In [72]:
```
# Packages
# from matplotlib import (pyplot as plt, lines)
# import seaborn as sns
import numpy as np
import os
import pandas as pd

```

In [63]:
```
# Globals
BASE_DIR = "/home/josh/PycharmProjects/eces-450/tutorial/data/algae-


# Given files
print("Assembly Dir:")
print('\n'.join(file for file in os.listdir(os.path.join(BASE_DIR,
print("\nRead Dir:")
print('\n'.join(file for file in os.listdir(os.path.join(BASE_DIR,
```

```
Assembly Dir:
simple.contig.fa

Read Dir:
CSJP002C_R1.fastq
CSJP002A_R2.fastq
CSJP002B_R2.fastq
CSJP002A_R1.fastq
CSJP002B_R1.fastq
CSJP002C_R2.fastq
```

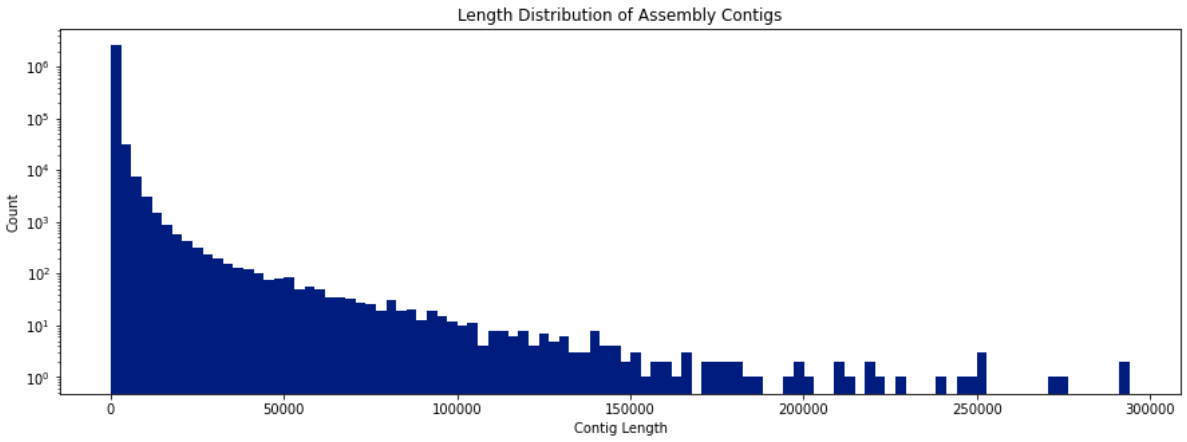
In [73]:
```
# Read Assembly File
fn = os.path.join(BASE_DIR, 'assembly', 'simple.contig.fa')
with open(fn, "r") as fh:
    lines = fh.readlines()

# Create list: contig_lengths
contig_lengths = []
contigs = []
i = 0
for line in lines:
    if line[0] == '>':
        contigs.append(line[1:])  # grab record contig id
    elif i < 1000:
        contig_lengths.append(len(line))  # grab record sequence le
    if i<2:
        print(line[0:250], end='')  # print the first record
        i+=1

# Plot the sequence lengths
plt.style.use('seaborn-dark-palette')
fig = plt.figure(figsize=(15, 5))
plt.hist(contig_lengths, bins=100, log=True)
plt.title("Length Distribution of Assembly Contigs")
plt.xlabel("Contig Length")
plt.ylabel("Count")
```

```
>contig-65_0
TGGCAGGCGATGTCGGAGCGCAAACTCCGCCGCCAGCGCGTCATCTTCTACATCTCACGTGGGTTGGAGA
ACGCCCCGACAAAGTTCCAGACCACCCCCGCCCGTCGCGACTACTACAACACGCTGCTCGACCAGCTTCA
GACCGAGTTCGAGCACGTTCATCGGCTGCTGCTGGAAATCTTCGGTTCAGGCGGCGCGCGCGTGTTGCCG
ATGACGGATGCCGACCACTTCCGGCACTACAAACGGTTTC
```

Out[73]: Text(0, 0.5, 'Count')




```
bwa index

Usage:   bwa index [options]

Options: -a STR    BWT construction algorithm: bwtsw, is or rb2
[auto]
         -p STR    prefix of the index [same as fasta name]
```

```
          -b INT    block size for the bwtsw algorithm (effectiv
e with -a bwtsw) [10000000]
          -6        index files named as .64.* instead of .*
```

Source: bwa man pages

A bit of googling and I found:
.amb is text file, to record appearance of N (or other non-ATG
C) in the ref fasta.
.ann is text file, to record ref sequences, name, length, etc.
.bwt is binary, the Burrows-Wheeler transformed sequence.
.pac is binary, packaged sequence (four base pairs encode one b
yte).
.sa is binary, suffix array index.

