

Подбор праймеров

Основные принципы

- Длина 18-30 нуклеотидов
- Температура отжига 45-65°C, максимально допустимая разница между температурами отжига праймеров 5°C
- 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 А/Т
- 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

The screenshot shows a web browser window with several tabs open: 'Nucleotide Sequence for EG', 'Primer-Blast results', 'NEB Tm Calculator', 'Почта Mail.ru', and 'Search NCBI databases - NC'. The address bar shows 'ncbi.nlm.nih.gov/search/'. The page header includes the NIH logo and 'National Library of Medicine National Center for Biotechnology Information'. A COVID-19 notice is displayed, along with a 'Log in' button. The search bar contains 'ett' and a dropdown menu with suggestions: 'energy-dependent translational throttle protein EttA', 'Arabidopsis thaliana ETT', 'Ettlia pseudoalveolaris chloroplast', and 'Ettlia pseudoalveolaris plastid'. Below the search bar, there are sections for 'News' and 'Recent blog posts'. The 'News' section features articles from 'The New York Times' and 'NIH Director's Blog'. The 'Recent blog posts' section includes 'NIH Director's Blog' and 'NLM Musings'. At the bottom, there are links to 'NCBI Virus', 'LitCovid', and 'BLAST'. The Windows taskbar is visible at the bottom with various application icons and a system tray showing the time as 15:27 on 08.10.2020.

[BLAST](#)
[SPARCLE](#)
[Download](#)

Literature

Bookshelf	0
MeSH	1
NLM Catalog	0
PubMed	2
PubMed Central	21

Genes

Gene	506
GEO DataSets	0
GEO Profiles	20
HomoloGene	0
PopSet	0

Proteins

Conserved Domains	0
Identical Protein Groups	30,147
Protein	72,140
Protein Clusters	6
Sparcle	1
Structure	2

Genomes

Assembly	0
BioCollections	0
BioProject	0
BioSample	0
Genome	0
<u>Nucleotide</u>	54,932
SRA	0
Taxonomy	0

Clinical

ClinicalTrials.gov	0
ClinVar	0
dbGaP	0
dbSNP	0
dbVar	0
GTR	0
MedGen	0
OMIM	0

PubChem

BioAssays	0
Compounds	0
Pathways	0
Substances	0

Nucleotide Nucleotide energy-dependent translational throttle protein Etta Search

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/

- Species Animals (7) Plants (2) Protists (24) Bacteria (54,815) Archaea (2) Viruses (3) Customize ...

- Molecule types genomic DNA/RNA (54,927) mRNA (2) Customize ...

- Source databases INSDC (GenBank) (52,912) RefSeq (2,016) Customize ...

- Sequence Type Nucleotide (54,932)

- Genetic compartments Plasmid (29)

- Sequence length Custom range...

- Release date Custom range...

- Revision date Custom range...

Clear all Show additional filters

Summary 20 per page Sort by Default order Send to Filters: Manage Filters

PROTEIN Was this helpful? energy-dependent translational throttle protein Etta Bacteria ABC transporter ATP-binding protein similar to EtA, 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 Protein family RefSeq protein Identical protein groups PubMed (22) BLAST SPARCLE Download

Items: 1 to 20 of 54932 << First < Prev Page 1 of 2747 Next > Last >>

- Desulfoluna spongiiphila isolate Desulfoluna spongiiphila strain DBB genome assembly, contig; 1. DBBSCAFFOLD_1_C2, whole genome shotgun sequence 6,296,034 bp linear DNA Accession: CABVLC010000002.1 GI: 1743652803 BioProject BioSample Protein Taxonomy GenBank FASTA Graphics
- Burkholderia cepacia strain BC16 chromosome 1, complete sequence 2. 3,688,624 bp circular DNA Accession: CP045235.1 GI: 1770636962 Assembly BioProject BioSample Protein Taxonomy GenBank FASTA Graphics

Results by taxon

- Top Organisms [Tree] Escherichia coli (7612) Salmonella enterica (4751) Klebsiella pneumoniae (3483) Pseudomonas aeruginosa (2295) Acinetobacter baumannii (2000) All other taxa (34791) More...

