Primer Design Exercise

Try it out for yourselves

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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

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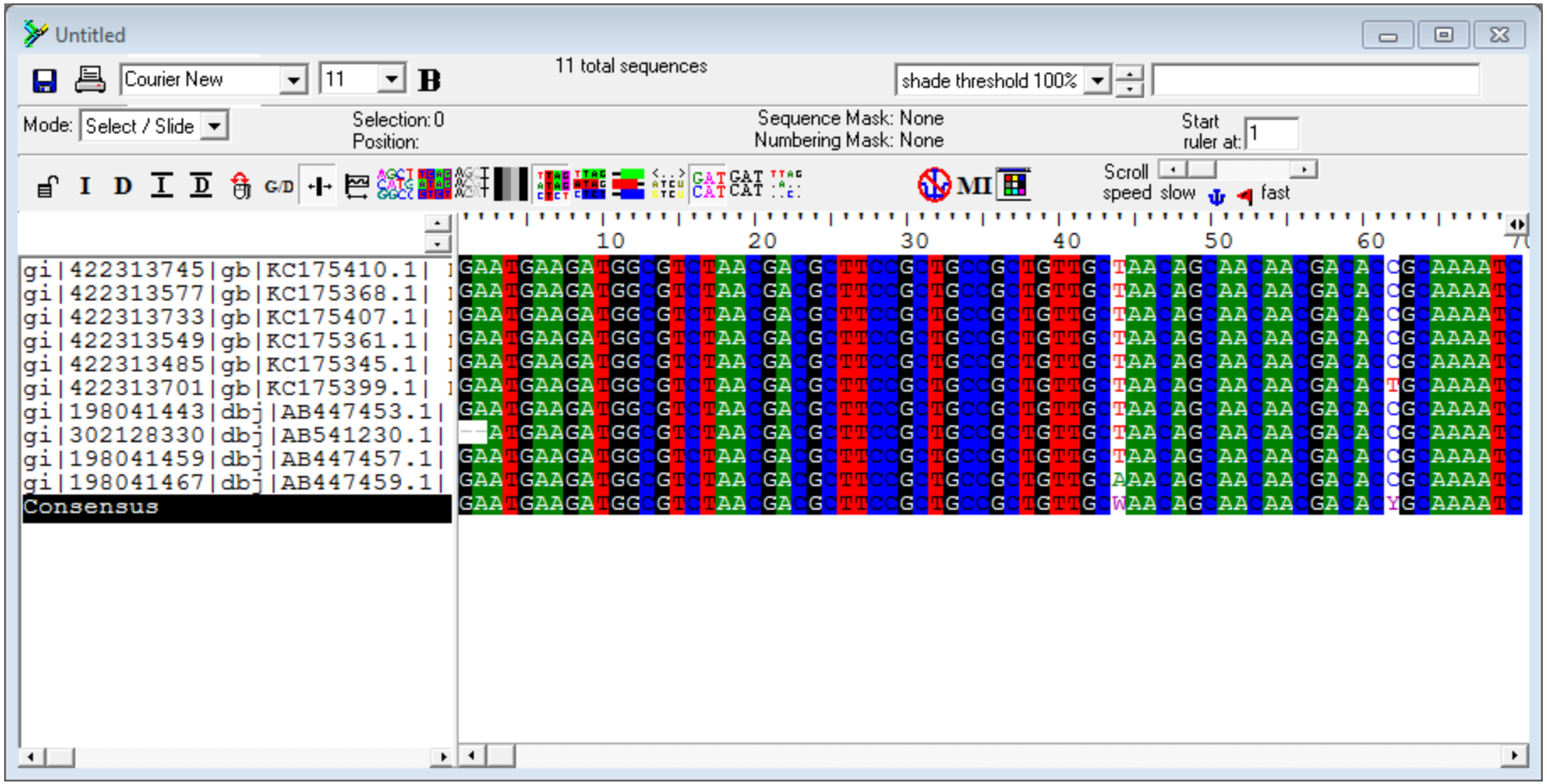
Get some sequences

- 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?



