Discussion Section 6

HW 5 tips and questions?

Motif-finding algorithms

 If time: using valgrind to find memory leaks/out of bounds bugs

HW 5 output

- What you report:
 - Nucleotide histogram
 - Background frequency
 - Count matrix (-10 to 10 nucleotides)
 - Frequency matrix (-10 to 10 nucleotides)
 - Weight matrix (-10 to 10 nucleotides)
 - Maximum score
 - Score histogram for CDS
 - Score histogram for all positions
 - List of non-CDS positions with score >= 10

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Precision matters! (use doubles in C++)

Watch out for multi-line joins

Weight matrix definition

 log₂(frequency of base in start site/background frequency of base)

 use -99 if frequency is zero (alternative to pseudocounts)

Score histogram for CDS and all sites

- Bins labeled with integer values
 - Round scores down to determine the bin

Print all bins with at least one count

Put all scores less than -50 into one bin

```
Score Histogram All:
-5 101880
-4 76413
-3 54704
-2 38081
-1 27202
0 21440
1 18671
2 18825
3 19072
4 18675
5 17308
6 14429
7 10595
8 6915
9 3886
10 1850
11 699
12 225
13 46
14 4
lt-50 6132782
```

HW 5 questions?

Sequence 1 G T A C T A T C C A G C T A T C G G T Sequence 2 T A G G G C A A C T T T T C A G T C A Sequence 3 A C G T C A T A T G G A T C T C G G A Sequence 4 T T C A A A G C A A C C C A A A T A G Sequence 5 C T T G G A A C T G G T T A T C A G T Sequence 6 A C G A T G C C A T T A C C A T A A T Sequence 7 A A A G A T C A G T A T G G C A C T A

Basic idea:

- Given a set of *t* sequences of length *n*
 - Find a set of k-mers with maximum consensus score
 - One *k*-mer from each sequence

Sequence 1 G T A C T A T C C A G C T A T C G G T Sequence 2 T A G G G C A A C T T T T C A G T C A Sequence 3 A C G T C A T A T G G A T C T C G G A Sequence 4 T T C A A A G C A A C C C A A A T A G Sequence 5 C T T G G A A C T G G T T A T C A G T Sequence 6 A C G A T G C C A T T A C C A T A A T Sequence 7 A A A G A T C A G T A T G G C A C T A

```
Sequence 1 A T C C A G C T
```

```
Sequence 2 G G G C A A C T
```

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Sequence 3 A T G G A T C T

Sequence 4 A A G C A A C C

Sequence 5 T T G G A A C T

Sequence 6 A T G C C A T T

Sequence 7 A T G G C A C T

A 5 1 0 0 5 5 0 0

Sequence 1 A T C C A G C T

Sequence 2 G G C A A C T

A 5 1 0 0 5 5 0 0 T 1 5 0 0 0 1 1 6

Sequence 3 A T G G A T C T

Sequence 4 A A G C A A C C

Sequence 5 T T G G A A C T

Sequence 6 A T G C C A T T

Sequence 7 A T G G C A C T

Sequence 1 A T C C A G C T

Sequence 2 G G G C A A C T

Sequence 3 A T G G A T C T

Sequence 4 A A G C A A C C

Sequence 5 T T G G A A C T

Sequence 6 A T G C C A T T

Sequence 7 A T G G C A C T

A 5 1 0 0 5 5 0 0 T 1 5 0 0 0 1 1 6 G 1 1 6 3 0 1 0 0

Sequence 1 A T C C A G C T

Sequence 2 G G G C A A C T

Sequence 3 A T G G A T C T

Sequence 4 A A G C A A C C

Sequence 5 T T G G A A C T

Sequence 6 A T G C C A T T

Sequence 7 A T G G C A C T

A 5 1 0 0 5 5 0 0 T 1 5 0 0 0 1 1 6 G 1 1 6 3 0 1 0 0 C 0 0 1 4 2 0 6 1

```
Sequence 1 A T C C A G C T
```

