

Primer Design Exercise

Try it out for yourselves

Where we are

- 13:30-14:00 – Primer Design to Amplify Microbial Genomes for Sequencing
- **14:00-14:15 – Primer Design Exercise**
- 14:15-14:45 – Molecular Barcoding to Allow Multiplexed NGS
- 14:45-15:15 – Processing NGS Data – de novo and mapping assembly
- 15:15-15:30 – Break
- 15:30-15:45 – Assembly Exercise
- 15:45-16:15 – Annotation
- 16:15-16:30 – Annotation Exercise
- 16:30-17:00 – Submitting Data to GenBank

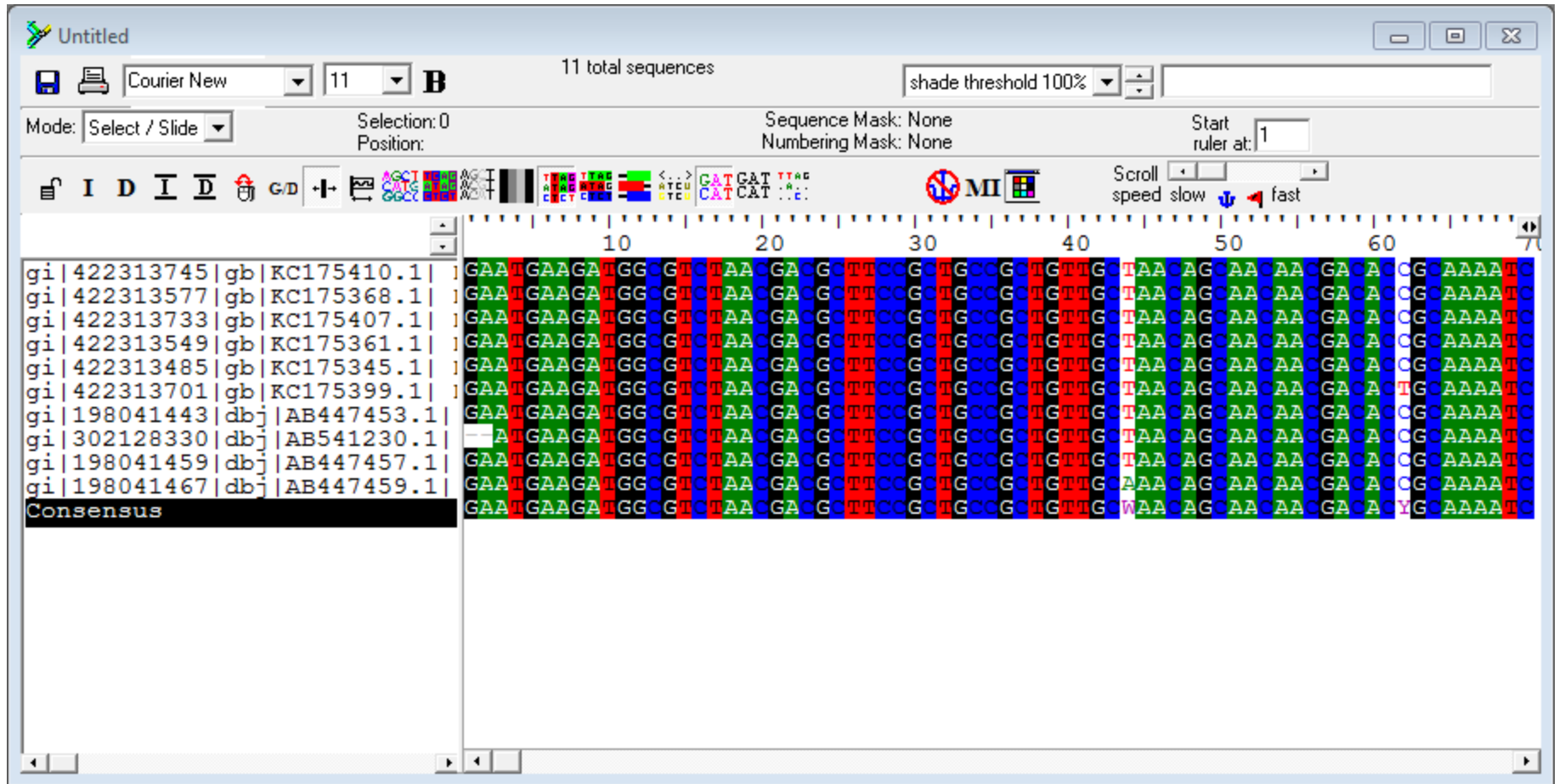
Get some sequences

- For those with Windows PC, if BioEdit isn't installed, download BioEdit, <http://www.mbio.ncsu.edu/bioedit/bioedit.html>
- Get 10 complete norovirus genomes from NCBI (try search of “nucleotide” database, using “norovirus”[organism] AND “complete genome”[title])
- Save them as a fasta file on the PC

Build a consensus sequence

- Start BioEdit and open the fasta file of viruses
- Select Edit->Select All Sequences
- Accessory Application->ClustalW Multiple Alignment
- Run, and wait, and wait
- Alignment->Create Consensus Sequence
- Click on consensus, and then use Edit->Copy sequences to clipboard (fasta)

Something like this?



