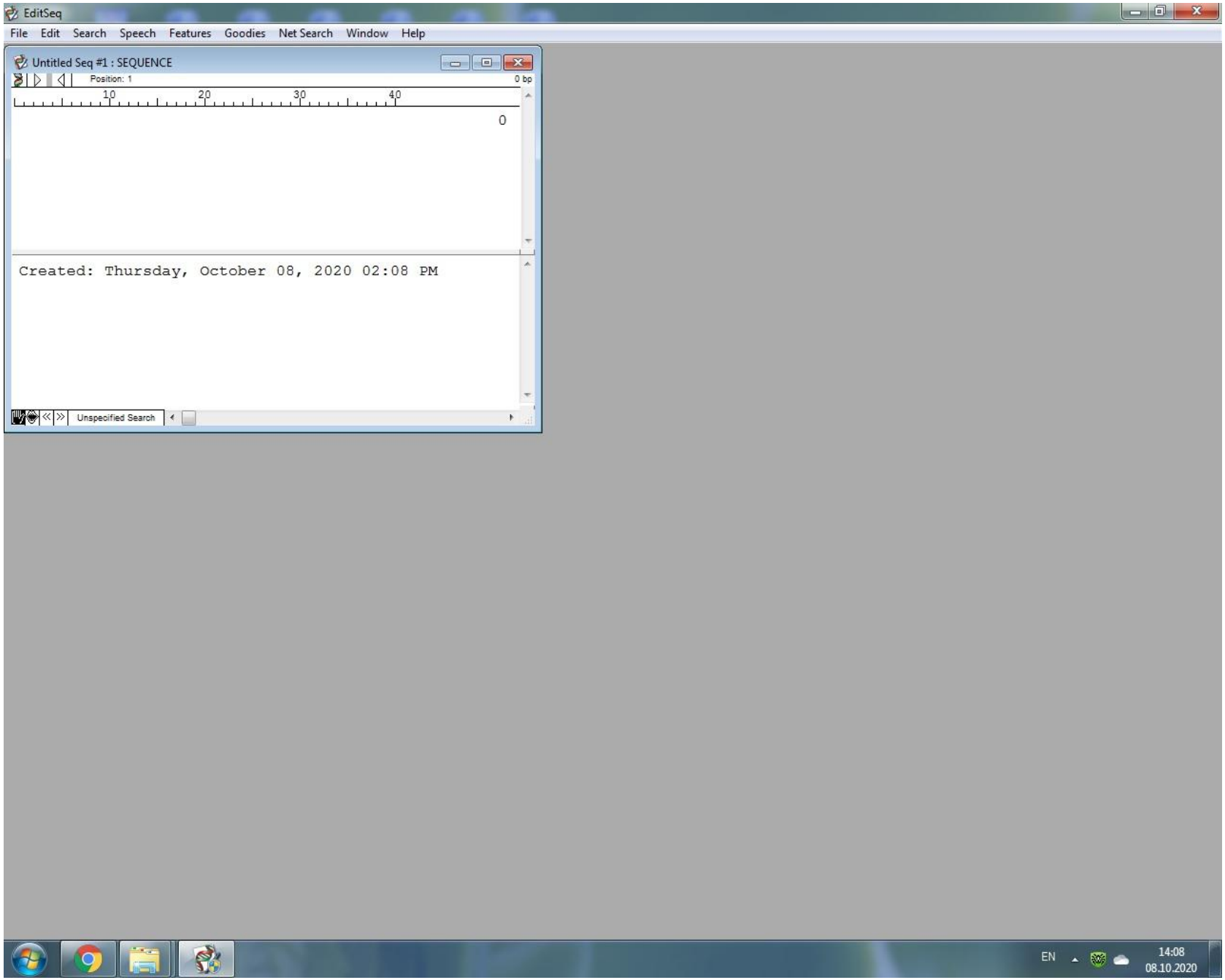
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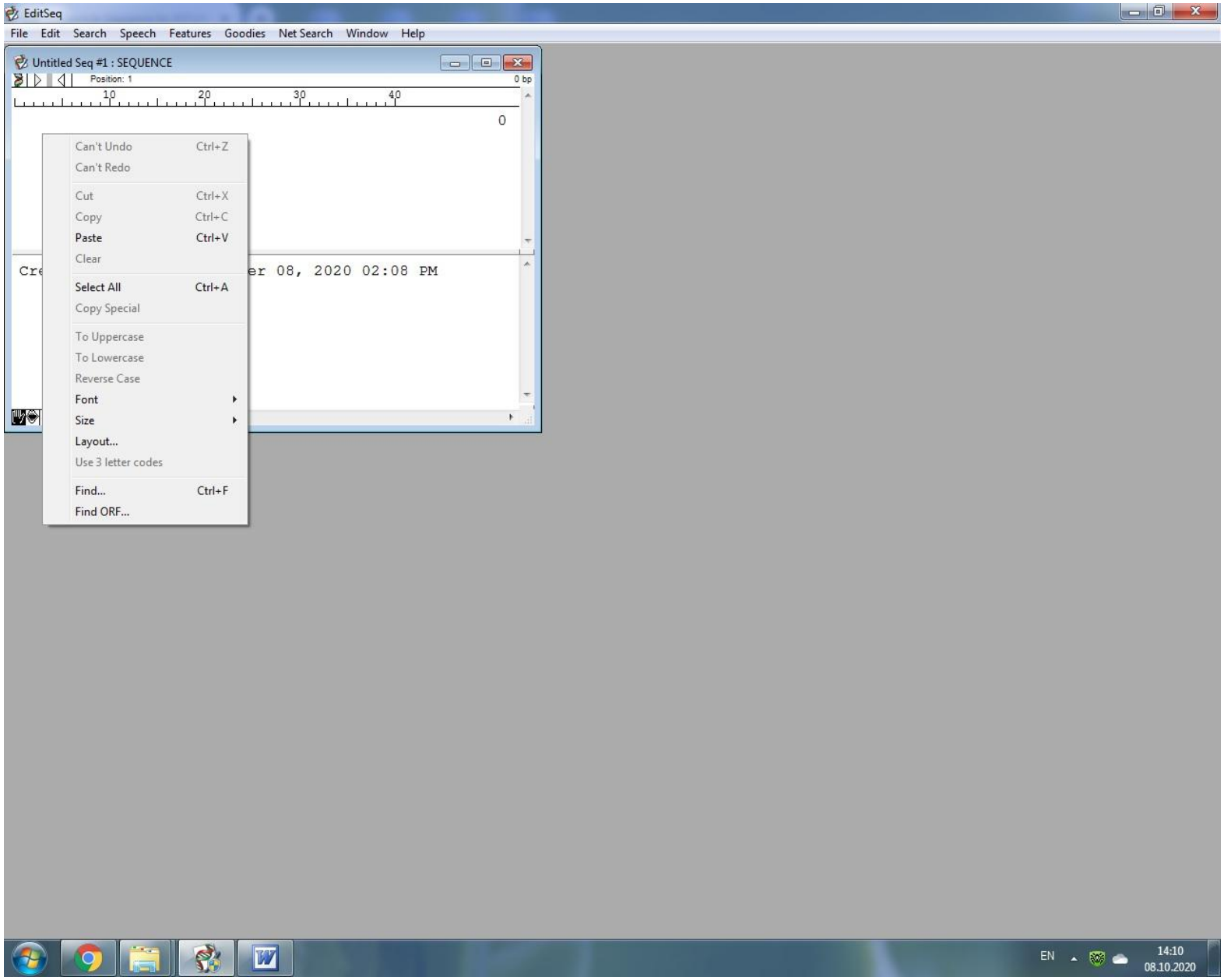
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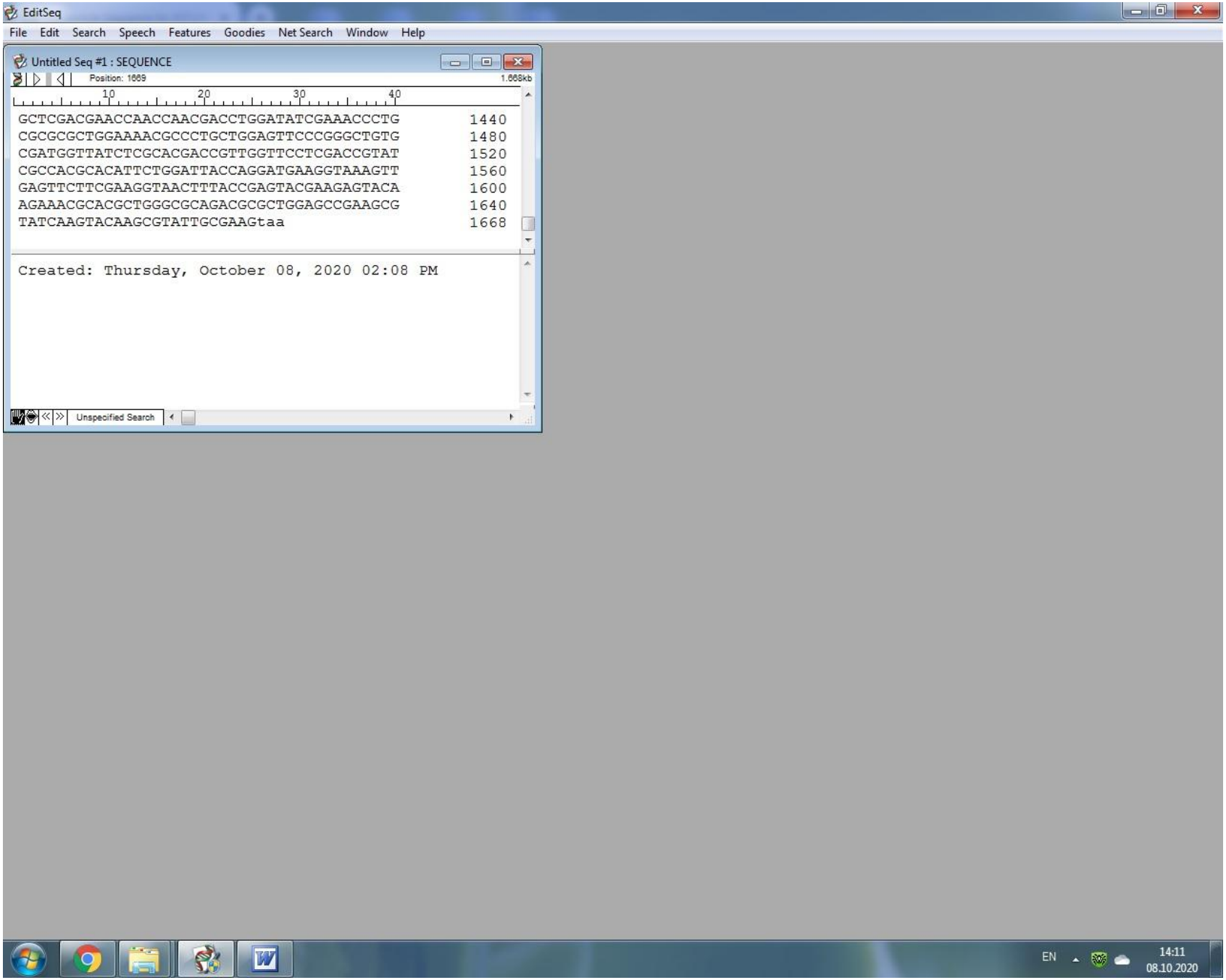
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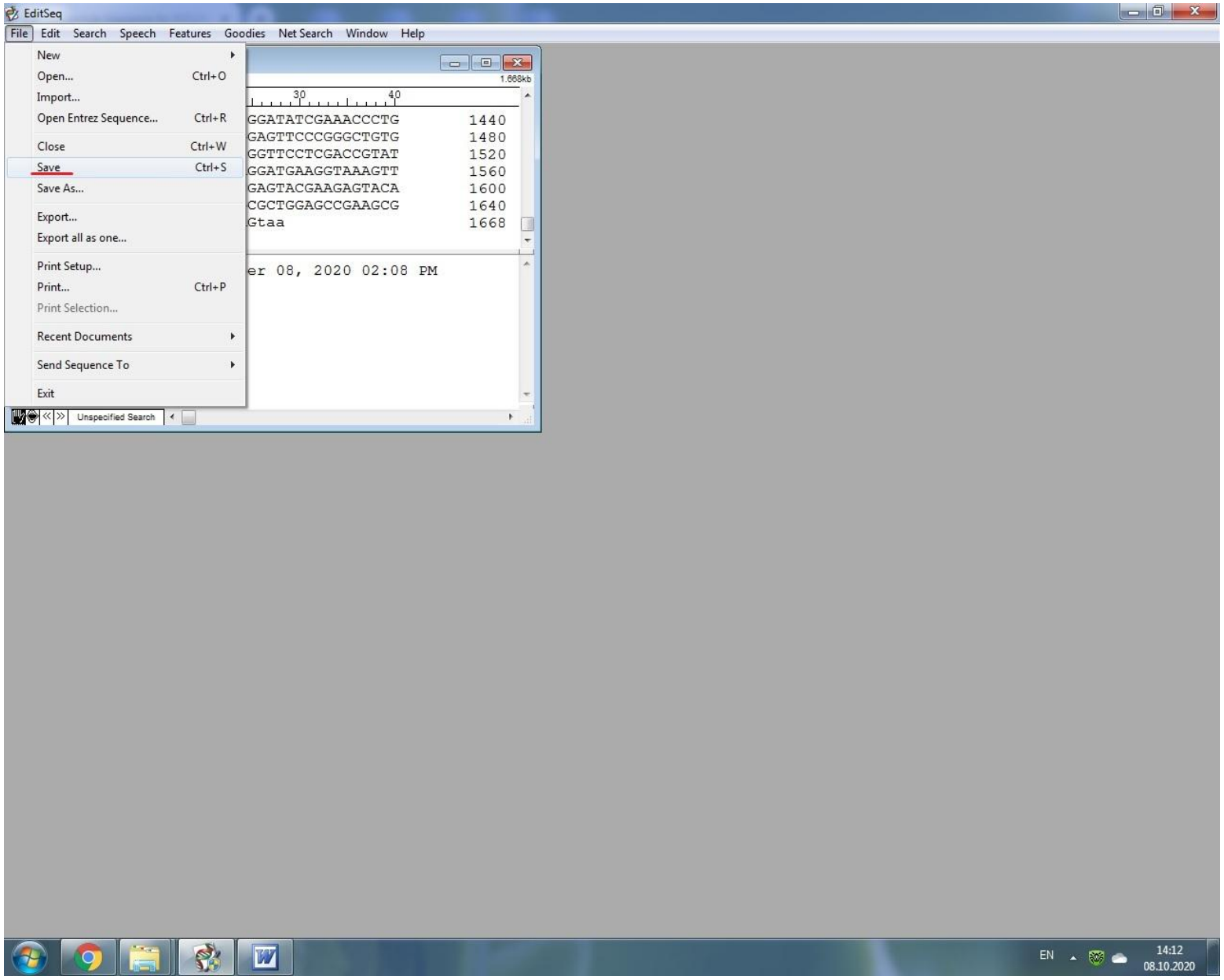
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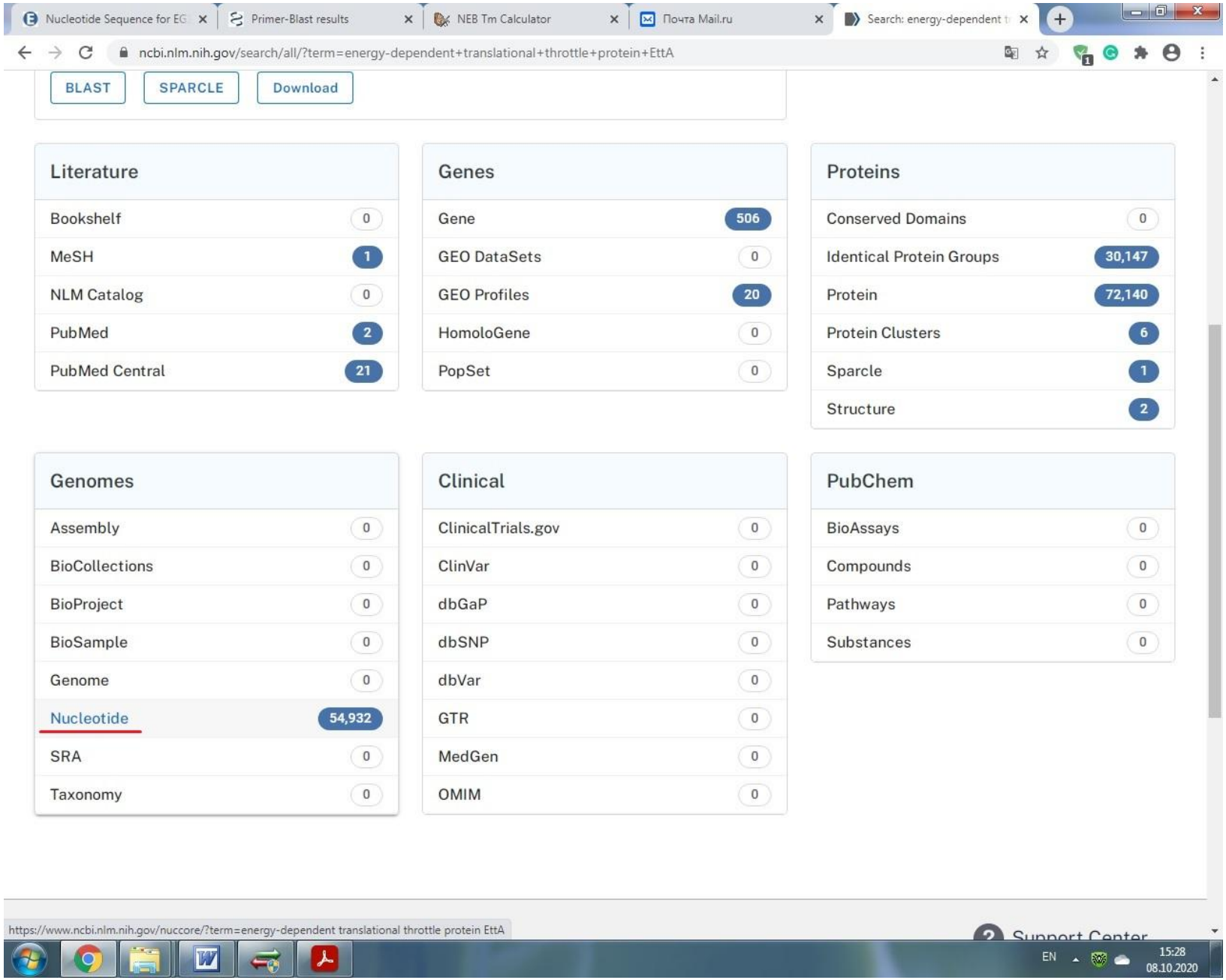
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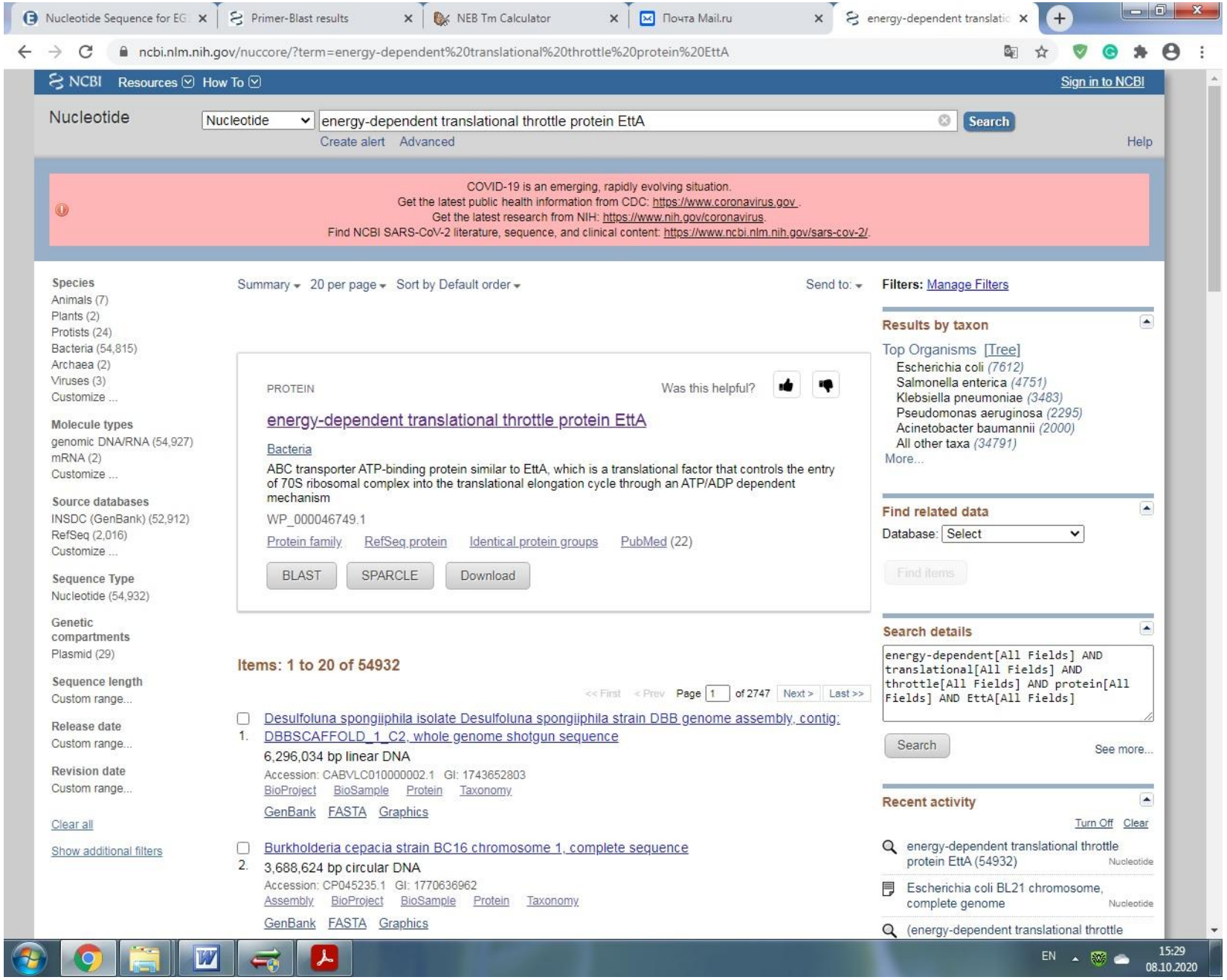
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Plants (2)
Protists (24)
Bacteria (54,815)
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energy-dependent translational throttle protein EttA
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ABC transporter ATP-binding protein similar to EttA, which is a translational factor that controls the entry of 70S ribosomal complex into the translational elongation cycle through an ATP/ADP dependent mechanism
WP_000046749.1
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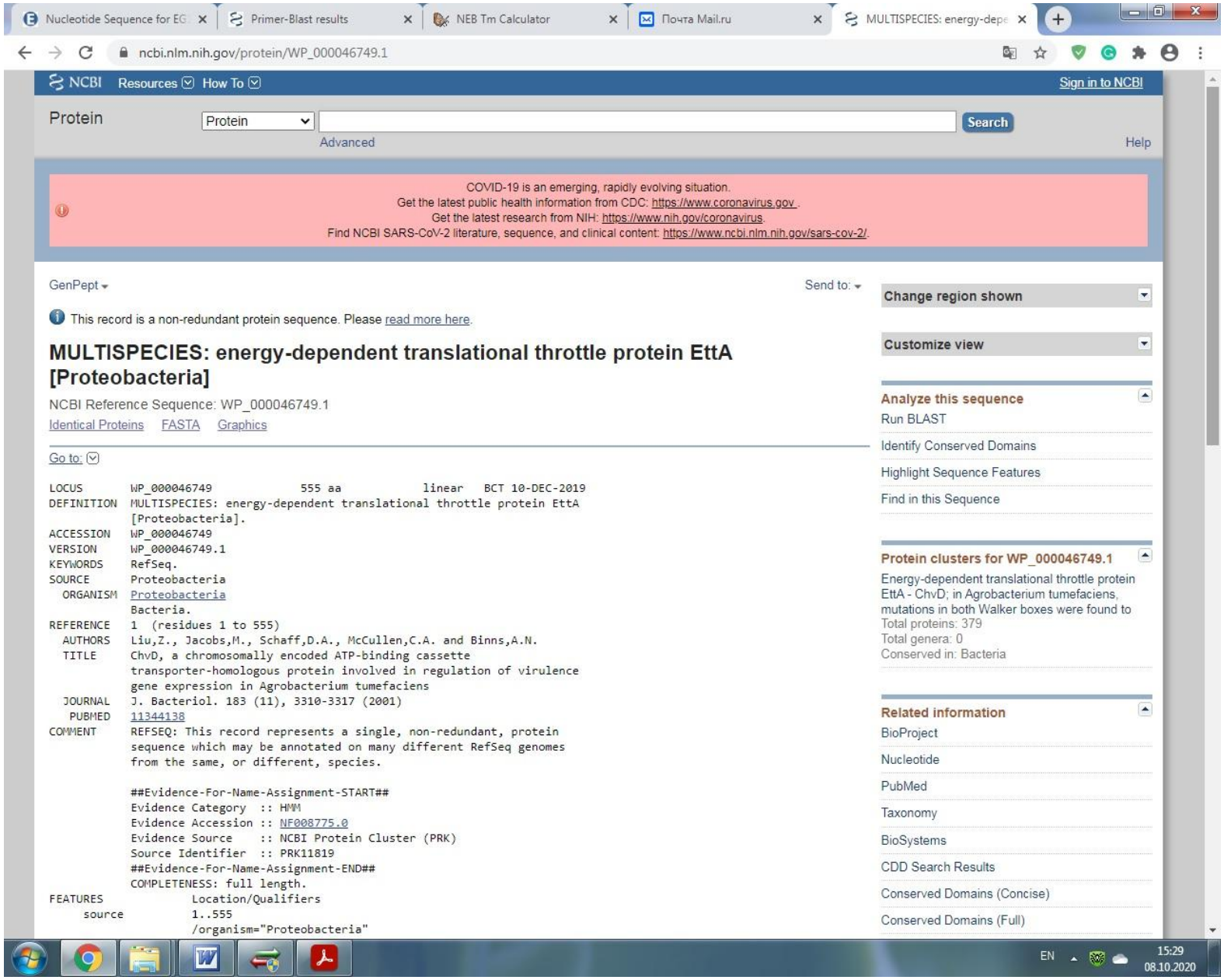
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Advanced
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Get the latest research from NIH: https://www.nih.gov/coronavirus.
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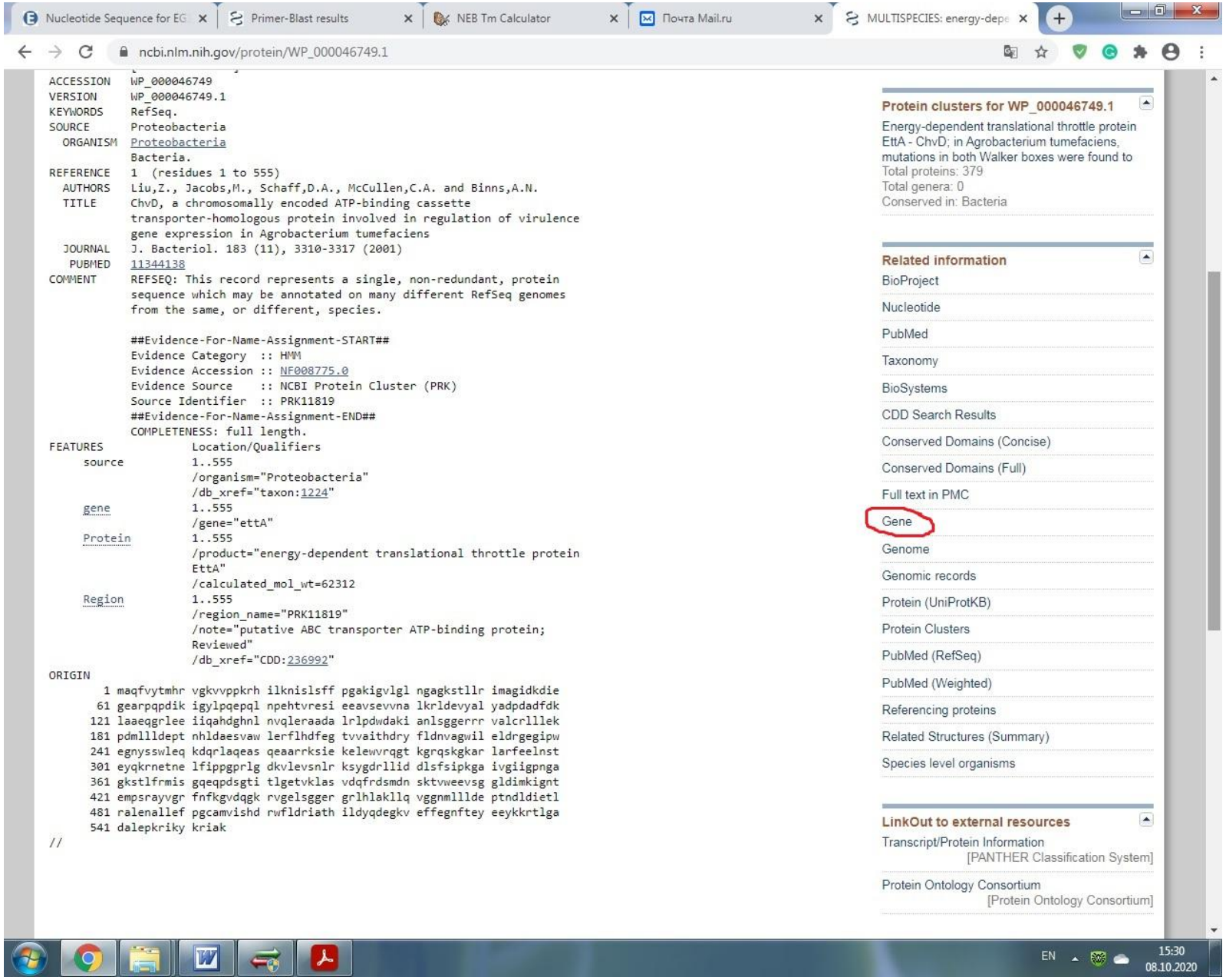
08.10.2020