Consensus:

```
ATGCAACT
```

```
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```
A 5 1 0 0 5 5 0 0
T 1 5 0 0 0 1 1 6
G 1 1 6 3 0 1 0 0
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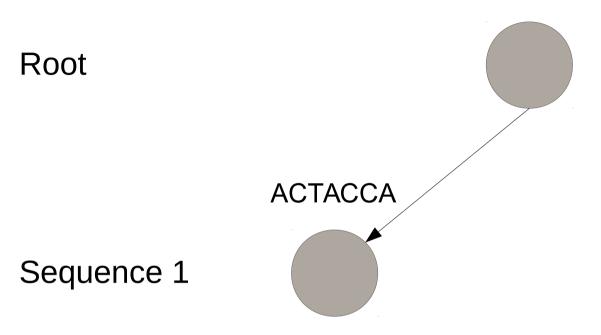
Root

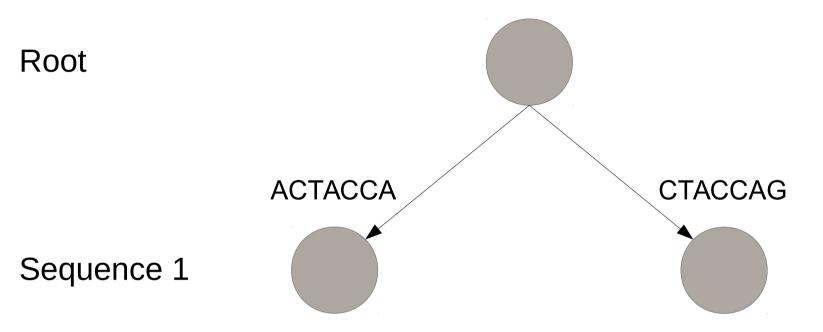


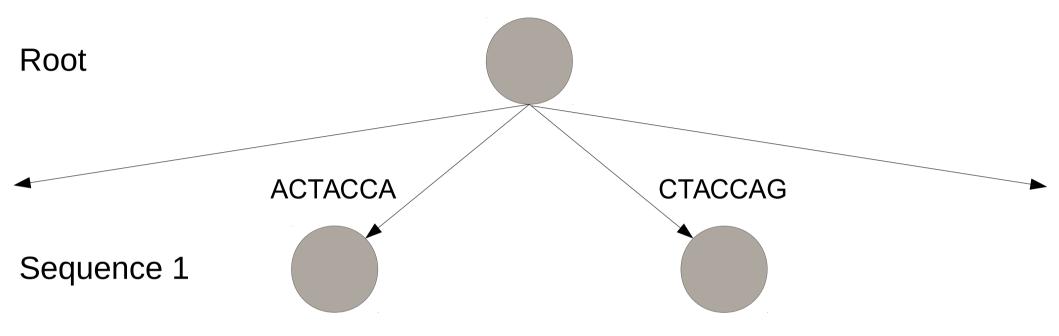
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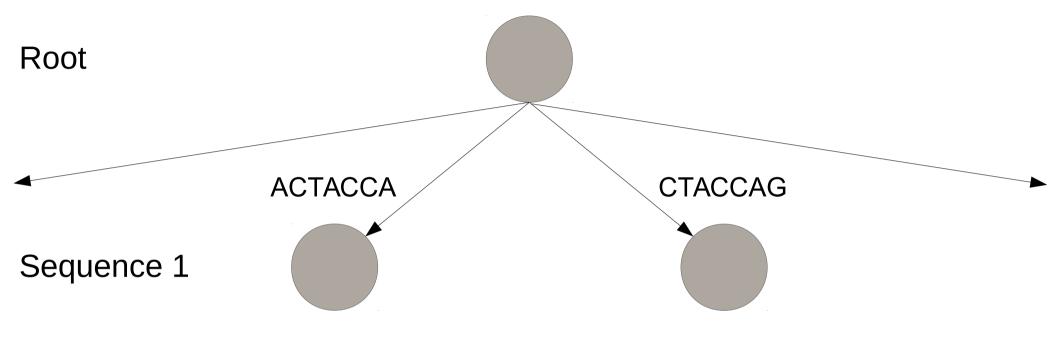


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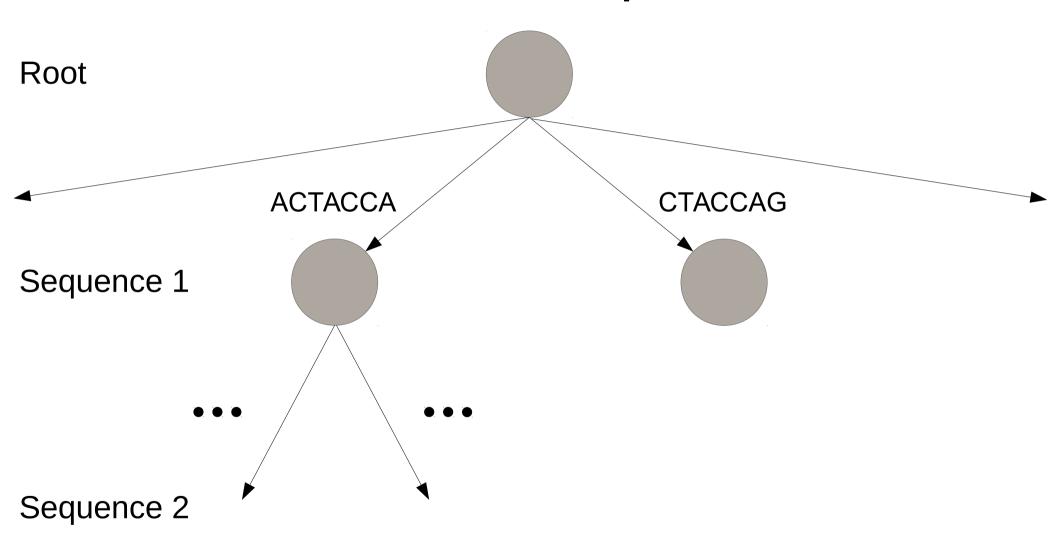


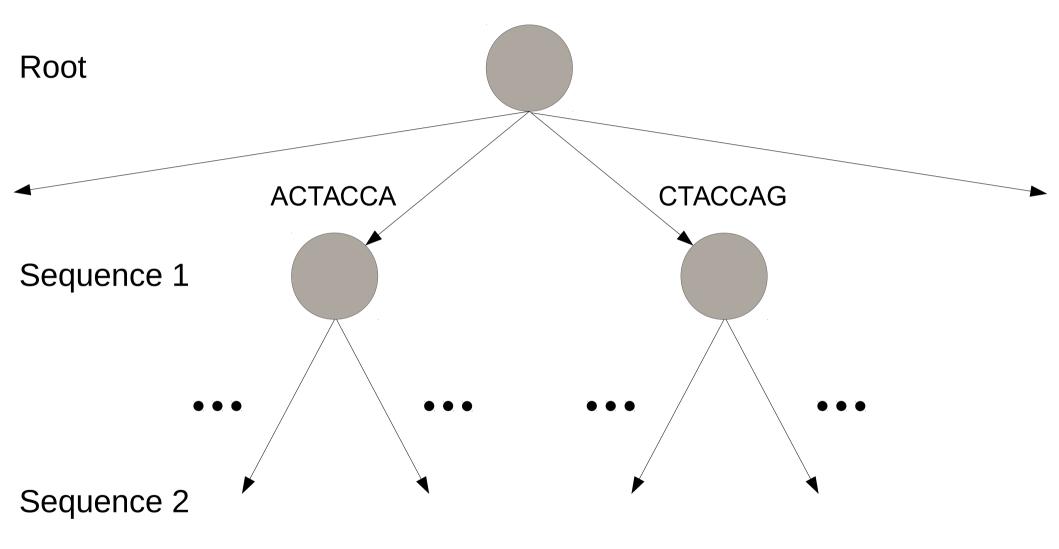


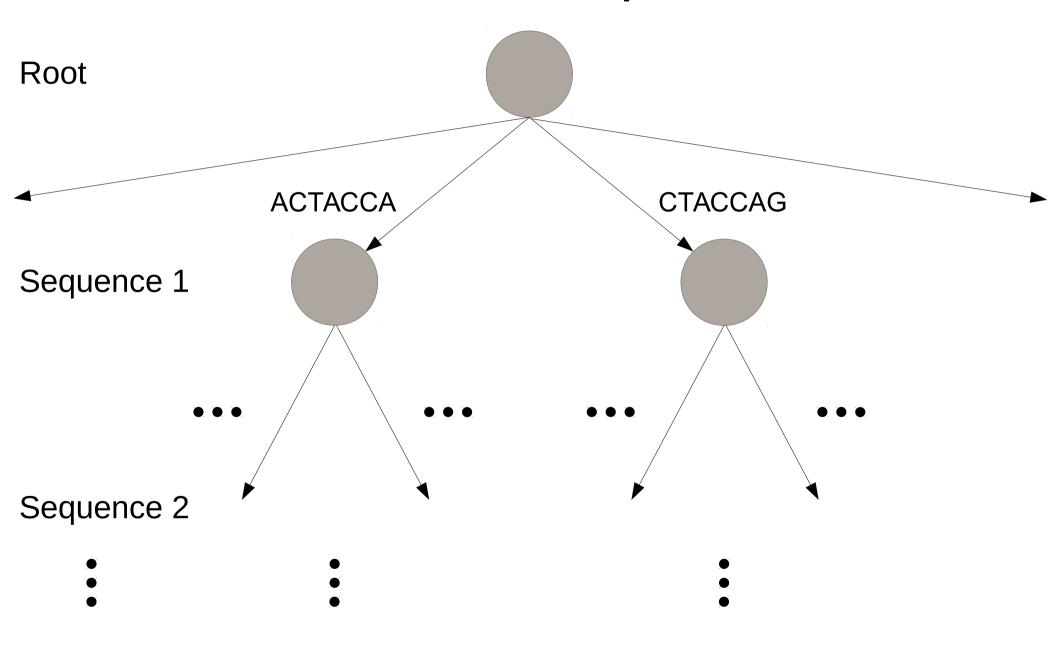




Sequence 2







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Leaf vertices are complete motif sets

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What could we do better?