Paste consensus into primer design website – Primer-BLAST

www.ncbi.nlm.nih.gov/tools/primer-blast/

Primer-BLAST

A tool for finding specific primers

NCBI Primer-BLAST: Finding primers specific to your PCR template (using Primer3 and BLAST). [More...](#) [Tips for finding specific primers](#)

PCR Template

[Reset page](#) [Save search parameters](#) [Retrieve recent results](#)

Enter accession, gi, or FASTA sequence (A refseq record is preferred) [Clear](#)

```
>Consensus
GAATGAAGATGGCGTCTAACGACGCTTCCGCTGCCGCTGTTCGWAACAGCAACAACGACACYGCAAAA
TCTTCAAGTGACAARATGTTTCTARCATGGCTGTCACTTTTAAACGCGCCCTYGGGGCGCGGCTAA
ACAGCCYCCCCGAGGAAAYACCACAAAGACCCCCACGACCACCTACYCCAGAACTGGTCAAAAAGA
TCCCYCCTCCCCRCYAAACGGAGAGGAYGAARYAGTGGTTTCTTATAGTGYYCAAAGATGGCGTTCC
GGTTTRCCTGAGCTTTCYACYGTCAGGCAACCGGAAGAAACCAATACGGCMTTCAGTGTCCCYCCACT
```

Range

Forward primer From To [Clear](#)
Reverse primer

Or, upload FASTA file

[Browse...](#)

Primer Parameters

Use my own forward primer (5'→3' on plus strand) [Clear](#)

Use my own reverse primer (5'→3' on minus strand) [Clear](#)

PCR product size
Min Max

of primers to return

Primer melting temperatures (T_m)
Min Opt Max Max T_m difference [Clear](#)

Don't amplify human DNA

Note: Parameter values that differ from the c

Primer Pair Specificity Checking Parameters

Specificity check	<input checked="" type="checkbox"/> Enable search for primer pairs specific to the intended PCR template
Database	Genome (chromosomes from all organisms)
Organism	Homo sapiens Enter an organism name, taxonomy id or select from the suggestion list as you type. Add more organisms
Exclusion (optional)	<input type="checkbox"/> Exclude predicted Refseq transcripts (accession with XM, XR prefix) <input type="checkbox"/> Exclude uncultured/er
Entrez query (optional)	<input type="text"/>
Primer specificity stringency	Primer must have at least <input type="text" value="2"/> total mismatches to unintended targets, including at least <input type="text" value="2"/> mismatches within the last <input type="text" value="5"/> bps at the 3' end. Ignore targets that have <input type="text" value="6"/> or more mismatches to the primer.
Misprimed product size deviation	<input type="text" value="4000"/>
Splice variant handling	<input type="checkbox"/> Allow primer to amplify mRNA splice variants (requires refseq mRNA sequence as PCR temp

Get Primers Show results in a new window Use new graphic view

[Advanced parameters](#) Note: Parameter values that differ from the c

Overview of amplicons

Primer-BLAST Primer-Blast results

▶ [NCBI/Primer-BLAST : results: Job id=JSID_01_45590_130.14.18.128_9003](#) [more...](#)

Input PCR template Consensus
Range 1 - 7511
Specificity of primers Primer pairs are specific to input template as no other targets were found in selected database: Reference chromosomes (Organism limited to Homo sapiens)
Other reports ▶ [Search Summary](#)

▼ **Graphical view of primer pairs**

1: 1..7.5K (7.5Kbp) | Find on Sequence: | Tools | Configure | ?

Template 500 | 1 K | 1,500 | 2 K | 2,500 | 3 K | 3,500 | 4 K | 4,500 | 5 K | 5,500 | 6 K | 6,500 | 7 K | 7,511

Primer pairs for submitted sequence

Primer 2 | Primer 5 | Primer 1 | Primer 3 | Primer 4 | Primer 6 | Primer 7

PCR 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	GTGCGCCAGAATCAGGTACT	Plus	20	2396	2415	60.11	55.00	5.00	3.00
Reverse primer	ATTAGGCCTCCGAATGCTCG	Minus	20	4177	4158	59.97	55.00	6.00	2.00
Product length	1782								

Primer pair 2

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCCTGTATGTTGAGCGAGGT	Plus	20	424	443	60.11	55.00	3.00	0.00
Reverse primer	GTCCCATAGGACGACCCTCT	Minus	20	1637	1618	60.11	60.00	8.00	2.00
Product length	1214								

Primer pair 3

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GCATTCGGAGGCCTAATGGA	Plus	20	4161	4180	59.89	55.00	7.00	3.00
Reverse primer	GTAGCTTTATGGCCACGGGT	Minus	20	6131	6112	60.11	55.00	6.00	2.00
Product length	1971								

Primer pair 4

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CCCTAGAAAACGCTCCAGGTG	Plus	20	5283	5302	60.11	60.00	4.00	3.00
Reverse primer	ATCCATGTTGGGATACCCGC	Minus	20	6426	6407	59.89	55.00	4.00	2.00
Product length	1144								