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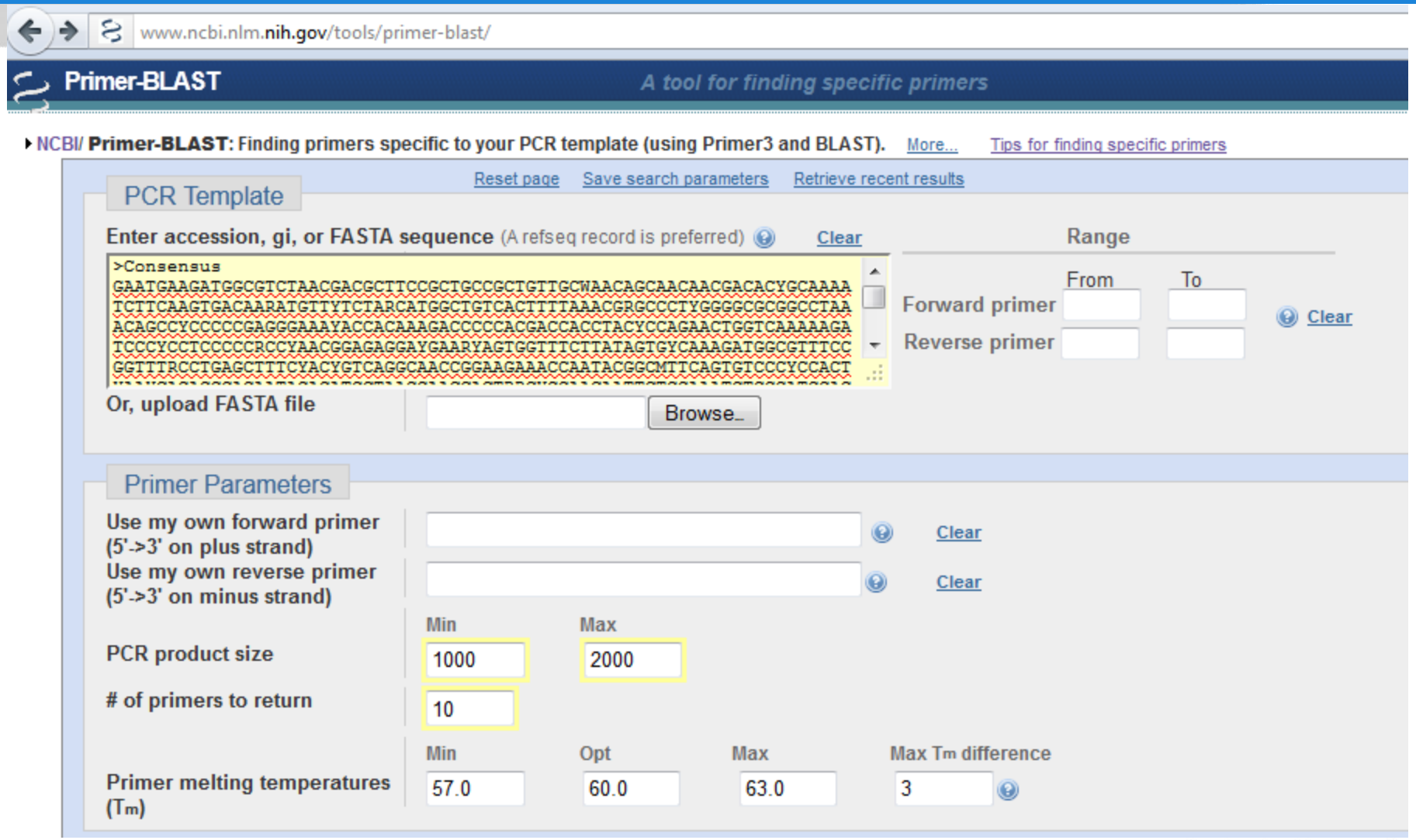
Paste consensus into primer design website – Primer-BLAST



