Cite: <mark>A.M. King, C.K. Vanderpool, and P.H. Degnan. sRNA-target Prediction Organizing Tool (SPOT) integrates computational and experimental data to facilitate functional characterization of bacterial small RNAs</mark>

1. Rationale

Computational approaches for sRNA target prediction have limitations but are relied upon to generate testable hypotheses for sRNA function. Some algorithms are available online or downloadable (e.g., TargetRNA2, IntaRNA), however these tools frequently yield distinct results, have different data output formats and default search parameters. Therefore, manually compiling results from these disparate tools and integrating the predictions with existing experimental data is not trivial. We have generated an innovative approach to streamline use of multiple existing sRNA target prediction algorithms and integrate predictions with experimental data to generate a unified set of target predictions. To this end, we have developed **SPOT** a flexible software pipeline that searches for sRNA-mRNA binding sites in parallel using separate search tools, collates the predictions, and integrates experimental data using customizable results filters.
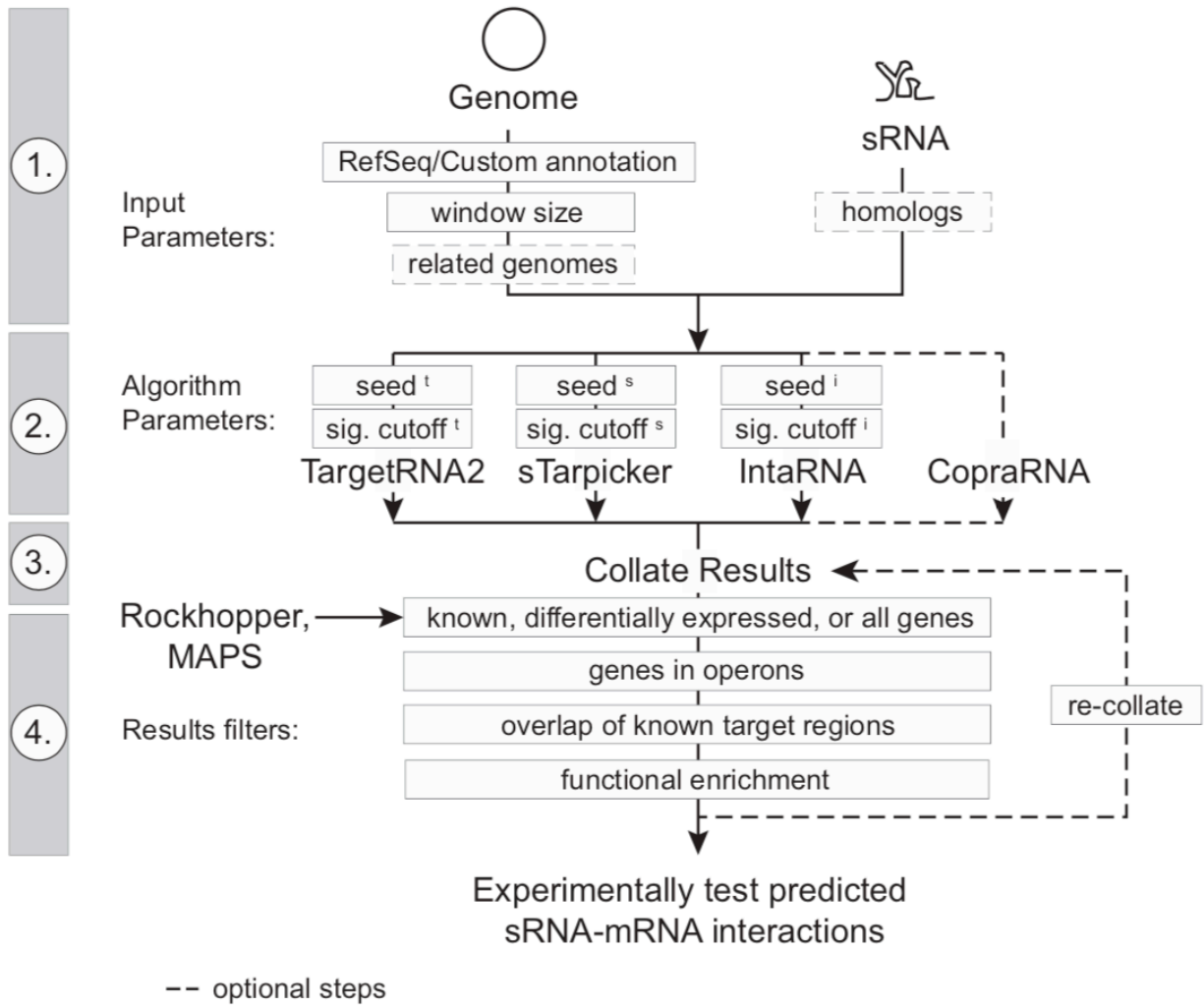
Figure 1. Schematic of **SPOT** pipeline analysis (King et al.)

2. Installing SPOT

SPOT is a PERL program that runs TargetRNA2, IntaRNA, StarPicker and CopraRNA in parallel, and collates the results to find consensus sRNA-mRNA targets (Figure 1). Furthermore, additional data types can be utilized to filter the results including expression differences, known binding sites, operon predictions and window size of possible binding sites.

As written the program can run on any Unix/Linux based system, however it has a number of dependencies. To facilitate its use we have set up an [Amazon Web Service (AWS)](#) cloud [Amazon Machine Image (AMI)](#) with all of the required software installed. Skip to sections 4-7 for setting up your own **SPOT** AMI. However, using the code available [here](#) you can set up and run **SPOT** on a local server.

