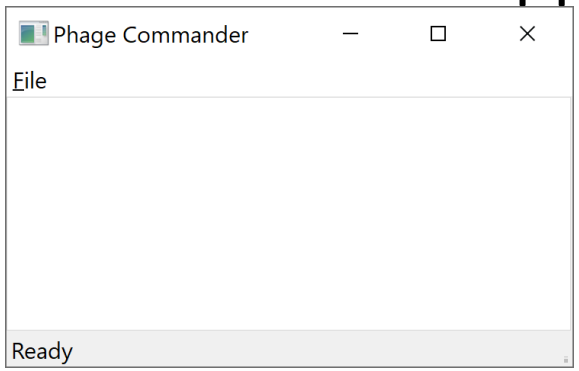
Introduction to Phage Commander

- **Phage Commander** is a software tool for identifying genes in phage genomes
- Phage Commander runs a phage's DNA sequence through gene identification tools and outputs a list of potential genes. These tools include:
  - Glimmer, Genemark, Genemark.hmm, Genemark S, Genemark S2, Genemark Heuristic, Prodigal, RAST, Metagene, and Aragorn (for tRNA genes)
- Phage Commander's output can be exported in Excel format (.xlsx) or NCBI GenBank format (.gb)
- A draft paper describing Phage Commander in detail is here: https://www.biorxiv.org/content/10.1101/2020.11.11.378802v1

How to run Phage Commander

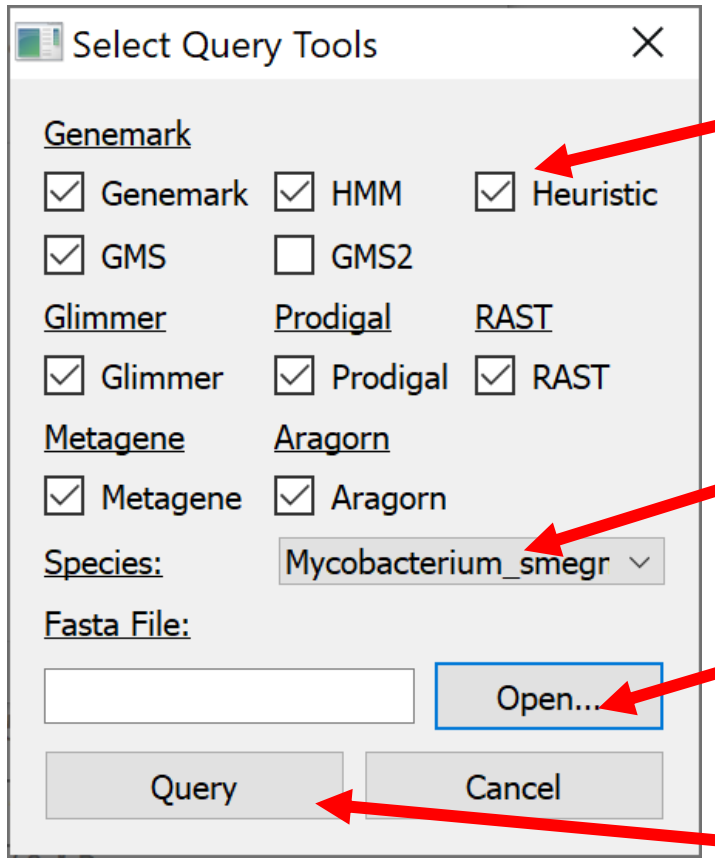
- Download Phage Commander from GitHub repository: **https://github.com/sarah-harris/PhageCommander**
- Run Phage Commander:
  - **Windows:** Navigate to phagecommander/bin. Click on the executable: phagecom-windows.exe
  - **Linux or Mac:** Open a shell. Navigate to folder where you have downloaded phagecommander. Type py phagecom.py. If you need to install any of the supporting packages, first type: 'pip install .' (in the PhageCommander-master directory – the directory that contains setup.py)
- A small Phage Commander window will appear:



How to use Phage Commander

- In the Phage Commander Window, click on the **File** menu, and select **New**
- A Select Query Tools window will open (see next slide). Select which tools you would like to use
  - If using **GeneMark.hmm** you will need to select your phage's bacterial host from the drop down menu under **Species**
  - To use **RAST**, you will need to create a login and password at https://rast.nmpdr.org
- Press **Open** and select your phage's fasta file - an example fasta file is included in the GitHub repository: Patience.fasta
- Press **Query** to run Phage Commander. Phage Commander will now run the phage genome through the selected tools – this may take several minutes
- When Phage Commander completes, press **OK**

