

# Discussion Section 5

- HW4 questions?
- BLAST algorithm
- If time: the Stack vs. the Heap
  - Using *new* and *delete* in C++

# HW4 Questions?

# Basic Local Alignment Search Tool (BLAST)

 **BLAST®** *Basic Local Alignment Search Tool*

[Home](#) [Recent Results](#) [Saved Strategies](#) [Help](#)

▶ [NCBI/ BLAST Home](#)

BLAST finds regions of similarity between biological sequences. [more...](#)

## BLAST Assembled Genomes

Find Genomic BLAST pages:

**GO**

- ▣ [Human](#)
- ▣ [Mouse](#)
- ▣ [Rat](#)
- ▣ [Cow](#)
- ▣ [Pig](#)
- ▣ [Dog](#)
- ▣ [Rabbit](#)
- ▣ [Chimp](#)
- ▣ [Guinea pig](#)
- ▣ [Fruit fly](#)
- ▣ [Honey bee](#)
- ▣ [Chicken](#)
- ▣ [Zebrafish](#)
- ▣ [Clawed frog](#)
- ▣ [Arabidopsis](#)
- ▣ [Rice](#)
- ▣ [Yeast](#)
- ▣ [Microbes](#)

## Basic BLAST

Choose a BLAST program to run.

[nucleotide blast](#)

Search a **nucleotide** database using a **nucleotide** query  
*Algorithms: blastn, megablast, discontinuous megablast*

[protein blast](#)

Search **protein** database using a **protein** query  
*Algorithms: blastp, psi-blast, phi-blast, delta-blast*

[blastx](#)

Search **protein** database using a **translated nucleotide** query

[tblastn](#)

Search **translated nucleotide** database using a **protein** query

[tblastx](#)

Search **translated nucleotide** database using a **translated nucleotide** query

# Basic Local Alignment Search Tool (BLAST)

 **BLAST®** *Basic Local Alignment Search Tool*

[Home](#) [Recent Results](#) [Saved Strategies](#) [Help](#)

▶ [NCBI/ BLAST Home](#)

BLAST finds regions of similarity between biological sequences. [more...](#)

## BLAST Assembled Genomes

Find Genomic BLAST pages:

**GO**

- ▣ [Human](#)
- ▣ [Mouse](#)
- ▣ [Rat](#)
- ▣ [Cow](#)
- ▣ [Pig](#)
- ▣ [Dog](#)
- ▣ [Rabbit](#)
- ▣ [Chimp](#)
- ▣ [Guinea pig](#)
- ▣ [Fruit fly](#)
- ▣ [Honey bee](#)
- ▣ [Chicken](#)
- ▣ [Zebrafish](#)
- ▣ [Clawed frog](#)
- ▣ [Arabidopsis](#)
- ▣ [Rice](#)
- ▣ [Yeast](#)
- ▣ [Microbes](#)

## Basic BLAST

Choose a BLAST program to run.

[nucleotide blast](#)

Search a **nucleotide** database using a **nucleotide** query  
*Algorithms: blastn, megablast, discontinuous megablast*

[protein blast](#)

Search **protein** database using a **protein** query  
*Algorithms: blastp, psi-blast, phi-blast, delta-blast*

[blastx](#)

Search **protein** database using a **translated nucleotide** query

[tblastn](#)

Search **translated nucleotide** database using a **protein** query

[tblastx](#)

Search **translated nucleotide** database using a **translated nucleotide** query

Original paper  
has been cited  
63,813 times

The general problem to solve

# The general problem to solve

- Given a reference string of length  $n$  and a query string of length  $p$

# The general problem to solve

- Given a reference string of length  $n$  and a query string of length  $p$

Reference: ABBBAABABBABAABABABAABBBAAAAABBABBA

# The general problem to solve

- Given a reference string of length  $n$  and a query string of length  $p$

Reference: ABBBAABABBABAABABABAABBBAAAAABBABBA

Query: ABBA



# The general problem to solve

- Given a reference string of length  $n$  and a query string of length  $p$
- Find matches to the query string in the reference string with up to  $e$  differences

Reference: ABBBAABABBABAABABABAABBBAAAAABBABBA

Query: ABBA

# The general problem to solve

- Given a reference string of length  $n$  and a query string of length  $p$
- Find matches to the query string in the reference string with up to  $e$  differences
  - Differences are the number of insertions, deletions, and substitutions

Reference: ABBBAABABBABAABABABAABBBAAAAABBABBA

Query: ABBA

# The general problem to solve

- Given a reference string of length  $n$  and a query string of length  $p$
- Find matches to the query string in the reference string with up to  $e$  differences
  - Differences are the number of insertions, deletions, and substitutions

Reference: ABBBAABABBABAABABABAABBBAAAAABBABBA

Query: ABBA

$e = 1$

# The general problem to solve

- Given a reference string of length  $n$  and a query string of length  $p$
- Find matches to the query string in the reference string with up to  $e$  differences
  - Differences are the number of insertions, deletions, and substitutions

Reference: **ABBB**AAB**ABB**AB**AABA**B**ABA**BBBAAAA**ABBA**BBA

Query: ABBA

$e = 1$

Some example matches



# Categories of approximate match algorithms

# Categories of approximate match algorithms

- Deterministic
  - Find the exact set of locations in the reference where the query matches given some threshold

# Categories of approximate match algorithms

- Deterministic
  - Find the exact set of locations in the reference where the query matches given some threshold
- Filter
  - Returns false positives but no false negatives

# Categories of approximate match algorithms

- Deterministic
  - Find the exact set of locations in the reference where the query matches given some threshold
- Filter
  - Returns false positives but no false negatives
- Heuristic
  - Some false negatives (misses some matches)
  - Some false positives (some incorrect matches)



# Neighborhoods are sets of approximate matches

Reference: ABBBAABABBABAABABABAABBBAAAAABBABBA

Query: ABBA

$e = 1$

# Neighborhoods are sets of approximate matches

Reference: ABBBAABABBABAABABABAABBBAAAAABBABBA

Query: ABBA

$e = 1$

1 difference neighborhood of ABBA:

$N(ABBA) = \{ABBA, BBBA, AABA, ABAA, ABBB, AABBA, BABBA, ABBBA, ABABA, ABBAA, ABBAB, BBA, ABA, ABB\}$

# Neighborhoods are sets of approximate matches

Reference: ABBBAABABBABAABAB**ABAA**BBBAAAAABBABBA

Query: ABBA

$e = 1$

Hey look, a match!

1 difference neighborhood of ABBA:

$N(ABBA) = \{ABBA, BBBA, AABA, \mathbf{ABAA}, AB BB, AABBA, BABBA, ABBBA, ABABA, ABBAA, ABBAB, BBA, ABA, ABB\}$

# Neighborhoods are sets of approximate matches

Reference: ABBBAABABBABAABAB**ABAA**BBBAAAAABBABBA

Query: ABBA

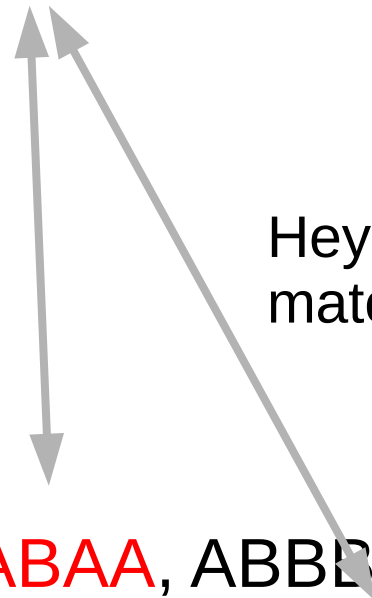
$e = 1$

Hey look, a match!

Hey look, another match?

1 difference neighborhood of ABBA:

$N(ABBA) = \{ABBA, BBBA, AABA, \text{ABAA}, ABBA, AABBA, BABBA, ABBBA, ABABA, ABBAA, ABBAB, BBA, \text{ABA}, ABB\}$



# Neighborhoods are sets of approximate matches

Reference: ABBBAABABBABAABAB**ABAA**BBBAAAAABBABBA

Query: ABBA

$e = 1$

1 difference neighborhood of ABBA:

$N(ABBA) = \{ABBA, BBBA, AABA, \text{ABAA}, AB BB, AABBA, BABBA, ABBBA, ABABA, ABBAA, ABBAB, BBA, \text{ABA}, ABB\}$

1 difference condensed neighborhood of ABBA:

$NC(ABBA) = \{BBBA, AABA, AABBA, BABBA, BBA, \text{ABA}, ABB\}$

# Neighborhoods are sets of approximate matches

Reference: ABBBAABABBABAABAB~~ABAA~~BBBAAAAABBABBA

Query: ABBA

$e = 1$

1 difference neighborhood of ABBA:

$N(ABBA) = \{ABBA, BBBA, AABA, \text{ABAA}, AB BB, AABBA, BABBA, ABBBA, ABABA, ABBAA, ABBAB, BBA, \text{ABA}, ABB\}$

1 difference condensed neighborhood of ABBA:

$NC(ABBA) = \{BBBA, AABA, AABBA, BABBA, BBA, \text{ABA}, ABB\}$

Only members without prefixes in the neighborhood (why?)