Nucleotide Sequence for EG
Primer-Blast results
NEB Tm Calculator
Почта Mail.ru
MULTISPECIES: energy-depe
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ACCESSION WP_000046749
VERSION WP_000046749.1
KEYWORDS RefSeq.
SOURCE Proteobacteria
ORGANISM Proteobacteria
Bacteria.
REFERENCE 1 (residues 1 to 555)
AUTHORS Liu,Z., Jacobs,M., Schaff,D.A., McCullen,C.A. and Binns,A.N.
TITLE ChvD, a chromosomally encoded ATP-binding cassette
transporter-homologous protein involved in regulation of virulence
gene expression in Agrobacterium tumefaciens
JOURNAL J. Bacteriol. 183 (11), 3310-3317 (2001)
PUBMED 11344138
COMMENT REFSEQ: This record represents a single, non-redundant, protein
sequence which may be annotated on many different RefSeq genomes
from the same, or different, species.
##Evidence-For-Name-Assignment-START##
Evidence Category :: HMM
Evidence Accession :: NF008775.0
Evidence Source :: NCBI Protein Cluster (PRK)
Source Identifier :: PRK11819
##Evidence-For-Name-Assignment-END##
COMPLETENESS: full length.
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Protein 1..555
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EttA"
/calculated_mol_wt=62312
Region 1..555
/region_name="PRK11819"
/note="putative ABC transporter ATP-binding protein;
Reviewed"
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121 laaeqgrlee iiqahdghnl nvqleraada lrlpdwdaki anlsggerrr valcrlllek
181 pdmllldept nhldaesvaw lerflhdfeg tvvaithdry fldnvagwil eldrgegipw
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301 eyqkrnetne lfippgprlg dkvlevsnlr ksygdrllid dlsfsipkga ivgiigpnga
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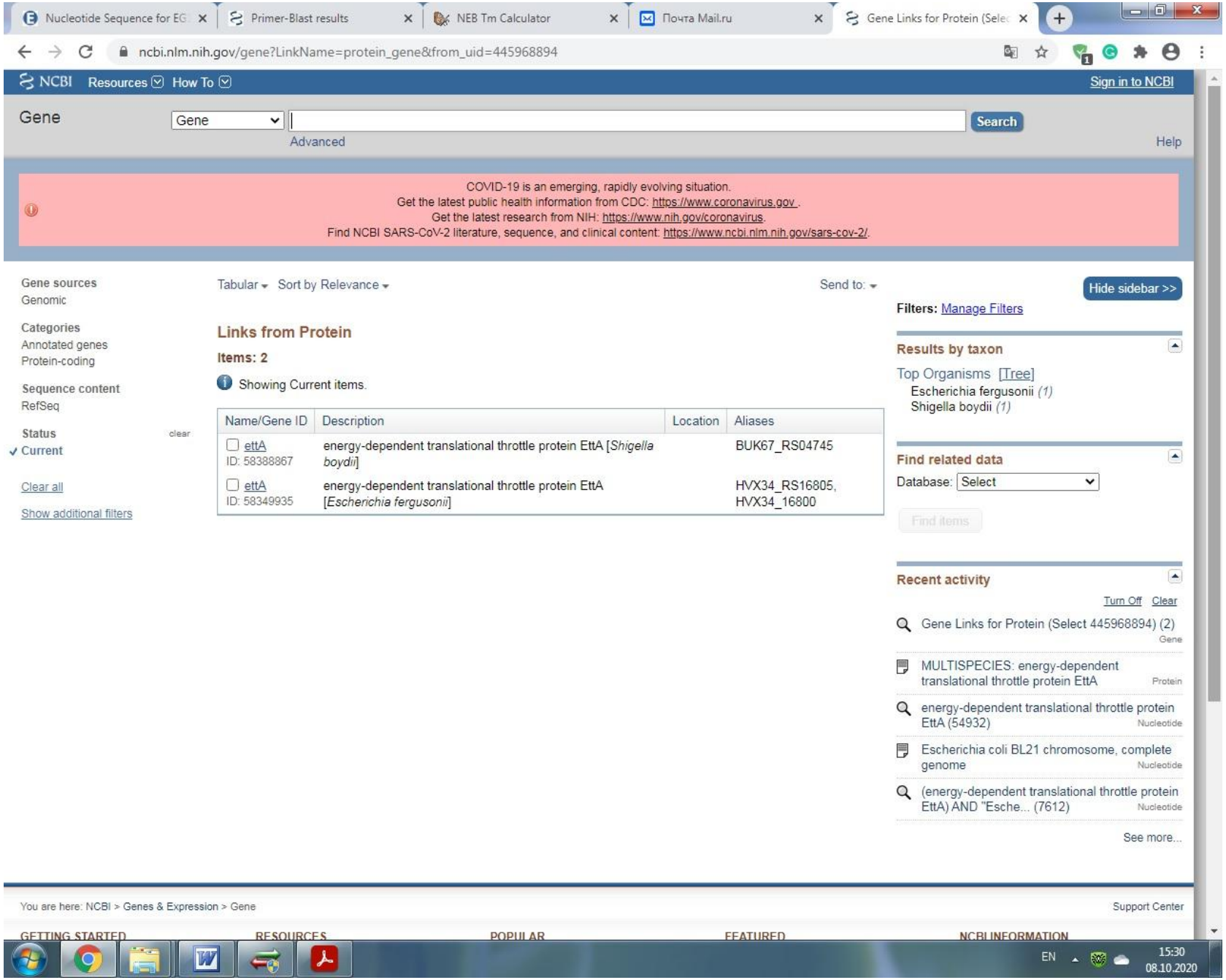
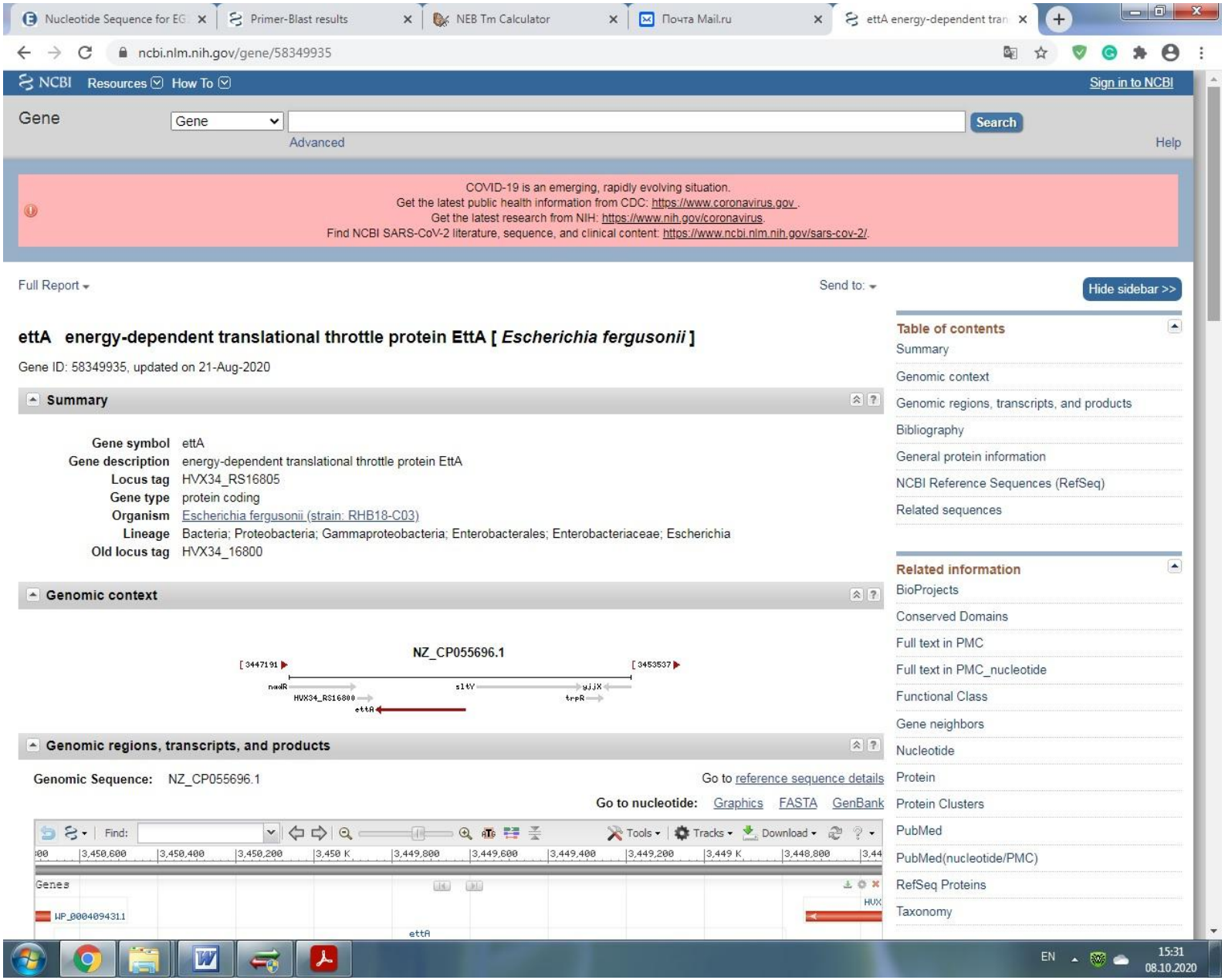
421 empsrayvgr fnfkgvdqgk rvgelsgger grlhlakllq vggnmlllde ptndldietl
481 ralenallef pgcamvishd rwfldriath ildyqdegkv effegnftey eeykkrtlga
541 dalepkriky kriak
//
Protein clusters for WP_000046749.1
Energy-dependent translational throttle protein EttA - ChvD; in Agrobacterium tumefaciens, mutations in both Walker boxes were found to
Total proteins: 379
Total genera: 0
Conserved in: Bacteria
Related information
BioProject
Nucleotide
PubMed
Taxonomy
BioSystems
CDD Search Results
Conserved Domains (Concise)
Conserved Domains (Full)
Full text in PMC
Gene
Genome
Genomic records
Protein (UniProtKB)
Protein Clusters
PubMed (RefSeq)
PubMed (Weighted)
Referencing proteins
Related Structures (Summary)
Species level organisms
LinkOut to external resources
Transcript/Protein Information
[PANTHER Classification System]
Protein Ontology Consortium
[Protein Ontology Consortium]
EN
15:30
08.10.2020


Nucleotide Sequence for EG
Primer-Blast results
NEB Tm Calculator
Почта Mail.ru
Gene Links for Protein (Sele
ncbi.nlm.nih.gov/gene?LinkName=protein_gene&from_uid=445968894
NCBI Resources How To
Sign in to NCBI
Gene
Gene
Search
Advanced
Help
COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: https://www.coronavirus.gov .
Get the latest research from NIH: https://www.nih.gov/coronavirus.
Find NCBI SARS-CoV-2 literature, sequence, and clinical content: https://www.ncbi.nlm.nih.gov/sars-cov-2/.
Gene sources
Genomic
Categories
Annotated genes
Protein-coding
Sequence content
RefSeq
Status
clear
Current
Clear all
Show additional filters
Tabular
Sort by Relevance
Send to:
Links from Protein
Items: 2
Showing Current items.
Name/Gene ID
Description
Location
Aliases
ettA
ID: 58388867
energy-dependent translational throttle protein EttA [Shigella boydii]
BUK67_RS04745
ettA
ID: 58349935
energy-dependent translational throttle protein EttA [Escherichia fergusonii]
HVX34_RS16805, HVX34_16800
Hide sidebar >>
Filters: Manage Filters
Results by taxon
Top Organisms [Tree]
Escherichia fergusonii (1)
Shigella boydii (1)
Find related data
Database: Select
Find items
Recent activity
Turn Off
Clear
Gene Links for Protein (Select 445968894) (2)
Gene
MULTISPECIES: energy-dependent translational throttle protein EttA
Protein
energy-dependent translational throttle protein EttA (54932)
Nucleotide
Escherichia coli BL21 chromosome, complete genome
Nucleotide
(energy-dependent translational throttle protein EttA) AND "Esche... (7612)
Nucleotide
See more...
You are here: NCBI > Genes & Expression > Gene
Support Center
GETTING STARTED
RESOURCES
POPULAR
FEATURED
NCBI INFORMATION
EN
15:30
08.10.2020