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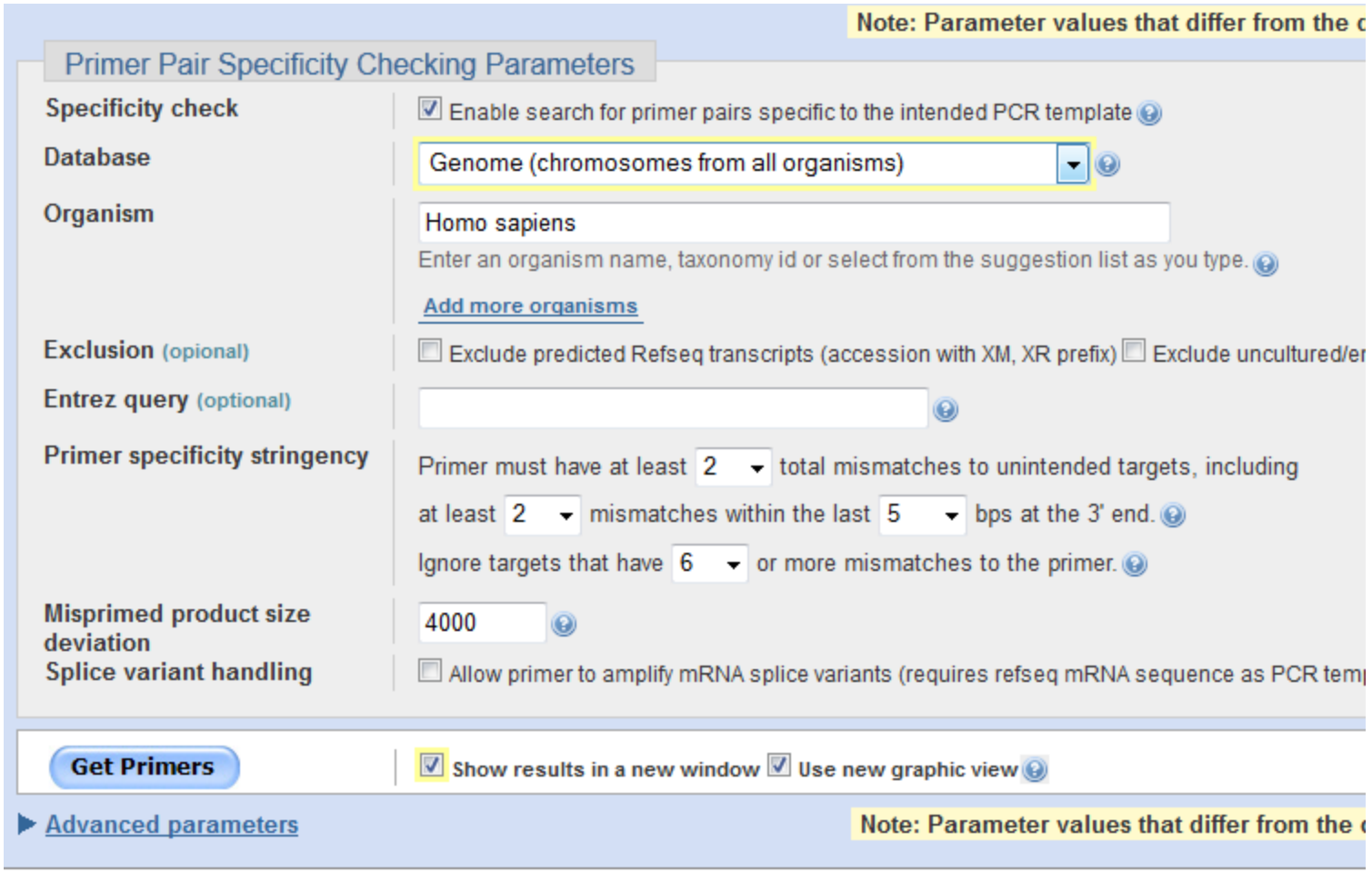
Don’t amplify human DNA