# Basic idea for BLAST

# Basic idea for BLAST

1. Partition query sequence in  $p/k$   $k$ -mers



# Basic idea for BLAST

1. Partition query sequence in  $p/k$   $k$ -mers
2. Generate the  $e$ -neighborhood of the query  $k$ -mers and find exact matches in the reference

# Basic idea for BLAST

1. Partition query sequence in  $p/k$   $k$ -mers
2. Generate the  $e$ -neighborhood of the query  $k$ -mers and find exact matches in the reference
3. Check each candidate match by extending from the ends of the  $k$ -mer

# Basic idea for BLAST

1. Partition query sequence in  $p/k$   $k$ -mers
2. Generate the  $e$ -neighborhood of the query  $k$ -mers and find exact matches in the reference
3. Check each candidate match by extending from the ends of the  $k$ -mer

What  $k$ -mer size?

# Basic idea for BLAST

1. Partition query sequence in  $p/k$   $k$ -mers
2. Generate the  $e$ -neighborhood of the query  $k$ -mers and find exact matches in the reference
3. Check each candidate match by extending from the ends of the  $k$ -mer

What  $k$ -mer size?

Something around  $\log n$

# BLAST in detail: Step 1

- Defining *k*-mers

# BLAST in detail: Step 1

- Defining *k*-mers
  - Should we use adjacent *k*-mers?

# BLAST in detail: Step 1

- Defining  $k$ -mers
  - Should we use adjacent  $k$ -mers?

Query: PPRHKKMFYAVG

$k = 6$

# BLAST in detail: Step 1

- Defining  $k$ -mers
  - Should we use adjacent  $k$ -mers?

Query: PPRHKKMFYAVG

$k = 6$

Adjacent  $k$ -mers: {PPRHKK, MFYAVG}



# BLAST in detail: Step 1

- Defining  $k$ -mers
  - Should we use adjacent  $k$ -mers?
    - There could be some matches to regions spanning  $k$ -mer boundaries

Query: PPRHKKMFYAVG

$k = 6$

Adjacent  $k$ -mers: {PPRHKK, MFYAVG}

# BLAST in detail: Step 1

- Defining  $k$ -mers
  - Should we use adjacent  $k$ -mers?
    - There could be some matches to regions spanning  $k$ -mer boundaries
  - Try  $p-k+1$  overlapping  $k$ -mers instead

Query: PPRHKKMFYAVG

$k = 6$

Adjacent  $k$ -mers: {PPRHKK, MFYAVG}

# BLAST in detail: Step 1

- Defining  $k$ -mers
  - Should we use adjacent  $k$ -mers?
    - There could be some matches to regions spanning  $k$ -mer boundaries
  - Try  $p-k+1$  overlapping  $k$ -mers instead

Query: PPRHKKMFYAVG

$k = 6$

Adjacent  $k$ -mers: {PPRHKK, MFYAVG}

Overlapping  $k$ -mers: {PPRHKK, PRHKKM, RHKKMF, HKKMFY, KKMFYA, KMFYAV, MFYAVG}

# BLAST in detail: Step 2

- Generating the *e*-neighborhood of each *k*-mer

# BLAST in detail: Step 2

- Generating the *e*-neighborhood of each *k*-mer
  - How should we define differences?

# BLAST in detail: Step 2

- Generating the *e*-neighborhood of each *k*-mer
  - How should we define differences?
    - BLOSUM62 matrix for protein similarity

# BLAST in detail: Step 2

- Generating the *e*-neighborhood of each *k*-mer
  - How should we define differences?
    - BLOSUM62 matrix for protein similarity
  - Given *k*-mer “PQG”, some possible neighbors

# BLAST in detail: Step 2

- Generating the *e*-neighborhood of each *k*-mer
  - How should we define differences?
    - BLOSUM62 matrix for protein similarity
  - Given *k*-mer “PQG”, some possible neighbors
    - PEG: score is 15
    - PQA: score is 12



# BLAST in detail: Step 2

- Generating the *e*-neighborhood of each *k*-mer
  - How should we define differences?
    - BLOSUM62 matrix for protein similarity
  - Given *k*-mer “PQG”, some possible neighbors
    - PEG: score is 15
    - PQA: score is 12
  - Use a cutoff score to define neighborhood

# BLAST in detail: Step 2

- Generating the *e*-neighborhood of each *k*-mer
  - How should we define differences?
    - BLOSUM62 matrix for protein similarity
  - Given *k*-mer “PQG”, some possible neighbors
    - PEG: score is 15
    - PQA: score is 12
  - Use a cutoff score to define neighborhood
- Use an efficient method for identifying exact matches

# BLAST in detail: Step 2

- Generating the  $e$ -neighborhood of each  $k$ -mer
  - How should we define differences?
    - BLOSUM62 matrix for protein similarity
  - Given  $k$ -mer “PQG”, some possible neighbors
    - PEG: score is 15
    - PQA: score is 12
  - Use a cutoff score to define neighborhood
- Use an efficient method for identifying exact matches
  - Finite automaton, like a Mealy machine

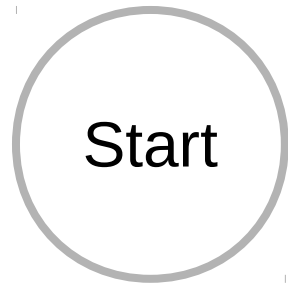
# Brief Mealy machine example

# Brief Mealy machine example

Input Sequence:  
0010110

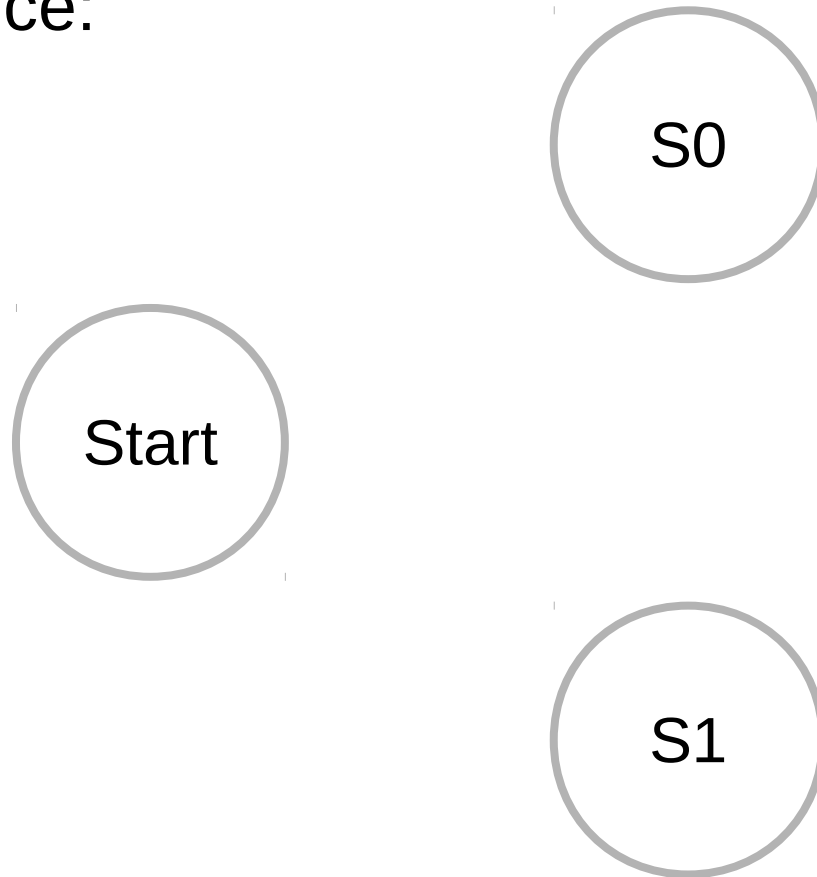
# Brief Mealy machine example

Input Sequence:  
0010110



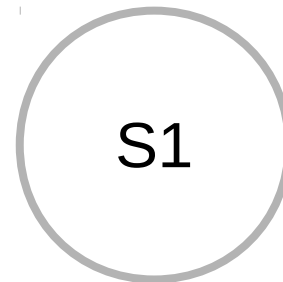
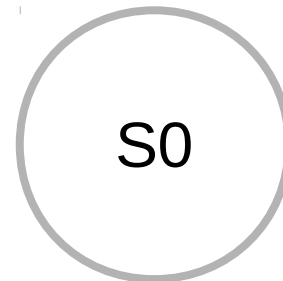
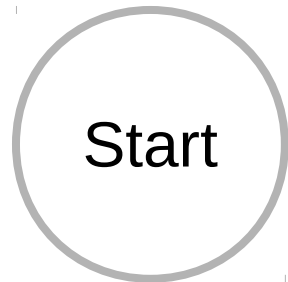
# Brief Mealy machine example