Find related data

Database: Select Find items

Search details

energy-dependent[All Fields] AND translational[All Fields] AND throttle[All Fields] AND protein[All Fields] AND Etta[All Fields]

Search See more...

Recent activity

- energy-dependent translational throttle protein Etta (54932) Nucleotide
- Escherichia coli BL21 chromosome, complete genome Nucleotide
- (energy-dependent translational throttle

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 :: HMW
 Evidence Accession :: [NF008775.0](#)
 Evidence Source :: NCBI Protein Cluster (PRK)
 Source Identifier :: PRK11819
 ##Evidence-For-Name-Assignment-END##
 COMPLETENESS: full length.

FEATURES Location/Qualifiers
 source 1..555
 /organism="Proteobacteria"
 /db_xref="taxon:1224"
 gene 1..555
 /gene="etta"
 Protein 1..555
 /product="energy-dependent translational throttle protein EttaA"
 /calculated_mol_wt=62312
 Region 1..555
 /region_name="PRK11819"
 /note="putative ABC transporter ATP-binding protein; Reviewed"
 /db_xref="CDD:236992"

ORIGIN
 1 maqfvymhr gkvvppkrh ilknislsff pgakigvlg ngagkstllr imagidkdie
 61 gearppdik igylpqepql npehtvresi eavsevna lkrldevyal yadpdadfdk
 121 laaeqgrlee iqahghnl nvqleraada lrlpdwdaki anlsggerrr valcrlillek
 181 pdmllldept nhldaesvaw lerflhdfeg tvvaithdry fldnvagwil eldrgegipw
 241 egnyswleq kdqrlaqaas qeaarrksie kelewvrgt kgrqskgkar larfeelnst
 301 eyqkrnetne lfippgprlg dkvlevsnlr ksygdrllid dlsfsipka ivgiigpnga
 361 gkstlrfmis gaeqpsdgti tlgetvklas vdqfrdsmdn sktwveevsg gldimkigt
 421 empsrayvgr fnfkgvdqgk rvgelsgger grhlakllq vggnmlllde ptndldietl
 481 ralenallef pgcamvishd rwfldriath ildyqdegkv effegnftay eeykkrtlga
 541 dalepkriky kriak

//

Protein clusters for WP_000046749.1

Energy-dependent translational throttle protein EttaA - 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]

Gene 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
 - Current
- [Clear all](#)
[Show additional filters](#)

Tabular Send to:

Links from Protein

Items: 2
 Showing Current items.

Name/Gene ID	Description	Location	Aliases
<input type="checkbox"/> ettA ID: 58388867	energy-dependent translational throttle protein EttA [<i>Shigella boydii</i>]		BUK67_RS04745
<input type="checkbox"/> ettA ID: 58349935	energy-dependent translational throttle protein EttA [<i>Escherichia fergusonii</i>]		HVX34_RS16805, HVX34_16800

Filters: [Manage Filters](#)

Results by taxon
 Top Organisms [\[Tree\]](#)
 Escherichia fergusonii (1)
 Shigella boydii (1)

Find related data
 Database:

- 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...](#)

Gene 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 v Send to: v Hide sidebar >>

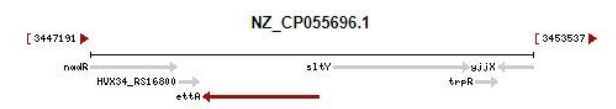
ettA energy-dependent translational throttle protein EttA [*Escherichia fergusonii*]

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



Genomic regions, transcripts, and products

Genomic Sequence: NZ_CP055696.1 [Go to reference sequence details](#)
[Go to nucleotide:](#) [Graphics](#) [FASTA](#) [GenBank](#)