Select Query Tools Window



Check the boxes of the tools you want to use

Select host from drop down menu if "HMM" box is checked (GeneMark.hmm)

Press open select phage fasta file

Press "Query"

In the Event of an Error

- If Phage Commander runs successfully, you will see a window saying "**Done! Query Successful**"
- Press **OK** and you will see the Phage Commander output in the form of a spreadsheet (expand the window)
- If phage commander encounters an error, the error message will display which program is causing the error (e.g. Glimmer, Aragorn, GMS2, etc.)
- To work around this, re-run Phage Commander without including the program causing the error

Total Calls column
Is the number of programs identifying a gene

Each row is a gene

Genes identified by GeneMark

Genes identified by GeneMark.hmm

Genes identified by GeneMark Heuristic

Genes identified by GeneMark S

TOTAL CALL	ALL	ONE	GM	GM	GM	GM	HMM	HMM	HMM	HMM	HEURISTIC	HEURISTIC	HEURISTIC	HEURISTIC	GMS	GMS	GMS	GMS	GM
7			+	733	1176	444	+	571	1176	606	+	487	1176	690	+	487	1176	690	
9	X		+	1089	1925	837	+	1332	1925	594	+	1332	1925	594	+	1089	1925	837	
9	X		+	1925	2146	222	+	1925	2146	222	+	1925	2146	222	+	1925	2146	222	
9	X		+	2482	2952	471	+	2569	2952	384	+	2482	2952	471	+	2482	2952	471	
9	X		+	3037	3999	963	+	3037	3999	963	+	3037	3999	963	+	3037	3999	963	
2							-	4053	4550	498	-	4053	4550	498					
7			+	3999	4664	666									+	3999	4664	666	
8			+	4661	5359	699					+	4661	5359	699	+	4661	5359	699	
9	X		+	5696	5833	138	+	5696	5833	138	+	5696	5833	138	+	5696	5833	138	
9	X		+	5833	7044	1212	+	5833	7044	1212	+	5833	7044	1212	+	5833	7044	1212	
9	X		+	7044	7475	432	+	7044	7475	432	+	7044	7475	432	+	7044	7475	432	
9	X		+	7512	9266	1755	+	7512	9266	1755	+	7512	9266	1755	+	7512	9266	1755	
9	X		+	9284	10726	1443	+	9323	10726	1404	+	9284	10726	1443	+	9284	10726	1443	
9	X		+	10801	11607	807	+	10801	11607	807	+	10801	11607	807	+	10801	11607	807	
9	X		+	11656	12186	531	+	11656	12186	531	+	11656	12186	531	+	11656	12186	531	
9	X		+	12219	13190	972	+	12219	13190	972	+	12219	13190	972	+	12219	13190	972	