Input Sequence:  
0010110



# Brief Mealy machine example

Input Sequence:  
0010110



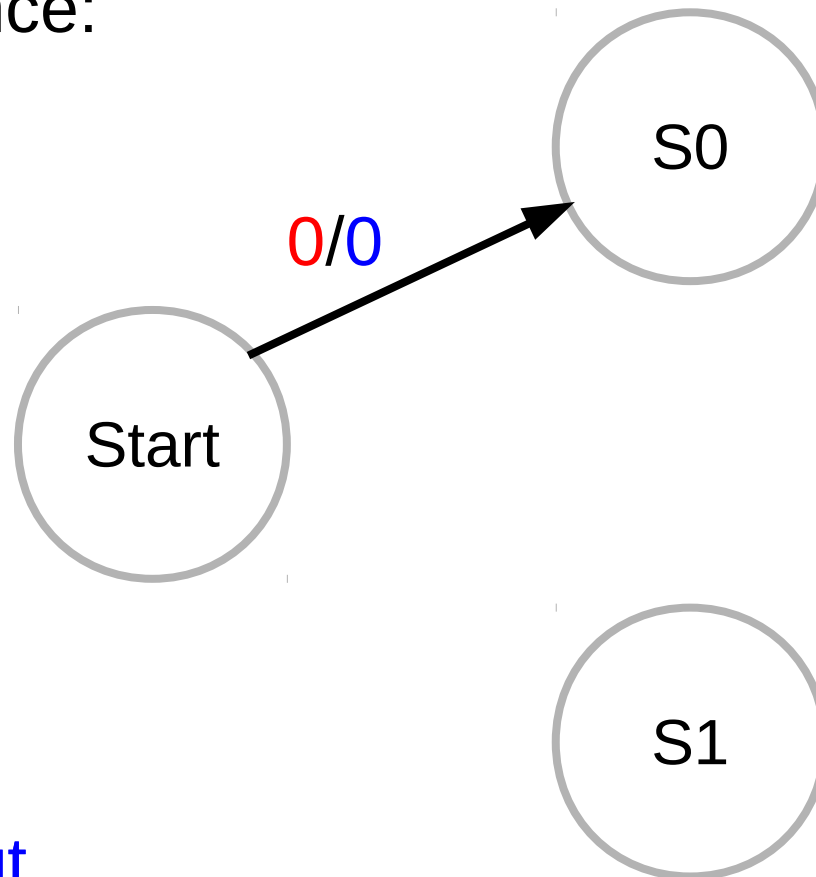
Input/Output





# Brief Mealy machine example

Input Sequence:  
0010110

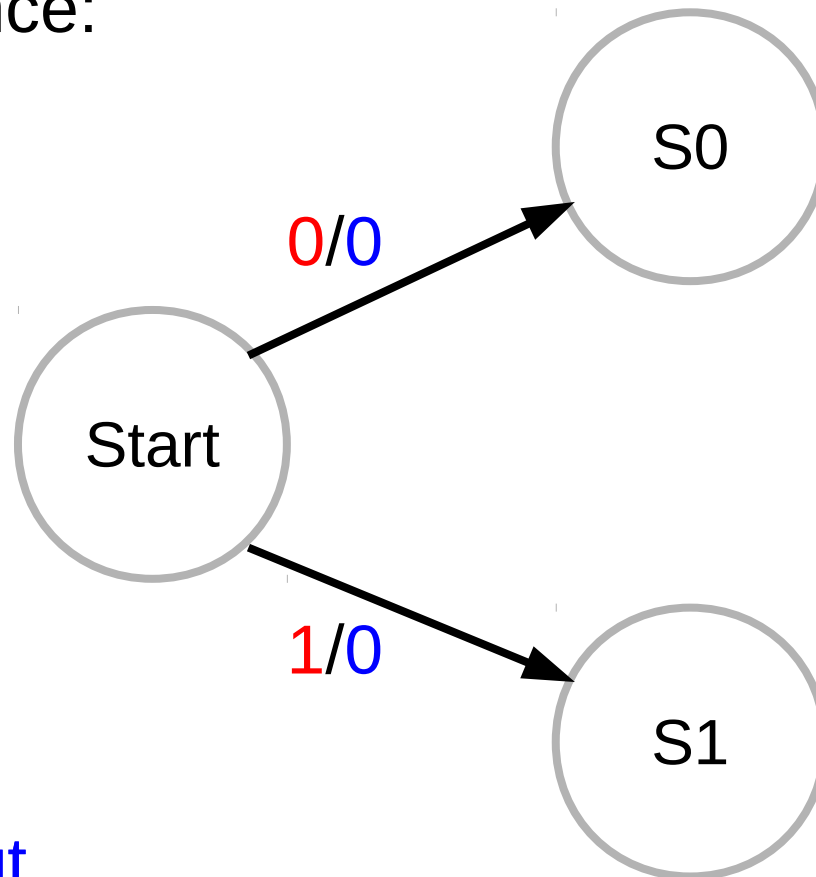


Input/Output



# Brief Mealy machine example

Input Sequence:  
0010110

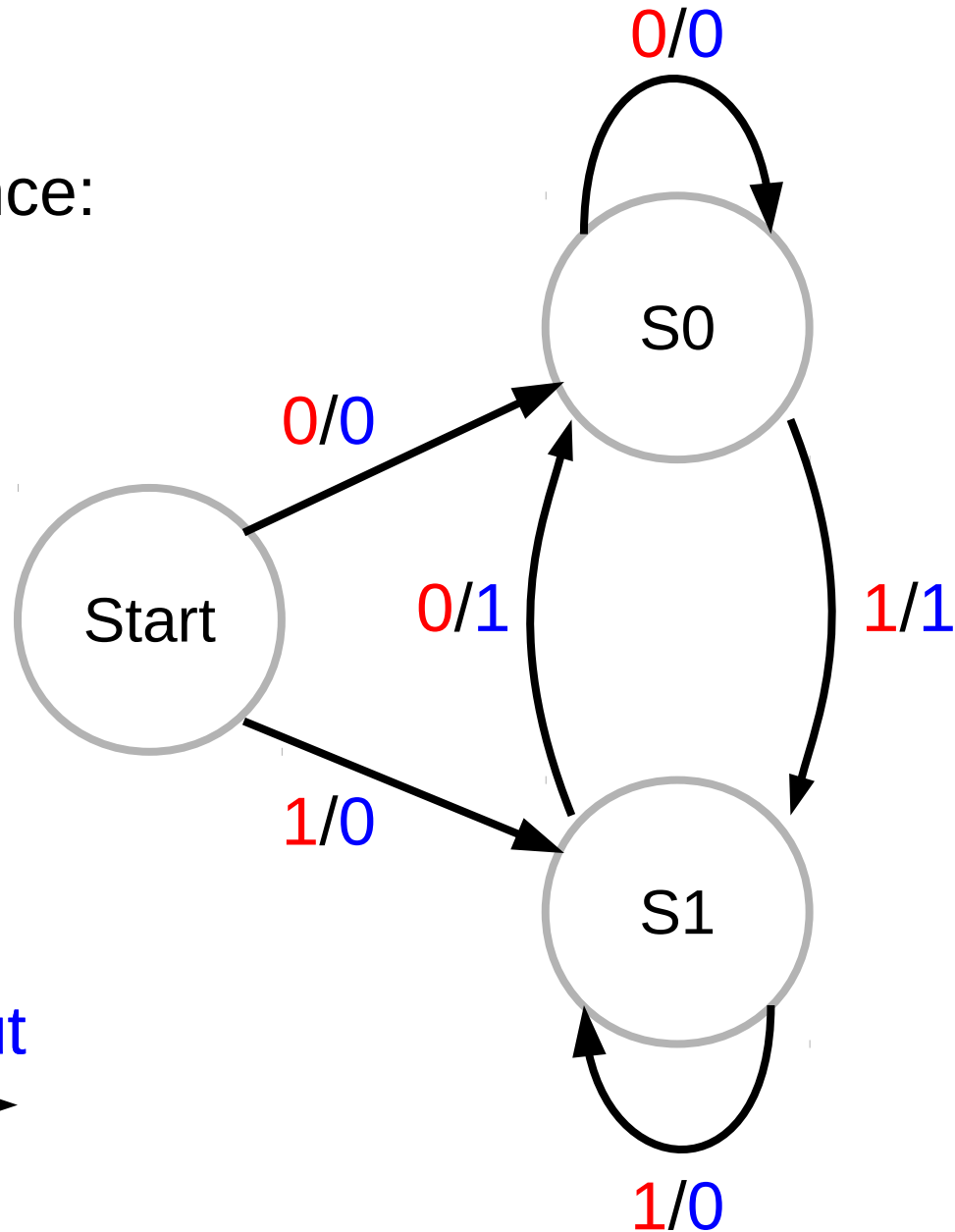


Input/Output



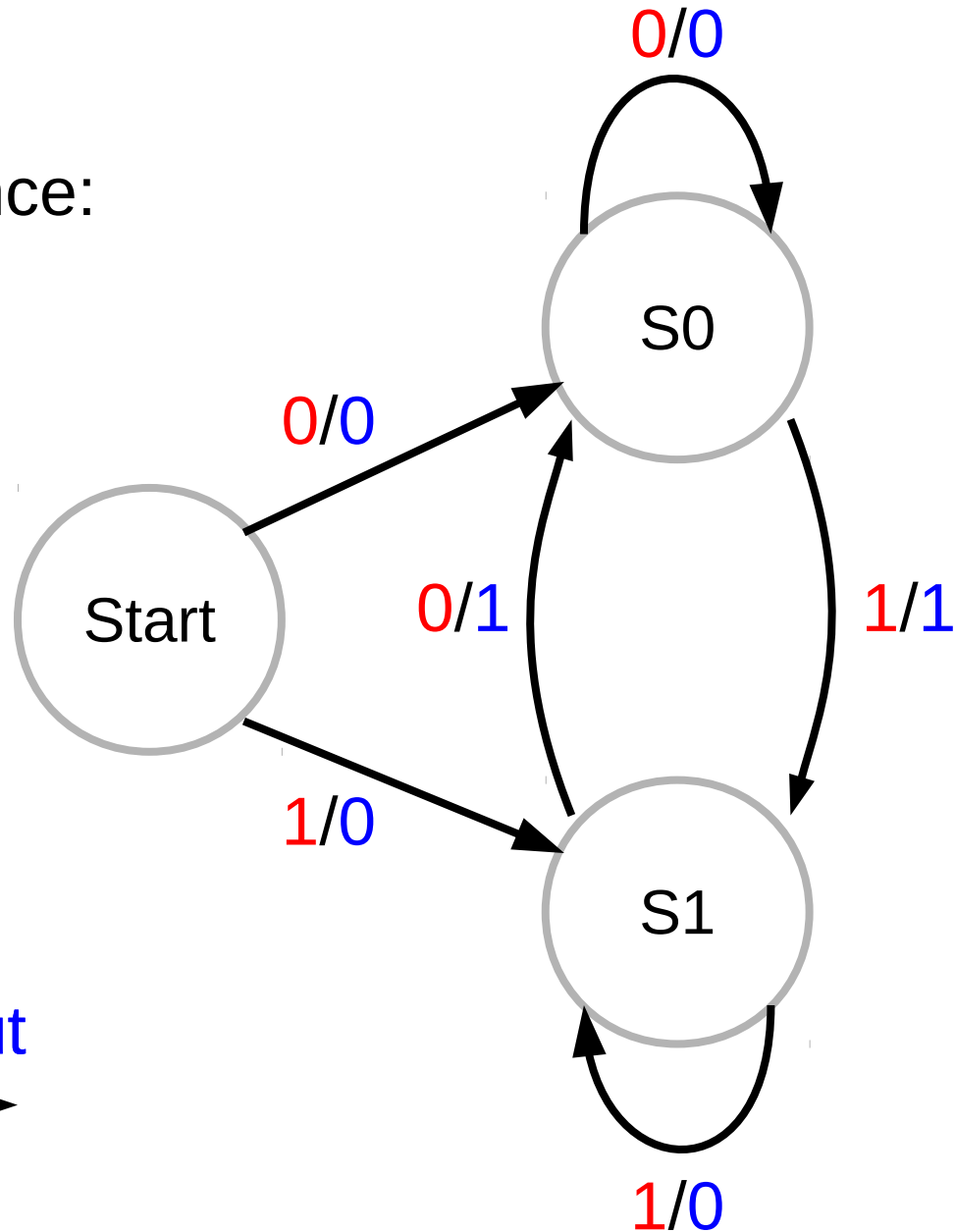
# Brief Mealy machine example

Input Sequence:  
0010110



# Brief Mealy machine example

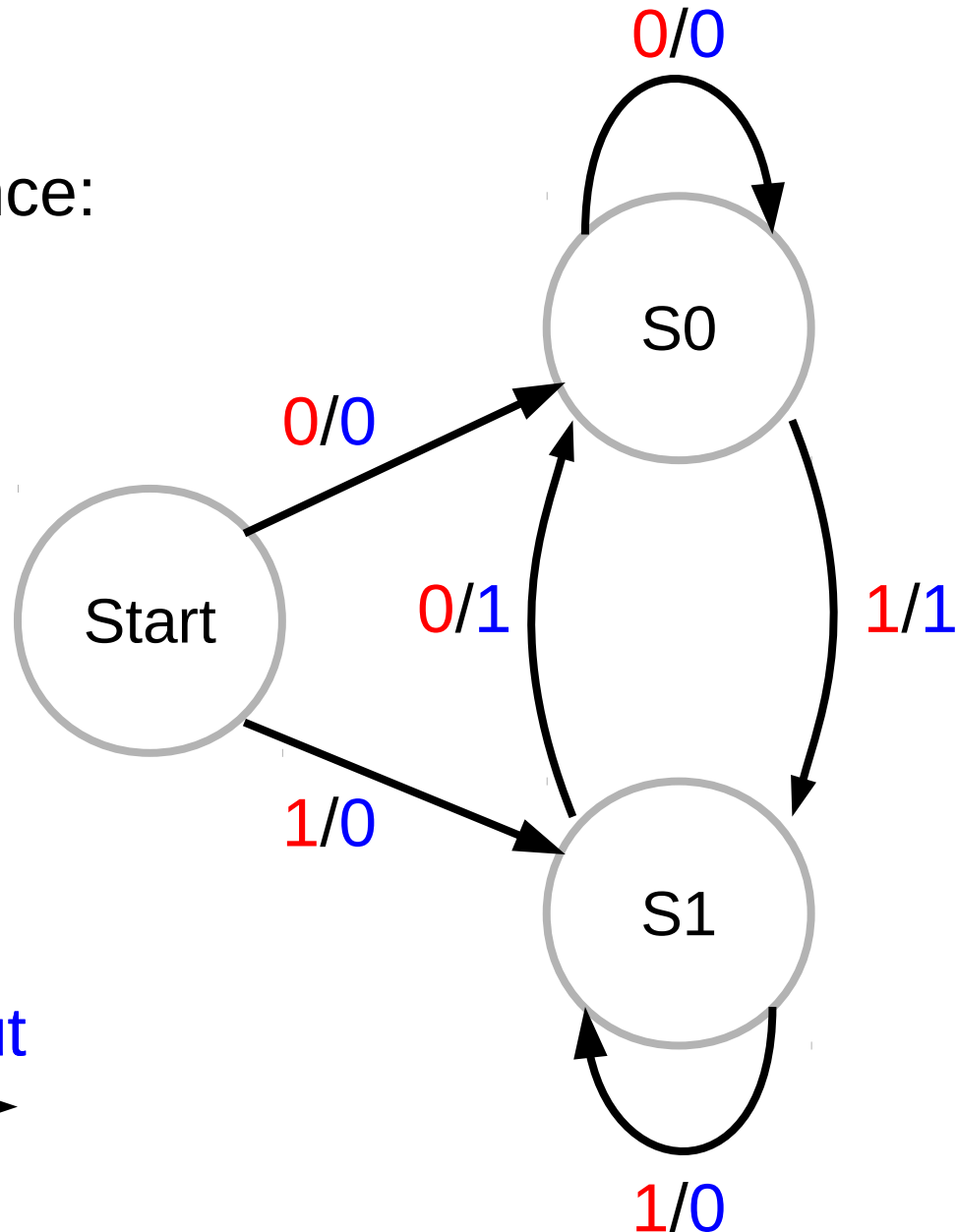
Input Sequence:  
0010110



When does it  
output a 1?

# Brief Mealy machine example

Input Sequence:  
0010110



When does it  
output a 1?

When the  
previous value  
is different

# BLAST in detail: Step 3

- Extend each 'seed' match into a local alignment

# BLAST in detail: Step 3

- Extend each 'seed' match into a local alignment
  - How do we extend?

# “Double and Check” extension



# “Double and Check” extension

Query: PPRHKKMFYAVG

$k = 3$ ,  $k$ -mer 'HKK'

# “Double and Check” extension

Query: PPRHKKMFYAVG

$k = 3$ ,  $k$ -mer 'HKK'

Candidate match: GAMPRHKKQFFM

# “Double and Check” extension

Query: PPRHKKMFYAVG

$k = 3$ ,  $k$ -mer 'HKK'

Candidate match: GAMPRHKKQFFM

- Look at  $2k$ -mers that span 'HKK' in the query and see if they have an e-match spanning the candidate match

# “Double and Check” extension

Query: PPRHKKMFYAVG

$k = 3$ ,  $k$ -mer 'HKK'

Candidate match: GAMPRHKKQFFM

- Look at  $2k$ -mers that span 'HKK' in the query and see if they have an e-match spanning the candidate match