Nucleotide Sequence for EG
Primer-Blast results
NEB Tm Calculator
Почта Mail.ru
ettA energy-dependent tran
ncbi.nlm.nih.gov/gene/58349935
NCBI Resources How To
Sign in to NCBI
Gene
Gene
Search
Advanced
Help
COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: https://www.coronavirus.gov .
Get the latest research from NIH: https://www.nih.gov/coronavirus.
Find NCBI SARS-CoV-2 literature, sequence, and clinical content: https://www.ncbi.nlm.nih.gov/sars-cov-2/.
Full Report
Send to:
Hide sidebar >>
ettA energy-dependent translational throttle protein EttA [Escherichia fergusonii]
Gene ID: 58349935, updated on 21-Aug-2020
Summary
Gene symbol ettA
Gene description energy-dependent translational throttle protein EttA
Locus tag HVX34_RS16805
Gene type protein coding
Organism Escherichia fergusonii (strain: RHB18-C03)
Lineage Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escherichia
Old locus tag HVX34_16800
Genomic context
NZ_CP055696.1
[3447191
[3453537
nadR
HVX34_RS16800
ettA
sltY
yjjX
trpR
Genomic regions, transcripts, and products
Genomic Sequence: NZ_CP055696.1
Go to reference sequence details
Go to nucleotide: Graphics FASTA GenBank
Find:
Tools
Tracks
Download
3,450,600
3,450,400
3,450,200
3,450 K
3,449,800
3,449,600
3,449,400
3,449,200
3,449 K
3,448,800
Genes
WP_000409431.1
ettA
Table of contents
Summary
Genomic context
Genomic regions, transcripts, and products
Bibliography
General protein information
NCBI Reference Sequences (RefSeq)
Related sequences
Related information
BioProjects
Conserved Domains
Full text in PMC
Full text in PMC_nucleotide
Functional Class
Gene neighbors
Nucleotide
Protein
Protein Clusters
PubMed
PubMed(nucleotide/PMC)
RefSeq Proteins
Taxonomy
EN
15:31
08.10.2020

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      841 aaaggccgtc agtcgaaagg taaagcacgt ctggcgcgct ttgaagagct gaacagcacc
      901 gaatatcaga aacgtaacga aaccaacgaa ctgtttattc cacctggacc gcgtctgggc
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     1141 actttaggtg aaacggtgaa actggcatct gtcgatcagt tccgtgactc aatggataac
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     1501 cgttggttcc tcgaccgtat cgccacgcac atcctggact accaggatga aggtaaagtt
     1561 gaattcttcg aaggtaactt tactgagtac gaagagtaca agaaacgcac gctgggcgca
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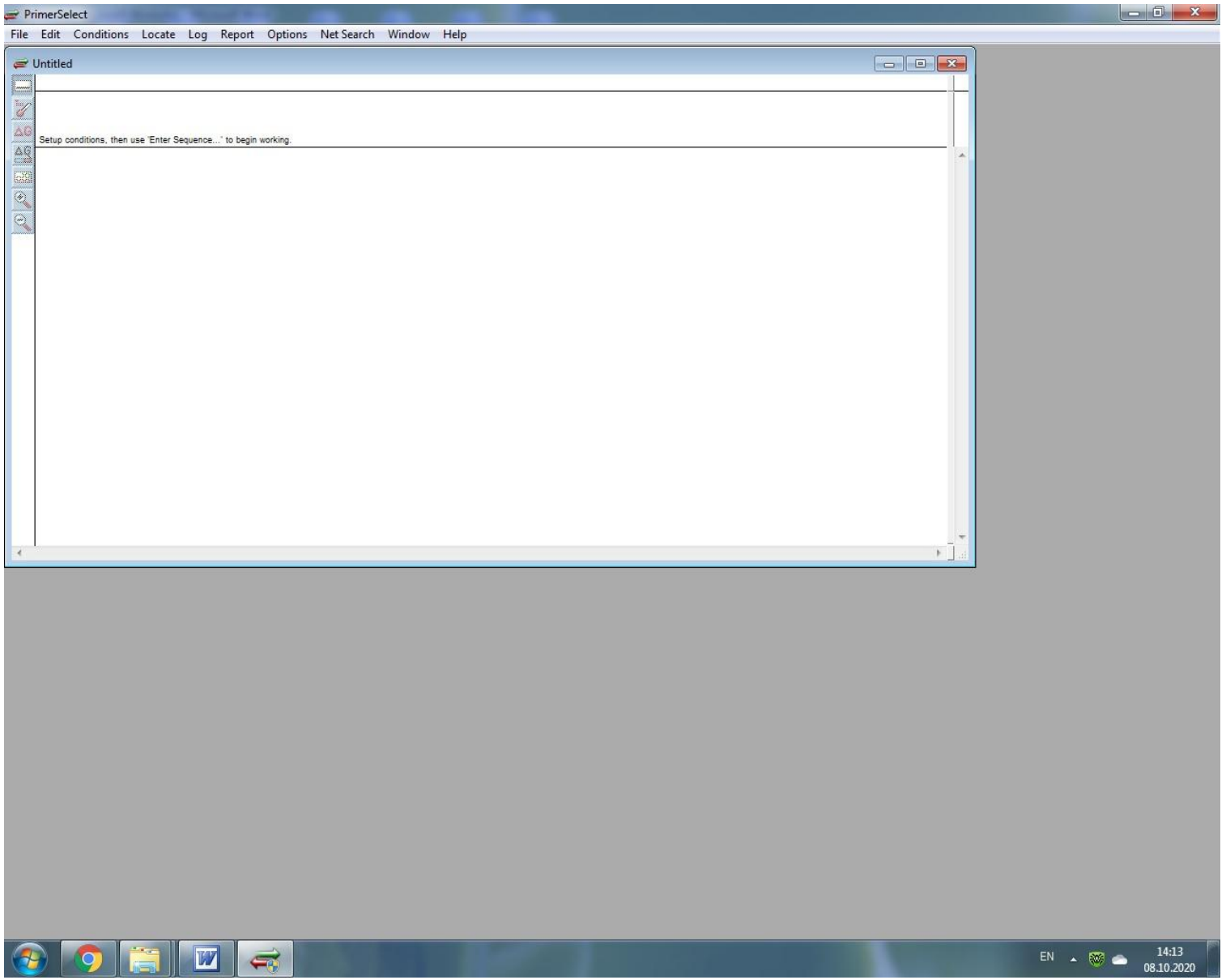
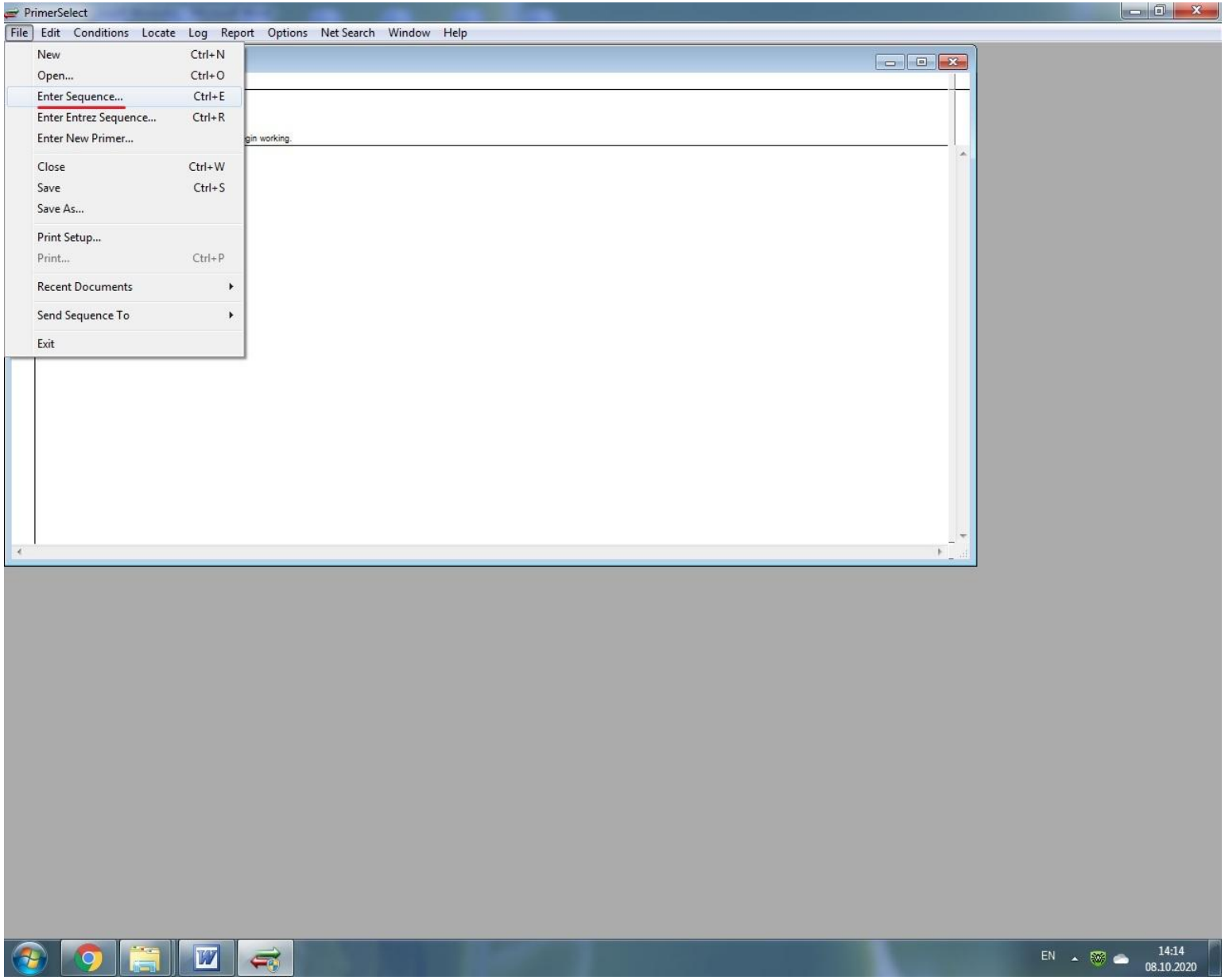
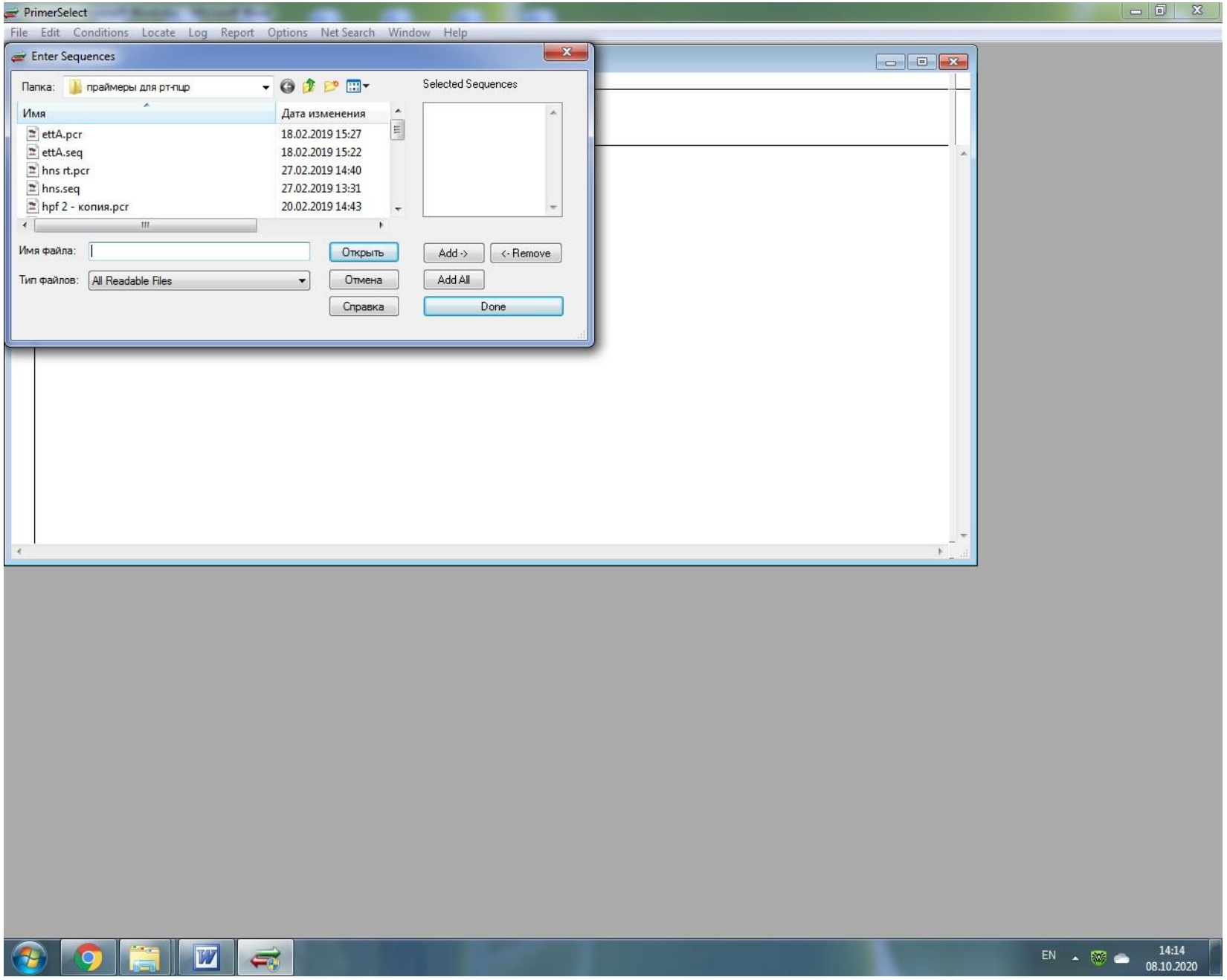
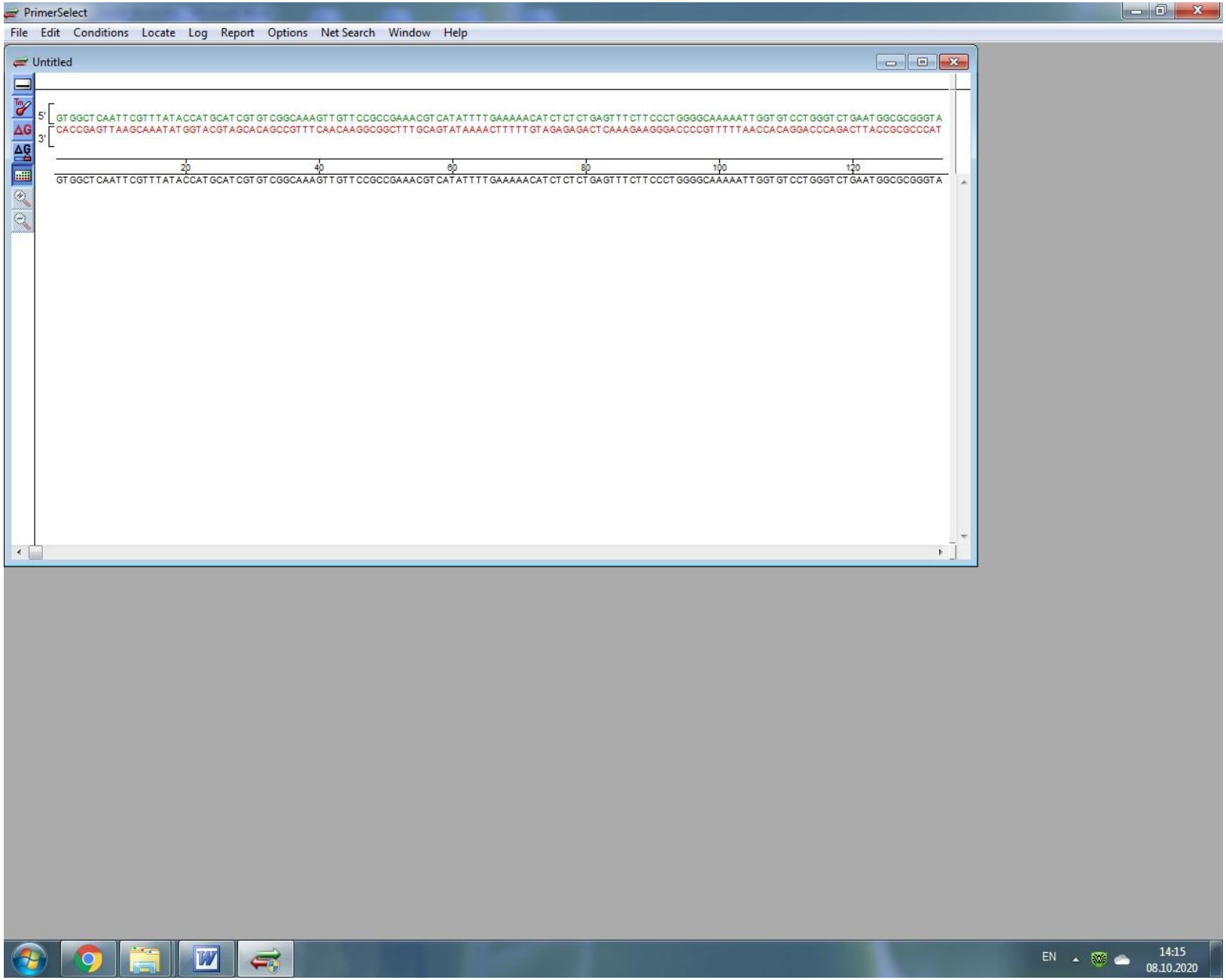
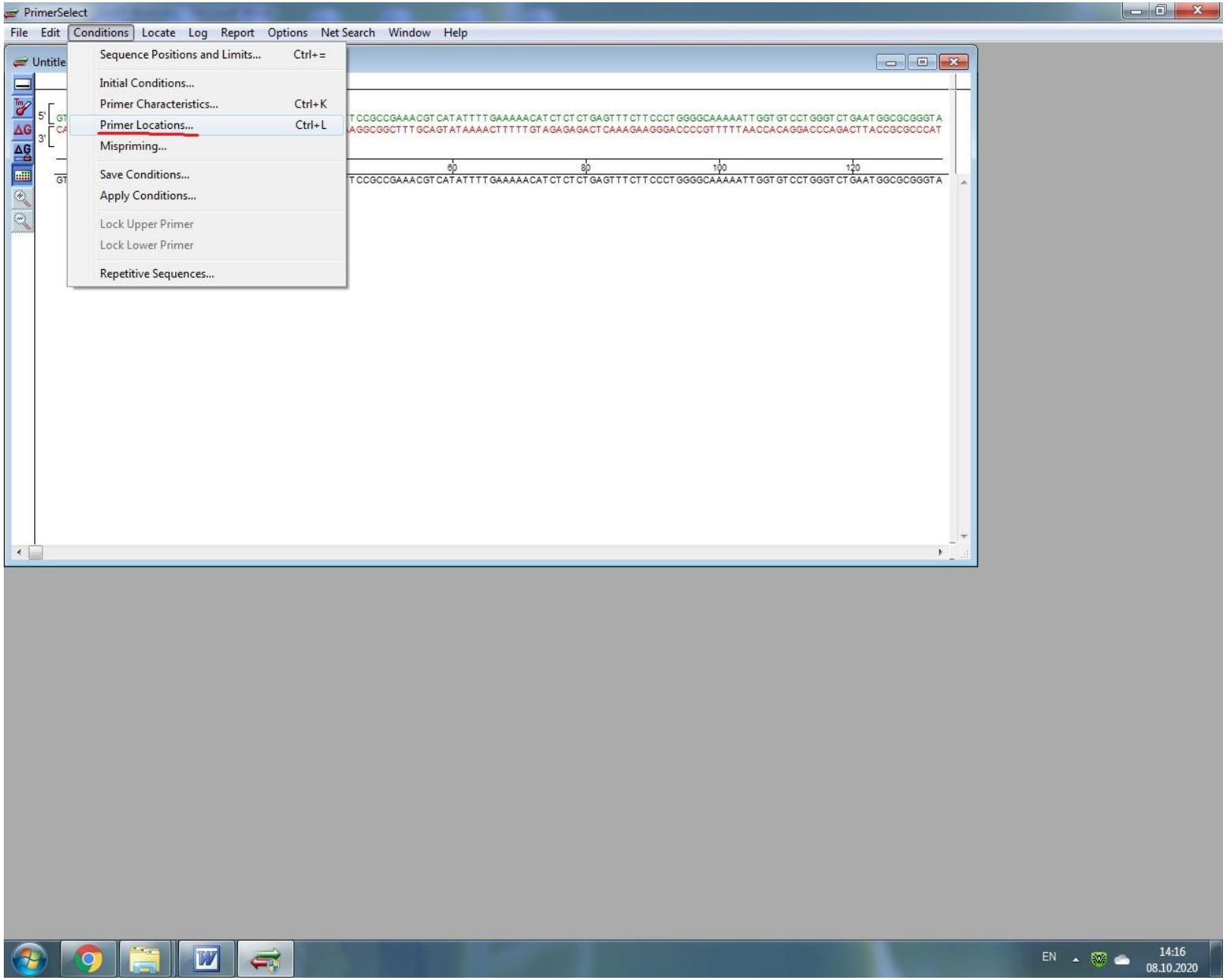
Подбор праймеров в программе “PrimerSelect”