9	X		+	13263	13460	198	+	13263	13460	198	+	13263	13460	198	+	13263	13460	198	
9	X		+	13463	13840	378	+	13463	13840	378	+	13463	13840	378	+	13463	13840	378	
9	X		+	13837	14028	192	+	13837	14028	192	+	13837	14028	192	+	13837	14028	192	
9	X		+	14028	14396	369	+	14088	14396	309	+	14028	14396	369	+	14028	14396	369	
9	X		+	14396	14749	354	+	14396	14749	354	+	14396	14749	354	+	14396	14749	354	
9	X		+	14773	15165	393	+	14929	15165	237	+	14746	15165	420	+	14746	15165	420	
9	X		+	15188	15790	603	+	15212	15790	579	+	15188	15790	603	+	15188	15790	603	
9	X		+	15901	16305	405	+	15901	16305	405	+	15901	16305	405	+	15901	16305	405	
9	X		+	16302	16721	420	+	16302	16721	420	+	16302	16721	420	+	16302	16721	420	
9	X		+	16714	18918	2205	+	16714	18918	2205	+	16714	18918	2205	+	16714	18918	2205	
9	X		+	18945	19958	1014	+	18945	19958	1014	+	18945	19958	1014	+	18945	19958	1014	
9	X		+	19931	21727	1797	+	19955	21727	1773	+	19955	21727	1773	+	19931	21727	1797	
9	X		+	21743	22195	453	+	21743	22195	453	+	21743	22195	453	+	21743	22195	453	
9	X		+	22192	22488	297	+	22192	22488	297	+	22192	22488	297	+	22192	22488	297	
9	X		+	22485	23906	1422	+	22485	23906	1422	+	22485	23906	1422	+	22485	23906	1422	
9	X		+	23934	24200	267	+	23934	24200	267	+	23916	24200	285	+	23934	24200	267	
3							+	24233	24391	159	+	24197	24391	195					
9	X		+	25052	25630	579	+	25070	25630	561	+	25052	25630	579	+	25052	25630	579	
9	X		+	25623	25898	276	+	25623	25898	276	+	25623	25898	276	+	25623	25898	276	
8			+	25984	26115	132	+	25984	26115	132	+	25954	26115	162	+	25954	26115	162	
6			-	26202	26360	159									-	26202	26360	159	
9	X		-	26587	26973	387	-	26587	26973	387	-	26587	26973	387	-	26587	26973	387	
9	X		-	26970	27272	303	-	26970	27269	300	-	26970	27269	300	-	26970	27272	303	
9	X		-	27269	27502	234	-	27269	27502	234	-	27269	27526	258	-	27269	27526	258	
9	X		-	27523	27741	219	-	27523	27687	165	-	27523	27741	219	-	27523	27741	219	
9	X		-	27764	29605	1842	-	27764	29605	1842	-	27764	29605	1842	-	27764	29605	1842	
9	X		-	29613	29801	189	-	29613	29801	189	-	29613	29801	189	-	29613	29801	189	
9	X		-	30057	30629	573	-	30057	30518	462	-	30057	30644	588	-	30057	30629	573	