  - If yes, continue doubling
  - If no, then stop (provably correct)

# “Double and Check” extension

Query: PPRHKKMFYAVG

$k = 3$ ,  $k$ -mer 'HKK'

Candidate match: GAMPRHKKQFFM

- Look at  $2k$ -mers that span 'HKK' in the query and see if they have an e-match spanning the candidate match
  - If yes, continue doubling
  - If no, then stop (provably correct)

Example:

$2k$ -mer: 'PRHKKM'

New candidate match: GAMPRHKKQFFM

# BLAST in detail: Step 3

- Extend each 'seed' match into a local alignment
  - How do we extend? “double and check”

# BLAST in detail: Step 3

- Extend each 'seed' match into a local alignment
  - How do we extend? “double and check”
  - Stop when score drops too far below the best score seen

# BLAST in detail: Step 3

- Extend each 'seed' match into a local alignment
  - How do we extend? “double and check”
  - Stop when score drops too far below the best score seen
    - Hmm, this sounds similar to something we've done



# BLAST in detail: Step 3

- Extend each 'seed' match into a local alignment
  - How do we extend? “double and check”
  - Stop when score drops too far below the best score seen
    - Hmm, this sounds similar to something we've done
- If the score is high enough, report the full match

# BLAST in detail: Step 3

- Extend each 'seed' match into a local alignment
  - How do we extend? “double and check”
  - Stop when score drops too far below the best score seen
    - Hmm, this sounds similar to something we've done
- If the score is high enough, report the full match
  - What is high enough?