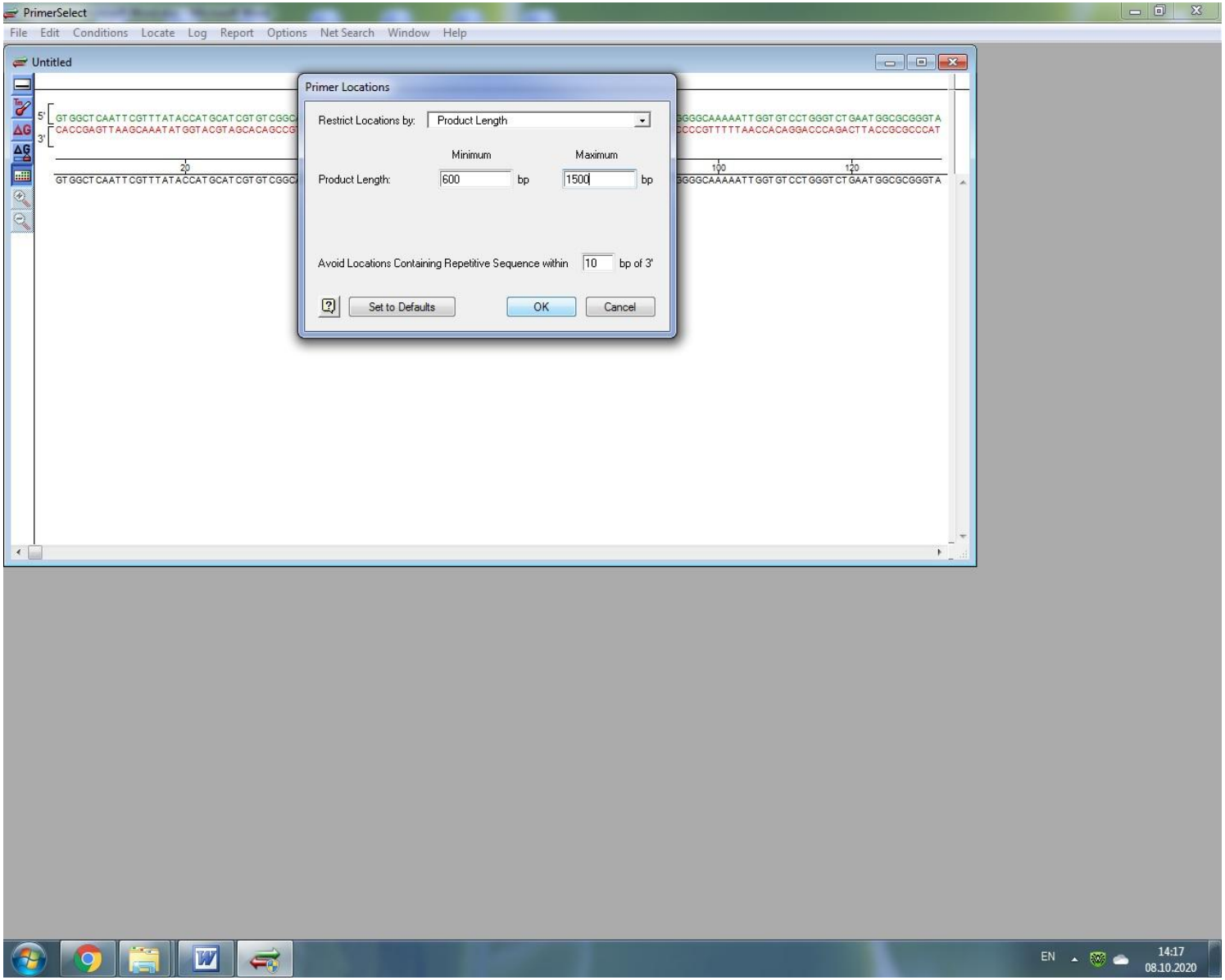


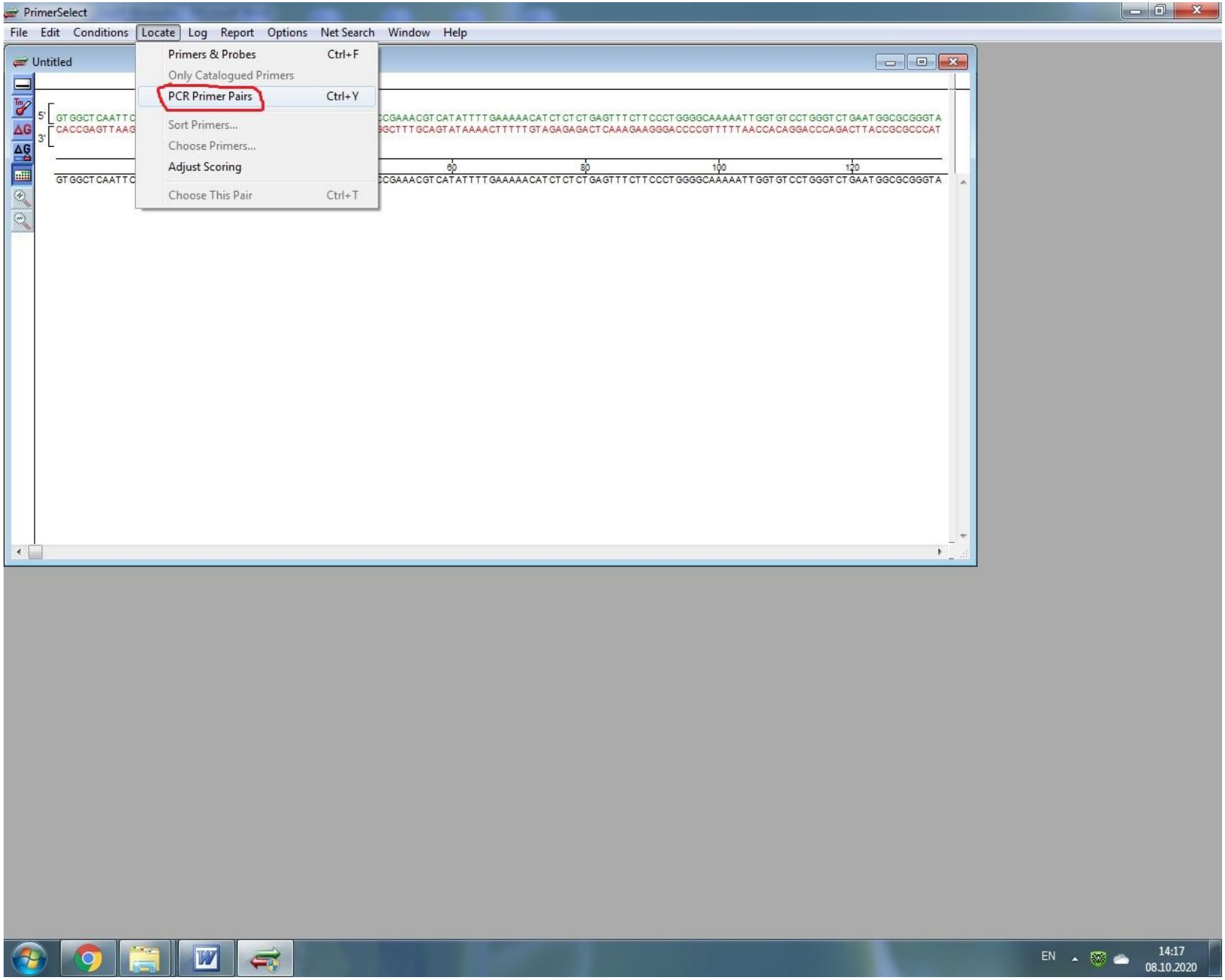

PrimerSelect
File
Edit
Conditions
Locate
Log
Report
Options
Net Search
Window
Help
New Ctrl+N
Open... Ctrl+O
Enter Sequence... Ctrl+E
Enter Entrez Sequence... Ctrl+R
Enter New Primer...
Close Ctrl+W
Save Ctrl+S
Save As...
Print Setup...
Print... Ctrl+P
Recent Documents
Send Sequence To
Exit
gin working.
EN
14:14
08.10.2020


PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Enter Sequences
Папка: праймеры для рт-пцр
Имя
Дата изменения
ettA.pcr 18.02.2019 15:27
ettA.seq 18.02.2019 15:22
hns rt.pcr 27.02.2019 14:40
hns.seq 27.02.2019 13:31
hpf 2 - копия.pcr 20.02.2019 14:43
Selected Sequences
Имя файла:
Тип файлов: All Readable Files
Открыть
Отмена
Справка
Add ->
<- Remove
Add All
Done
EN
14:14
08.10.2020


PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Untitled
5' GT GGCT CAATT CGTTT ATACCAT GCAT CGT GT CGGCAAAGTT GTT CCGCCGAAACGT CAT ATTTT GAAAAACAT CT CT CT GAGTTT CTT CCCT GGGGCAAAAATT GGT GT CCT GGGT CT GAAT GGCGCGGGT A
3' CACCGAGTT AAGCAAATAT GGT ACGT AGCACAGCCGTTT CAACAAGGCGGCTTT GCAGT ATAAAACTTTTT GT AGAGAGACT CAAAGAAGGGACCCCGTTTTT AACCACAGGACCCAGACTT ACCGCGCCCAT
20
40
60
80
100
120
GT GGCT CAATT CGTTT ATACCAT GCAT CGT GT CGGCAAAGTT GTT CCGCCGAAACGT CAT ATTTT GAAAAACAT CT CT CT GAGTTT CTT CCCT GGGGCAAAAATT GGT GT CCT GGGT CT GAAT GGCGCGGGT A
EN
14:15
08.10.2020


PrimerSelect
File
Edit
Conditions
Locate
Log
Report
Options
Net Search
Window
Help
Sequence Positions and Limits... Ctrl+=
Initial Conditions...
Primer Characteristics... Ctrl+K
Primer Locations... Ctrl+L
Mispriming...
Save Conditions...
Apply Conditions...
Lock Upper Primer
Lock Lower Primer
Repetitive Sequences...
5'
3'
60
80
100
120
EN
14:16
08.10.2020


PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Untitled
Primer Locations
Restrict Locations by:
Product Length
Minimum
Maximum
Product Length:
600
bp
1500
bp
Avoid Locations Containing Repetitive Sequence within
10
bp of 3'
Set to Defaults
OK
Cancel
EN
14:17
08.10.2020


PrimerSelect
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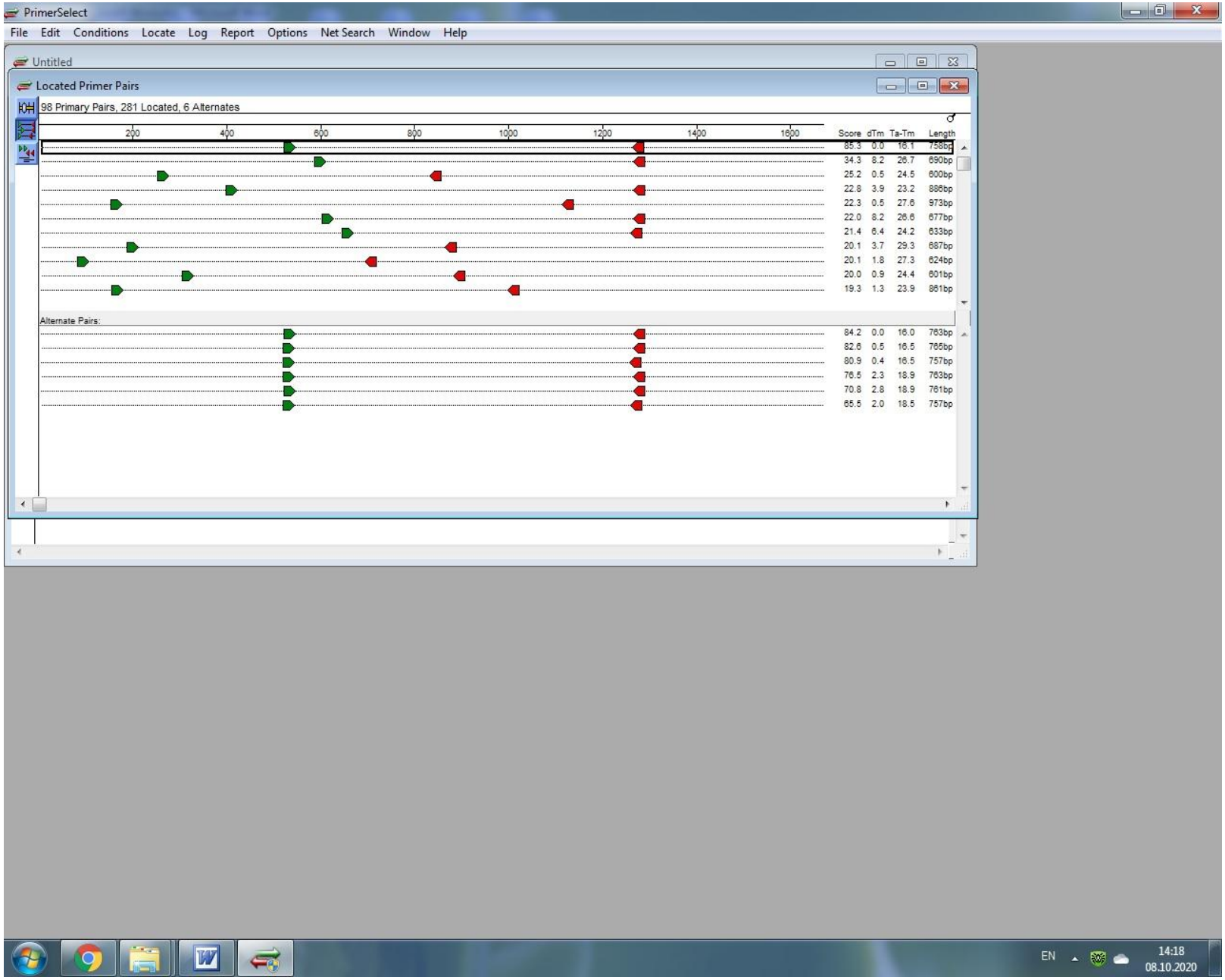
Untitled
Primers & Probes Ctrl+F
Only Catalogued Primers
PCR Primer Pairs Ctrl+Y
Sort Primers...
Choose Primers...
Adjust Scoring
Choose This Pair Ctrl+T
5'
3'
60
80
100
120
EN
14:17
08.10.2020


PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Untitled
Located Primer Pairs
98 Primary Pairs, 281 Located, 6 Alternates
200 400 600 800 1000 1200 1400 1600
Score dTm Ta-Tm Length
85.3 0.0 16.1 758bp
34.3 8.2 26.7 690bp
25.2 0.5 24.5 600bp
22.8 3.9 23.2 886bp
22.3 0.5 27.6 973bp
22.0 8.2 26.6 677bp
21.4 6.4 24.2 633bp
20.1 3.7 29.3 687bp
20.1 1.8 27.3 624bp
20.0 0.9 24.4 601bp
19.3 1.3 23.9 861bp
Alternate Pairs:
84.2 0.0 16.0 763bp
82.6 0.5 16.5 765bp
80.9 0.4 16.5 757bp
76.5 2.3 18.9 763bp
70.8 2.8 18.9 761bp
65.5 2.0 18.5 757bp
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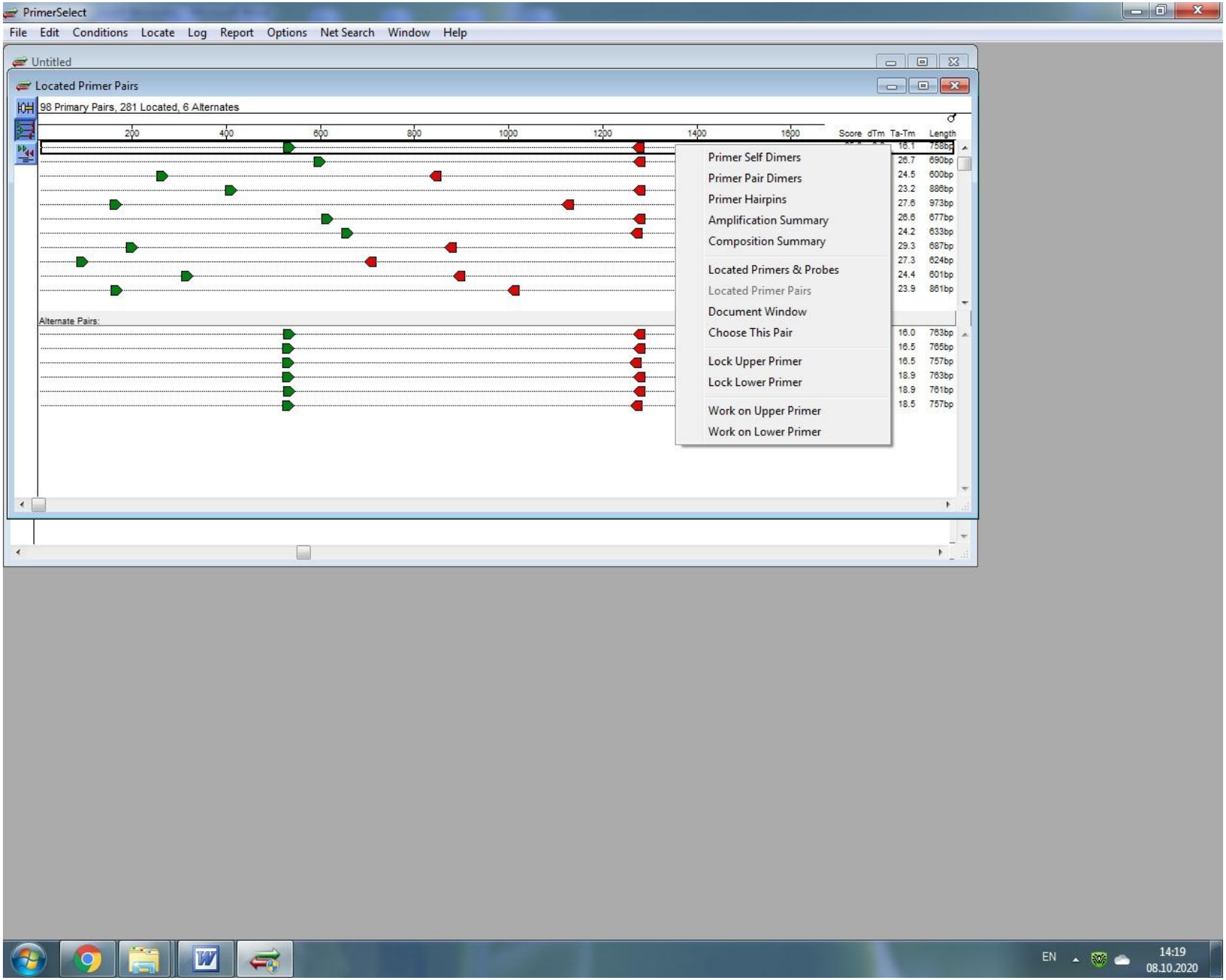
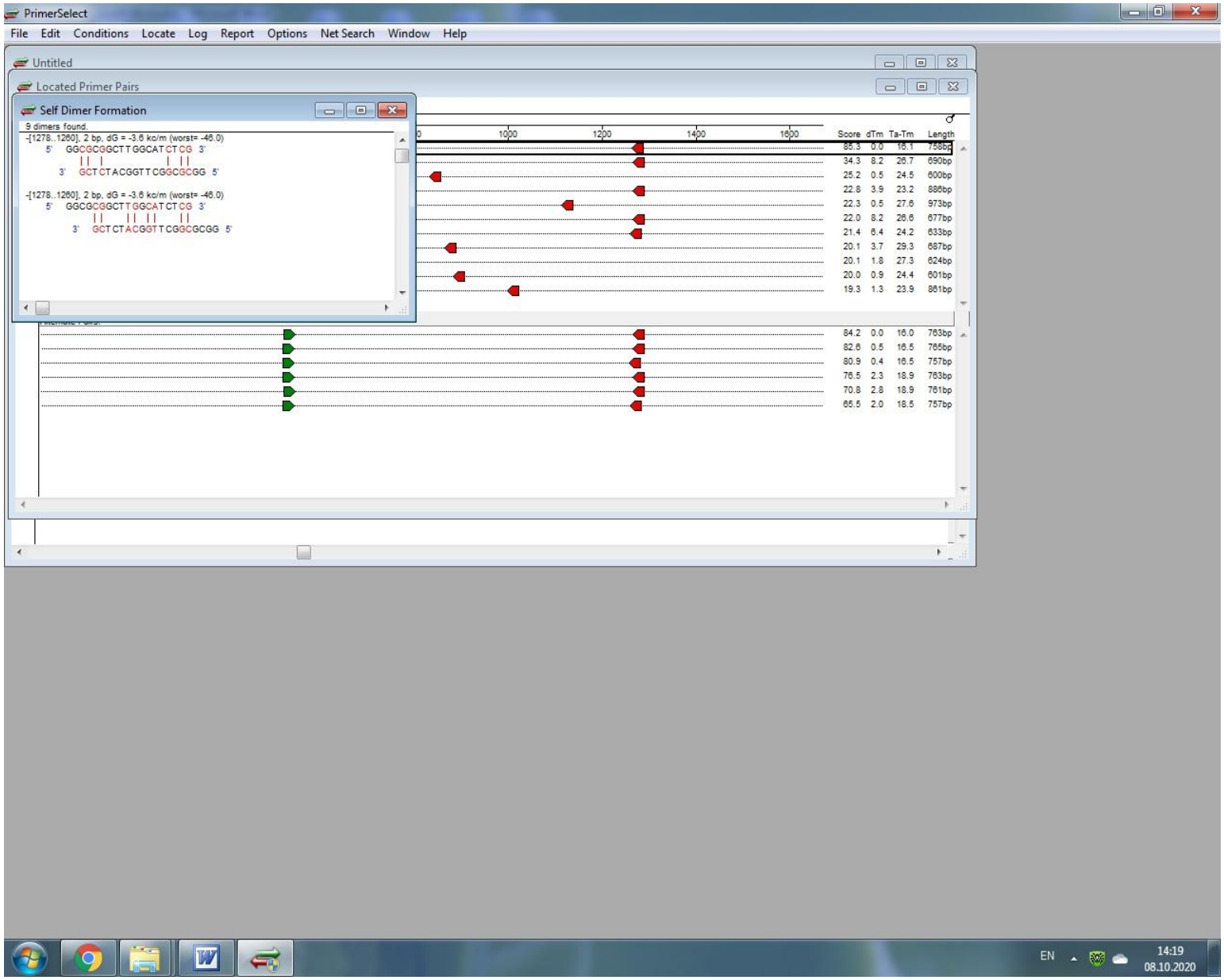
08.10.2020


PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Untitled
Located Primer Pairs
98 Primary Pairs, 281 Located, 6 Alternates
200 400 600 800 1000 1200 1400 1600
Score dTm Ta-Tm Length
Alternate Pairs:
Primer Self Dimers
Primer Pair Dimers
Primer Hairpins
Amplification Summary
Composition Summary
Located Primers & Probes
Located Primer Pairs
Document Window
Choose This Pair
Lock Upper Primer
Lock Lower Primer
Work on Upper Primer
Work on Lower Primer
EN
14:19
08.10.2020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File Edit Conditions Locate Log Report Options Net Search Window Help
Untitled
Located Primer Pairs
Self Dimer Formation
9 dimers found.
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3' GCTCTACGGTTCGGCGCGG 5'
-[1278..1260], 2 bp, dG = -3.6 kc/m (worst= -46.0)
5' GGCGCGGCTTGGCATCTCG 3'
3' GCTCTACGGTTCGGCGCGG 5'
1000
1200
1400
1600
Score dTm Ta-Tm Length
85.3 0.0 16.1 758bp
34.3 8.2 26.7 690bp
25.2 0.5 24.5 600bp
22.8 3.9 23.2 886bp
22.3 0.5 27.6 973bp
22.0 8.2 26.6 677bp
21.4 6.4 24.2 633bp
20.1 3.7 29.3 687bp
20.1 1.8 27.3 624bp
20.0 0.9 24.4 601bp
19.3 1.3 23.9 861bp
84.2 0.0 16.0 763bp
82.6 0.5 16.5 765bp
80.9 0.4 16.5 757bp
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70.8 2.8 18.9 761bp
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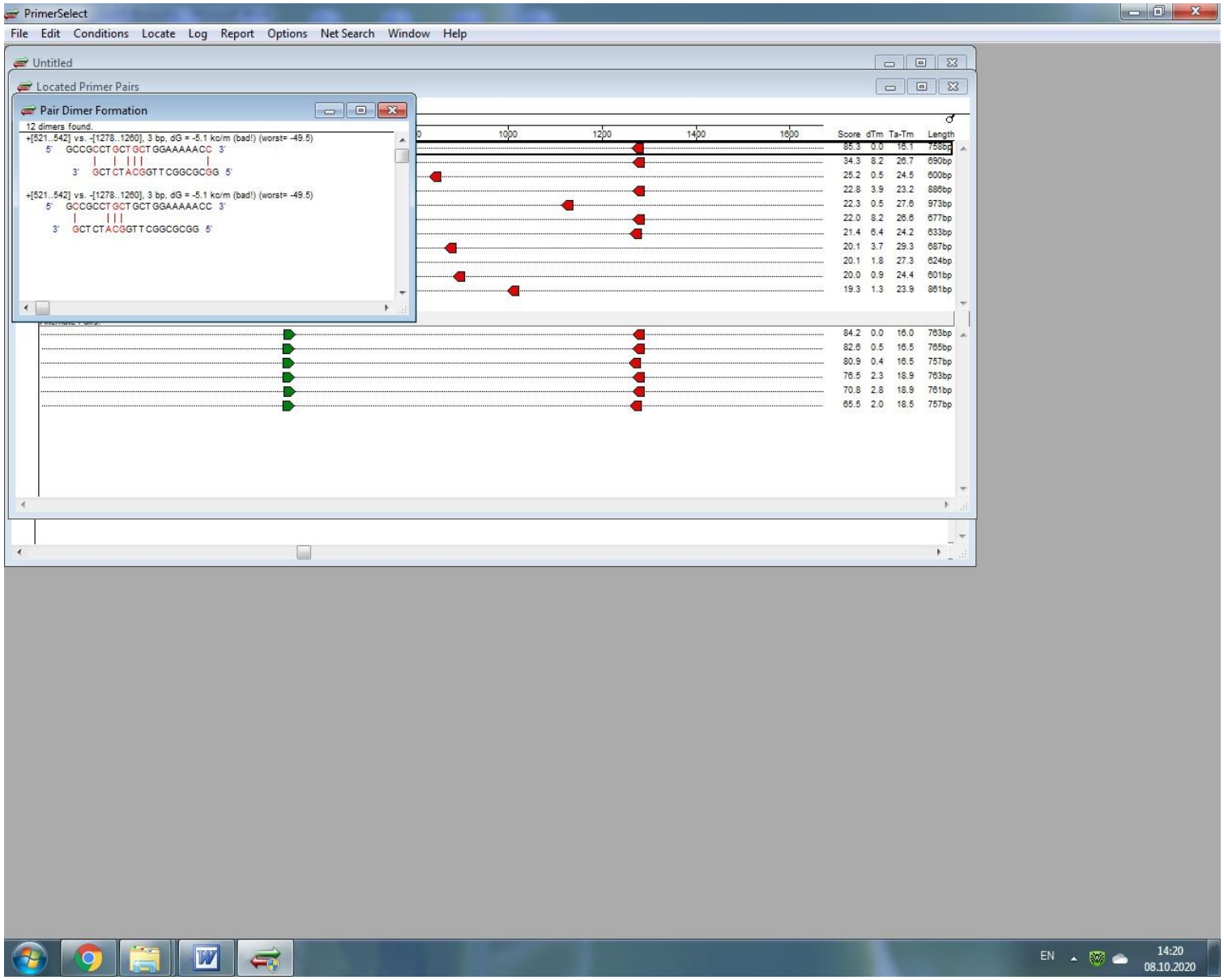
14:19
08.10.2020


PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Untitled
Located Primer Pairs
Pair Dimer Formation
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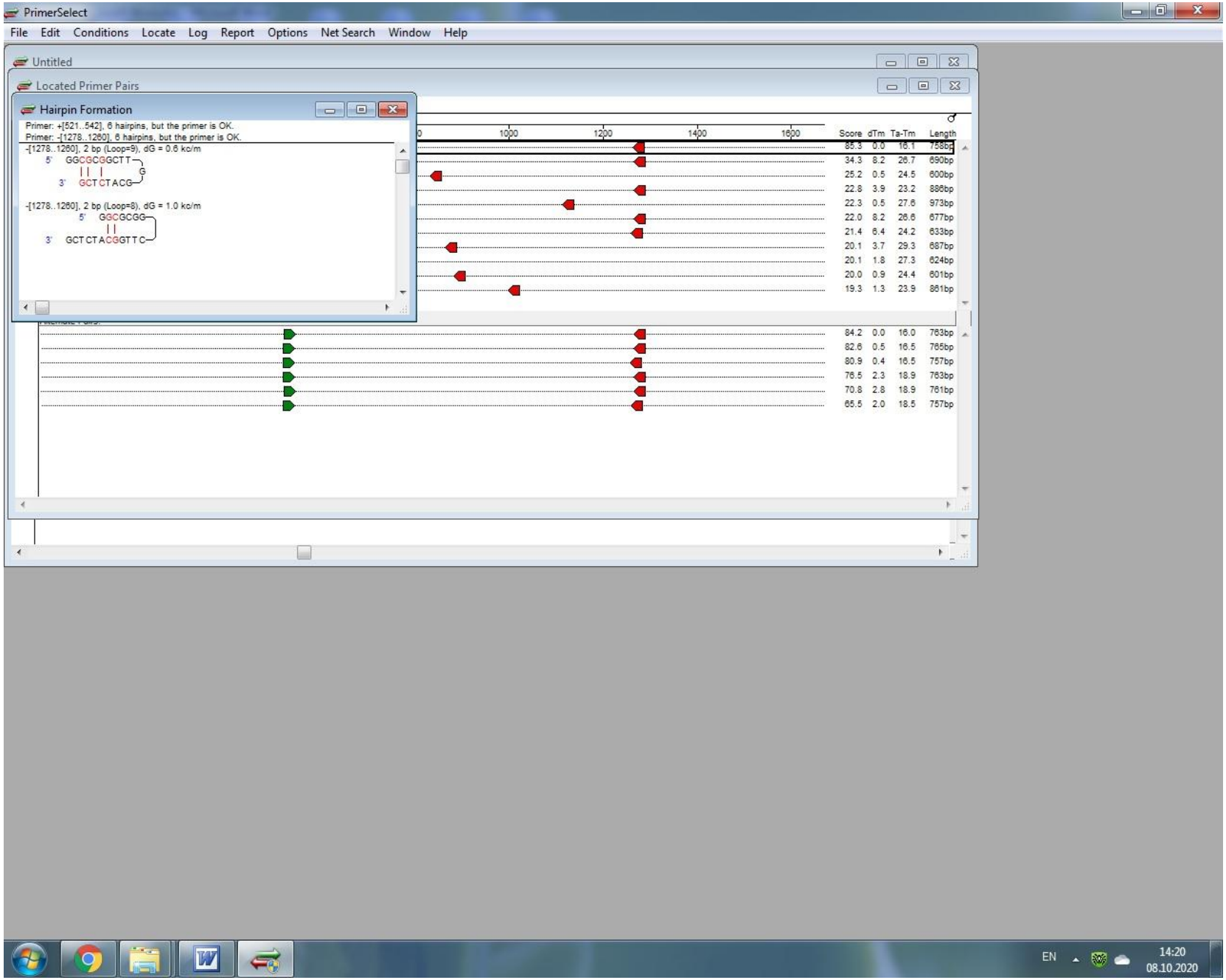
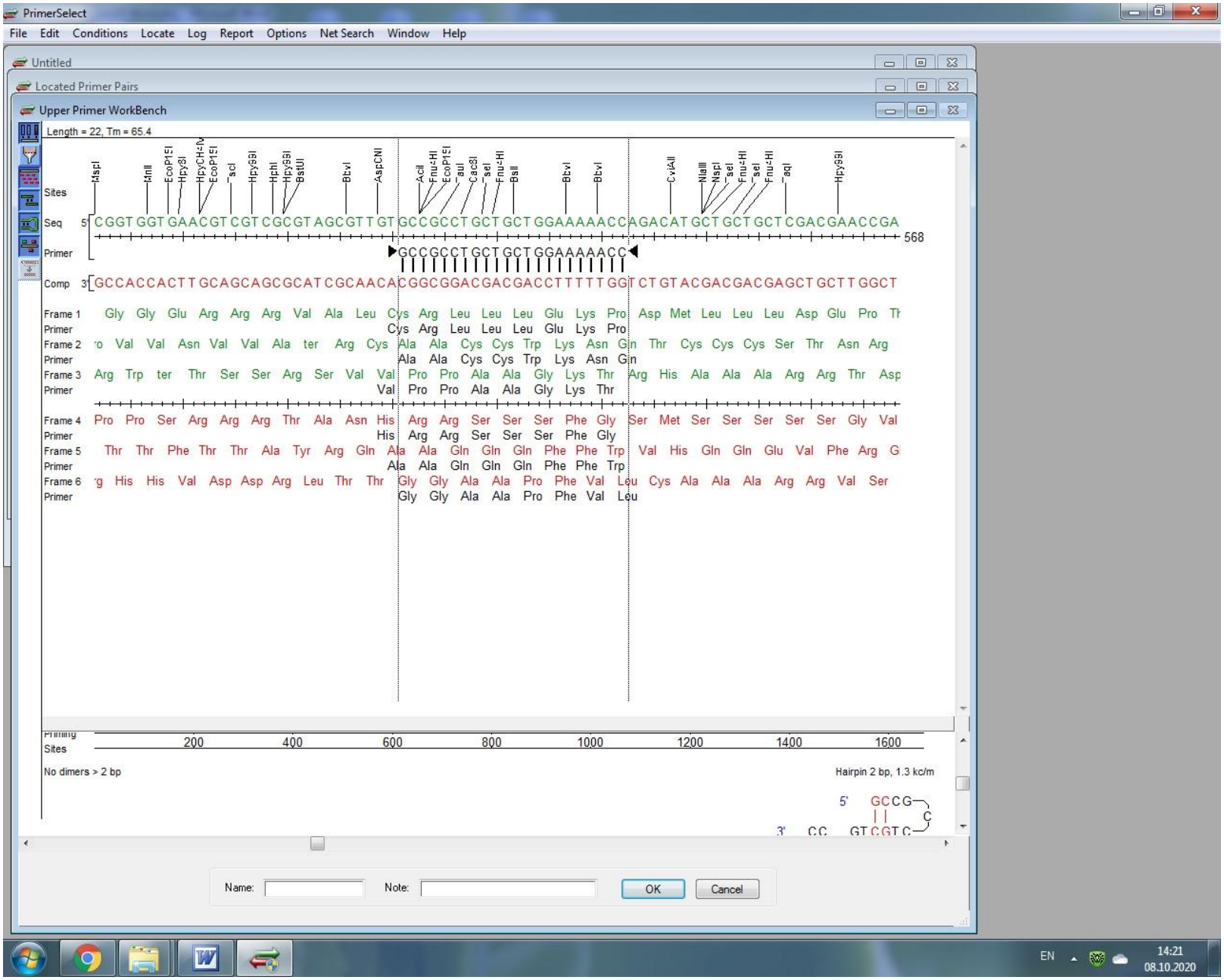
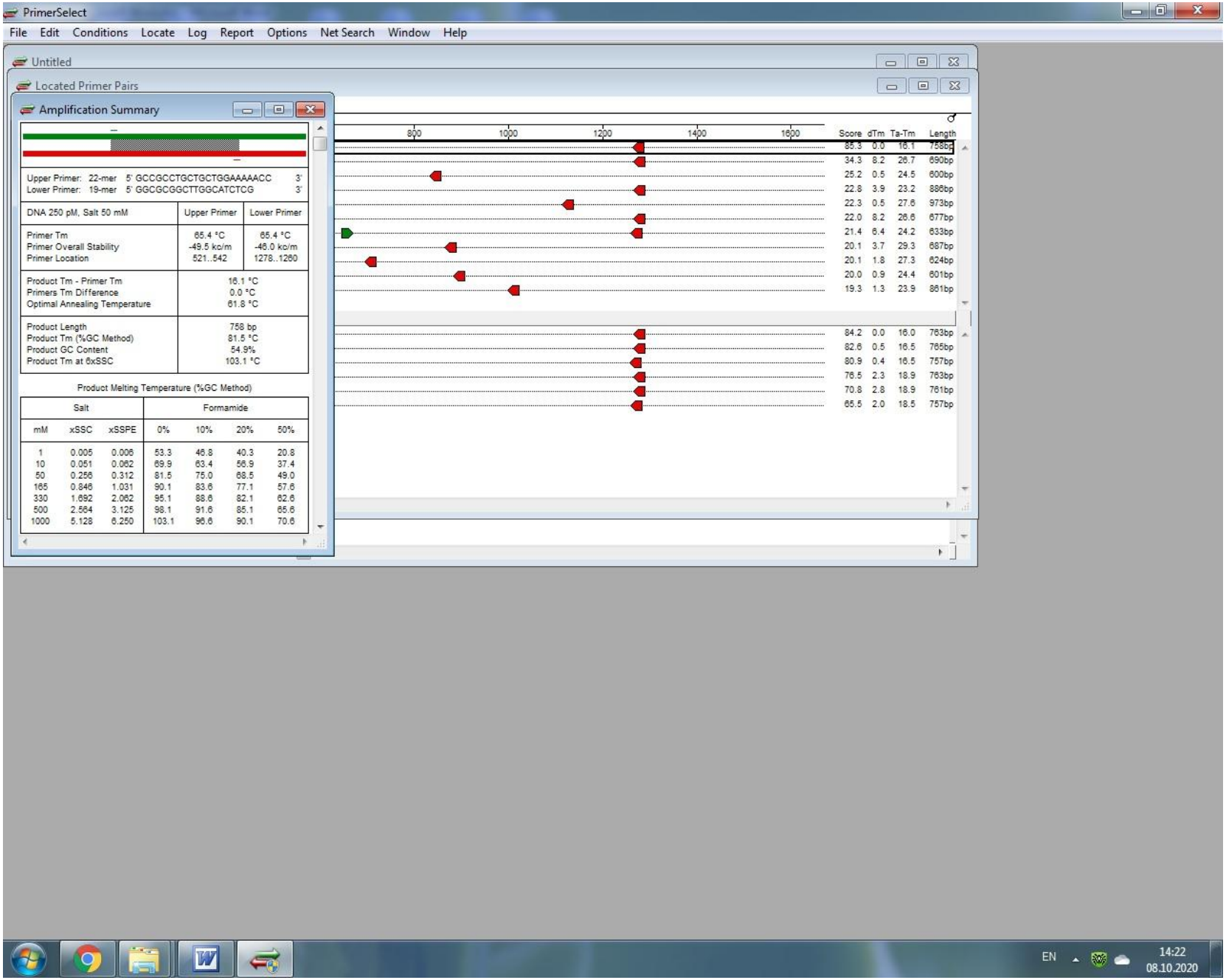
+[521..542] vs. -[1278..1260], 3 bp, dG = -5.1 kc/m (bad!) (worst= -49.5)
5' GCCGCCTGCTGCTGGAAAAACC 3'
3' GCTCTACGGTTCGGCGCGG 5'
+[521..542] vs. -[1278..1260], 3 bp, dG = -5.1 kc/m (bad!) (worst= -49.5)
5' GCCGCCTGCTGCTGGAAAAACC 3'
3' GCTCTACGGTTCGGCGCGG 5'
1000
1200
1400
1600
Score dTm Ta-Tm Length
85.3 0.0 16.1 758bp
34.3 8.2 26.7 690bp
25.2 0.5 24.5 600bp
22.8 3.9 23.2 886bp
22.3 0.5 27.6 973bp
22.0 8.2 26.6 677bp
21.4 6.4 24.2 633bp
20.1 3.7 29.3 687bp
20.1 1.8 27.3 624bp
20.0 0.9 24.4 601bp
19.3 1.3 23.9 861bp
84.2 0.0 16.0 763bp
82.6 0.5 16.5 765bp
80.9 0.4 16.5 757bp
76.5 2.3 18.9 763bp
70.8 2.8 18.9 761bp
65.5 2.0 18.5 757bp
EN
14:20
08.10.2020


PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Untitled
Located Primer Pairs
Hairpin Formation
Primer: +[521..542], 6 hairpins, but the primer is OK.
Primer: -[1278..1260], 6 hairpins, but the primer is OK.
-[1278..1260], 2 bp (Loop=9), dG = 0.6 kc/m
5' GGCGCGGCTT
3' GCTCTACG
-[1278..1260], 2 bp (Loop=8), dG = 1.0 kc/m
5' GGCGCGG
3' GCTCTACGGTTC
1000
1200
1400
1600
Score dTm Ta-Tm Length
85.3 0.0 16.1 758bp
34.3 8.2 26.7 690bp
25.2 0.5 24.5 600bp
22.8 3.9 23.2 886bp
22.3 0.5 27.6 973bp
22.0 8.2 26.6 677bp
21.4 6.4 24.2 633bp
20.1 3.7 29.3 687bp
20.1 1.8 27.3 624bp
20.0 0.9 24.4 601bp
19.3 1.3 23.9 861bp
84.2 0.0 16.0 783bp
82.6 0.5 16.5 765bp
80.9 0.4 16.5 757bp
76.5 2.3 18.9 763bp
70.8 2.8 18.9 761bp
65.5 2.0 18.5 757bp
EN
14:20
08.10.2020


PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Untitled
Located Primer Pairs
Upper Primer WorkBench
Length = 22, Tm = 65.4
Sites
Seq 5' CGGT GGT GAA CGT CGT CGC GT AGC GTT GT GCC GCC T GCT GCT GGA AAA ACC AGA CAT GCT GCT GCT CGA CGA ACC GA
568
Primer GCC GCC T GCT GCT GGA AAA ACC
Comp 3' GCC ACC ACT T GCA GCA GCG CAT CGC AAC A CGG CGG ACG ACG ACC TTT TT GG T CT GT ACG ACG ACG AGC T GCT T GGC T
Frame 1 Gly Gly Glu Arg Arg Arg Val Ala Leu Cys Arg Leu Leu Leu Glu Lys Pro Asp Met Leu Leu Leu Asp Glu Pro Th
Primer Cys Arg Leu Leu Leu Glu Lys Pro
Frame 2 'o Val Val Asn Val Val Ala ter Arg Cys Ala Ala Cys Cys Trp Lys Asn Gln Thr Cys Cys Cys Ser Thr Asn Arg
Primer Ala Ala Cys Cys Trp Lys Asn Gln
Frame 3 Arg Trp ter Thr Ser Ser Arg Ser Val Val Pro Pro Ala Ala Gly Lys Thr Arg His Ala Ala Ala Arg Arg Thr Asp
Primer Val Pro Pro Ala Ala Gly Lys Thr
Frame 4 Pro Pro Ser Arg Arg Arg Thr Ala Asn His Arg Arg Ser Ser Ser Phe Gly Ser Met Ser Ser Ser Ser Ser Gly Val
Primer His Arg Arg Ser Ser Ser Phe Gly
Frame 5 Thr Thr Phe Thr Thr Ala Tyr Arg Gln Ala Ala Gln Gln Gln Phe Phe Trp Val His Gln Gln Glu Val Phe Arg G
Primer Ala Ala Gln Gln Gln Phe Phe Trp
Frame 6 'g His His Val Asp Asp Arg Leu Thr Thr Gly Gly Ala Ala Pro Phe Val Leu Cys Ala Ala Ala Arg Arg Val Ser
Primer Gly Gly Ala Ala Pro Phe Val Leu
Priming Sites
200 400 600 800 1000 1200 1400 1600
No dimers > 2 bp
Hairpin 2 bp, 1.3 kc/m
5' GCCG
C
3' CC GTCGTC
Name:
Note:
OK
Cancel
EN
14:21
08.10.2020


PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Untitled
Located Primer Pairs
Amplification Summary
Upper Primer: 22-mer 5' GCCGCCTGCTGCTGGAAAAACC 3'
Lower Primer: 19-mer 5' GGCGCGGCTTGGCATCTCG 3'
DNA 250 pM, Salt 50 mM | Upper Primer | Lower Primer
Primer Tm | 65.4 °C | 65.4 °C
Primer Overall Stability | -49.5 kc/m | -46.0 kc/m
Primer Location | 521..542 | 1278..1260
Product Tm - Primer Tm 16.1 °C
Primers Tm Difference 0.0 °C
Optimal Annealing Temperature 61.8 °C
Product Length 758 bp
Product Tm (%GC Method) 81.5 °C
Product GC Content 54.9%
Product Tm at 6xSSC 103.1 °C
Product Melting Temperature (%GC Method)
Salt | Formamide
mM | xSSC | xSSPE | 0% | 10% | 20% | 50%
1 | 0.005 | 0.006 | 53.3 | 46.8 | 40.3 | 20.8
10 | 0.051 | 0.062 | 69.9 | 63.4 | 56.9 | 37.4
50 | 0.256 | 0.312 | 81.5 | 75.0 | 68.5 | 49.0
165 | 0.846 | 1.031 | 90.1 | 83.6 | 77.1 | 57.6
330 | 1.692 | 2.062 | 95.1 | 88.6 | 82.1 | 62.6
500 | 2.564 | 3.125 | 98.1 | 91.6 | 85.1 | 65.6
1000 | 5.128 | 6.250 | 103.1 | 96.6 | 90.1 | 70.6
800 1000 1200 1400 1600
Score dTm Ta-Tm Length
85.3 0.0 16.1 758bp
34.3 8.2 26.7 690bp
25.2 0.5 24.5 600bp
22.8 3.9 23.2 886bp
22.3 0.5 27.6 973bp
22.0 8.2 26.6 677bp
21.4 6.4 24.2 633bp
20.1 3.7 29.3 687bp
20.1 1.8 27.3 624bp
20.0 0.9 24.4 601bp
19.3 1.3 23.9 861bp
84.2 0.0 16.0 763bp
82.6 0.5 16.5 765bp
80.9 0.4 16.5 757bp
76.5 2.3 18.9 763bp
70.8 2.8 18.9 761bp
65.5 2.0 18.5 757bp
EN
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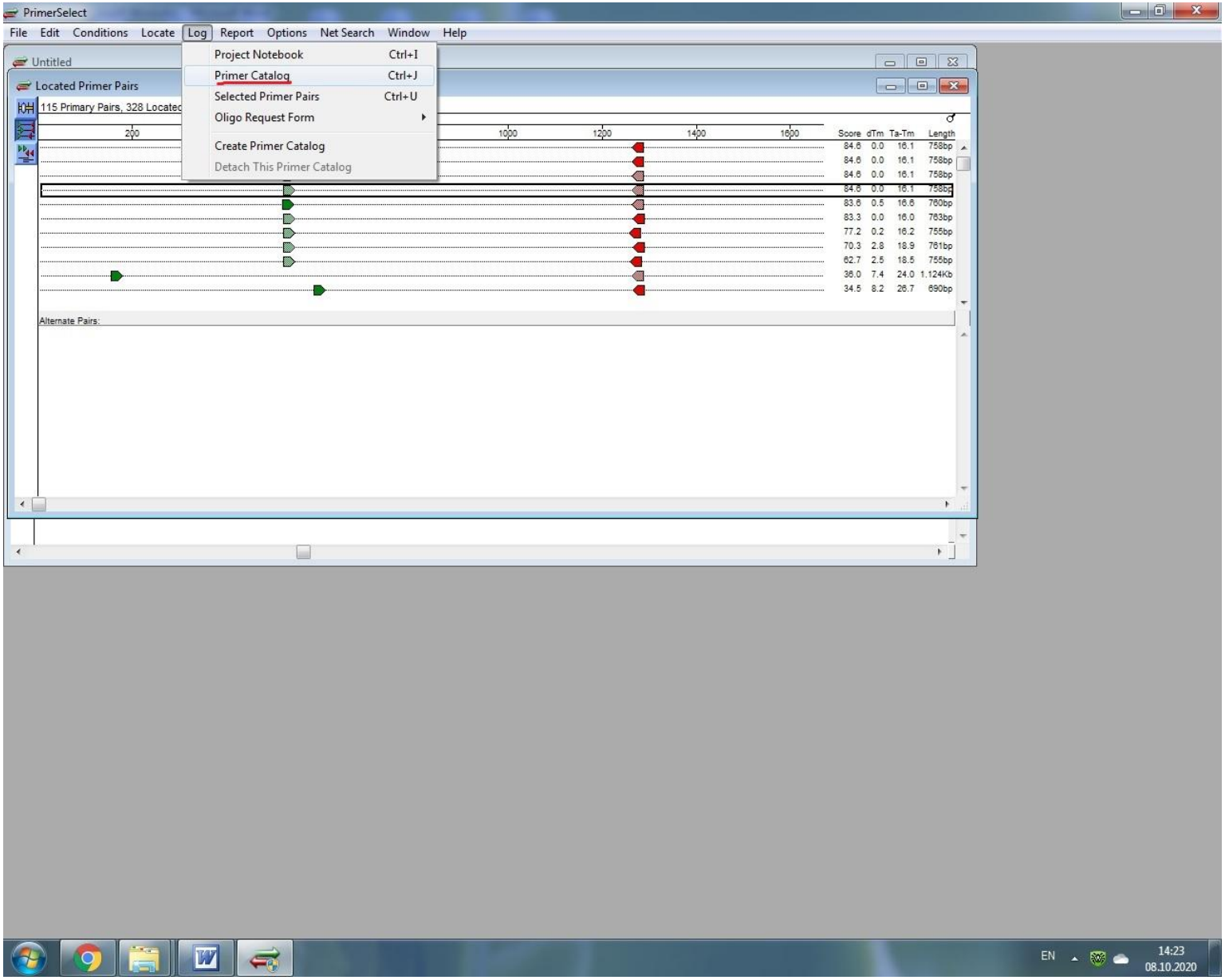
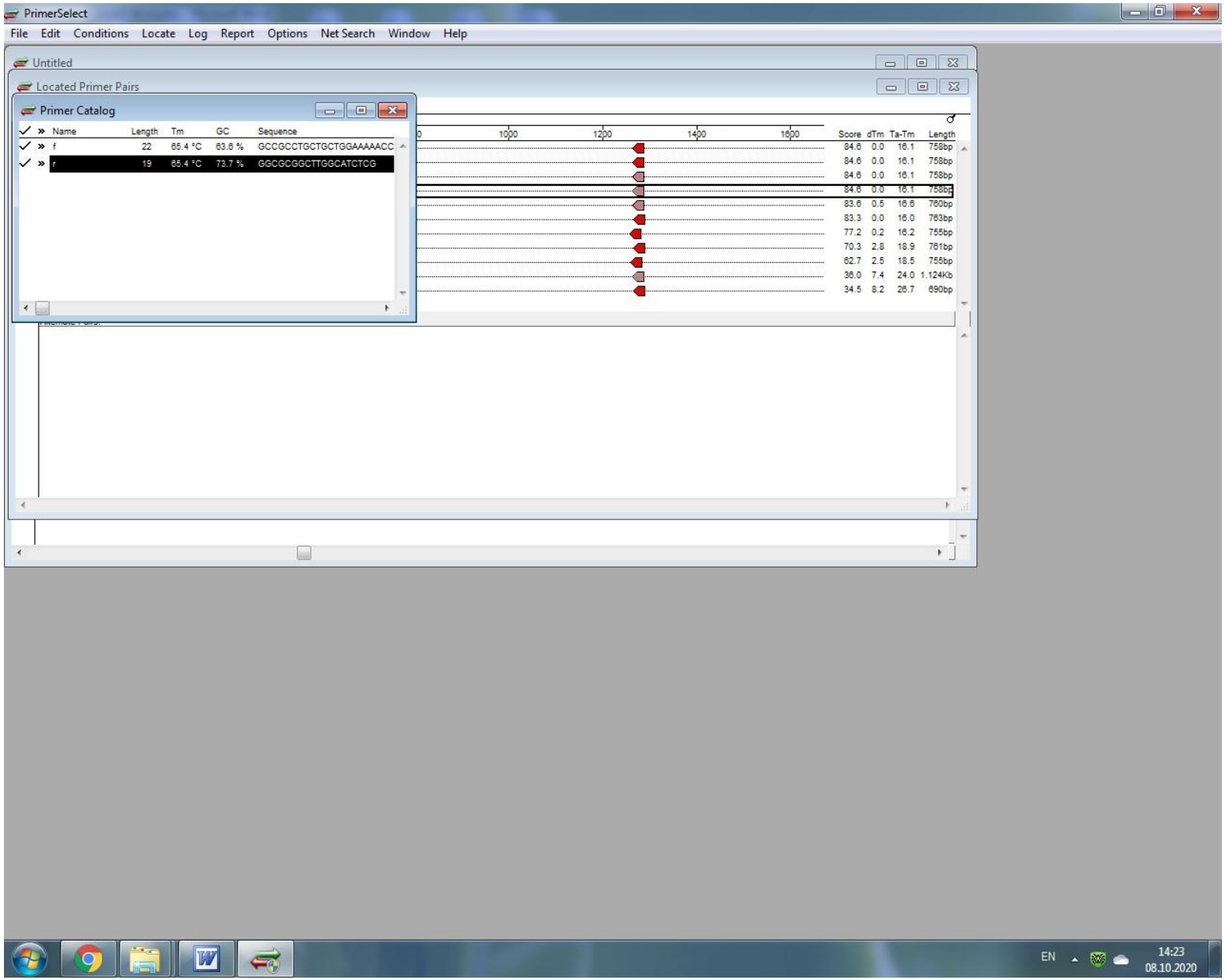
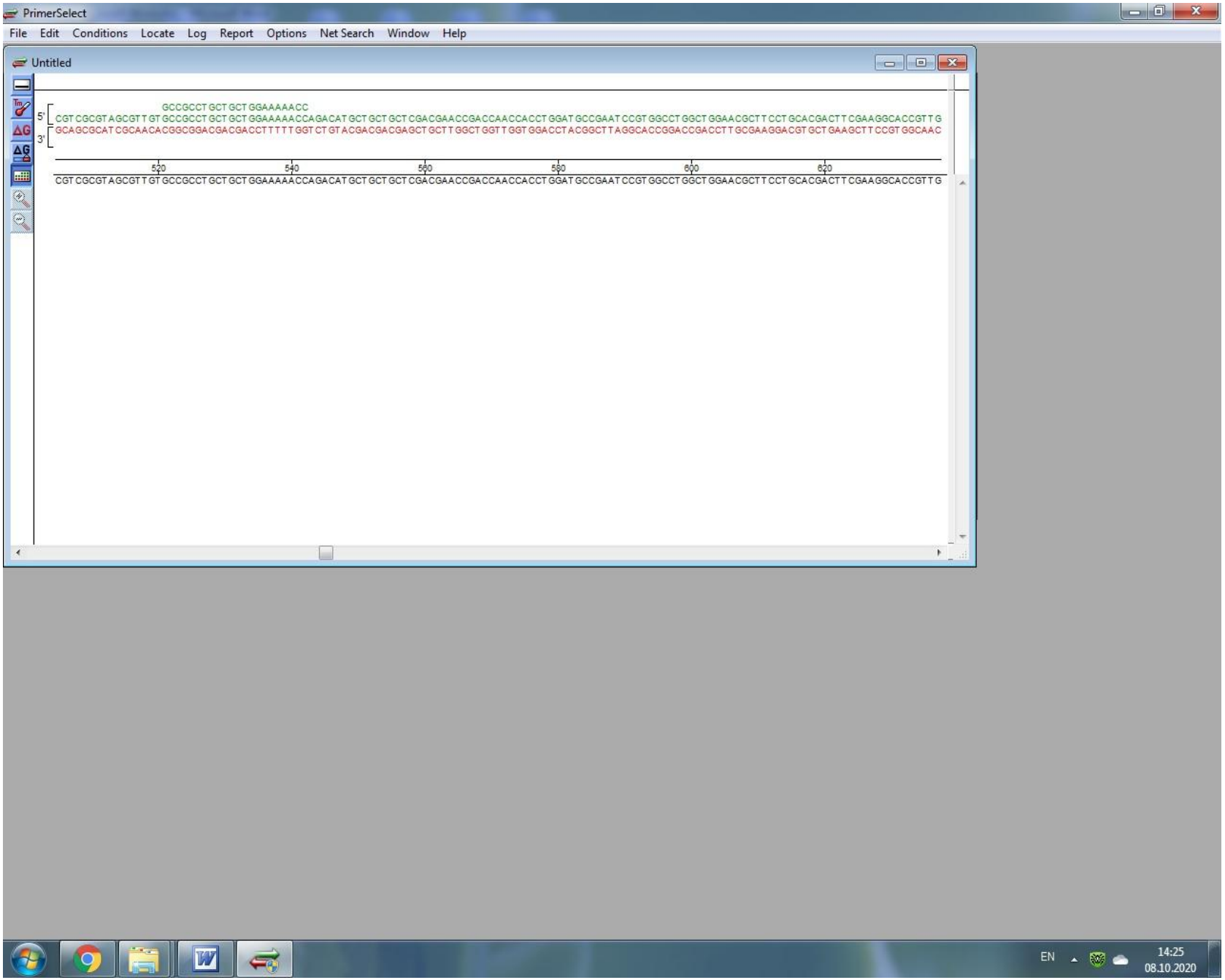
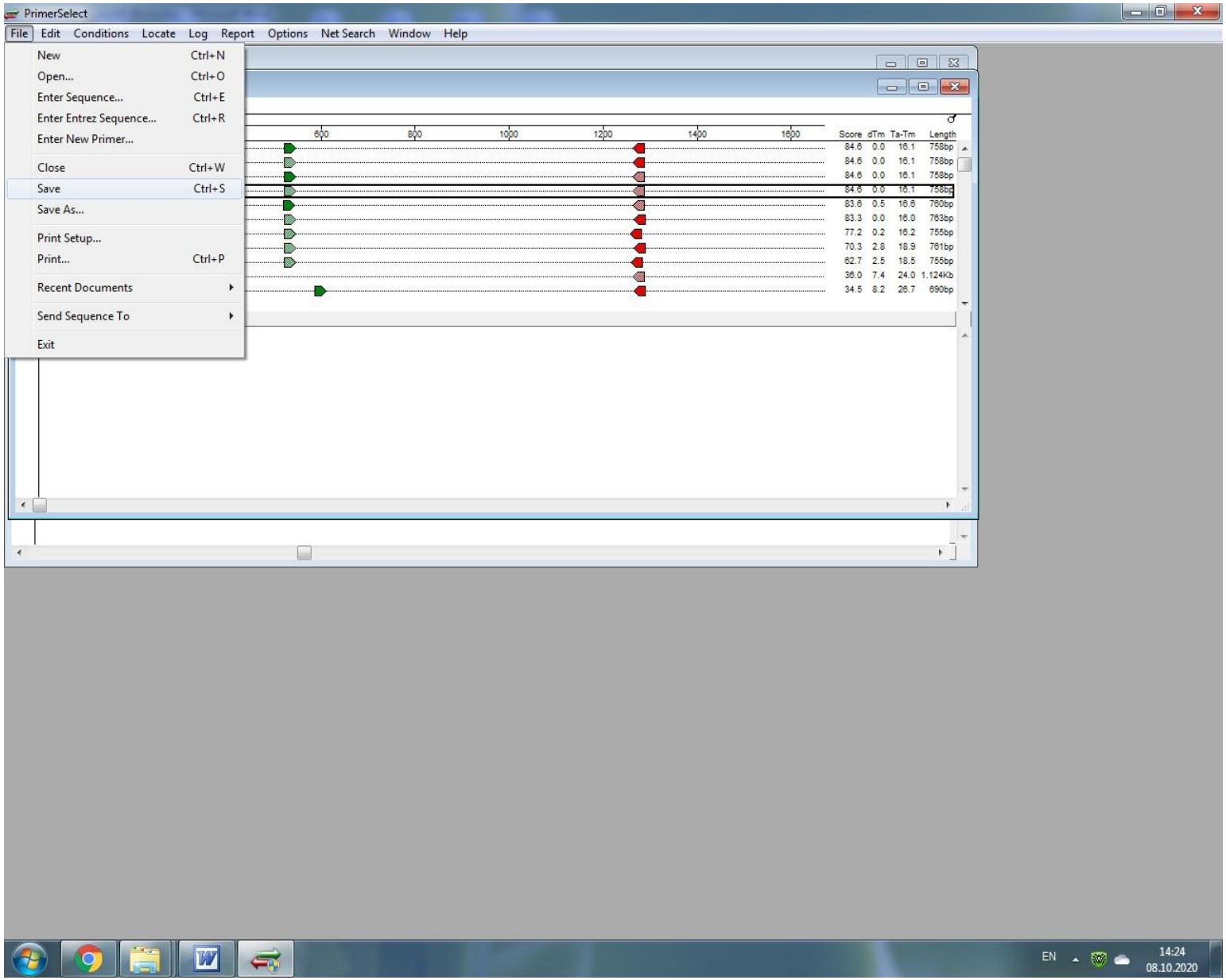
08.10.2020

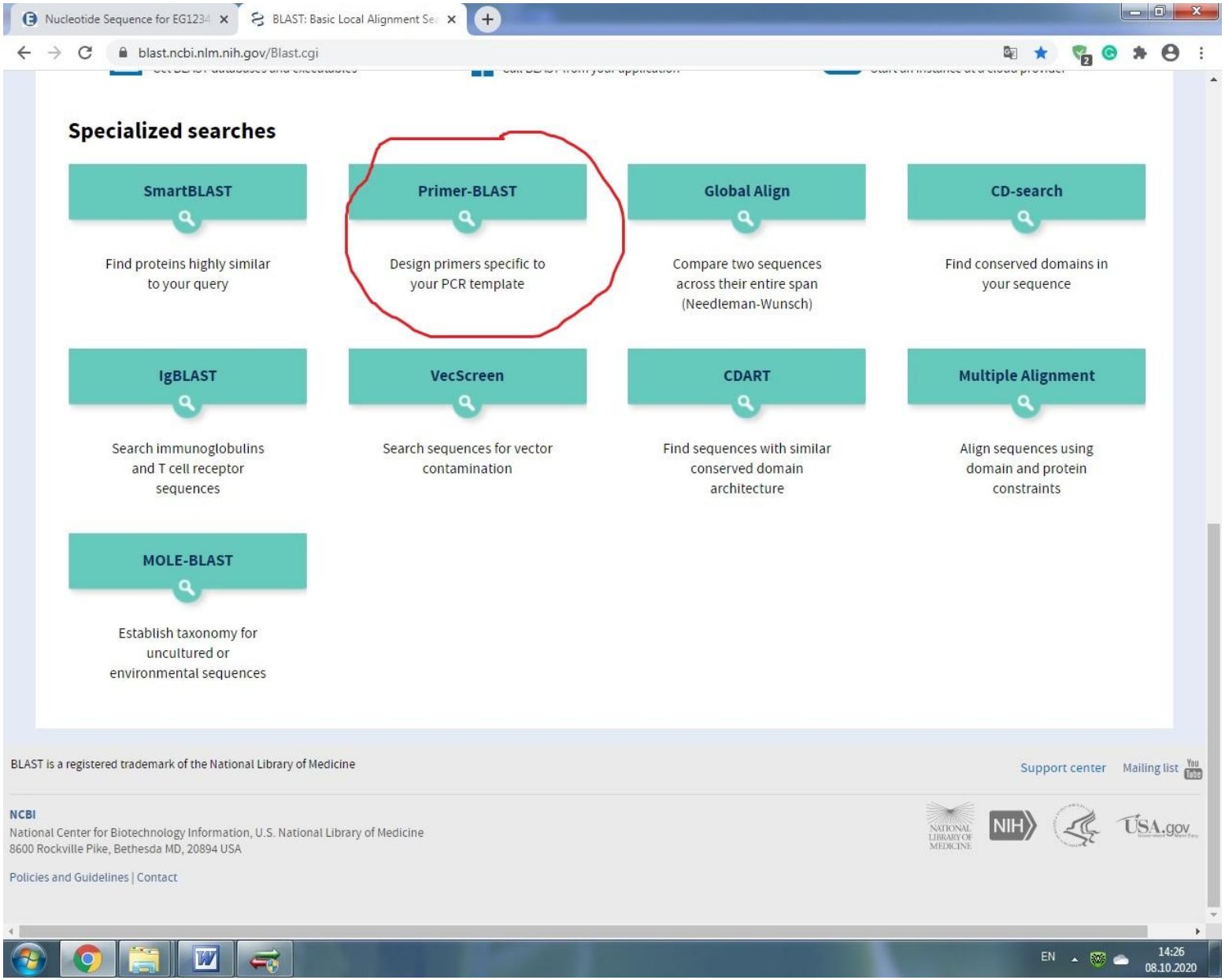

PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Project Notebook Ctrl+I
Primer Catalog Ctrl+J
Selected Primer Pairs Ctrl+U
Oligo Request Form
Create Primer Catalog
Detach This Primer Catalog
Untitled
Located Primer Pairs
115 Primary Pairs, 328 Located
200
1000
1200
1400
1600
Score dTm Ta-Tm Length
84.6 0.0 16.1 758bp
84.6 0.0 16.1 758bp
84.6 0.0 16.1 758bp
84.6 0.0 16.1 758bp
83.6 0.5 16.6 760bp
83.3 0.0 16.0 763bp
77.2 0.2 16.2 755bp
70.3 2.8 18.9 761bp
62.7 2.5 18.5 755bp
36.0 7.4 24.0 1,124Kb
34.5 8.2 26.7 690bp
Alternate Pairs:
EN
14:23
08.10.2020