TOTAL CALL	ALL	ONE	GM	GM	GM	GM		HMM	HMM	HMM	HMM		HEURISTIC	HEURISTIC	HEURISTIC	HEURISTIC		GMS	GMS	GMS	GMS		GM
7			+	733	1176	444		+	571	1176	606		+	487	1176	690		+	487	1176	690		
9	X		+	1089	1925	837		+	1332	1925	594		+	1332	1925	594		+	1089	1925	837		
9	X		+	1925	2146	222		+	1925	2146	222		+	1925	2146	222		+	1925	2146	222		
9	X		+	2482	2952	471		+	2569	2952	384		+	2482	2952	471		+	2482	2952	471		
9	X		+	3037	3999	963		+	3037	3999	963		+	3037	3999	963		+	3037	3999	963		
2								-	4053	4550	498		-	4053	4550	498							
7			+	3999	4664	666												+	3999	4664	666		
8			+	4661	5359	699							+	4661	5359	699		+	4661	5359	699		
9	X		+	5696	5833	138		+	5696	5833	138		+	5696	5833	138		+	5696	5833	138		
9	X		+	5833	7044	1212		+	5833	7044	1212		+	5833	7044	1212		+	5833	7044	1212		
9	X		+	7044	7475	432		+	7044	7475	432		+	7044	7475	432		+	7044	7475	432		
9	X		+	7512	9266	1755		+	7512	9266	1755		+	7512	9266	1755		+	7512	9266	1755		
9	X		+	9284	10726	1443		+	9323	10726	1404		+	9284	10726	1443		+	9284	10726	1443		
9	X		+	10801	11607	807		+	10801	11607	807		+	10801	11607	807		+	10801	11607	807		
9	X		+	11656	12186	531		+	11656	12186	531		+	11656	12186	531		+	11656	12186	531		
9	X		+	12219	13190	972		+	12219	13190	972		+	12219	13190	972		+	12219	13190	972		
9	X		+	13263	13460	198		+	13263	13460	198		+	13263	13460	198		+	13263	13460	198		
9	X		+	13463	13840	378		+	13463	13840	378		+	13463	13840	378		+	13463	13840	378		
9	X		+	13837	14028	192		+	13837	14028	192		+	13837	14028	192		+	13837	14028	192		
9	X		+	14028	14396	369		+	14088	14396	309		+	14028	14396	369		+	14028	14396	369		
9	X		+	14396	14749	354		+	14396	14749	354		+	14396	14749	354		+	14396	14749	354		
9	X		+	14773	15165	393		+	14929	15165	237		+	14746	15165	420		+	14746	15165	420		
9	X		+	15188	15790	603		+	15212	15790	579		+	15188	15790	603		+	15188	15790	603		
9	X		+	15901	16305	405		+	15901	16305	405		+	15901	16305	405		+	15901	16305	405		
9	X		+	16302	16721	420		+	16302	16721	420		+	16302	16721	420		+	16302	16721	420		
9	X		+	16714	18918	2205		+	16714	18918	2205		+	16714	18918	2205		+	16714	18918	2205		
9	X		+	18945	19958	1014		+	18945	19958	1014		+	18945	19958	1014		+	18945	19958	1014		
9	X		+	19931	21727	1797		+	19955	21727	1773		+	19955	21727	1773		+	19931	21727	1797		
9	X		+	21743	22195	453		+	21743	22195	453		+	21743	22195	453		+	21743	22195	453		
9	X		+	22192	22488	297		+	22192	22488	297		+	22192	22488	297		+	22192	22488	297		
9	X		+	22485	23906	1422		+	22485	23906	1422		+	22485	23906	1422		+	22485	23906	1422		
9	X		+	23934	24200	267		+	23934	24200	267		+	23916	24200	285		+	23934	24200	267		
3								+	24233	24391	159		+	24197	24391	195							
9	X		+	25052	25630	579		+	25070	25630	561		+	25052	25630	579		+	25052	25630	579		
9	X		+	25623	25898	276		+	25623	25898	276		+	25623	25898	276		+	25623	25898	276		
8			+	25984	26115	132		+	25984	26115	132		+	25954	26115	162		+	25954	26115	162		
6			-	26202	26360	159												-	26202	26360	159		
9	X		-	26587	26973	387		-	26587	26973	387		-	26587	26973	387		-	26587	26973	387		
9	X		-	26970	27272	303		-	26970	27269	300		-	26970	27269	300		-	26970	27272	303		
9	X		-	27269	27502	234		-	27269	27502	234		-	27269	27526	258		-	27269	27526	258		
9	X		-	27523	27741	219		-	27523	27687	165		-	27523	27741	219		-	27523	27741	219		
9	X		-	27764	29605	1842		-	27764	29605	1842		-	27764	29605	1842		-	27764	29605	1842		
9	X		-	29613	29801	189		-	29613	29801	189		-	29613	29801	189		-	29613	29801	189		
9	X		-	30057	30629	573		-	30057	30518	462		-	30057	30644	588		-	30057	30629	573		
9	X		-	30641	32665	2025		-	30641	32479	1839		-	30641	32665	2025		-	30641	32665	2025		