First, download and install the following software tools and all of their dependencies according the authors' instructions:

- [TargetRNA v2](#)
- [StarPicker](#)
- [IntaRNA v1.0.4](#)
- [CopraRNA v1.2.9](#)

Several modifications were made to the StarPicker and IntaRNA code to accommodate demands of the pipeline.

Replace the following programs with those provided in the GitHub link. Modifications in the code are marked with ## comments and/or initials (PHD). Descriptions of edits made are listed briefly below.

StarPicker:
> `sTarPicker_global2.pl` : changes made to input of command line arguments

IntaRNA v1.0.4:
> `add_GI_genename_annotation.pl` : distinguish GeneIDs vs GI Nos
> `get_refseq_from_ftp.pl` : Replacement code for get_refseq_from_ftp.sh
> `IntaRNA_wrapper.pl` : Option added to use local GenBank files, use get_refseq_from_ftp.pl
> `rerun_enrichment.pl` : code snippet re-running enrichment analysis from IntaRNA_Wrapper.pl
> `termClusterReport.pl` : code modified to handle GeneIDs vs GI Nos

CopraRNA v1.2.9:
> `get_refseq_from_ftp.pl` : Replacement code for get_refseq_from_ftp.sh
> `termClusterReport.pl` : code modified to handle GeneIDs vs GI Nos
> `get_CDS_from_gbk.pl` : code modified to skip and flag GenBank files not present in kegg2refseqnew.csv list

Note: D3 Javascript libraries may or may not be accessible using existing framework to generate functional enrichment heatmaps (http://d3js.org/d3.v3.min.js). If problems are encountered, it is possible to edit the master html files in IntaRNA and CopraRNA to use a local version of d3.v3.min.js .

Be sure all programs are added to the user path and all path references in StarPicker, IntaRNA, and CopraRNA match your system installation.
The statistics program R is installed as a requirement for IntaRNA and CopraRNA. As such add the following two packages:

- RColorBrewer
- gplots

```
$ sudo R
> install.packages(c('RColorBrewer', 'gplots'))
```

Some of the output from the **SPOT** program will be written in an xlsx format using the Excel Writer PERL module:

- Excel-Writer-XLSX-0.98

```
$ sudo cpan Excel::Writer::XLSX
```

Most existing Unix/Linux installations should have sendmail installed. If not, install the appropriate package

- sendmail

```
$ sudo apt install sendmail-bin
```

SPOT can work with local copies of genomes and annotations. However, to access genomes from NCBI install the efetch program from the Entrez Direct (edirect) toolkit.

- edirect

Retrieve and decompress the **SPOT** directory from GitHub containing core pipeline script and its additional required support PERL scripts.

- SPOT

Make sure **SPOT** and all of the programs are in your user path. Modify the core pipeline script with the absolute path locations for TargetRNA2, IntaRNA, StarPicker and CopraRNA, and other support PERL scripts.

3. Running SPOT - Quick Start

SPOT is a pipeline script that when run without arguments will print all of the possible program options:

```
$ spot.pl
Usage ./spot.pl
Input parameters:
-r    Fasta file of sRNA query
-a    RefSeq Accession number (assumes any local files have RefSeq
      number as their prefix)
...
```

The minimum data required for a **SPOT** search are:
1. A fasta file of the small RNA sequence
2. A RefSeq genome accession number

```
$ spot.pl –r sgrS.fasta –a NC_000913
```

This will initiate a job using the SgrS as the sRNA query and the *E. coli* str. K12 (NC_000913) as the reference genome. Progress of the search will be printed to the screen. Run time will depend on the number of processors available as each search tool is distributed to a separate sub process. By default CopraRNA **is not run** unless specifically requested.

4. Running SPOT

SPOT has an array of actions that control the input, algorithm parameters, and results filtering.

```
$ spot.pl
Usage ./spot.pl
Input parameters:
      -r    Fasta file of sRNA query
      -a    RefSeq Accession number (assumes any local files have RefSeq
            number as their prefix)
      -o    output file prefix (default = TEST)
      -g    Use local GenBank or PTT&FNA files for all Programs? (default = N
            use latest from GenBank, CopraRNA cannot use local files)
      -n    Other genome RefSeq ids for CopraRNA listed in quotes '' ,
            current max is 5 genomes (default ='')
      -m    Multisequence sRNA file for each genome in CopraRNA list
             (default ='')
      -x    Email address for job completion notification (default ='')

Algorithm parameters:
      -u    Number of nt upstream of start site to search (default = 60)
      -d    Number of nt downstream of start site to search (default = 60)
      -s    seed sizes for I, T, S e.g., '6 7 6' (defaults TargetRNA = 7,
            IntaRNA & Starpicker = 6)
      -c    P/Threshold value Cutoff for T, S, I e.g., '0.5 .001 un'
      (defaults Target = 0.05, Starpicker = 0.5, IntaRNA = top)

Results Filters:
      -b    Number of nt upstream of start site to filter results
             (default = -20)
      -e    Number of nt downstream of start site to filter results
             (default = 20)
      Note: -b and -e ignored if using a list (-l) or Rockhopper
            results (-t)
      Note: Set -b and -e to -u and -d to get all possible matches in
            results
      -l    List of up and/or down regulated genes, include binding coord if
            known e.g.,
      b1101\tdown\n
      b3826\tup\tsRNA_start\tsRNA_stop\tmRNA_start\tmRNA_stop\n
                         OR
      -t    transcriptome expression file from Rockhopper *_transcripts.txt
      -f    Rockhopper fold change cutoff (default = 1.5)
      -q    Rockhopper q value cutoff (default = 0.01)
      -k    Rockhopper Expression cutoff value (default = 100)
      -p    Operon file from DOOR-2 (http://csbl.bmb.uga.edu/DOOR/index.php)
             (optional)
      -w    Report all genes even if List or Rockhopper provided?
             (default = No)
      -y    Exclude target predictions by only 1 method? (default = Yes)
            Note: Does not apply to genes on List or significantly expressed
            from Rockhopper
      -z    Skip sRNA-mRNA detection steps, and just re-analyze data [Yy]es
            (default = No) (Run in the same directory & requires original
            results files from each program)
```