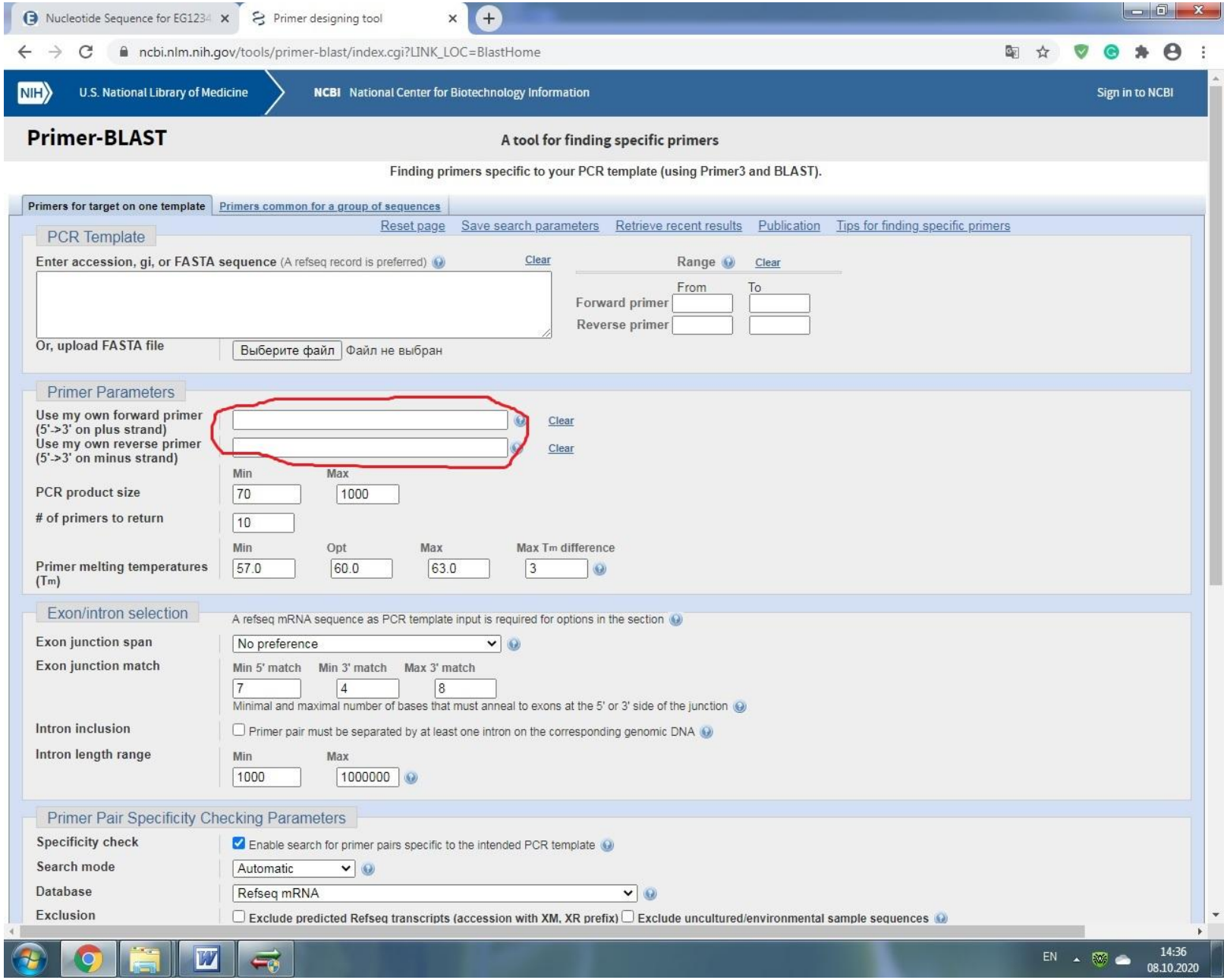

PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Untitled
Located Primer Pairs
Primer Catalog
Name Length Tm GC Sequence
f 22 65.4 °C 63.6 % GCCGCCTGCTGCTGGAAAAACC
r 19 65.4 °C 73.7 % GGCGCGGCTTGGCATCTCG
1000 1200 1400 1600
Score dTm Ta-Tm Length
84.6 0.0 16.1 758bp
84.6 0.0 16.1 758bp
84.6 0.0 16.1 758bp
84.6 0.0 16.1 758bp
83.6 0.5 16.6 760bp
83.3 0.0 16.0 763bp
77.2 0.2 16.2 755bp
70.3 2.8 18.9 761bp
62.7 2.5 18.5 755bp
36.0 7.4 24.0 1.124Kb
34.5 8.2 26.7 690bp
EN
14:23
08.10.2020

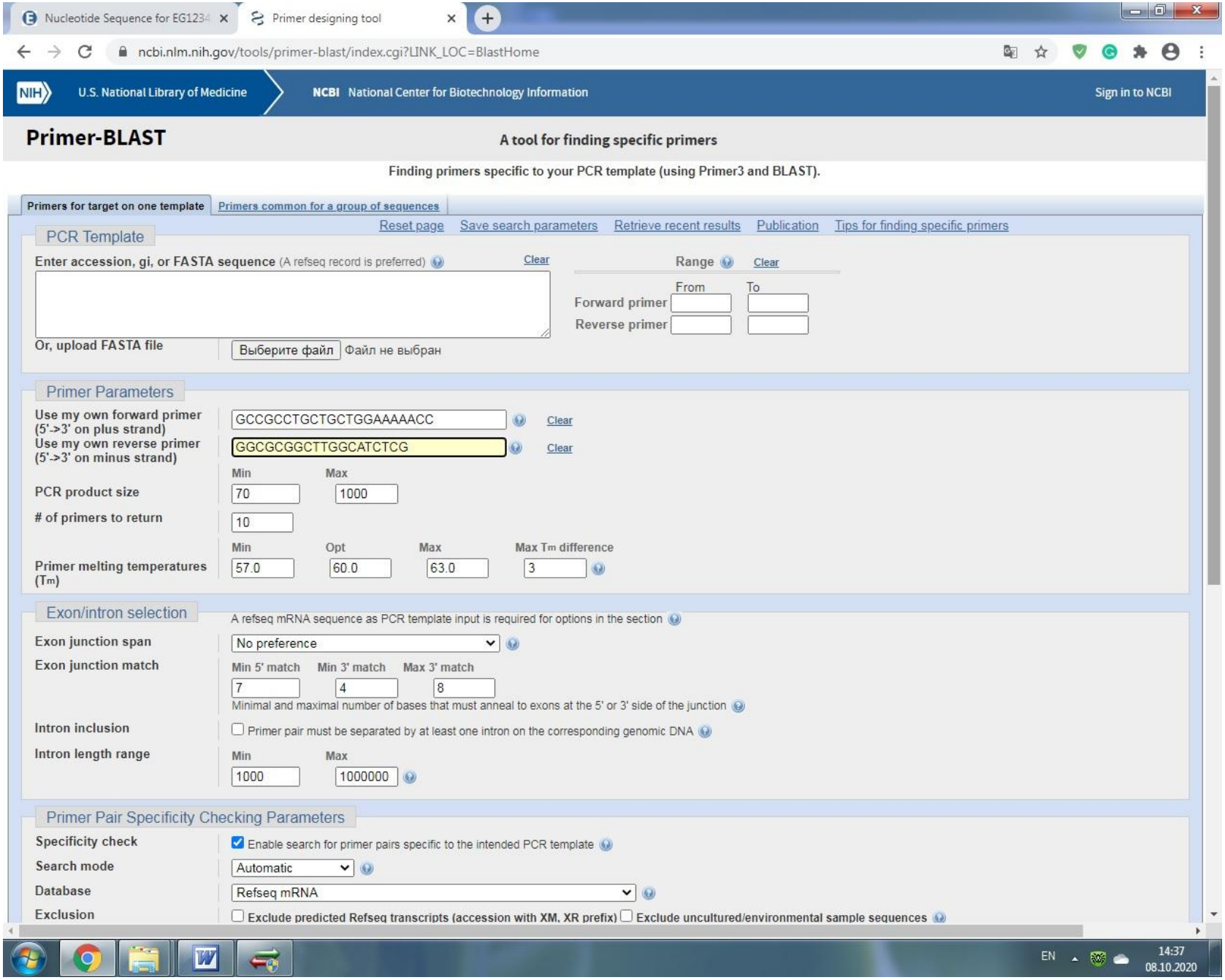

PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
Untitled
GCCGCCT GCT GCT GGAAAAACC
5'
CGT CGCGT AGCGT T GT GCCGCCT GCT GCT GGAAAAACCAGACAT GCT GCT GCT CGACGAACCGACCAACCACCT GGAT GCCGAAT CCGT GGCCT GGCT GGAACGCTT CCT GCACGACTT CGAAGGCACCGTT G
3'
GCAGCGCAT CGCAACACGGCGGACGACGACCTTTTT GGT CT GT ACGACGACGAGCT GCTT GGCT GGTT GGT GGACCT ACGGCTT AGGCACCGGACCGACCTT GCGAAGGACGT GCT GAAGCTT CCGT GGCAAC
520
540
560
580
600
620
CGT CGCGT AGCGT T GT GCCGCCT GCT GCT GGAAAAACCAGACAT GCT GCT GCT CGACGAACCGACCAACCACCT GGAT GCCGAAT CCGT GGCCT GGCT GGAACGCTT CCT GCACGACTT CGAAGGCACCGTT G
EN
14:25
08.10.2020

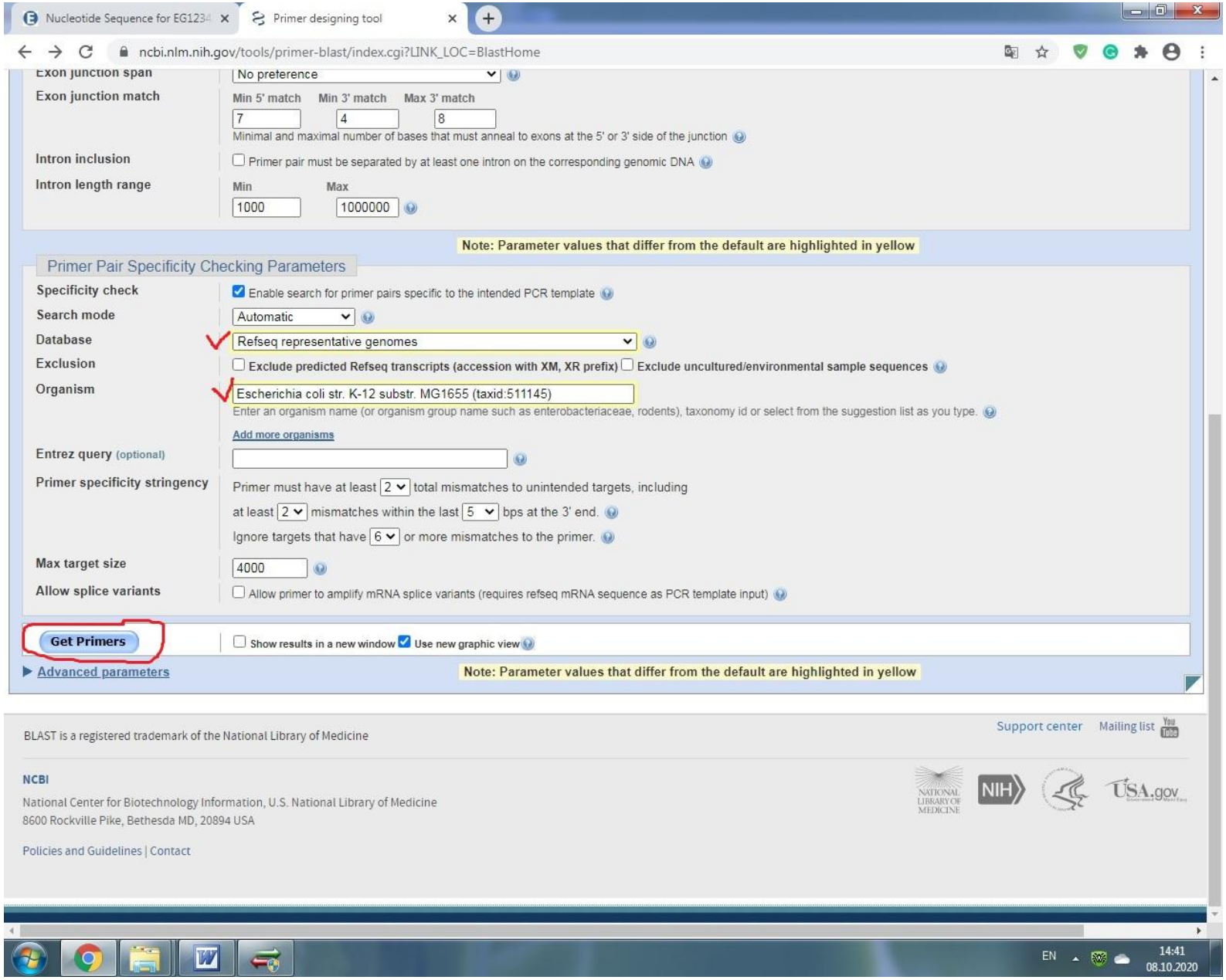

PrimerSelect
File Edit Conditions Locate Log Report Options Net Search Window Help
New Ctrl+N
Open... Ctrl+O
Enter Sequence... Ctrl+E
Enter Entrez Sequence... Ctrl+R
Enter New Primer...
Close Ctrl+W
Save Ctrl+S
Save As...
Print Setup...
Print... Ctrl+P
Recent Documents
Send Sequence To
Exit
600 800 1000 1200 1400 1600
Score dTm Ta-Tm Length
84.6 0.0 16.1 758bp
84.6 0.0 16.1 758bp
84.6 0.0 16.1 758bp
84.6 0.0 16.1 758bp
83.6 0.5 16.6 760bp
83.3 0.0 16.0 763bp
77.2 0.2 16.2 755bp
70.3 2.8 18.9 761bp
62.7 2.5 18.5 755bp
36.0 7.4 24.0 1.124Kb
34.5 8.2 26.7 690bp
EN
14:24
08.10.2020

Выравнивание праймеров




Nucleotide Sequence for EG1234
Primer designing tool
ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome
NIH U.S. National Library of Medicine
NCBI National Center for Biotechnology Information
Sign in to NCBI
Primer-BLAST
A tool for finding specific primers
Finding primers specific to your PCR template (using Primer3 and BLAST).
Primers for target on one template
Primers common for a group of sequences
Reset page
Save search parameters
Retrieve recent results
Publication
Tips for finding specific primers
PCR Template
Enter accession, gi, or FASTA sequence (A refseq record is preferred)
Clear
Range
Clear
From
To
Forward primer
Reverse primer
Or, upload FASTA file
Выберите файл
Файл не выбран
Primer Parameters
Use my own forward primer (5'->3' on plus strand)
Use my own reverse primer (5'->3' on minus strand)
Clear
Clear
Min
Max
PCR product size
70
1000
# of primers to return
10
Min
Opt
Max
Max Tm difference
Primer melting temperatures (Tm)
57.0
60.0
63.0
3
Exon/intron selection
A refseq mRNA sequence as PCR template input is required for options in the section
Exon junction span
No preference
Exon junction match
Min 5' match
Min 3' match
Max 3' match
7
4
8
Minimal and maximal number of bases that must anneal to exons at the 5' or 3' side of the junction
Intron inclusion
Primer pair must be separated by at least one intron on the corresponding genomic DNA
Intron length range
Min
Max
1000
1000000
Primer Pair Specificity Checking Parameters
Specificity check
Enable search for primer pairs specific to the intended PCR template
Search mode
Automatic
Database
Refseq mRNA
Exclusion
Exclude predicted Refseq transcripts (accession with XM, XR prefix)
Exclude uncultured/environmental sample sequences
EN
14:36
08.10.2020


Nucleotide Sequence for EG1234
Primer designing tool
ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome
NIH U.S. National Library of Medicine
NCBI National Center for Biotechnology Information
Sign in to NCBI
Primer-BLAST
A tool for finding specific primers
Finding primers specific to your PCR template (using Primer3 and BLAST).
Primers for target on one template
Primers common for a group of sequences
Reset page
Save search parameters
Retrieve recent results
Publication
Tips for finding specific primers
PCR Template
Enter accession, gi, or FASTA sequence (A refseq record is preferred)
Clear
Range
Clear
From
To
Forward primer
Reverse primer
Or, upload FASTA file
Выберите файл
Файл не выбран
Primer Parameters
Use my own forward primer (5'->3' on plus strand)
GCCGCCTGCTGCTGGAAAAACC
Clear
Use my own reverse primer (5'->3' on minus strand)
GGCGCGGCTTGGCATCTCG
Clear
Min
Max
PCR product size
70
1000
# of primers to return
10
Min
Opt
Max
Max Tm difference
Primer melting temperatures (Tm)
57.0
60.0
63.0
3
Exon/intron selection
A refseq mRNA sequence as PCR template input is required for options in the section
Exon junction span
No preference
Exon junction match
Min 5' match
Min 3' match
Max 3' match
7
4
8
Minimal and maximal number of bases that must anneal to exons at the 5' or 3' side of the junction
Intron inclusion
Primer pair must be separated by at least one intron on the corresponding genomic DNA
Intron length range
Min
Max
1000
1000000
Primer Pair Specificity Checking Parameters
Specificity check
Enable search for primer pairs specific to the intended PCR template
Search mode
Automatic
Database
Refseq mRNA
Exclusion
Exclude predicted Refseq transcripts (accession with XM, XR prefix)
Exclude uncultured/environmental sample sequences
EN
14:37
08.10.2020


Nucleotide Sequence for EG1234
Primer designing tool
ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome
Exon junction span
No preference
Exon junction match
Min 5' match
Min 3' match
Max 3' match
7
4
8
Minimal and maximal number of bases that must anneal to exons at the 5' or 3' side of the junction
Intron inclusion
Primer pair must be separated by at least one intron on the corresponding genomic DNA
Intron length range
Min
Max
1000
1000000
Note: Parameter values that differ from the default are highlighted in yellow
Primer Pair Specificity Checking Parameters
Specificity check
Enable search for primer pairs specific to the intended PCR template
Search mode
Automatic
Database
Refseq representative genomes
Exclusion
Exclude predicted Refseq transcripts (accession with XM, XR prefix)
Exclude uncultured/environmental sample sequences
Organism
Escherichia coli str. K-12 substr. MG1655 (taxid:511145)
Enter an organism name (or organism group name such as enterobacteriaceae, rodents), taxonomy id or select from the suggestion list as you type.
Add more organisms
Entrez query (optional)
Primer specificity stringency
Primer must have at least 2 total mismatches to unintended targets, including
at least 2 mismatches within the last 5 bps at the 3' end.
Ignore targets that have 6 or more mismatches to the primer.
Max target size
4000
Allow splice variants
Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)
Get Primers
Show results in a new window
Use new graphic view
Advanced parameters
Note: Parameter values that differ from the default are highlighted in yellow
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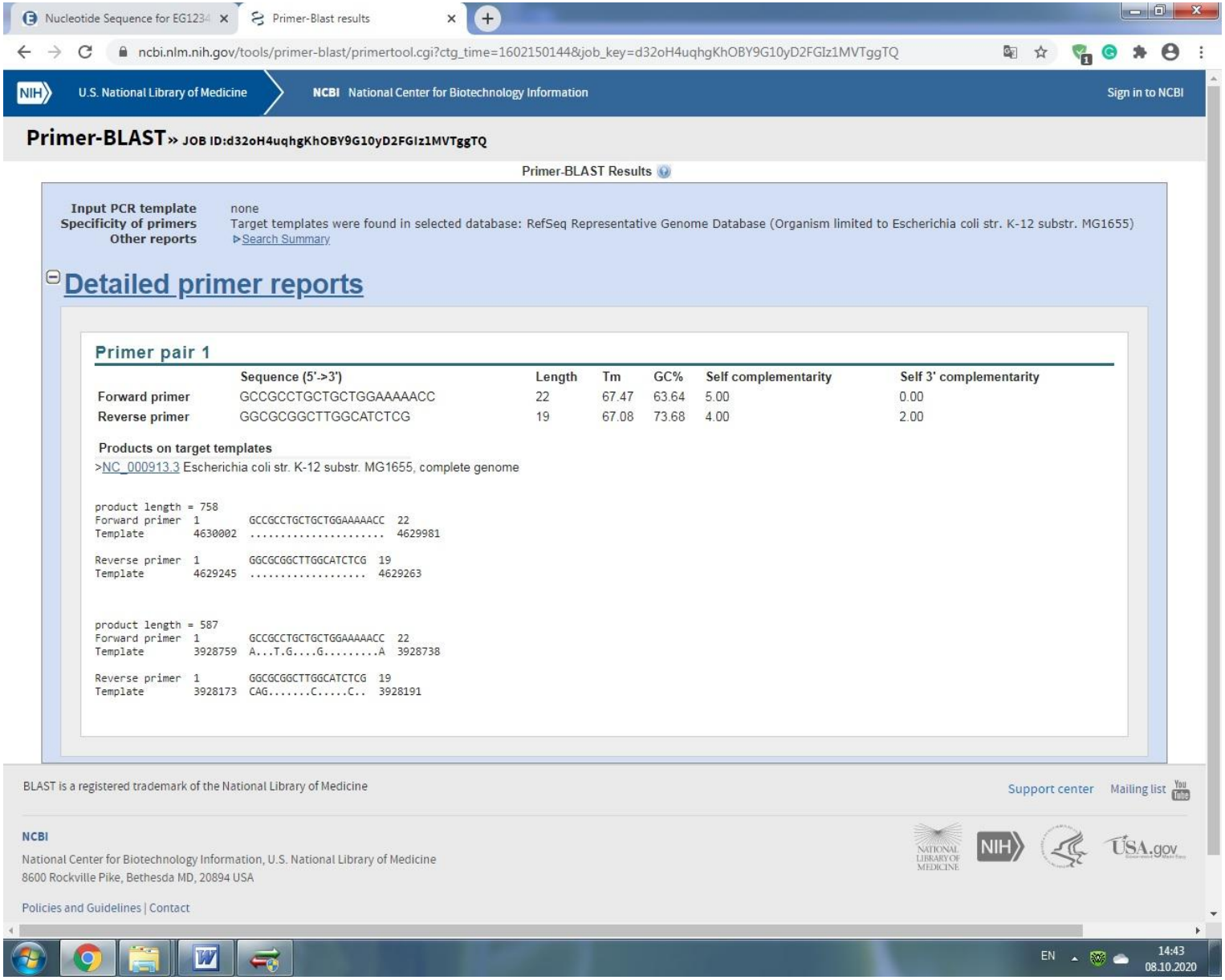
08.10.2020