# BLAST in detail: Step 3

- Extend each 'seed' match into a local alignment
  - How do we extend? “double and check”
  - Stop when score drops too far below the best score seen
    - Hmm, this sounds similar to something we've done
- If the score is high enough, report the full match
  - What is high enough?
  - Need some measure of significance

# BLAST in detail: Step 3

- Extend each 'seed' match into a local alignment
  - How do we extend? “double and check”
  - Stop when score drops too far below the best score seen
    - Hmm, this sounds similar to something we've done
- If the score is high enough, report the full match
  - What is high enough?
  - Need some measure of significance
    - E-values based on Gumbel extreme value distribution

BLAST time complexity?

# BLAST time complexity?

- Costly operations

# BLAST time complexity?

- Costly operations
  - Generating the condensed  $k$ -mer neighborhoods

# BLAST time complexity?

- Costly operations
  - Generating the condensed  $k$ -mer neighborhoods
  - Identifying candidate matches



# BLAST time complexity?

- Costly operations
  - Generating the condensed  $k$ -mer neighborhoods
  - Identifying candidate matches
  - Extending candidate matches

# BLAST time complexity?

- Costly operations
  - Generating the condensed  $k$ -mer neighborhoods
  - Identifying candidate matches
  - Extending candidate matches
- Generating neighborhoods and extending matches can be done in sublinear time (in terms of the size of the reference)

# BLAST time complexity?

- Costly operations
  - Generating the condensed  $k$ -mer neighborhoods
  - Identifying candidate matches
  - Extending candidate matches
- Generating neighborhoods and extending matches can be done in sublinear time (in terms of the size of the reference)
  - Use a precomputed hash index to look up  $k$ -mer matches

# What about gapped alignment?

- Similar for steps 1 and 2 (list  $k$ -mers neighborhoods, find candidate matches)

# What about gapped alignment?

- Similar for steps 1 and 2 (list  $k$ -mers neighborhoods, find candidate matches)
- Instead of extending
  - Look for candidate match pairs closer together than some distance threshold

# What about gapped alignment?

- Similar for steps 1 and 2 (list  $k$ -mers neighborhoods, find candidate matches)
- Instead of extending
  - Look for candidate match pairs closer together than some distance threshold
  - Then extend from ends of paired matches

# What about gapped alignment?

- Similar for steps 1 and 2 (list  $k$ -mers neighborhoods, find candidate matches)
- Instead of extending
  - Look for candidate match pairs closer together than some distance threshold
  - Then extend from ends of paired matches
- Can also consider continuing to combine paired matches

# Alternatively, you could have Gene Myers explain BLAST

- <https://www.youtube.com/watch?v=pVFX3V0Q2Rg>
- [http://myerslab.mpi-cbg.de/wp-content/uploads/2014/06/behind.blast\\_.pdf](http://myerslab.mpi-cbg.de/wp-content/uploads/2014/06/behind.blast_.pdf)



# Stack vs. Heap

## Using *new* and *delete* in C++

# Stack vs. Heap

## Using *new* and *delete* in C++

- The stack is a preallocated piece of memory given to your program when it is run

# Stack vs. Heap

## Using *new* and *delete* in C++

- The stack is a preallocated piece of memory given to your program when it is run



# Stack vs. Heap

## Using *new* and *delete* in C++

- The stack is a preallocated piece of memory given to your program when it is run
- Variables get put on the stack as your program runs



# Stack vs. Heap

## Using *new* and *delete* in C++

- The stack is a preallocated piece of memory given to your program when it is run
- Variables get put on the stack as your program runs



# Stack vs. Heap

## Using *new* and *delete* in C++

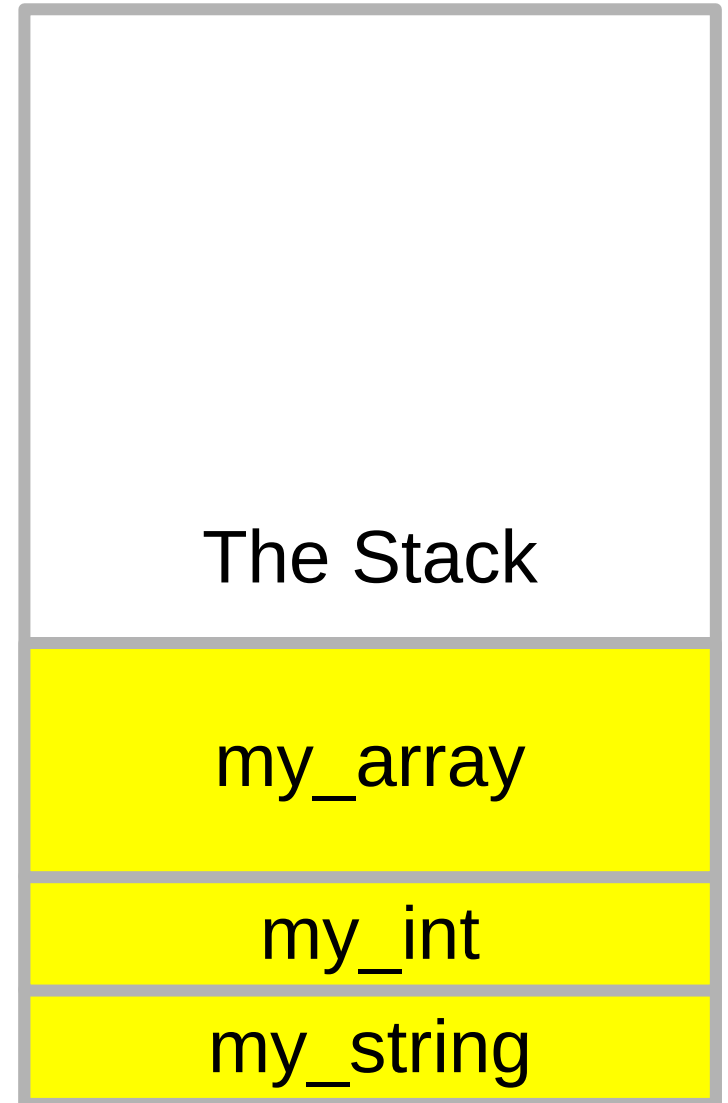
- The stack is a preallocated piece of memory given to your program when it is run
- Variables get put on the stack as your program runs



# Stack vs. Heap

## Using *new* and *delete* in C++

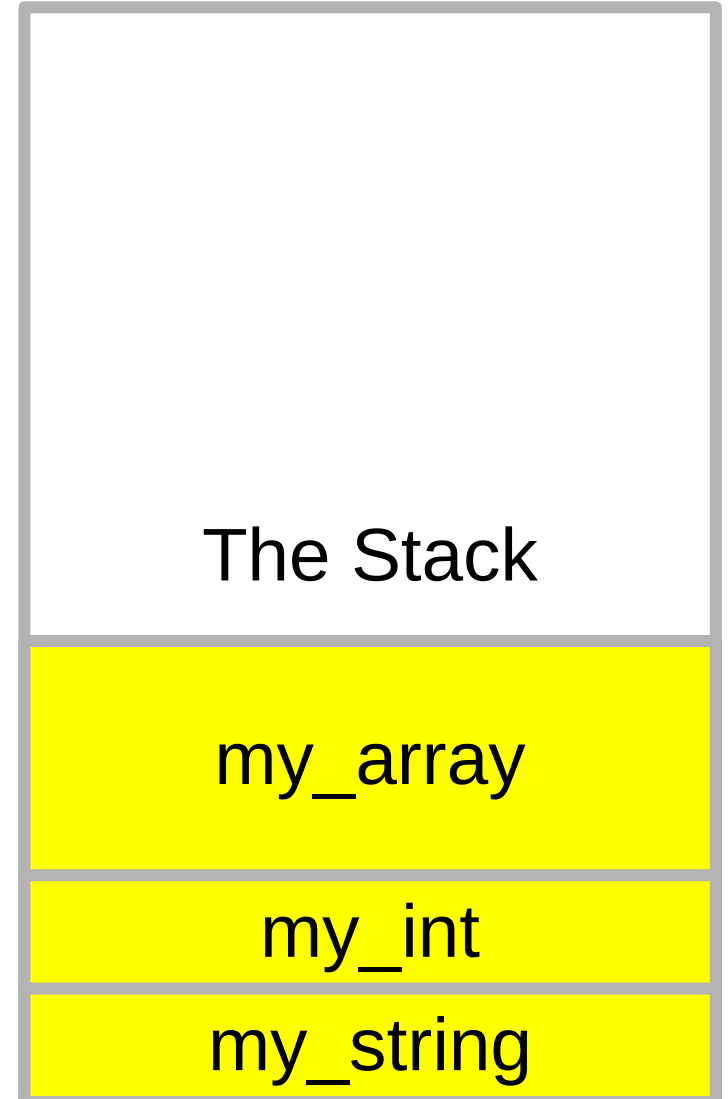
- The stack is a preallocated piece of memory given to your program when it is run
- Variables get put on the stack as your program runs