DNA strand (+ or -)

Gene start (stop if strand "-")

Gene stop (start if strand is "-")

Gene length

Row shading is proportional to how many programs identify a gene (darker = more programs, white = only one program)

TOTAL CALL	ALL	ONE	GM	GM	GM	GM		HMM	HMM	HMM	HMM		HEURISTIC	HEURISTIC	HEURISTIC	HEURISTIC		GMS	GMS	GMS	GMS		GM
7			+	733	1176	444		+	571	1176	606		+	487	1176	690		+	487	1176	690		
9	X		+	1089	1925	837		+	1332	1925	594		+	1332	1925	594		+	1089	1925	837		
9	X		+	1925	2146	222		+	1925	2146	222		+	1925	2146	222		+	1925	2146	222		
9	X		+	2482	2952	471		+	2569	2952	384		+	2482	2952	471		+	2482	2952	471		
9	X		+	3037	3999	963		+	3037	3999	963		+	3037	3999	963		+	3037	3999	963		
2								-	4053	4550	498		-	4053	4550	498							
7			+	3999	4664	666												+	3999	4664	666		
8			+	4661	5359	699							+	4661	5359	699		+	4661	5359	699		
9	X		+	5696	5833	138		+	5696	5833	138		+	5696	5833	138		+	5696	5833	138		
9	X		+	5833	7044	1212		+	5833	7044	1212		+	5833	7044	1212		+	5833	7044	1212		
9	X		+	7044	7475	432		+	7044	7475	432		+	7044	7475	432		+	7044	7475	432		
9	X		+	7512	9266	1755		+	7512	9266	1755		+	7512	9266	1755		+	7512	9266	1755		
9	X		+	9284	10726	1443		+	9323	10726	1404		+	9284	10726	1443		+	9284	10726	1443		
9	X		+	10801	11607	807		+	10801	11607	807		+	10801	11607	807		+	10801	11607	807		
9	X		+	11656	12186	531		+	11656	12186	531		+	11656	12186	531		+	11656	12186	531		
9	X		+	12219	13190	972		+	12219	13190	972		+	12219	13190	972		+	12219	13190	972		
9	X		+	13263	13460	198		+	13263	13460	198		+	13263	13460	198		+	13263	13460	198		
9	X		+	13463	13840	378		+	13463	13840	378		+	13463	13840	378		+	13463	13840	378		
9	X		+	13837	14028	192		+	13837	14028	192		+	13837	14028	192		+	13837	14028	192		
9	X		+	14028	14396	369		+	14088	14396	309		+	14028	14396	369		+	14028	14396	369		
9	X		+	14396	14749	354		+	14396	14749	354		+	14396	14749	354		+	14396	14749	354		
9	X		+	14773	15165	393		+	14929	15165	237		+	14746	15165	420		+	14746	15165	420		
9	X		+	15188	15790	603		+	15212	15790	579		+	15188	15790	603		+	15188	15790	603		
9	X		+	15901	16305	405		+	15901	16305	405		+	15901	16305	405		+	15901	16305	405		
9	X		+	16302	16721	420		+	16302	16721	420		+	16302	16721	420		+	16302	16721	420		
9	X		+	16714	18918	2205		+	16714	18918	2205		+	16714	18918	2205		+	16714	18918	2205		
9	X		+	18945	19958	1014		+	18945	19958	1014		+	18945	19958	1014		+	18945	19958	1014		
9	X		+	19931	21727	1797		+	19955	21727	1773		+	19955	21727	1773		+	19931	21727	1797		
9	X		+	21743	22195	453		+	21743	22195	453		+	21743	22195	453		+	21743	22195	453		
9	X		+	22192	22488	297		+	22192	22488	297		+	22192	22488	297		+	22192	22488	297		
9	X		+	22485	23906	1422		+	22485	23906	1422		+	22485	23906	1422		+	22485	23906	1422		
9	X		+	23934	24200	267		+	23934	24200	267		+	23916	24200	285		+	23934	24200	267		
3								+	24233	24391	159		+	24197	24391	195							
9	X		+	25052	25630	579		+	25070	25630	561		+	25052	25630	579		+	25052	25630	579		
9	X		+	25623	25898	276		+	25623	25898	276		+	25623	25898	276		+	25623	25898	276		
8			+	25984	26115	132		+	25984	26115	132		+	25954	26115	162		+	25954	26115	162		
6			-	26202	26360	159												-	26202	26360	159		
9	X		-	26587	26973	387		-	26587	26973	387		-	26587	26973	387		-	26587	26973	387		
9	X		-	26970	27272	303		-	26970	27269	300		-	26970	27269	300		-	26970	27272	303		
9	X		-	27269	27502	234		-	27269	27502	234		-	27269	27526	258		-	27269	27526	258		
9	X		-	27523	27741	219		-	27523	27687	165		-	27523	27741	219		-	27523	27741	219		
9	X		-	27764	29605	1842		-	27764	29605	1842		-	27764	29605	1842		-	27764	29605	1842		
9	X		-	29613	29801	189		-	29613	29801	189		-	29613	29801	189		-	29613	29801	189		
9	X		-	30057	30629	573		-	30057	30518	462		-	30057	30644	588		-	30057	30629	573		

Alternative starts shown in different color font (starts by majority of programs show in black or white font)

tRNA genes
Identified by Aragorn
in “TRNA” tab

	TOTAL CALLS	ALL	ONE	ARAGORN	ARAGORN	ARAGORN	ARAGORN
1	1	X		+	5403	5475	73
2	1	X		+	5480	5553	74
3	1	X		+	5595	5667	73

Total calls is always 1
since Aragorn is the only
program for identifying
tRNA genes