Given the time **SPOT** runs can take it is recommended to use a queueing tool on large distributed servers (`qsub, slurm`). Alternatively, on the AWS server, laptop or other smaller computers it is recommended to use `screen` to ensure that jobs are not prematurely aborted if the user account is logged out of.

```
$ screen -L spot.pl -r sgrS.fasta -a NC_000913
```

Four test datasets and precomputed output files are included in the folder `example_files`. The following examples correspond to the four provided test datasets.

test01 - Examine entire *E. coli* str. K12 genome for SgrS sRNA target mRNAs. This folder only has the sRNA sequence in a `fasta` file, uses the individual program default SEED size and significance settings and retrieves the genome sequence for *E. coli* from GenBank. The final option is to have an email sent to the user after the job has completed.

```
$ cd test01
$ ls
sgrS.fasta
$ spot.pl -r sgrS.fasta -o stringent -a NC_000913 -x username@email.edu

=========Prepping RefSeq Files====================
[Thu Aug 23 23:14:27 UTC 2018]
...
```

test02 - Examine *E. coli* str. K12 genome for SgrS sRNA target mRNA matches among a set of defined differentially expressed genes (`sgrS_diff.txt`). In this case the user has a `fasta` file and a traditional GenBank protein translation file (`PTT`). The user also indicates a larger window size 150 nt upstream of the CDS start position and 100 nt downstream to search for binding sites.

```
$ cd test02
$ ls
sgrS.fasta
sgrS_diff.txt
NC_000913.fna
NC_000913.ptt
$ spot.pl -r sgrS.fasta -l sgrS_diff.txt -u 150 -d 100 -c '0.5 0.001 un' -o
relaxed -a NC_000913 -g Y
```

Note: `PTT` files can be easily generated in Excel. Allowing for *customization* of gene annotations and subsequent analyses. A script included with **SPOT** is `fnaptt2gbk.pl` which can be used to generate GenBank files using the genome `PTT` and `fasta` files as inputs. However, always make sure that MAC or DOS line breaks are converted into UNIX line breaks.

test03 - SPOT was designed to allow re-analysis of existing results. This example code block is rung in a folder containing the results of **test02**'s search. In this case even though the upstream/downstream region searched was 150nt and 100nt, the reanalysis eliminates any binding sites found outside of 50nt upstream and 30nt downstream. This search also does not use the list of differentially expressed genes.

```
$ cd test03
$ ls
sgrS.fasta
sgrS_diff.txt
NC_000913.fna
NC_000913.ptt
...
$ spot.pl -r sgrS.fasta -u 150 -d 100 -c '0.5 0.001 un' -o changed_50_30 -a
NC_000913 -g Y -b -50 -e 30 -z Y
```

test04 – SPOT can also be run using a `*transcript.txt` file generated by the RNAseq analysis program Rockhopper directly (instead of list as in example **test02**). In this example default expression cutoffs are used, however these can be specified by the user. In addition, when provided a set of sRNA homologs and target genomes CopraRNA can be run. In these instances only genomes in RefSeq can be used. Custom genome annotations cannot be utilized.

```
$ cd test04
$ ls
NC_000913_SgrS_transcripts.txt
sgrS.fasta
sgrS_homologs.fasta
$ spot.pl -r sgrS.fasta -t NC_000913_SgrS_transcripts.txt -o express -a
NC_000913 -m sgrS_homologs.fasta -n 'NC_002695 NC_011740' -u 150 -d 100
```

When the jobs have completed compare your results to the files in the corresponding `_results` folder.

5. Data input formats

sRNA fasta file – DNA sequence of sRNA in a standard fasta file. File extension does not matter (`.fasta, .fa, .fna, .frn, .ffn`)

RefSeq ID – Standard RefSeq IDs can be used and GenBank files (`.gbff`) will be retrieved using `efetch`. Program will retrieve additional replicons (e.g., plasmids) or scaffolds associated with the provided RefSeq IDs, however, the search will only be

carried out on the file with a name corresponding to the input RefSeq ID. By default the `.gbff` is renamed to a `.gb` file, and `.fna` and `.ptt` files are generated.