# Stack vs. Heap

## Using *new* and *delete* in C++

- The stack is a preallocated piece of memory given to your program when it is run
- Variables get put on the stack as your program runs
- Different functions may define part of the stack for local variables

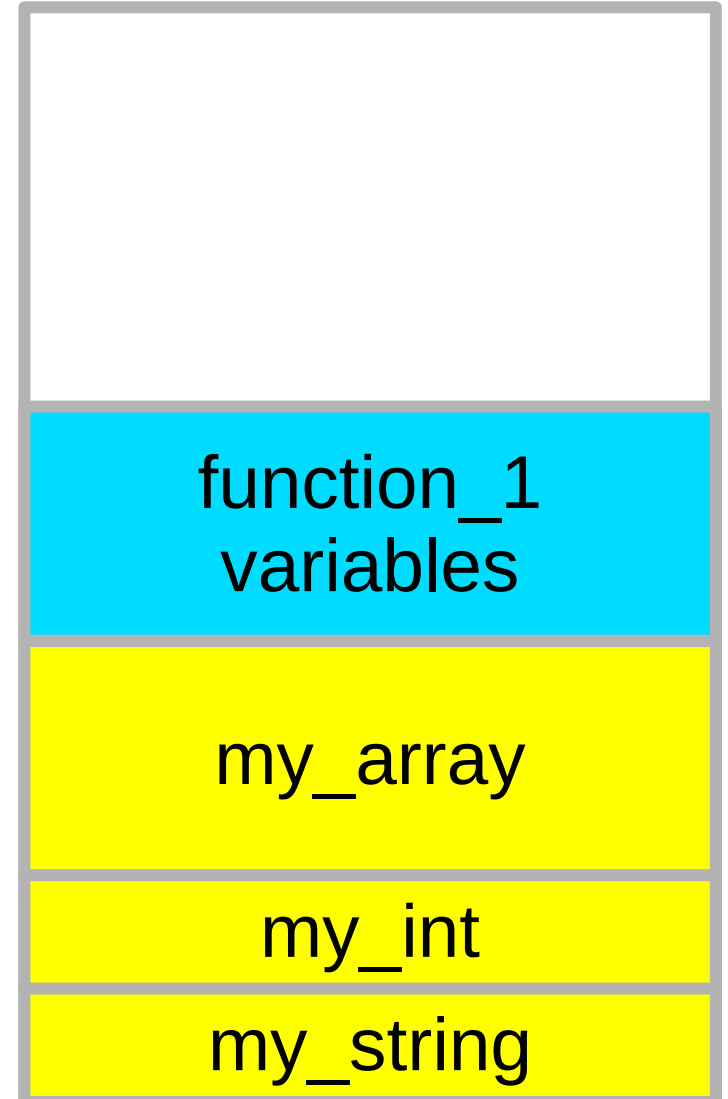




# Stack vs. Heap

## Using *new* and *delete* in C++

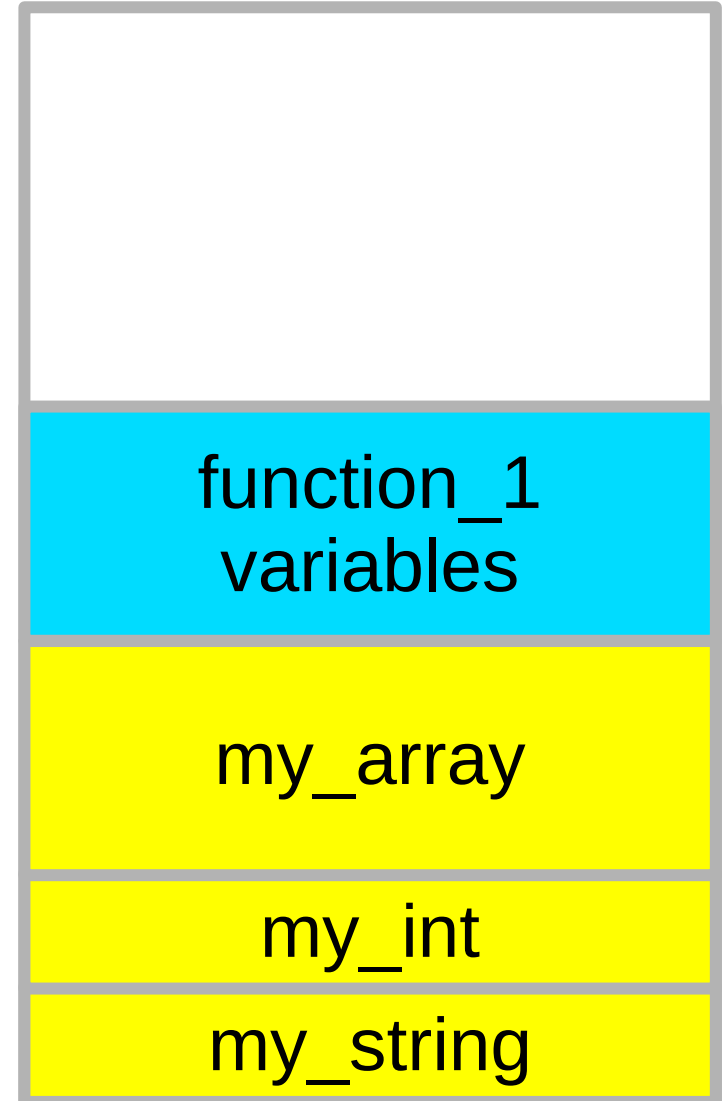
- The stack is a preallocated piece of memory given to your program when it is run
- Variables get put on the stack as your program runs
- Different functions may define part of the stack for local variables



# Stack vs. Heap

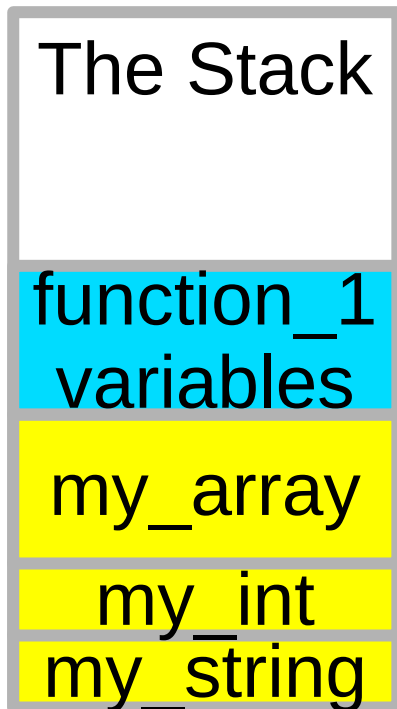
## Using *new* and *delete* in C++

- The stack is a preallocated piece of memory given to your program when it is run
- Variables get put on the stack as your program runs
- Different functions may define part of the stack for local variables
  - This is how scoping gets determined