Exporting Phage Commander Results

- To export as excel spreadsheet, select:
  - **File → Export → Excel**
- To export as GenBank (.gb) format file, select:
  - **File → Export → GenBank**
  - Set the threshold number of programs for exporting genes
  - “Less than or equal to” will export those genes identified by an equal or lower number of programs than the threshold (e.g. genes identified by 3 or fewer programs. Set the threshold to maximum to export all genes identified)
  - “Greater than” will export those genes identified by a number of programs greater than the threshold (e.g. genes identified by more than 2 programs. Use “0” as the threshold and this setting to export all genes identified)
  - Press “Save as” and enter desired filename
  - Press “Export”

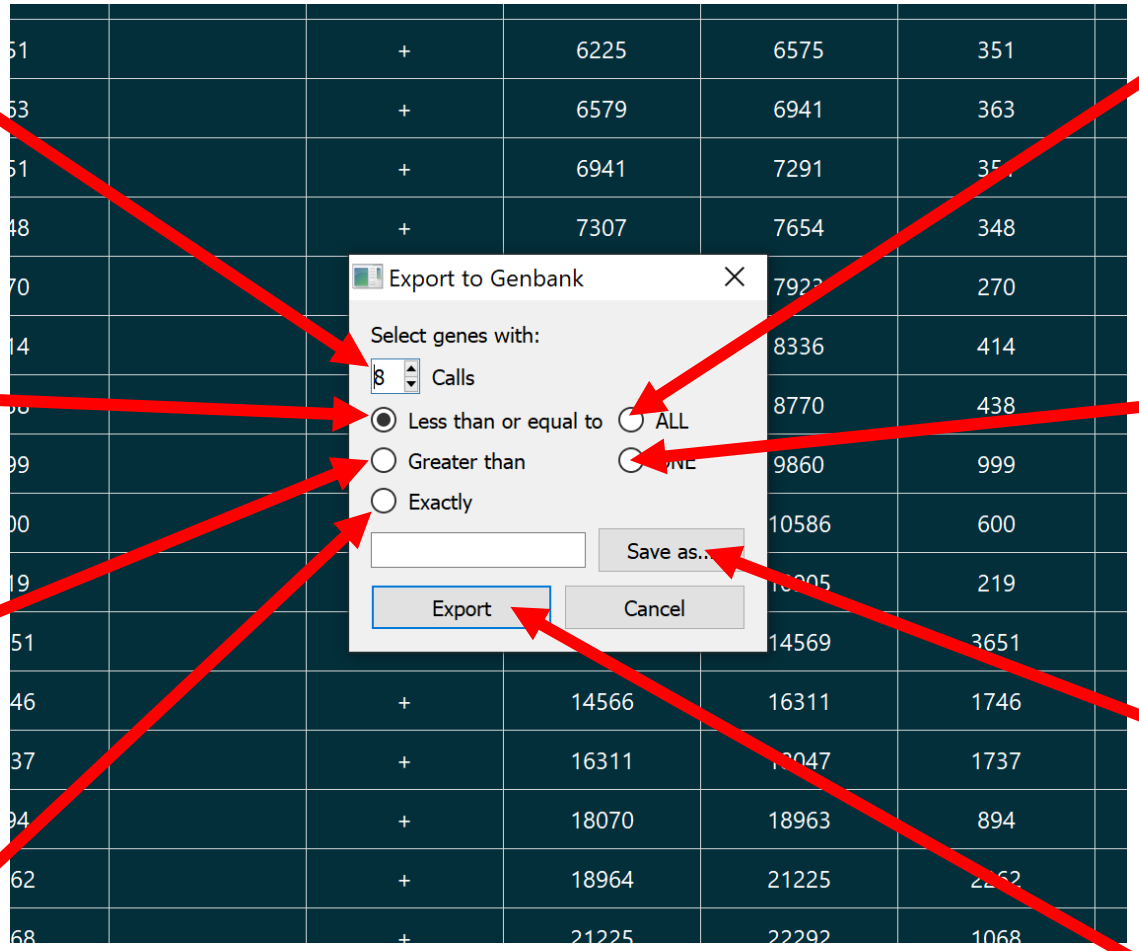
Exporting Phage Commander results in .gb format

User-set threshold number of programs for exporting genes

Export genes identified by no more than the set number of programs

Export genes identified by at least the set number of programs

Export genes identified exactly by the set number of programs



Export only those genes identified by <u>all</u> programs (most stringent)

Export genes identified by at least <u>one</u> program (i.e. all genes, least stringen)

Press "Save as" and enter desired filename

Press "Export"