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 - If the bound is too low, don't use this branch

- More specifically
 - Given the partial consensus for i sequences chosen
 - The rest of the sequences can improve the score by at most (t - i) x k
 - When does this happen? The rest match the partial consensus
 - So if current score + $(t i) \times k$ is less than the best score so far, don't bother checking

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- How many possibilities for V?
 - -4^k

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Can we use branch-and-bound again?

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- What can we do while checking scores for a candidate median string?
 - If we've found the smallest distance match for a sequence, what does that tell us about the best total score for the candidate?
 - In general, how does the score change as we look at more sequences?
 - As soon as the current score for the candidate is greater than the best (lowest) score seen, move on to the next candidate

Branch-and-bound methods can help in practice, but don't actually improve the worst-case time

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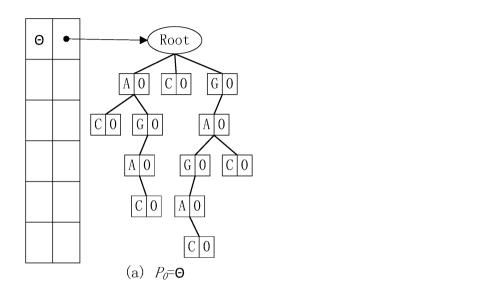
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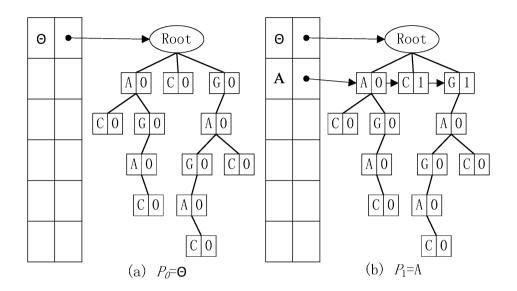
CONSENSUS

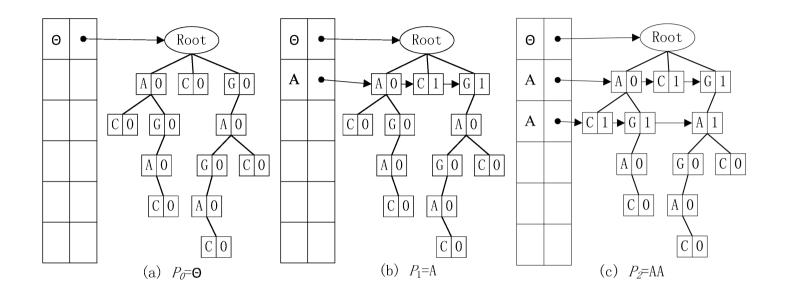
- Uses a greedy search as described except it stores m k-mers at each step
 - Less likely to miss better ones

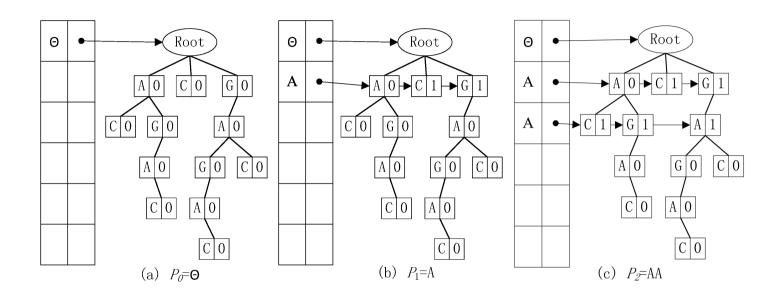
The WEEDER algorithm (2014)