Local Files – Different combinations of local files can be used. They all **must** have the same prefix and end in the following suffixes:

`.fna`	Genome fasta sequence
`.ptt`	Protein translation table – gene annotation
`.gb` or `.gbk`	Genbank file

Files *without* these suffixes will be ignored. All must have Unix linebreaks and the `.ptt` file must be tab separated. Allowed input combinations include:

	`.fna`	`.ptt`	`.gb` or `.gbk`	Status	Action
1.	√	√	√	okay	Start run
2.	√	√		okay	Make `.gb` file, start run
3.	√		√	okay	Make `.ptt` file, start run
4.		√	√	okay	Make `.fna` file, start run
5.			√	okay	Make `.fna` and `.ptt` file, start run
6.	√			bad	Abort run
7.		√		bad	Abort run

`.ptt` *Files* – This is a legacy GenBank annotation format. However, the StarPicker algorithm used here requires this format. This format is very easy to generate in Excel and can allow users of **SPOT** to customize their annotations. See example:

Escherichia coli str. K-12 substr. MG1655, complete genome - 1..4641652								
4141 proteins								
Location	Strand	Length	PID	Gene	Synonym	Code	COG	Product
190..255	+	21		thrL	b0001	-	-	thr operon leader peptide
337..2799	+	820		thrA	b0002	-	-	Bifunctional aspartokinase
2801..3733	+	310		thrB	b0003	-	-	homoserine kinase
3734..5020	+	428		thrC	b0004	-	-	L-threonine synthase

Note: As indicated above, customization of `PTT` files allows users to correct or change annotations based on new data. Furthermore, by modifying `PTT` files **RNAs can be included** in the annotation. First, this allows for sRNA – RNA interactions to be identified. Second, this approach was used in the manuscript to perform a 'reverse' search. For a 'reverse' search the `PTT` file is edited to ONLY include the known sRNAs. Then, the user supplies the UTR or putative sRNA binding region to **SPOT** as a `fasta` file if it were the sRNA. 'Reverse' searches cannot use CopraRNA and as sRNAs do not have GI numbers and may not have GeneIDs - no functional enrichment plots will be produced. This may result in several warnings when the **SPOT** is run, however it should not influence the final composite predictions.

Differentially expressed genes – Lists of differential genes can be formatted as tab separated files one of two ways. **Do not include a header line**

Simple:

Locus	Expression
b1101	down
b3826	up

With known binding sites:

Locus	Expression	sRNA_start	sRNA_stop	mRNA_start	mRNA_stop
b1101	down	168	187	-30	-9
b3826	up	168	187	-96	-76

Rockhopper `*transcript.txt` *files* – **SPOT** can read default output files of Rockhopper from simple pairwise RNAseq experiments. Files generated with the verbose output option in Rockhopper cannot be read. Files should have 12 columns including the normalized expression values for the treatment and control, the *q* Values and the estimated fold-change.

sRNA Multisequence Fasta Files – If running CopraRNA, sRNA files must conform to expectations of the CopraRNA program:
1. RNA sequence must have Us instead of Ts
2. The sequence names must correspond to the individual genome RefSeq IDs
3. Must include the focal genome sRNA sequence as well

6. Data output formats

Data from each individual algorithm is preserved in the output folder for manual investigation.

```
TargetRNA2_*txt      = TargetRNA2 Primary report
*.output             = Starpicker Primary report
intarna_websrv_table_truncated.csv = IntaRNA Primary report
*_hIntaRNA.csv       = CopraRNA Primary report
```

SPOT generates several output files for further analysis:

XLSX file – Primary file containing consensus table of sRNA-mRNA predictions from the 3 or 4 tools used in the run. File name prefix corresponds to run output prefix that was assigned (`-o`, `default= TEST`).
- Sheet 1 (complete.txt) shows the aligned predictions, *p* values, and coordinates for the predicted interaction for each gene.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Locus	Gene	T-Energy	T-Pvalue	T-sRNA_start	T-sRNA_stop	T-mRNA_start	T-mRNA_stop	T-Structure	S-Energy	S-Pvalue	S-sRNA_start	S-sRNA_stop	S-mRNA_start	S-mRNA_stop	S-Structure
2	b1101	ptsG	-10.51	0.018	167	187	-29	-9	Ec_sgrS 187 3' U-GUGGUUAUGAGUCAGUGUGU 5' 167 ┆┆┆┆┆ ┆┆┆┆┆┆┆┆ :┆┆┆ ptsG -29 5' AGCACCCAUACUCAGGAGCAC- 3' -9 gene product:fused glucose-specific PTS enzymes: IIB component/IIC component; PTS system, glucose-specific IIBC component	-20.8	0.004	169	187	-28	-8	sRNA(Ec_sgrS) 169 --uguga ┆┆· Target(ptsG_b1101) -8 ucacgag
3	b0060	polB	-9.86	0.025	151	169	-2	17	Ec_sgrS 169 3' UGUACUACGUCCGUUCAGU 5' 151 :┆:┆┆: ┆┆┆┆┆┆:┆┆: polB -2 5' GCGUGGCGCAGGCAGGUUU 3' 17 gene product:DNA polymerase II							

- Sheet 2 (summary.txt) has the counts predicted by each gene, and a summary letter and ranking based location and on the number of algorithms that found the same prediction.

A　　Prediction overlaps a known binding site

B→E　Predictions that are not coincident with a known binding site when one was provided for that gene. Shared letters overlap the same site.

F→I　Predictions when no known binding site was provided. Shared letters overlap the same site.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Locus	Gene	TargetRNA2	Star	IntaRNA	Count_ALL	TargetRNA2_20_20	Star_-20_20	IntaRNA_-20_20	Count_-20_20	TargetRNA2_M	Star_M	IntaRNA_M	Rank
2	b1101	ptsG	1	1	1	3	1	1	1	3	F	F	F	4
3	b0060	polB	1		1	2	1		1	2	F		F	4

* summary.pdf file* – This file has a R generated plot that corresponds to Sheet 2 (summary.txt) which can be imported to Illustrator.