# Stack vs. Heap

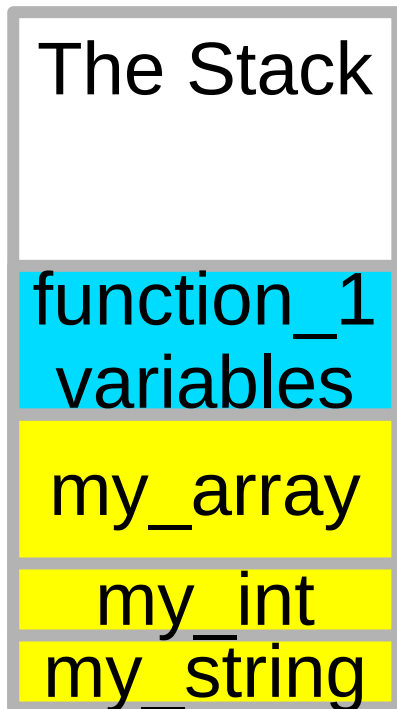
## Using *new* and *delete* in C++



# Stack vs. Heap

## Using *new* and *delete* in C++

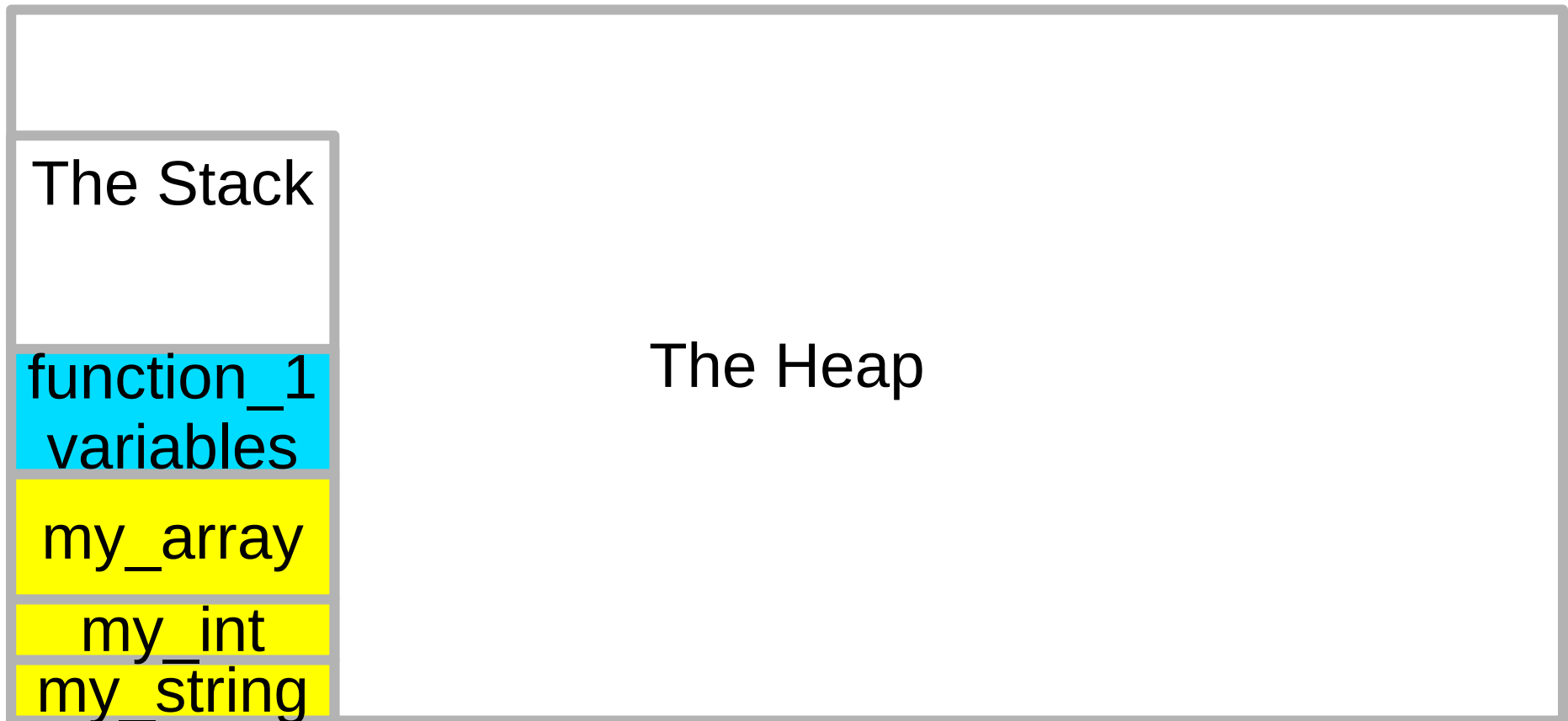
- The heap is all non-allocated space in memory



# Stack vs. Heap

## Using *new* and *delete* in C++

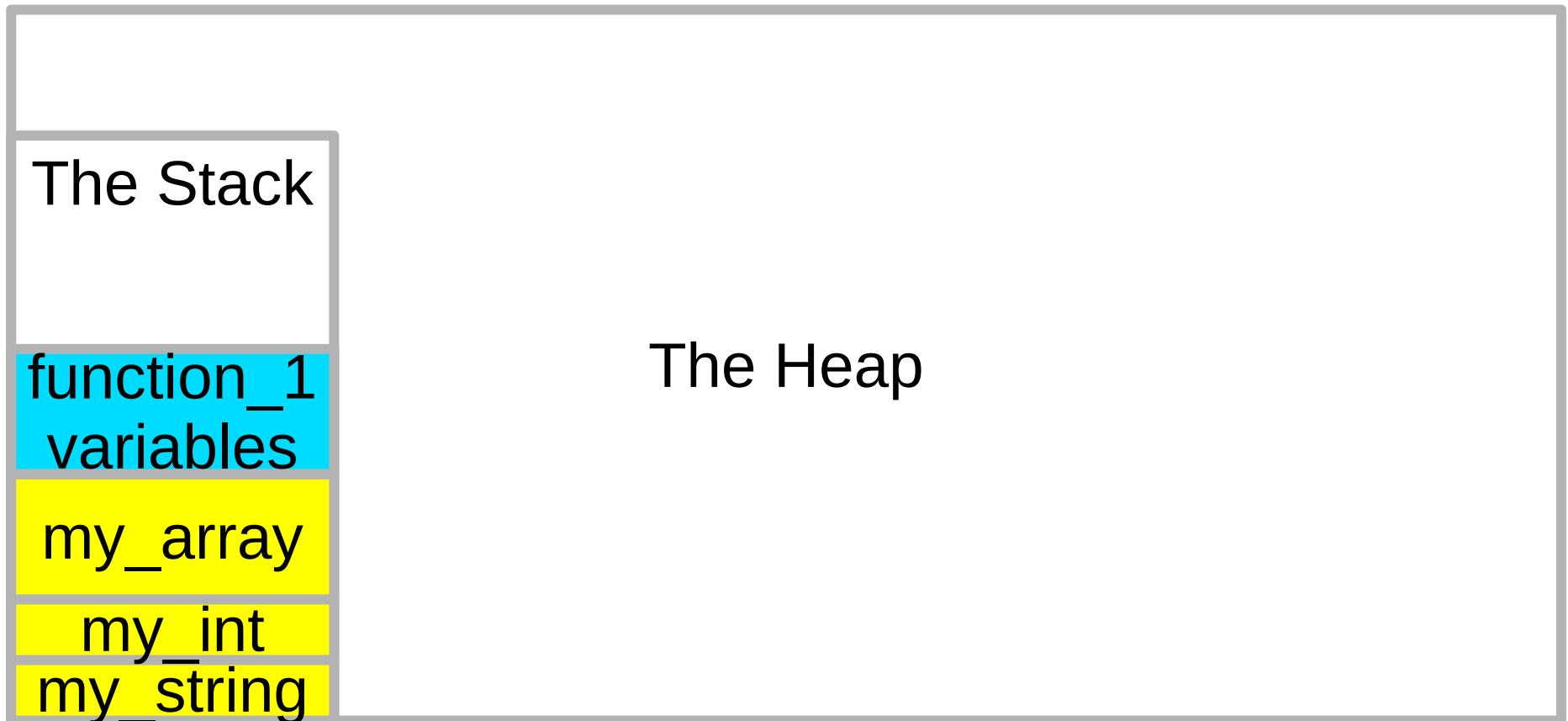
- The heap is all non-allocated space in memory



# Stack vs. Heap

## Using *new* and *delete* in C++

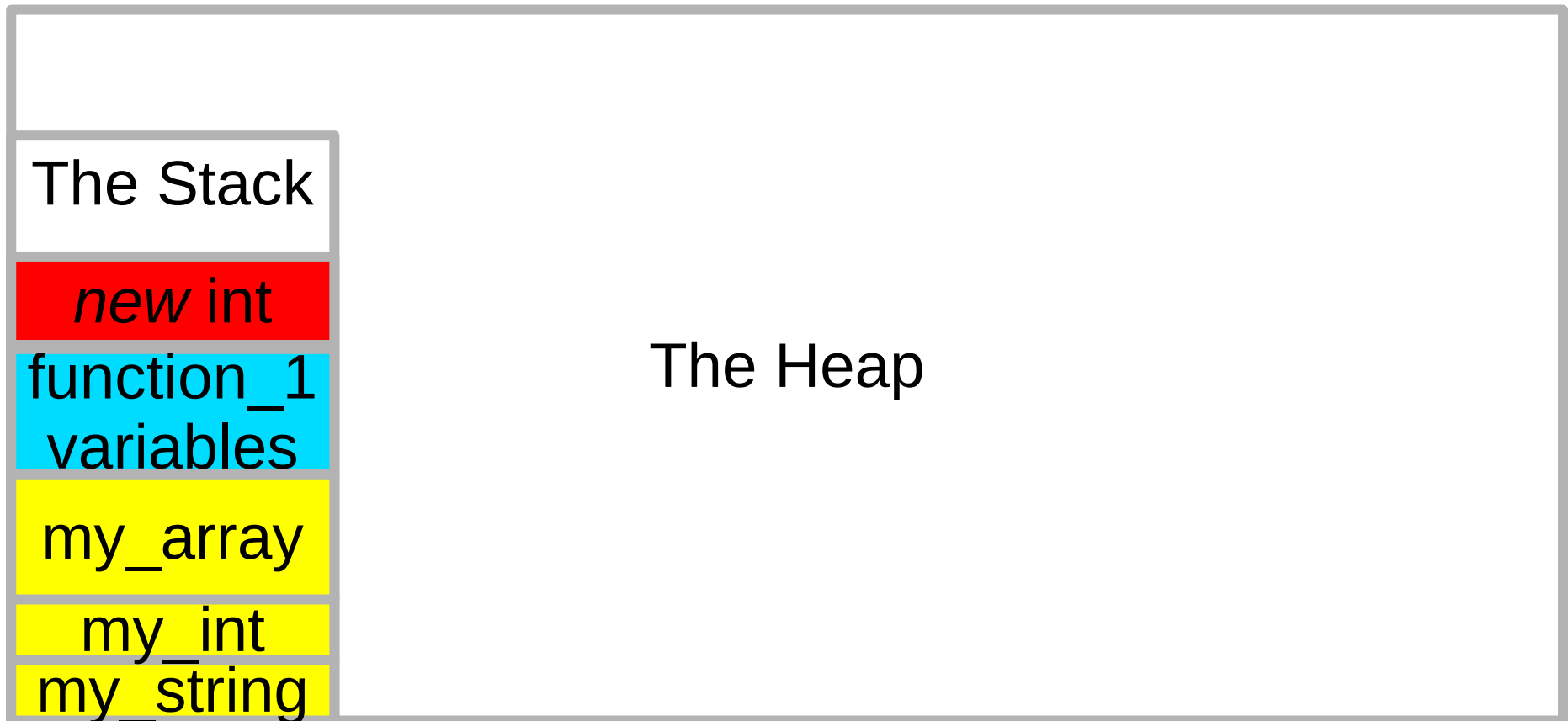
- The heap is all non-allocated space in memory
- Using *new* allocates space on the heap for a variable