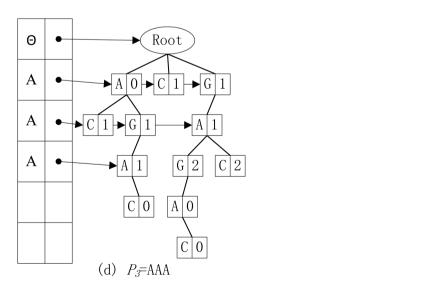
- Specifically looking for transcription factor (TF) binding sites
- Uses a range of motif sizes similar to observed
 TF binding sites
- Allows a specified number of differences (mutations) d
- Uses a 'mismatched' suffix tree to search sequences for candidate motif occurrences

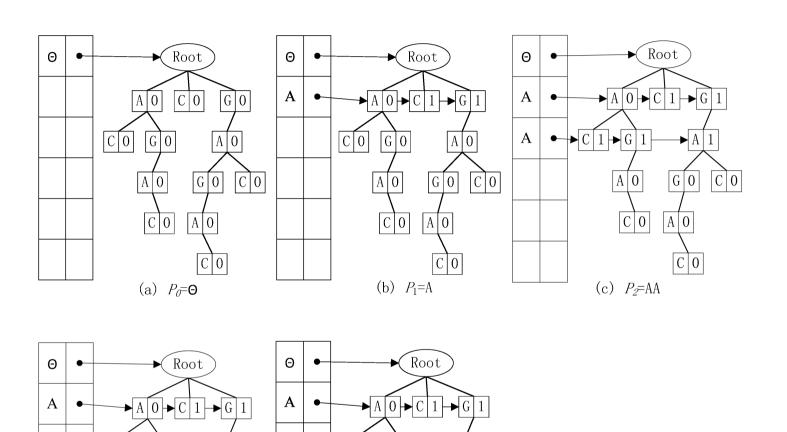












G 2

(e) $P_{\mathcal{A}}$ =AAAC

A

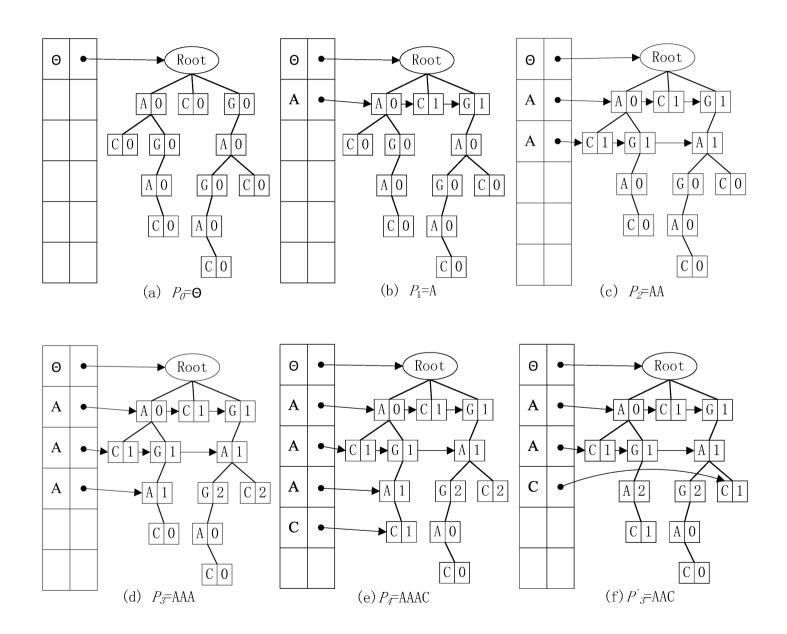
Α

G 2

A 0

(d) $P_3 = AAA$

 $C \mid 0 \mid$



Using valgrind to check for memory bugs

 Valgrind is a command line tool for profiling and checking program memory use

 If you compile with g++, then you just add the '-g' flag when compiling

- You can then run your program with valgrind and it gives detailed memory usage info
 - Sometimes a bit too detailed