Color Key

0　2　4
Value

b0060 polB
b2966 yqgA
b2721 hycE
b3520 yhjB
b4036 lamB
b1808 yoaA
b0128 yadH
b0272 yagI
b3499 rlmJ
b2206 napA
b4268 idnK
b1055 yceA
b1312 ycjP
b4184 yjfL
b0031 dapB
b1101 ptsG
b2314 dedD
b3215 yhcA
b1238 tdk
b2983 yghQ
b3253 acuI
b2416 ptsI
b3902 rhaD
b0340 cynS
b1785 yeaI
b0810 glnP
b0824 ybiY
b2128 yehW
blank

t s i
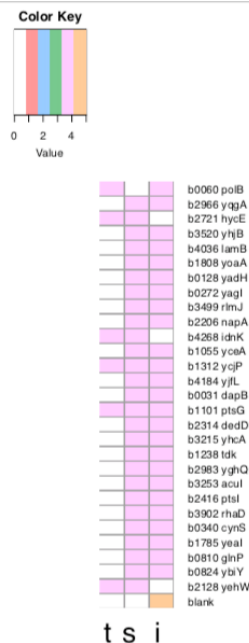
COLLATED_RESULTS folder – This folder contains plots generated based on IntaRNA tools showing the localization of binding sites of the mRNA and sRNA as *pdf, *png and *ps files. In addition, a functional enrichment heatmap is included as a *pdf file similar to those individually provided by IntaRNA and CopraRNA - however it represents the collated results.

Predicted interaction regions sRNA
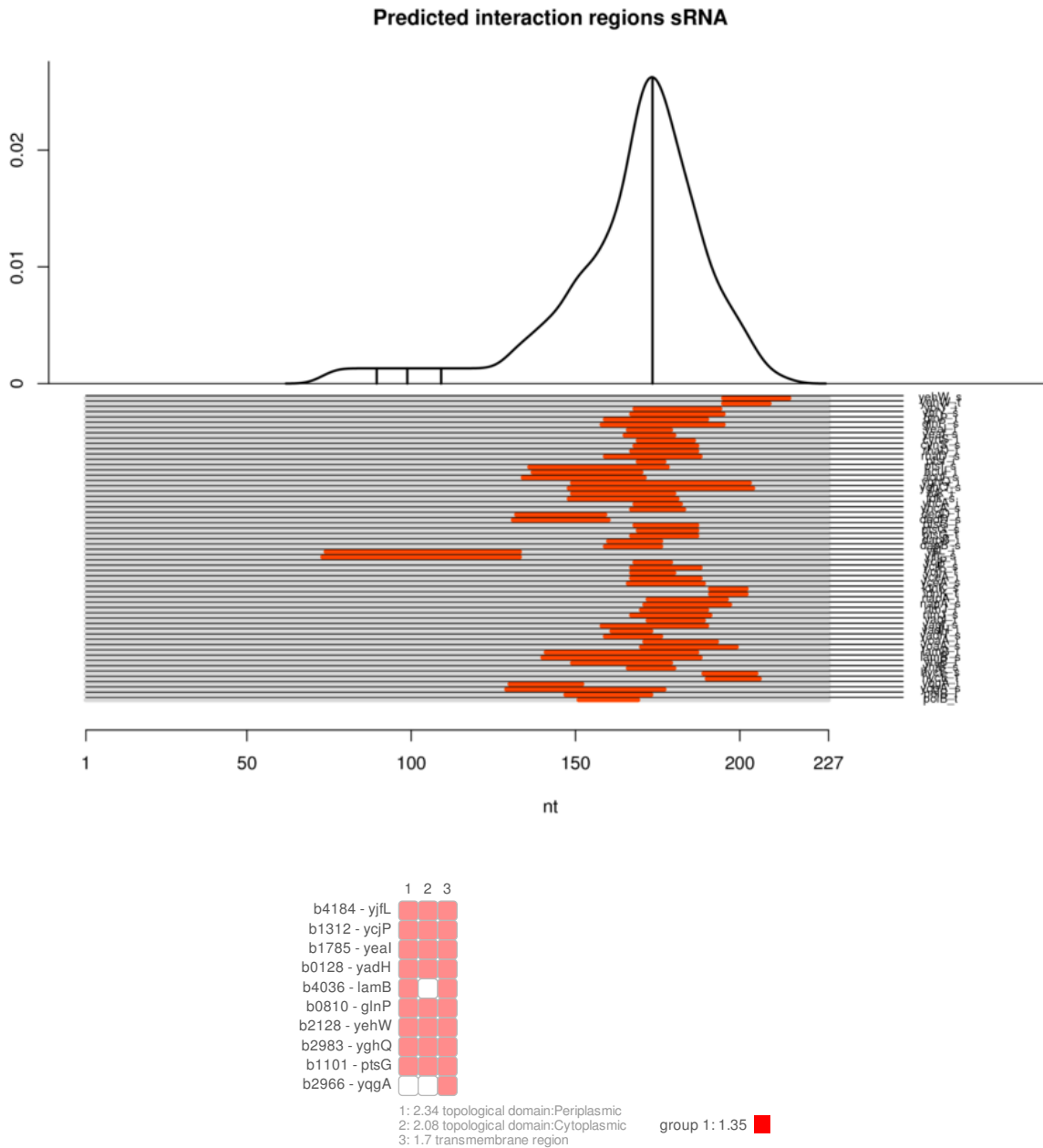
Example result files for the sRNA SgrS and corresponding test datasets are available with the **SPOT** software distribution.