# Stack vs. Heap

## Using *new* and *delete* in C++

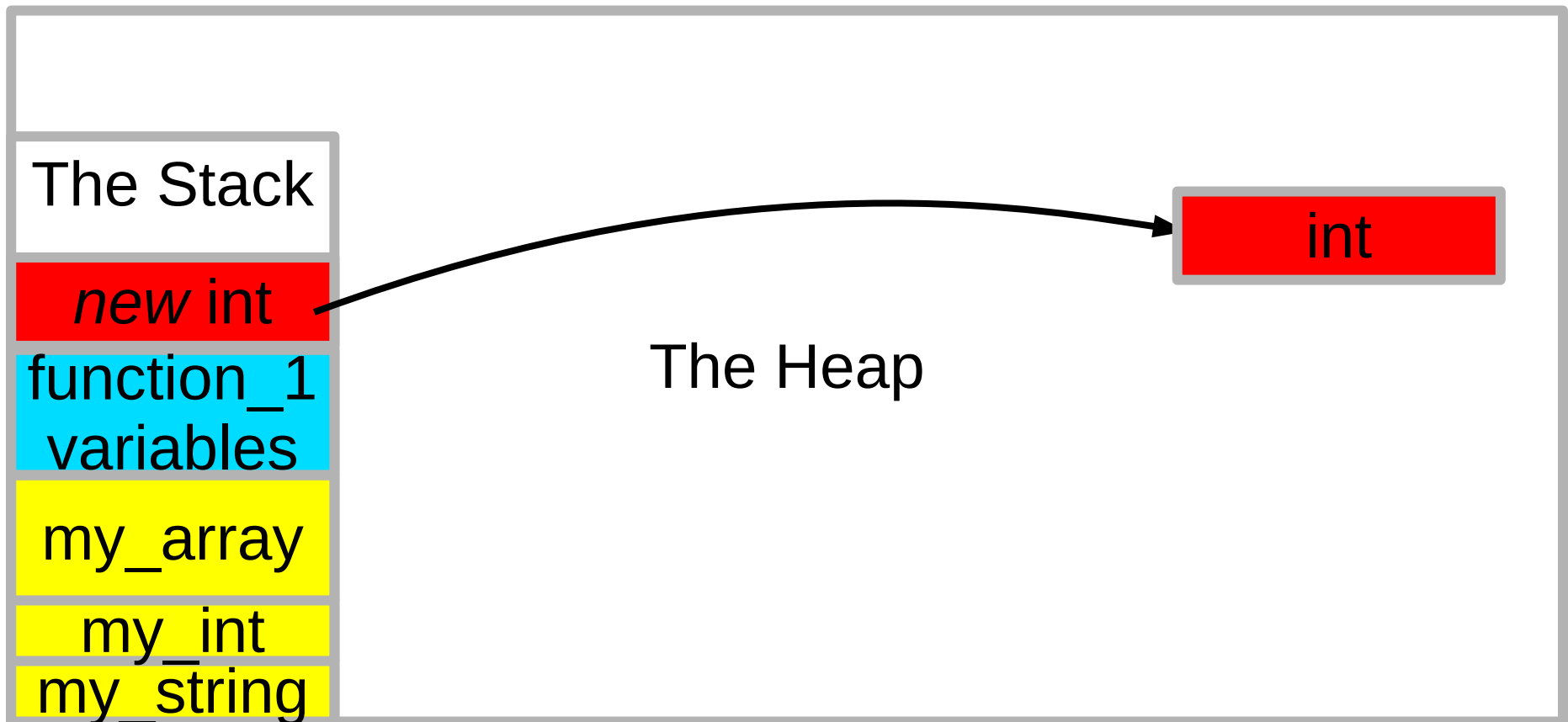
- The heap is all non-allocated space in memory
- Using *new* allocates space on the heap for a variable



# Stack vs. Heap

## Using *new* and *delete* in C++

- The heap is all non-allocated space in memory
- Using *new* allocates space on the heap for a variable

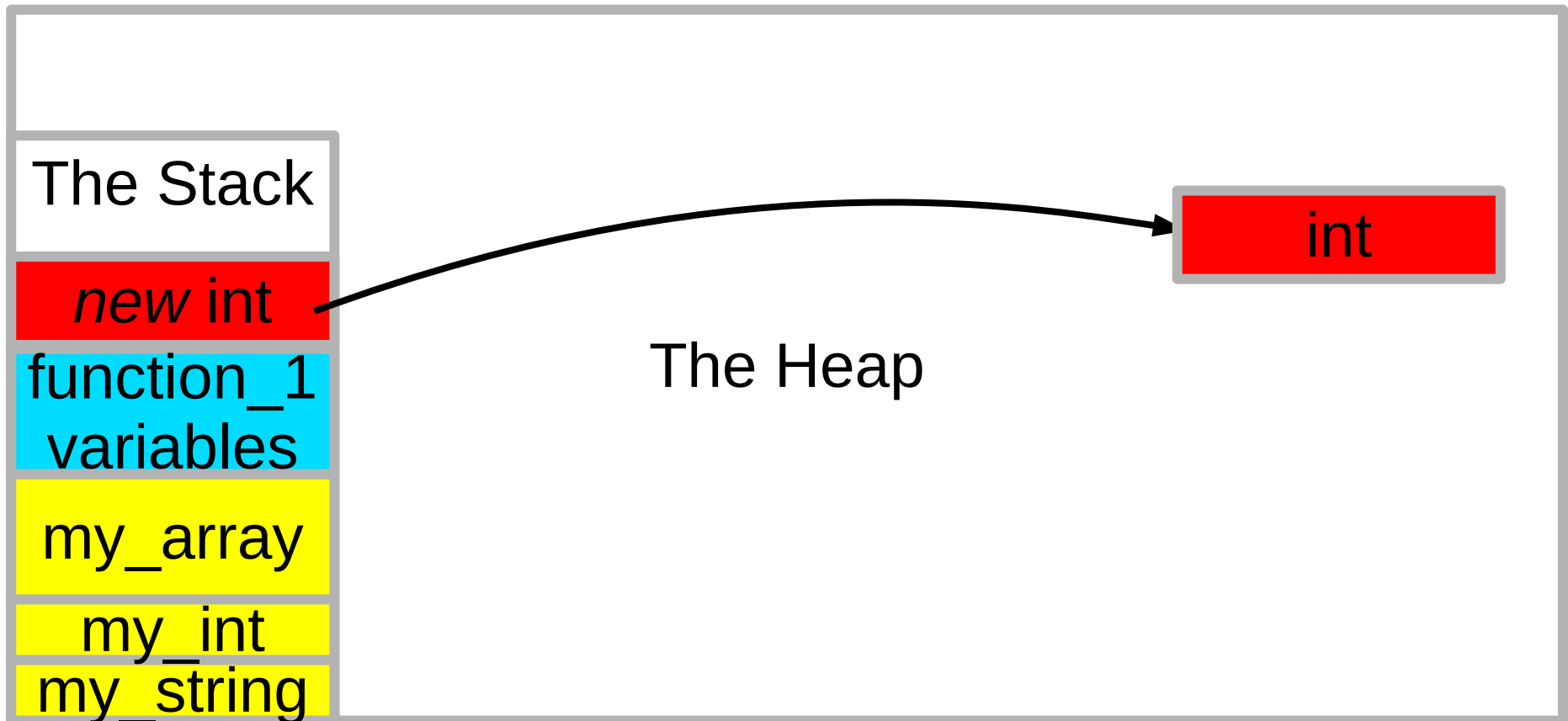




# Stack vs. Heap

## Using *new* and *delete* in C++

- The heap is all non-allocated space in memory
- Using *new* allocates space on the heap for a variable
- *delete* frees the space allocated by *new*



# Stack vs. Heap

## Using *new* and *delete* in C++

- Technically, you don't have to use *delete*

# Stack vs. Heap

## Using *new* and *delete* in C++

- Technically, you don't have to use *delete*
  - But what problems could this cause?

# Stack vs. Heap

## Using *new* and *delete* in C++

- Technically, you don't have to use *delete*
  - But what problems could this cause?
    - Memory can't be used again while your program is running

# Stack vs. Heap

## Using *new* and *delete* in C++

- Technically, you don't have to use *delete*
  - But what problems could this cause?
    - Memory can't be used again while your program is running
      - It can't even be used by other programs

# Stack vs. Heap

## Using *new* and *delete* in C++

- Technically, you don't have to use *delete*
  - But what problems could this cause?
    - Memory can't be used again while your program is running
      - It can't even be used by other programs
  - It's a good idea to *delete* things when you're done using them so you're not wasting memory