```
1  #include <fstream>
2  #include <iostream>
3  using namespace std;
4
5  int main(){
6    int num_counts = 4;
7    int counts[num_counts] = {1, 2, 3, 4};
8    for (int i = 0; i <= num_counts; ++i){
9       cout<<counts[i]<<endl;
10    }
11 }</pre>
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```
[2017-02-09 11:18:14 alex@Rincewind valgrind_examples]$ g++ test_out_of_bounds.c
pp -o test_out_of_bounds.o
[2017-02-09 11:18:30 alex@Rincewind valgrind_examples]$ ./test_out_of_bounds.o
1
2
3
4
```

```
[2017-02-09 11:18:34 alex@Rincewind valgrind_examples]$ g++ -g test_out_of_bound
s.cpp -o memcheck test out of bounds.o
[2017-02-09 11:19:16 alex@Rincewind valgrind_examples]$ valgrind ./memcheck test
out of bounds.o
==14777== Memcheck, a memory error detector
==14777== Copyright (C) 2002-2015, and GNU GPL'd, by Julian Seward et al.
==14777== Using Valgrind-3.12.0.SVN and LibVEX; rerun with -h for copyright info
==14777== Command: ./memcheck_test_out_of_bounds.o
==14777==
==14777== Conditional jump or move depends on uninitialised value(s)
             at 0x4F3F4BA: std::ostreambuf iterator<char, std::char traits<char>
> std::num_put<char, std::ostreambuf_iterator<char, std::char_traits<char> > >:
:_M_insert_int<long>(std::ostreambuf_iterator<char, std::char_traits<char> >, st
d::ios base&, char, long) const (in /usr/lib/x86 64-linux-gnu/libstdc++.so.6.0.2
            by 0x4F3F6EC: std::num_put<char, std::ostreambuf_iterator<char, std
::char_traits<char> > >::do_put(std::ostreambuf iterator<char, std::char traits<
char> >, std::ios_base&, char, long) const (in /usr/lib/x86_64-linux-gnu/libstdc
++.so.6.0.22)
             by 0x4F4BF19: std::ostream& std::ostream:: M insert<long>(long) (in
/usr/lib/x86 64-linux-gnu/libstdc++.so.6.0.22)
            by 0x108A50: main (test out of bounds.cpp:9)
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:_M_insert_int<long>(std::ostreambuf_iterator<char, std::char_traits<char> >, st
d::ios base&, char, long) const (in /usr/lib/x86 64-linux-gnu/libstdc++.so.6.0.2
            by 0x4F3F6EC: std::num_put<char, std::ostreambuf_iterator<char, std
::char_traits<char> > >::do_put(std::ostreambuf iterator<char, std::char traits<
char> >, std::ios_base&, char, long) const (in /usr/lib/x86_64-linux-gnu/libstdc
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d::ios base&, char, long) const (in /usr/lib/x86 64-linux-gnu/libstdc++.so.6.0.2
            by 0x4F3F6EC: std::num_put<char, std::ostreambuf_iterator<char, std
::char traits<char> > >::do put(std::ostreambuf iterator<char, std::char traits<
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```
[2017-02-09 11:19:24 alex@Rincewind valgrind_examples]$ g++ test_no_delete.cpp -
o test_no_delete.o
[2017-02-09 11:20:11 alex@Rincewind valgrind_examples]$ _/test_no_delete.o
```