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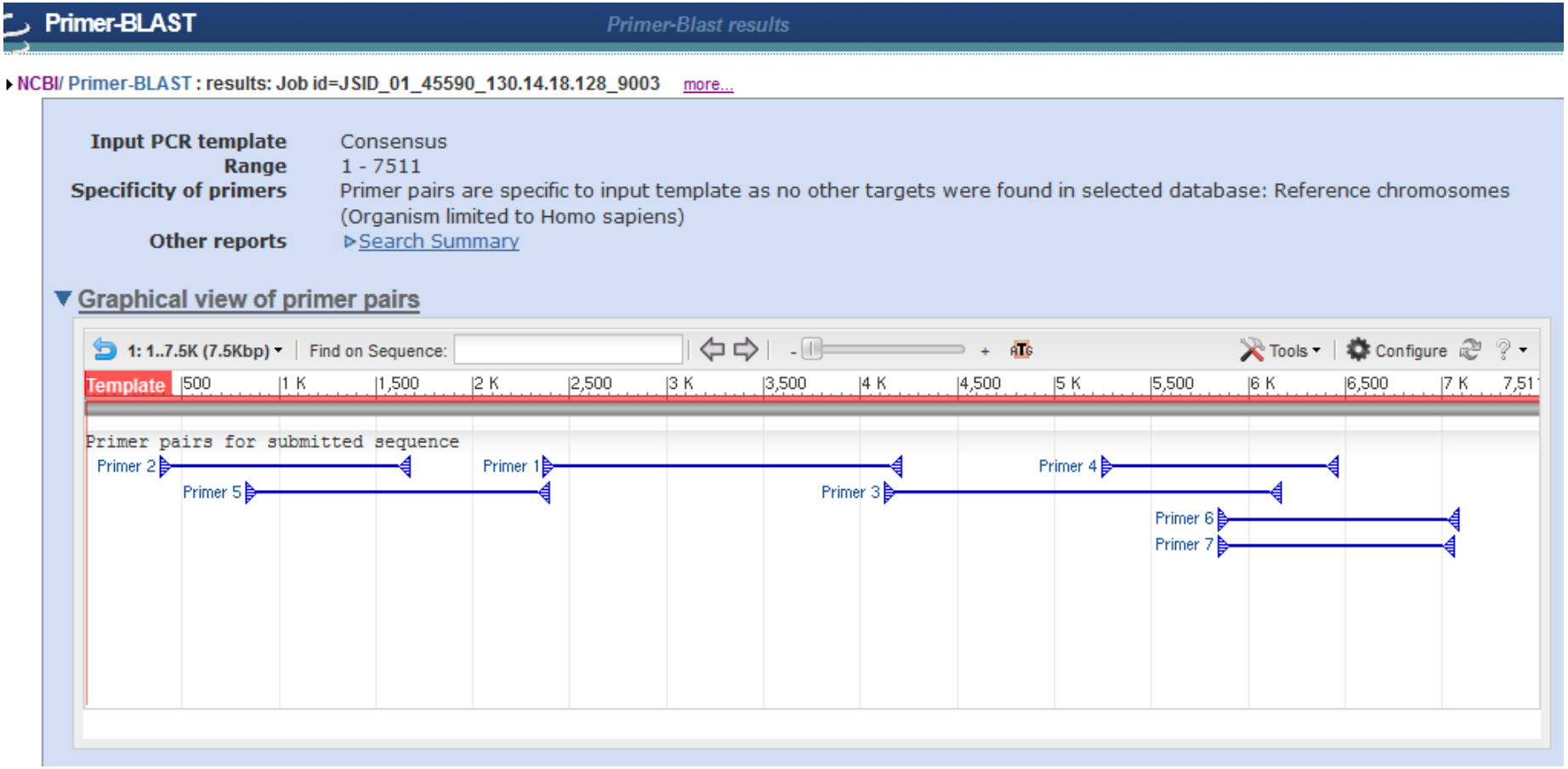
Overview of amplicons



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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	CCCTAGAAACGCTCCAGGTG	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								