7. Setup an AWS account

Navigate to the new account setup page:
https://portal.aws.amazon.com/billing/signup#/start

For now, set up your home region as "**U.S. East (W. Virginia)"** later you can switch this as necessary.

Unfortunately, when setting up an account you will need a credit card number

Input Education credit - Depending on your application it may be possible to apply for education credits to defray the cost of the AWS server time:

https://aws.amazon.com/education/awseducate/
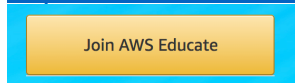
8. Setup personal AWS interface on your laptop

People with MACs:

Terminal will already be installed /Applications/Utilities

Download & Install **XQuartz** if not already installed
http://xquartz.macosforge.org/landing/

Download & Install **Cyberduck** https://cyberduck.io/?l=en

People with PCs:

Download & Install **PuTTY**
http://www.chiark.greenend.org.uk/~sgtatham/putty/download.html

Download & Install **xMing**
http://sourceforge.net/project/downloading.php?group_id=156984&filename=Xming-6-9-0-31-setup.exe

How to setup **xMing** : http://www.geo.mtu.edu/geoschem/docs/putty_install.html

Download & Install **WinSCP** http://winscp.net/eng/download.php
or
Download & Install **Cyberduck** https://cyberduck.io/?l=en

9. Starting an AWS instance

For in-depth instructions regarding starting an AWS instance please see:

https://docs.aws.amazon.com/AWSEC2/latest/UserGuide/launching-instance.html

1. After making and logging into your AWS account find your way to the EC2 (Elastic Computing Cloud) page. You can find it under "Services" menu on the upper left-hand corner of the page:

https://console.aws.amazon.com/ec2/v2/home?region=us-east-1#Home:

2. Make sure your home region as "**U.S. East (W. Virginia)"**. Your region is indicated in the upper right-hand corner of the page (circled above)

3. On the right-hand side bar under "**IMAGES**" select "**AMIs**"
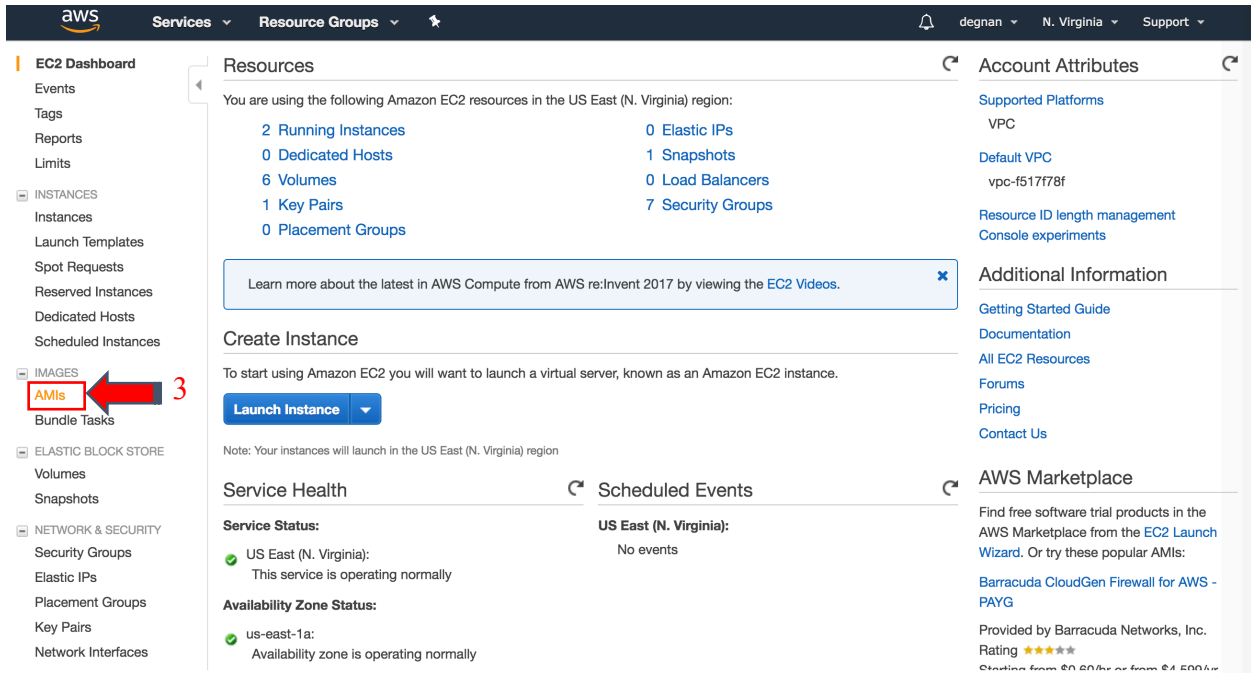
4. In the search bar switch from "Owned by me" to "**Public images**" and search for "**SPOTv1**"
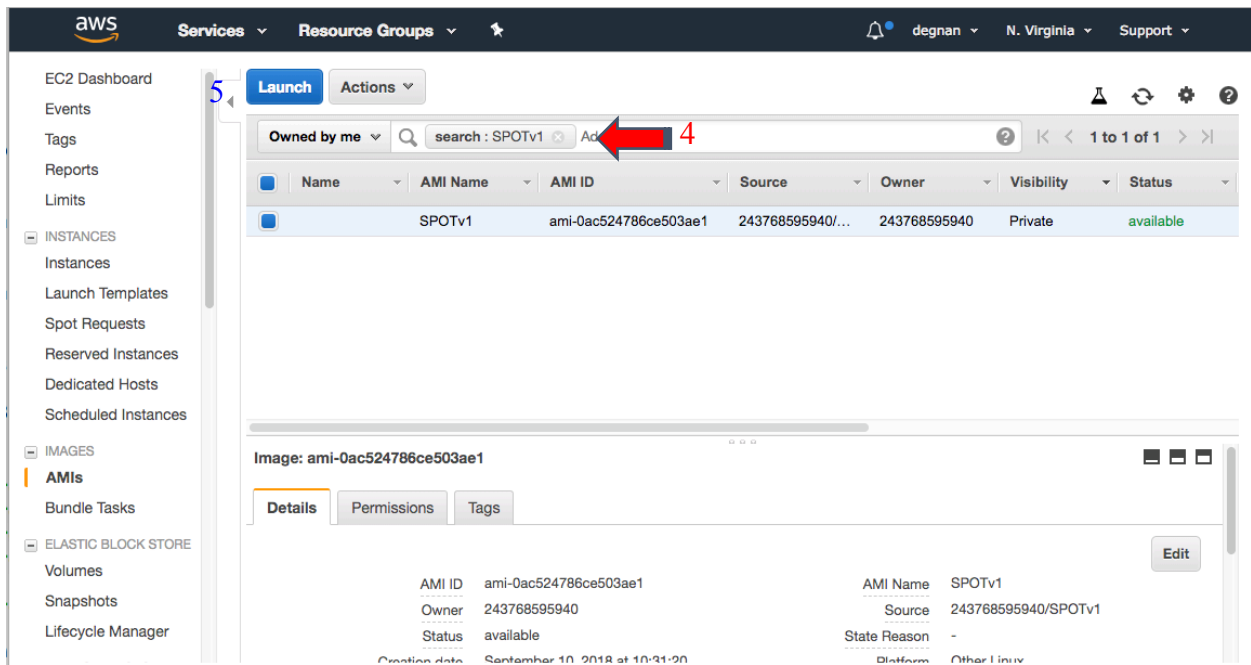
5. Select the blue "**Launch**" button