Source: [http://seqanswers.com/forums/showthread.php?t=25553 (ht
tp://seqanswers.com/forums/showthread.php?t=25553)](http://seqanswers.com/forums/showthread.php?t=25553)

In [74]:
```
# After running bwa index on the assembly file, several new files ar
print("Assembly Dir:")
print('\n'.join(file for file in os.listdir(os.path.join(BASE_DIR,
```

```
Assembly Dir:
simple.contig.fa.amb
simple.contig.fa.ann
simple.contig.fa.pac
simple.contig.fa.sa
simple.contig.fa.bwt
simple.contig.fa
```

```
bwa mem

The  BWA-MEM  algorithm performs local alignment. It may produce
multiple primary alignments for different part of a query
sequence. This is a crucial feature for long sequences. However,
some tools such as Picard's markDuplicates does not work with
split alignments. One may consider to use option -M to flag
shorter split hits as secondary.

```

In [ ]:
```
#!/bin/bash
#### Create a map from the reads to the newly indexed assembly-file
#### This took 10.5 hours to complete on proteus

BASE_DIR="./"
samples=(2A 2B 2C)

for sample in ${samples[@]}
    do
        echo CSJP00${sample}_R1.fastq
        bwa mem ${BASE_DIR}assembly/simple.contig.fa ${BASE_DIR}read
done
```

In [4]:
```

print("\nMapped:")
print('\n'.join(file for file in os.listdir(os.path.join(BASE_DIR,
```

```
Mapped:
2C.bam
2A.bam
2B.bam


Usage: samtools sort [options...] [in.bam]
Description:

    Sort  alignments  by leftmost coordinates, or by read name
when -n is used.  An appropriate @HD-SO sort order header tag w
ill be added or an existing one updated if necessary.

    The sorted output is written to standard output by default,
or to the specified file (out.bam) when -o is used.  This  comm
and  will  also  create  temporary files tmpprefix.%d.bam as ne
eded when the entire alignment data cannot fit into memory (as
controlled via the -m option).

Options:
  -l INT     Set compression level, from 0 (uncompressed) to 9
(best)
  -m INT     Set maximum memory per thread; suffix K/M/G recogn
ized [768M]
  -n         Sort by read name
  -t TAG     Sort by value of TAG. Uses position as secondary i
ndex (or read name if -n is set)
  -o FILE    Write final output to FILE rather than standard ou
tput
  -T PREFIX  Write temporary files to PREFIX.nnnn.bam
  --no-PG    do not add a PG line
      --input-fmt-option OPT[=VAL]
```

```
                Specify a single input file format option in the
form
                of OPTION or OPTION=VALUE
  -O, --output-fmt FORMAT[,OPT[=VAL]]...
                Specify output format (SAM, BAM, CRAM)
      --output-fmt-option OPT[=VAL]
                Specify a single output file format option in th
e form
                of OPTION or OPTION=VALUE
      --reference FILE
                Reference sequence FASTA FILE [null]
  -@, --threads INT
                Number of additional threads to use [0]
      --verbosity INT
                Set level of verbosity


-------------------------------------------------------------------
---------------------------------------
samtools sort -o ./sorted/2A.sorted.bam ./mapped/2A.bam
samtools sort -o ./sorted/2B.sorted.bam ./mapped/2B.bam
samtools sort -o ./sorted/2C.sorted.bam ./mapped/2C.bam
-------------------------------------------------------------------
---------------------------------------
```

In [48]:

```
# Sort the bam files for rapid processing, can also be run on proteu

print("\nSorted:")
print('\n'.join(file for file in os.listdir(os.path.join(BASE_DIR,'s
```

```
Sorted:
2B.sorted.bam
2C.sorted.bam
2A.sorted.bam
```

```
[js3973@proteusa01 Tutorial6_data]$ ls -al mapped
total 23896668
drwxrwsr-x 2 js3973 rosenclassGrp       4096 May 18 10:35 .
drwxrwsr-x 5 sk3389 rosenclassGrp       4096 May 18 10:32 ..
-rw-r--r-- 1 js3973 rosenclassGrp 5866752241 May 18 03:34 2A.bam
-rw-r--r-- 1 js3973 rosenclassGrp 6771359549 May 18 07:16 2B.bam
-rw-r--r-- 1 js3973 rosenclassGrp 5346570250 May 18 10:13 2C.bam
-rw-r--r-- 1 js3973 rosenclassGrp  581851433 May 18 10:37
2C.sorted.bam
-rw-r--r-- 1 js3973 rosenclassGrp  361791128 May 18 10:26
2C.sorted.bam.tmp.0000.bam
-rw-r--r-- 1 js3973 rosenclassGrp  361769335 May 18 10:27
2C.sorted.bam.tmp.0001.bam
-rw-r--r-- 1 js3973 rosenclassGrp  363248908 May 18 10:27
2C.sorted.bam.tmp.0002.bam
```

```
-rw-r--r-- 1 js3973 rosenclassGrp  364979550 May 18 10:28 2C.sorted.bam.tmp.0003.bam
-rw-r--r-- 1 js3973 rosenclassGrp  362689520 May 18 10:28 2C.sorted.bam.tmp.0004.bam
-rw-r--r-- 1 js3973 rosenclassGrp  360639096 May 18 10:29 2C.sorted.bam.tmp.0005.bam
-rw-r--r-- 1 js3973 rosenclassGrp  363002478 May 18 10:30 2C.sorted.bam.tmp.0006.bam
-rw-r--r-- 1 js3973 rosenclassGrp  363244902 May 18 10:30 2C.sorted.bam.tmp.0007.bam
-rw-r--r-- 1 js3973 rosenclassGrp  355685132 May 18 10:31 2C.sorted.bam.tmp.0008.bam
-rw-r--r-- 1 js3973 rosenclassGrp  357044755 May 18 10:32 2C.sorted.bam.tmp.0009.bam
-rw-r--r-- 1 js3973 rosenclassGrp  359320772 May 18 10:32 2C.sorted.bam.tmp.0010.bam
-rw-r--r-- 1 js3973 rosenclassGrp  358477722 May 18 10:33 2C.sorted.bam.tmp.0011.bam
-rw-r--r-- 1 js3973 rosenclassGrp  355302152 May 18 10:33 2C.sorted.bam.tmp.0012.bam
-rw-r--r-- 1 js3973 rosenclassGrp  358575023 May 18 10:34 2C.sorted.bam.tmp.0013.bam
-rw-r--r-- 1 js3973 rosenclassGrp  360891810 May 18 10:35 2C.sorted.bam.tmp.0014.bam