```
[2017-02-09 11:20:13 alex@Rincewind valgrind examples]$ g++ -g test no delete.cp
p -o memcheck test no delete.o
[2017-02-09 11:20:47 alex@Rincewind valgrind_examples]$ valgrind ./memcheck_test
 no delete.o
==14925== Memcheck, a memory error detector
==14925== Copyright (C) 2002-2015, and GNU GPL'd, by Julian Seward et al.
==14925== Using Valgrind-3.12.0.SVN and LibVEX; rerun with -h for copyright info
==14925== Command: ./memcheck test no delete.o
==14925==
==14925==
==14925== HEAP SUMMARY:
              in use at exit: 20 bytes in 2 blocks
==14925==
            total heap usage: 3 allocs, 1 frees, 72,724 bytes allocated
==14925==
==14925==
==14925== LEAK SUMMARY:
             definitely lost: 20 bytes in 2 blocks
==14925==
            indirectly lost: 0 bytes in 0 blocks
==14925==
               possibly lost: 0 bytes in 0 blocks
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             still reachable: 0 bytes in 0 blocks
==14925==
                  suppressed: 0 bytes in 0 blocks
==14925==
==14925== Rerun with --leak-check=full to see details of leaked memory
==14925==
==14925== For counts of detected and suppressed errors, rerun with: -v
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==14925==
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==14925== LEAK SUMMARY:
            definitely lost: 20 bytes in 2 blocks
==14925==
            indirectly lost: 0 bytes in 0 blocks
==14925==
               possibly lost: 0 bytes in 0 blocks
==14925==
             still reachable: 0 bytes in 0 blocks
==14925==
                  suppressed: 0 bytes in 0 blocks
==14925==
==14925== Rerun with --leak-check=full to see details of leaked memory
==14925==
==14925== For counts of detected and suppressed errors, rerun with: -v
==14925== ERROR SUMMARY: 0 errors from 0 contexts (suppressed: 0 from 0)
```