6. Now you are on AWS "Step 2: Choose and Instance Type" – Select your computer:

> **t2.micro** is the only free option, however it is maxed out at 1GiB of RAM, 1
>   processor and 30GiB of storage. *Very slow*
> **m5.2xlarge** 8 virtual processors, 64 GiB of RAM

7. Select "Next: Configure Instance Details" button on bottom-right

8. On "Step 3: Configure Instance Details" page – *leave defaults as-is*

9. Select the "Next: Add Storage" button on bottom-right, to move to the next step

10. On the "Step 4: Add Storage" adjust local disk size to 30 GiB

11. Select the "Next: Add Tags" button on bottom-right, to move to the next step

12. On the "Step 5: Add Tags" *optionally* hit the "Add Tag" button OR skip to step 14

13. For example Add a *key* = "Name" and *value* = "my-SPOT" or "SPOT-server"

14. Select the "Next: Configure Security Group" button on bottom-right, to move to the next step

15. On "Step 6: Configure Security Group" page – *leave defaults as-is*

Note: For now, we will ignore the *Warning*. In the future consider making your instances harder to access by non-users in your lab/group

16. Select the "Review and Launch" button on bottom-right, to move to the next step

17. You can inspect the settings before hitting the "**Launch**" button. As before ignore warnings.

18. Now it asks you to select or create a key pair.

Select an existing key pair or create a new key pair   ✕

A key pair consists of a **public key** that AWS stores, and a **private key file** that you store. Together, they allow you to connect to your instance securely. For Windows AMIs, the private key file is required to obtain the password used to log into your instance. For Linux AMIs, the private key file allows you to securely SSH into your instance.

Note: The selected key pair will be added to the set of keys authorized for this instance. Learn more about removing existing key pairs from a public AMI.

Proceed without a key pair   ▲▼
☑ I acknowledge that I will not be able to connect to this instance unless I already know the password built into this AMI.

Cancel   **Launch Instances**

19. You will need to download the key and save it to a private location on your computer (e.g., the folder ~/.ssh/) .

aws   Services ⌄   Resource Groups ⌄   ★   🔔   degnan ⌄   N. Virginia ⌄   Support ⌄

Launch Status

✅ **Your instances are now launching**
The following instance launches have been initiated: i-0f99c725900b146ce   View launch log

ℹ️ **Get notified of estimated charges**
Create billing alerts to get an email notification when estimated charges on your AWS bill exceed an amount you define (for example, if you exceed the free usage tier).

How to connect to your instances

Your instances are launching, and it may take a few minutes until they are in the **running** state, when they will be ready for you to use. Usage hours on your new instances will start immediately and continue to accrue until you stop or terminate your instances.

Click **View Instances** to monitor your instances' status. Once your instances are in the **running** state, you can **connect** to them from the Instances screen. Find out how to connect to your instances.