```

Create Depth Matrix:

First generate depth matrix from sorted bam files using jgi_summarize_bam_contig_depths (included with metabat2)

In [86]:

```
# Read Depth Matrix
fn = os.path.join(BASE_DIR, 'depth', 'depth_matrix.tab')
with open(fn, 'r') as fh:
    df = pd.read_csv(fh, delimiter='\t')
df.head(50)  # Show
```

17	contig-65_17	200413	30.7011	0.036851	0.048552	10.963000
18	contig-65_18	199480	29.6988	0.029925	0.036689	10.645100
19	contig-65_19	198319	23.7893	0.028052	0.044290	13.478700
20	contig-65_20	195195	16.9654	0.006224	0.008799	15.488800
21	contig-65_21	185758	22.6358	0.006659	0.010197	19.639300
22	contig-65_22	182600	23.3662	0.013412	0.020377	13.125900
23	contig-65_23	182193	14.7668	0.017331	0.025937	14.648100

Input to metabat2:
sorted bam files
depth matrix

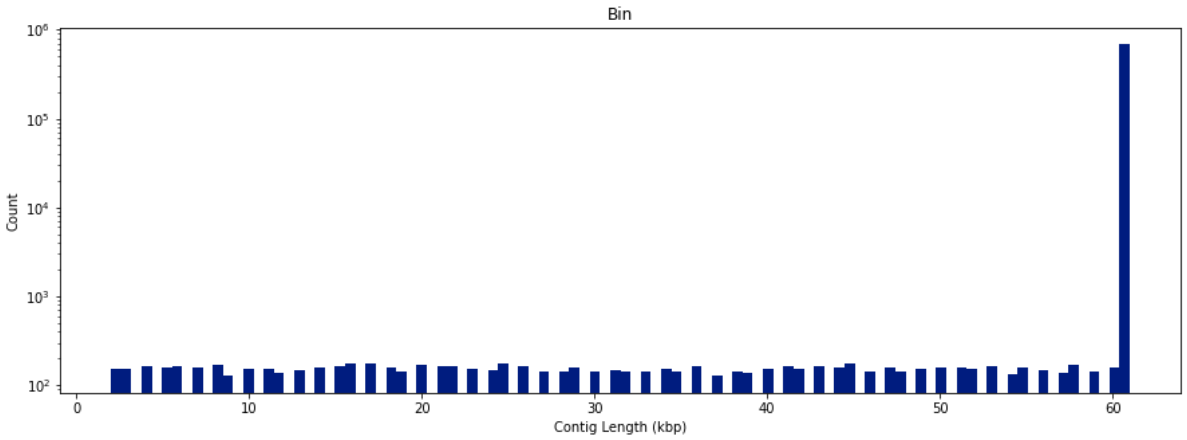
Output:
130 bins

In [85]:
```
# Visualize Contig lengths in largest bin
fn = os.path.join(BASE_DIR, 'bins',"bin.10.fa")
# fn = os.path.join(BASE_DIR, 'bins',"bin.22.fa")
with open(fn, "r") as fh:
    lines = fh.readlines()

# Create list: contig_lengths
contig_lengths = []
for line in lines:
    if not line[0] == '>':
        contig_lengths.append(len(line))

# Plot the sequence lengths
plt.style.use('seaborn-dark-palette')
fig = plt.figure(figsize=(15, 5))
plt.hist(contig_lengths, bins=100, log=True)
plt.title("Bin")
plt.xlabel("Contig Length (kbp)")
plt.ylabel("Count")
```

Out[85]: Text(0, 0.5, 'Count')



In [80]:
```
# Binned data
fn = os.path.join(BASE_DIR, 'bins',"bin.10.fa")
with open(fn, "r") as fh:
    lines = fh.readlines()

# Create list: contigs
contigs = []
for line in lines:
    if line[0] == '>':
        contigs.append(line[1:].strip())

```