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

Найденную последовательность нуклеотидов скопировать в документ Word (удалить пробелы, переносы строки)

подбор праймеров

The screenshot shows the NCBI BLAST website interface. The browser address bar displays 'blast.ncbi.nlm.nih.gov/Blast.cgi'. The main content area is titled 'Specialized searches' and features several search options, each with a magnifying glass icon:

- SmartBLAST**: Find proteins highly similar to your query
- Primer-BLAST**: Design primers specific to your PCR template (highlighted with a red circle)
- Global Align**: Compare two sequences across their entire span (Needleman-Wunsch)
- CD-search**: Find conserved domains in your sequence
- IgBLAST**: Search immunoglobulins and T cell receptor sequences
- VecScreen**: Search sequences for vector contamination
- CDART**: Find sequences with similar conserved domain architecture
- Multiple Alignment**: Align sequences using domain and protein constraints
- MOLE-BLAST**: Establish taxonomy for uncultured or environmental sequences

At the bottom of the page, there is a footer with the following information:

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[Support center](#) [Mailing list](#) [YouTube](#)

NCBI
National Center for Biotechnology Information, U.S. National Library of Medicine
8600 Rockville Pike, Bethesda MD, 20894 USA

[Policies and Guidelines](#) | [Contact](#)

Logos for the National Library of Medicine, NIH, and USA.gov are also present.

The Windows taskbar at the bottom shows the system tray with the date and time: 14:26, 08.10.2020.

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](#)

```
GTTGATCAGGGTAAACGCGTTGGTGAACCTCTCCGGTGGTGAAGCGGGTCTGCTGCATCTGGCGAAGCTGCTGCAGGTTGGCGGCAAC
ATGCTGCTGCTCGACGAACCAACCAACGACCTGGATATCGAAACCTGCGCGCGCTGGAAAACGCCCTGCTGGAGTTCCCGGGCTGT
GCGATGGTTATCTCGACGACCGCTTGGTTCCTCGACCGTATCGCCACGCAATTCTGGATTACCAAGGTAAGGTAAGTTGAGTTC
TTGAAAGTAACTTTACCGAGTACGAAAGGATACAGAAAACGCGACGCTGGGCGCAGACGCGCTGGAGCCGGAAGCGTATCAAGTACAAG
CGTATTGCGAAGtaa
```

Or, upload FASTA file

Выберите файл Файл не выбран

Range [Clear](#)

Forward primer	From	To
Reverse primer		

ввести последовательность нуклеотидов из документа Word

Primer Parameters

Use my own forward primer (5'->3' on plus strand)

Use my own reverse primer (5'->3' on minus strand)

PCR product size

Min: 600 Max: 1500

of primers to return

Primer melting temperatures (T_m)

Min: 50.0 Opt: 55.0 Max: 63.0 Max T_m difference: 5

установить параметры праймеров, длину ПЦР-продукта

Exon/intron selection

A refseq mRNA sequence as PCR template input is required for options in the section

Exon junction span

Exon junction match

Min 5' match: 7 Min 3' match: 4 Max 3' match: 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: 1000 Max: 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

Database

выравнивание праймеров

Exon junction span No preference

Exon junction match
Min 5' match: 7, Min 3' match: 4, Max 3' match: 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: 1000, Max: 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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Primer-BLAST

A tool for finding specific primers

Finding primers specific to your PCR template (using Primer3 and BLAST)

Input PCR template Id|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
<input type="checkbox"/> NC_000913.3	Escherichia coli str. K-12 substr. MG1655, complete genome	100%	1668	4628855	4630522	ettA