▼ Here are some helpful resources to get you started

• How to connect to your Linux instance   • Amazon EC2: User Guide
• Learn about AWS Free Usage Tier   • Amazon EC2: Discussion Forum

While your instances are launching you can also

Create status check alarms to be notified when these instances fail status checks. (Additional charges may apply)
Create and attach additional EBS volumes (Additional charges may apply)
Manage security groups

20. From here you can navigate using the left-hand side bar to your "**Instances**"
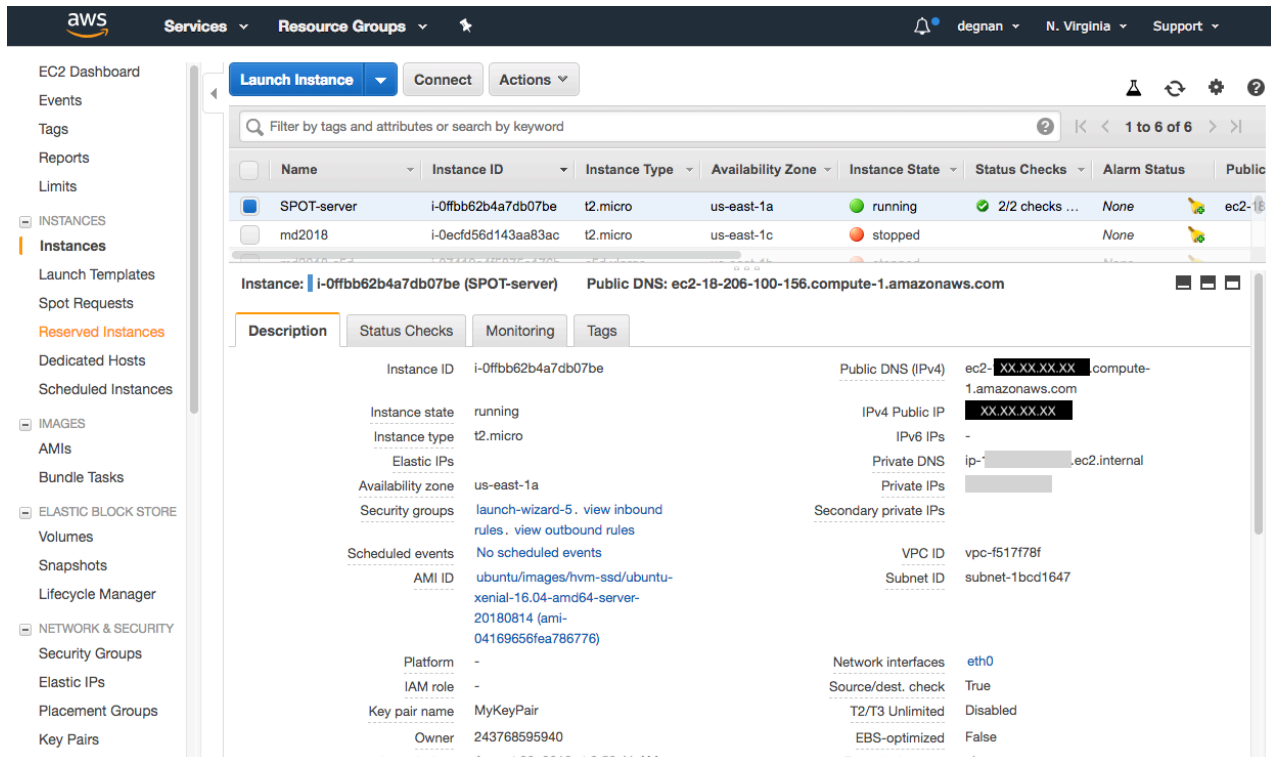
21. Instance state will be "*Initializing"* until the computer has "booted" up.

22. Once the Instance state switches to "*running*" and you select the instance, details of the instance will be shown below.

23. Find and copy the "IPv4 Public IP" address for your instance. You will use this to login to your server.

IPv4 Public IP:	

10. Logging into you AWS instance

To log into the server you will need your:

1. Private ssh key yourid_key.pem
2. username = first name and last initial as one word (e.g., Jane Doe = janed)
3. XX-XX-XX-XX = Your specific IPv4 Public IP from above

Login using Terminal on a **MAC or UNIX.**

$ ssh -Y -i ~/.ssh/yourid_key.pem username@XX-XX-XX-XX

Login from **Windows** using PuTTY

a. Open PuTTY
b. Under Category, click on SSH > Auth
c. Click browse

d. Find your private key (yourid_key.pem) and select it
e. Under Category, click Session and input address of your EC2 instance (XX-XX-XX-XX) in the "host name" box
f. Type "SPOT" in the box under saved sessions and click save.
g. Double-click on the "SPOT" that appears under saved sessions.
h. Log in with your username. Your key should be used automatically.
i. For future logins, just double-click the "SPOT" saved session.

Once entered you will find yourself on the command line interface:

```
delta7:Desktop degnan$ ssh -Y -i ~/.ssh/MyKeyPair.pem ubuntu@18.206.100.156
Warning: No xauth data; using fake authentication data for X11 forwarding.
Welcome to Ubuntu 16.04.5 LTS (GNU/Linux 4.4.0-1065-aws x86_64)

 * Documentation:  https://help.ubuntu.com
 * Management:     https://landscape.canonical.com
 * Support:        https://ubuntu.com/advantage

  Get cloud support with Ubuntu Advantage Cloud Guest:
    http://www.ubuntu.com/business/services/cloud

2 packages can be updated.
0 updates are security updates.

New release '18.04.1 LTS' available.
Run 'do-release-upgrade' to upgrade to it.


Last login: Thu Aug 23 21:50:22 2018 from 138.23.161.215
ubuntu@ip-172-31-38-185:~$
```

11. References

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McClure R, Balasubramanian D, Sun Y, Bobrovskyy M, Sumby P, Genco CA, Vanderpool CK, Tjaden B. 2013. Computational analysis of bacterial RNA-Seq data. Nucleic Acids Research 41:e140.

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