```
[2017-02-09 11:22:04 alex@Rincewind valgrind examples]$ valgrind --leak-check=fu
ll ./memcheck test no delete.o
==15519== Memcheck, a memory error detector
==15519== Copyright (C) 2002-2015, and GNU GPL'd, by Julian Seward et al.
==15519== Using Valgrind-3.12.0.SVN and LibVEX; rerun with -h for copyright info
==15519== Command: ./memcheck test no delete.o
==15519==
==15519==
==15519== HEAP SUMMARY:
==15519== in use at exit: 20 bytes in 2 blocks
          total heap usage: 3 allocs, 1 frees, 72,724 bytes allocated
==15519==
==15519==
==15519== 4 bytes in 1 blocks are definitely lost in loss record 1 of 2
            at 0x4C2D1AF: operator new(unsigned long) (in /usr/lib/valgrind/vgp
==15519==
reload memcheck-amd64-linux.so)
            by 0x1088A1: main (test no delete.cpp:6)
==15519==
==15519==
==15519== 16 bytes in 1 blocks are definitely lost in loss record 2 of 2
            at 0x4C2D8CF: operator new[](unsigned long) (in /usr/lib/valgrind/v
==15519==
gpreload memcheck-amd64-linux.so)
==15519==
            by 0x1088CE: main (test no delete.cpp:7)
==15519==
==15519== LEAK SUMMARY:
==15519== definitely lost: 20 bytes in 2 blocks
==15519== indirectly lost: 0 bytes in 0 blocks
              possibly lost: 0 bytes in 0 blocks
==15519==
==15519==
            still reachable: 0 bytes in 0 blocks
                  suppressed: 0 bytes in 0 blocks
==15519==
==15519==
==15519== For counts of detected and suppressed errors, rerun with: -v
==15519== ERROR SUMMARY: 2 errors from 2 contexts (suppressed: 0 from 0)
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```
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```

```
#include <fstream>
#include <iostream>
using namespace std;

int main(){
    int* num_counts = new int(4);
    int* counts = new int[*num_counts];
    for (int i = 0; i < *num_counts; ++i){
        counts[i] = i;
    }

delete[] num_counts;
delete counts;
}</pre>
```

```
#include <fstream>
#include <iostream>
using namespace std;

int main(){
    int* num_counts = new int(4);
    int* counts = new int[*num_counts];
    for (int i = 0; i < *num_counts; ++i){
        counts[i] = i;
    }

delete[] num_counts;
delete counts;
}</pre>
```

```
[2017-02-09 11:20:53 alex@Rincewind valgrind_examples]$ g++ test_wrong_delete.cp
p -o test_wrong_delete.o
[2017-02-09 11:21:27 alex@Rincewind valgrind_examples]$ _/test_wrong_delete.o
```

```
[2017-02-09 11:21:29 alex@Rincewind valgrind examples]$ q++ -q test wrong delete
.cpp -o memcheck test wrong delete.o
[2017-02-09 11:21:59 alex@Rincewind valgrind examples]$ valgrind ./memcheck test
wrong delete.o
==15095== Memcheck, a memory error detector
==15095== Copyright (C) 2002-2015, and GNU GPL'd, by Julian Seward et al.
==15095== Using Valgrind-3.12.0.SVN and LibVEX; rerun with -h for copyright info
==15095== Command: ./memcheck test wrong delete.o
==15095==
==15095== Mismatched free() / delete / delete []
             at 0x4C2E76B: operator delete[](void*) (in /usr/lib/valgrind/vgprel
==15095==
oad memcheck-amd64-linux.so)
             by 0x1089BD: main (test wrong delete.cpp:11)
==15095==
==15095== Address 0x5ab9c80 is 0 bytes inside a block of size 4 alloc'd
==15095==
             at 0x4C2D1AF: operator new(unsigned long) (in /usr/lib/valgrind/vgp
reload memcheck-amd64-linux.so)
            by 0x108941: main (test wrong delete.cpp:6)
==15095==
==15095==
==15095== Mismatched free() / delete / delete []
             at 0x4C2E26B: operator delete(void*) (in /usr/lib/valgrind/vgpreloa
==15095==
d memcheck-amd64-linux.so)
==15095==
             by 0x1089CE: main (test wrong delete.cpp:12)
==15095== Address 0x5ab9cd0 is 0 bytes inside a block of size 16 alloc'd
             at 0x4C2D8CF: operator new[](unsigned long) (in /usr/lib/valgrind/v
==15095==
gpreload memcheck-amd64-linux.so)
             by 0x10896E: main (test wrong delete.cpp:7)
==15095==
==15095==
==15095==
==15095== HEAP SUMMARY:
             in use at exit: 0 bytes in 0 blocks
==15095==
            total heap usage: 3 allocs, 3 frees, 72,724 bytes allocated
==15095==
==15095==
==15095== All heap blocks were freed -- no leaks are possible
==15095==
==15095== For counts of detected and suppressed errors, rerun with: -v
==15095== ERROR SUMMARY: 2 errors from 2 contexts (suppressed: 0 from 0)
```

```
[2017-02-09 11:21:29 alex@Rincewind valgrind examples]$ q++ -q test wrong delete
.cpp -o memcheck test wrong delete.o
[2017-02-09 11:21:59 alex@Rincewind valgrind examples]$ valgrind ./memcheck test
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