Show results in a new window

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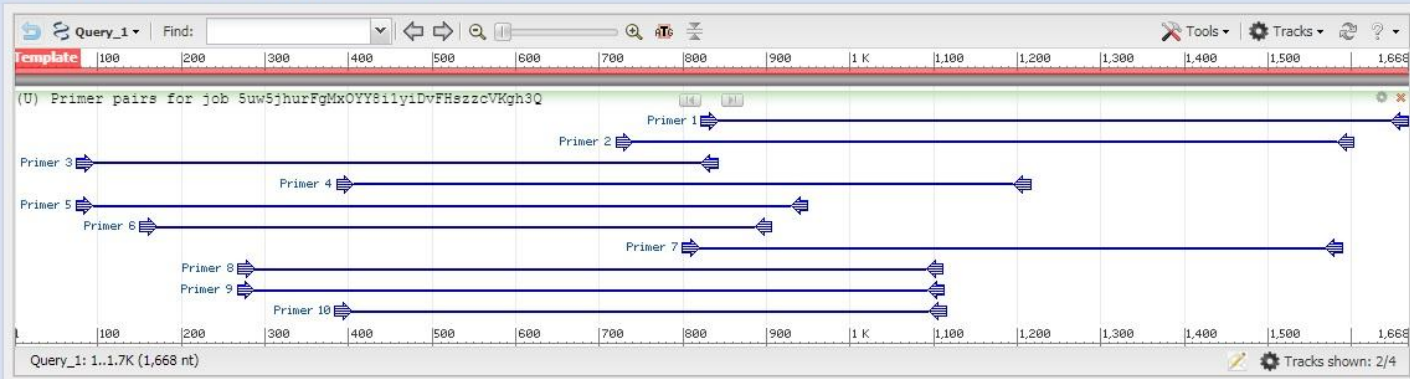
[Policies and Guidelines](#) | [Contact](#)

Primer-BLAST » JOB ID:5uw5jhurFgMxOYY8i1yiDvFHszzcVKgh3Q

Primer-BLAST Results

Input PCR template Icd|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

Выбрать одну-две пары праймеров из предложенных,
последовательность праймеров скопировать в уже созданный вами
документ Word

Помимо документа Word, также отправьте скриншот страницы с подобранными
праймерами

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 4629781 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	GGAAGGTAAGTACTCCTCCT	Plus	20	720	739	54.95	50.00	6.00	2.00
Reverse primer	CTTGACTCTTCGTAAGTCGG	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 GGAAGGTAAGTACTCCTCCT 20
Template 4629803 4629784
Reverse primer 1 CTTGACTCTTCGTAAGTCGG 20
Template 4628921 4628940

Primer pair 3

	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTCTCTGAGTTTCTCCCTG	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

product length = 767

Tm калькулятор

The screenshot shows a web browser window with the URL `tmcalculator.neb.com/#!/main`. The page title is "Tm Calculator" and the version is "1.13.0". The BioLabs logo is in the top right corner, along with the tagline "INSPIRED drive DISCOVERY BY GENUINE".

Instructions:

- Select the product group of the polymerase or kit you plan to use.
- Select the polymerase or kit from the list of products.
- If needed, modify the recommended primer concentration.
- Enter primer sequences (with up to 3 ambiguous bases). Spaces allowed.

Note that an annealing temperature will only be displayed if both primer sequences are entered.

Form Fields:

- Product Group:** Taq DNA Polymerase
- Polymerase/Kit:** Taq DNA Polymerase with Standard Taq Buffer
- Primer Concentration (nM):** 400 (with a "Reset concentration" button)
- Primer 1:** GCCGCCTGCTGCTGAAAAACC
- Primer 2:** GGCGCGGCTTGGCATCTCG (with "Switch to batch mode", "Clear", and "Use example input" buttons)

Results:

- Anneal at:** 63 °C
- Primer 1:** 22 nt, 64% GC, Tm: 68°C
- Primer 2:** 19 nt, 74% GC, Tm: 70°C

Footer:

- ABOUT THIS TOOL | HISTORY | ALL TOOLS | TECH SUPPORT
- © Copyright 2020 New England Biolabs. All Rights Reserved.

The Windows taskbar at the bottom shows the system tray with the date 08.10.2020 and time 14:58.