Nucleotide Sequence for EG1234
Primer-Blast results
ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi?ctg_time=1602150144&job_key=d32oH4uqhgKhOBY9G10yD2FGIz1MVTggTQ
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NCBI National Center for Biotechnology Information
Sign in to NCBI
Primer-BLAST» JOB ID:d32oH4uqhgKhOBY9G10yD2FGIz1MVTggTQ
Primer-BLAST Results
Input PCR template none
Specificity of primers Target templates were found in selected database: RefSeq Representative Genome Database (Organism limited to Escherichia coli str. K-12 substr. MG1655)
Other reports Search Summary
Detailed primer reports
Primer pair 1
Sequence (5'->3') Length Tm GC% Self complementarity Self 3' complementarity
Forward primer GCCGCCTGCTGCTGGAAAAACC 22 67.47 63.64 5.00 0.00
Reverse primer GGCGCGGCTTGGCATCTCG 19 67.08 73.68 4.00 2.00
Products on target templates
>NC_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome
product length = 758
Forward primer 1 GCCGCCTGCTGCTGGAAAAACC 22
Template 4630002 4629981
Reverse primer 1 GGCGCGGCTTGGCATCTCG 19
Template 4629245 4629263
product length = 587
Forward primer 1 GCCGCCTGCTGCTGGAAAAACC 22
Template 3928759 A...T.G....G.....A 3928738
Reverse primer 1 GGCGCGGCTTGGCATCTCG 19
Template 3928173 CAG.....C.....C.. 3928191
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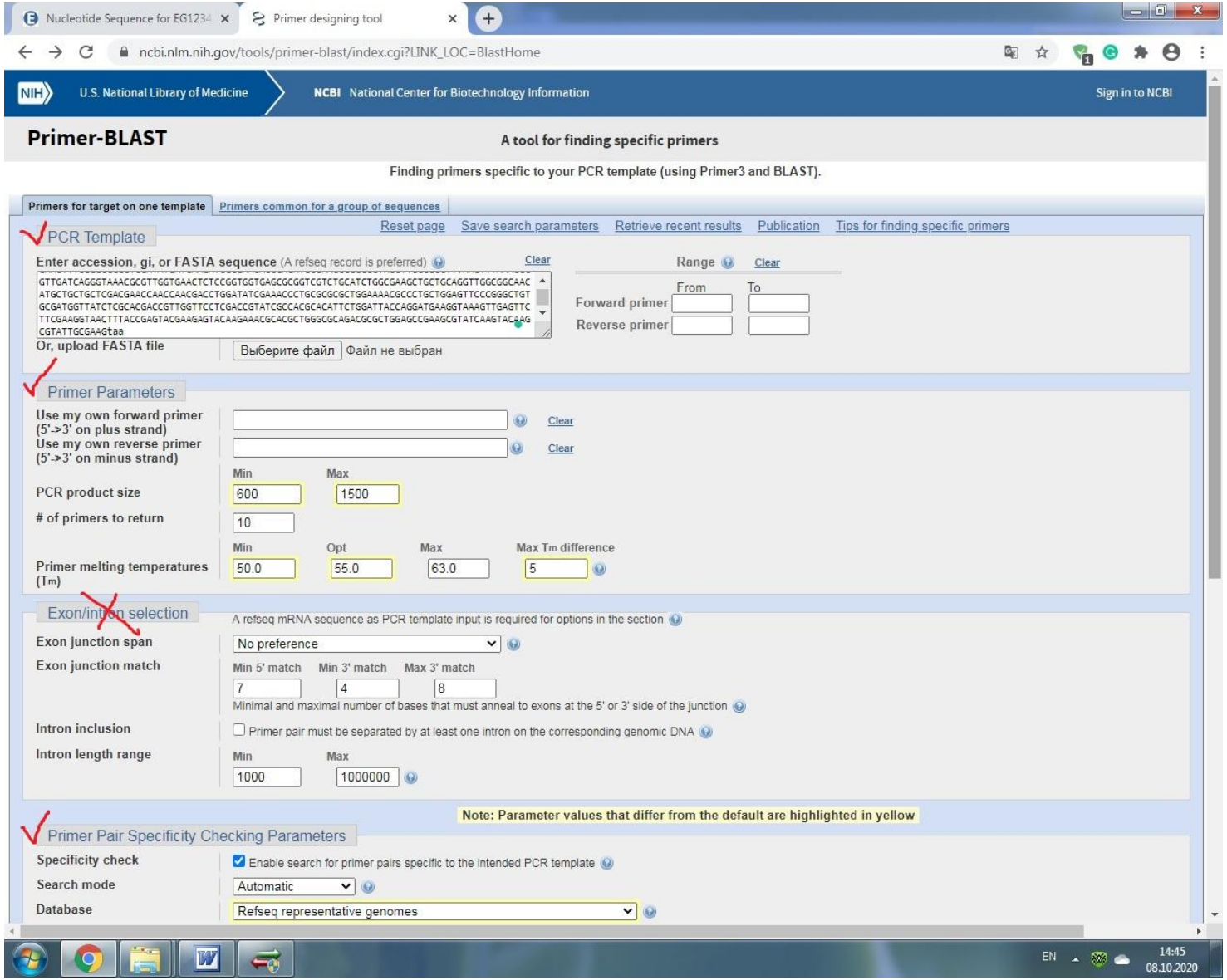
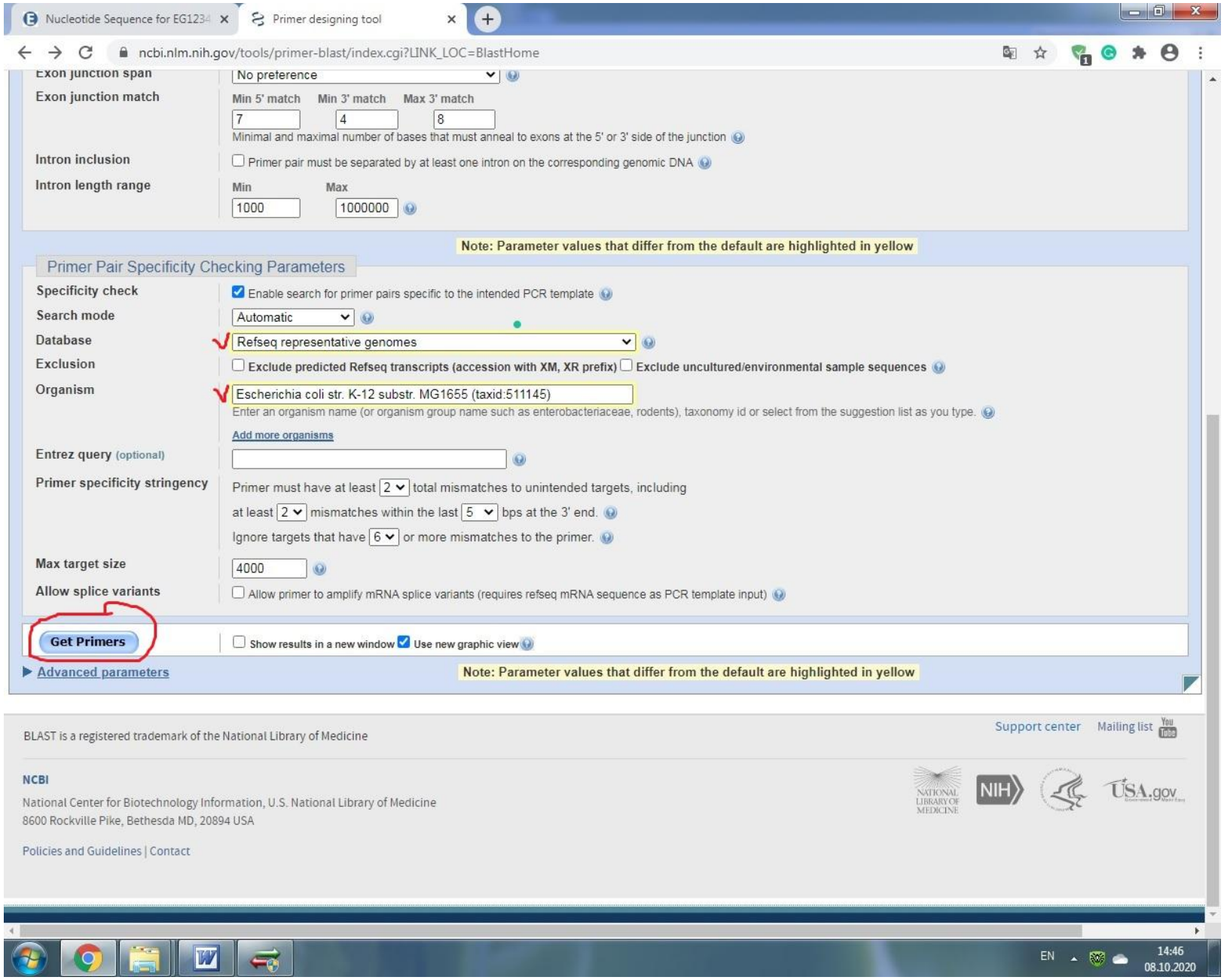
EN
14:43
08.10.2020

Подбор праймеров в GeneBank




Nucleotide Sequence for EG1234
Primer designing tool
ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome
Exon junction span
No preference
Exon junction match
Min 5' match
Min 3' match
Max 3' match
7
4
8
Minimal and maximal number of bases that must anneal to exons at the 5' or 3' side of the junction
Intron inclusion
Primer pair must be separated by at least one intron on the corresponding genomic DNA
Intron length range
Min
Max
1000
1000000
Note: Parameter values that differ from the default are highlighted in yellow
Primer Pair Specificity Checking Parameters
Specificity check
Enable search for primer pairs specific to the intended PCR template
Search mode
Automatic
Database
Refseq representative genomes
Exclusion
Exclude predicted Refseq transcripts (accession with XM, XR prefix)
Exclude uncultured/environmental sample sequences
Organism
Escherichia coli str. K-12 substr. MG1655 (taxid:511145)
Enter an organism name (or organism group name such as enterobacteriaceae, rodents), taxonomy id or select from the suggestion list as you type.
Add more organisms
Entrez query (optional)
Primer specificity stringency
Primer must have at least 2 total mismatches to unintended targets, including
at least 2 mismatches within the last 5 bps at the 3' end.
Ignore targets that have 6 or more mismatches to the primer.
Max target size
4000
Allow splice variants
Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR template input)
Get Primers
Show results in a new window
Use new graphic view
Advanced parameters
Note: Parameter values that differ from the default are highlighted in yellow
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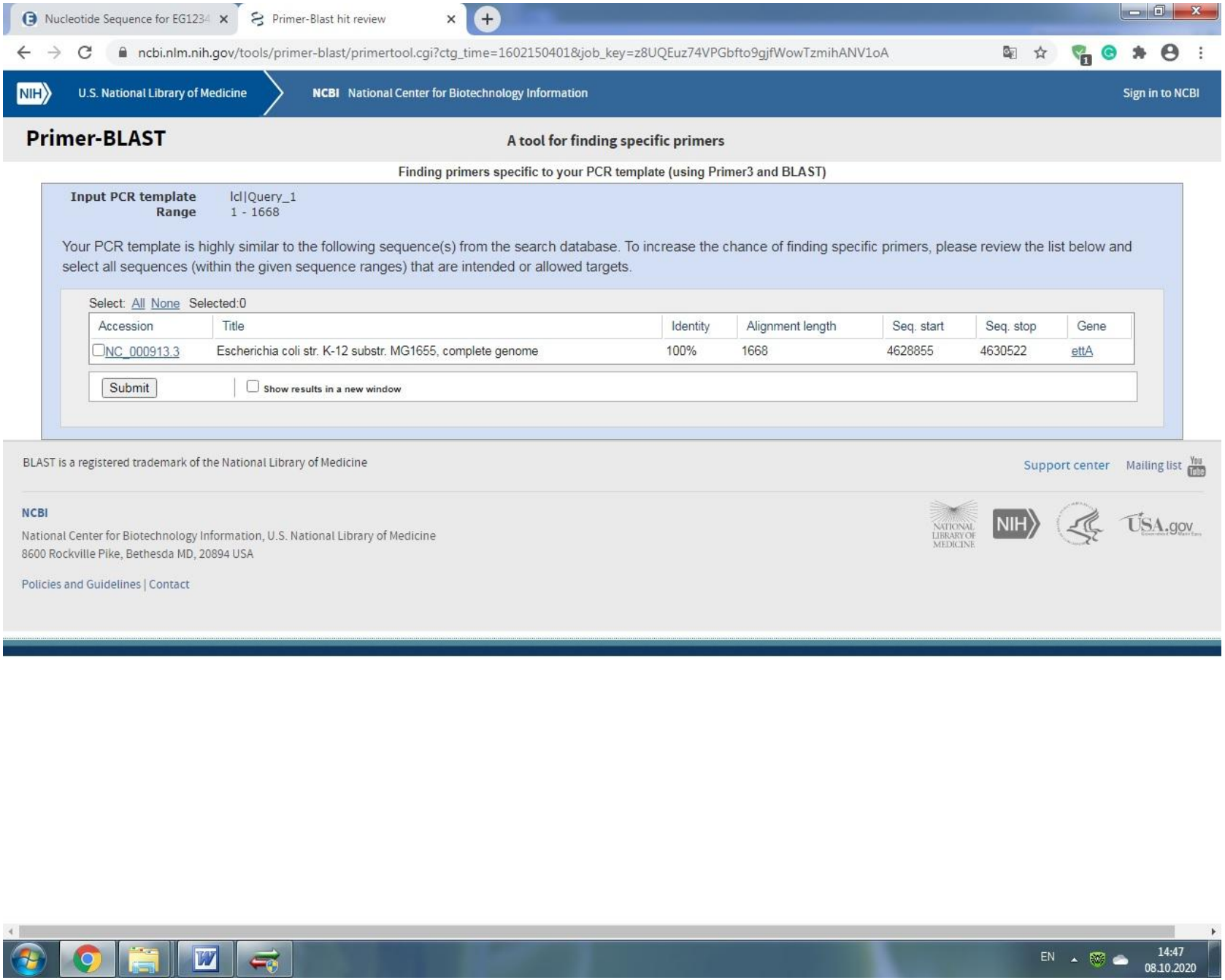
EN
14:46
08.10.2020


Nucleotide Sequence for EG1234
Primer-Blast hit review
ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi?ctg_time=1602150401&job_key=z8UQEuz74VPGbfto9gjfWowTzmihANV1oA
U.S. National Library of Medicine
NCBI National Center for Biotechnology Information
Sign in to NCBI
Primer-BLAST
A tool for finding specific primers
Finding primers specific to your PCR template (using Primer3 and BLAST)
Input PCR template lcl|Query_1
Range 1 - 1668
Your PCR template is highly similar to the following sequence(s) from the search database. To increase the chance of finding specific primers, please review the list below and select all sequences (within the given sequence ranges) that are intended or allowed targets.
Select: All None Selected:0
Accession
Title
Identity
Alignment length
Seq. start
Seq. stop
Gene
NC_000913.3
Escherichia coli str. K-12 substr. MG1655, complete genome
100%
1668
4628855
4630522
ettA
Submit
Show results in a new window
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EN
14:47
08.10.2020

Nucleotide Sequence for EG1234 | Primer-Blast results

ncbi.nlm.nih.gov/tools/primer-blast/primertool.cgi?ctg_time=1602150459&job_key=5uw5jhurFgMxOYY8i1yiDvFHszzcVKgh3Q

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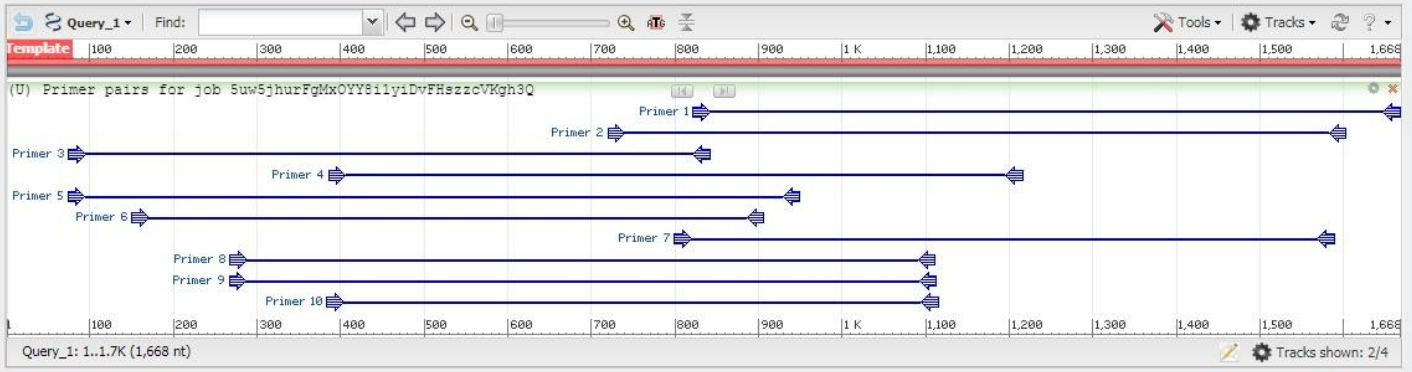
Primer-BLAST » JOB ID:5uw5jhurFgMxOYY8i1yiDvFHszzcVKgh3Q

Primer-BLAST Results

Input PCR template	lcl|Query_1
Range	1 - 1668
Specificity of primers	Primers may **not** be specific to the input PCR template as targets were found in selected database:RefSeq Representative Genome Database (Organism limited to Escherichia coli str. K-12 substr. MG1655)...help on specific primers
Other reports	▹Search Summary

Graphical view of primer pairs



Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGGGTACGTCAAGGTACTA	Plus	20	822	841	54.98	45.00	6.00	3.00
Reverse primer	TACTTCGCAATACGCTTGTA	Minus	20	1667	1648	54.95	40.00	4.00	2.00
Product length	846								

Products on potentially unintended templates

>NC_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome

product length = 846

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Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	ATGGGTACGTCAAGGTACTA	Plus	20	822	841	54.98	45.00	6.00	3.00
Reverse primer	TACTTCGCAATACGCTTGTA	Minus	20	1667	1648	54.95	40.00	4.00	2.00
Product length	846								

Products on potentially unintended templates

>NC_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome

```
product length = 846
Forward primer  1        ATGGGTACGTCAAGGTACTA  20
Template        4629701  ....................  4629682

Reverse primer  1        TACTTCGCAATACGCTTGTA  20
Template        4628856  ....................  4628875
```

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GGAAGGTAACTACTCCTCCT	Plus	20	720	739	54.95	50.00	6.00	2.00
Reverse primer	CTTGTACTCTTCGTACTCGG	Minus	20	1602	1583	55.09	50.00	4.00	1.00
Product length	883								

Products on potentially unintended templates

>NC_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome

```
product length = 883
Forward primer  1        GGAAGGTAACTACTCCTCCT  20
Template        4629803  ....................  4629784

Reverse primer  1        CTTGTACTCTTCGTACTCGG  20
Template        4628921  ....................  4628940
```

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTCTCTGAGTTTCTTCCCTG	Plus	20	75	94	54.81	50.00	5.00	1.00
Reverse primer	TAGTACCTTGACGTACCCAT	Minus	20	841	822	54.98	45.00	6.00	2.00
Product length	767								

Products on potentially unintended templates

>NC_000913.3 Escherichia coli str. K-12 substr. MG1655, complete genome

Tm калькулятор