2.3 () Generalized dynamic programming for multiple sequence alignment

[edit] (https://github.com/gregcaporaso/An-Introduction-to-Applied-Bioinformatics/edit/master/book/fundamentals/multiple-sequence-alignment.md#L2)

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Until now we worked with alignments between two sequences, but it is likely that you will want to align many sequences at the same time. For example, if you are trying to gain insight on the evolutionary relationships between all of the 16S bacterial genes in a given sample, it would be time consuming and very inefficient to compare them two at a time. It would be more efficient and useful to compare all of the 16S sequences from the bacteria in the same alignment. In the pariwise sequence alignment chapter, we went over dynamic programming algorithms. It's possible to generalize Smith-Waterman and Needleman-Wunsch, the dynamic programming algorithms that we explored for pairwise sequence aligment, to identify the optimal alignment of more than two sequences. Remember that our scoring scheme for pairwise alignment with Smith-Waterman looked like the following:

$$F(0,0) = 0$$
$$F(i,0) = F(i-1,0) - d$$
$$F(0,j) = F(0,j-1) - d$$

$$F(i,j) = max \begin{pmatrix} F(i-1,j-1) + s(c_i, c_j) \\ F(i-1,j) - d \\ F(i,j-1) - d) \end{pmatrix}$$

To generalize this to three sequences, we could create 3×3 scoring, dynamic programming, and traceback matrices. Our scoring scheme would then look like the following:

$$F(0,0,0) = 0$$
$$F(i,0,0) = F(i-1,0,0) - d$$
$$F(0,j,0) = F(0,j-1,0) - d$$
$$F(0,0,k) = F(0,0,k-1) - d$$

$$F(i,j,k) = max \begin{pmatrix} F(i-1,j-1,k-1) + s(c_i,c_j) + s(c_i,c_k) + s(c_j,c_k) \\ F(i,j-1,k-1) + s(c_j,c_k) - d \\ F(i-1,j,k-1) + s(c_i,c_k) - d \\ F(i-1,j-1,k) + s(c_i,c_j) - d \\ F(i,j,k-1) - 2d \\ F(i,j-1,k) - 2d \\ F(i-1,j,k) - 2d \end{pmatrix}$$

However the complexity of this algorithm is much worse than for pairwise alignment. For pairwise alignment, remember that if aligning two sequences of lengths m and n, the runtime of the algorithm will be proportional to $m \times n$. If n is longer than or as long as m, we simplify the statement to say that the runtime of the algorithm will be be proportional to n^2. This curve has a pretty scary trajectory: runtime for pairwise alignment with dynamic programming is said to scale quadratically.

In [1]:

```
%pylab inline
from __future__ import division, print_function
from functools import partial
from IPython.core import page
page.page = print
```

```
Populating the interactive namespace from numpy and matplotlib
```

In [2]:

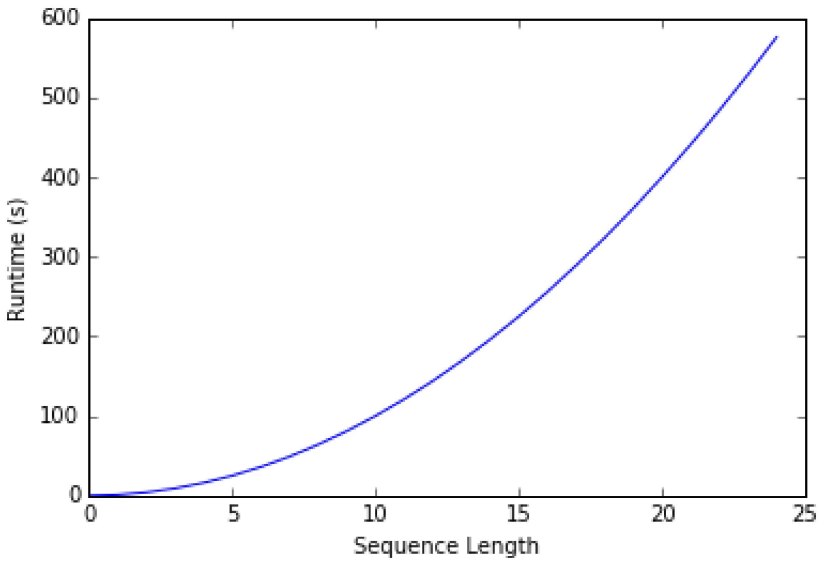
```
import matplotlib.pyplot as plt

seq_lengths = range(25)
s2_times = [t ** 2 for t in range(25)]

plt.plot(range(25), s2_times)
plt.xlabel('Sequence Length')
plt.ylabel('Runtime (s)')
```

Out[2]:

```
<matplotlib.text.Text at 0x7f0516f155f8>
```



The exponent in the n^2 term comes from the fact that, in pairwise alignment, if we assume our sequences are both of length n, there are $n \times n$ cells to fill in in the dynamic programming matrix. If we were to generalize either Smith-Waterman or Needleman-Wunsch to three sequences, we would need to create a 3 dimensional array to score and trace back the alignment. For sequences of length n, we would therefore have $n \times n \times n$ cells to fill in, and our runtime versus sequence length curve would look like the following.

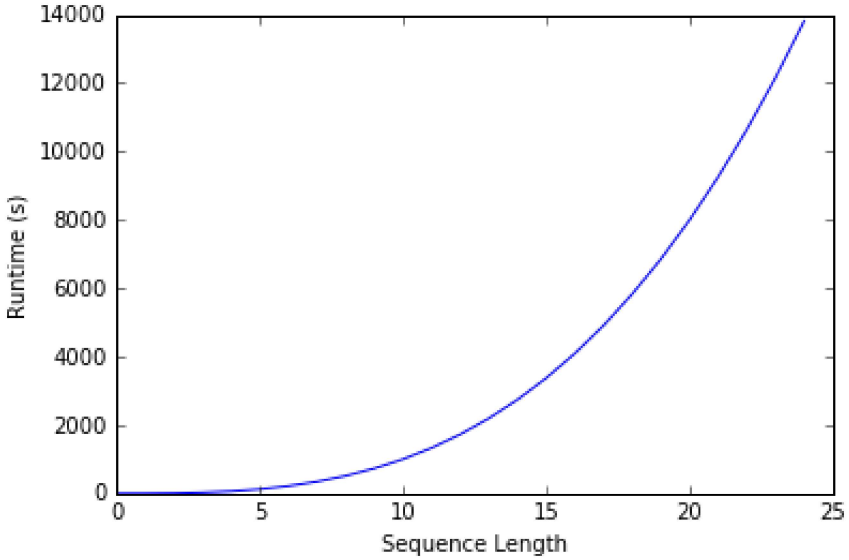
In [3]:

```
s3_times = [t ** 3 for t in range(25)]

plt.plot(range(25), s3_times)
plt.xlabel('Sequence Length')
plt.ylabel('Runtime (s)')
```

Out[3]:

```
<matplotlib.text.Text at 0x7f0516a342b0>
```



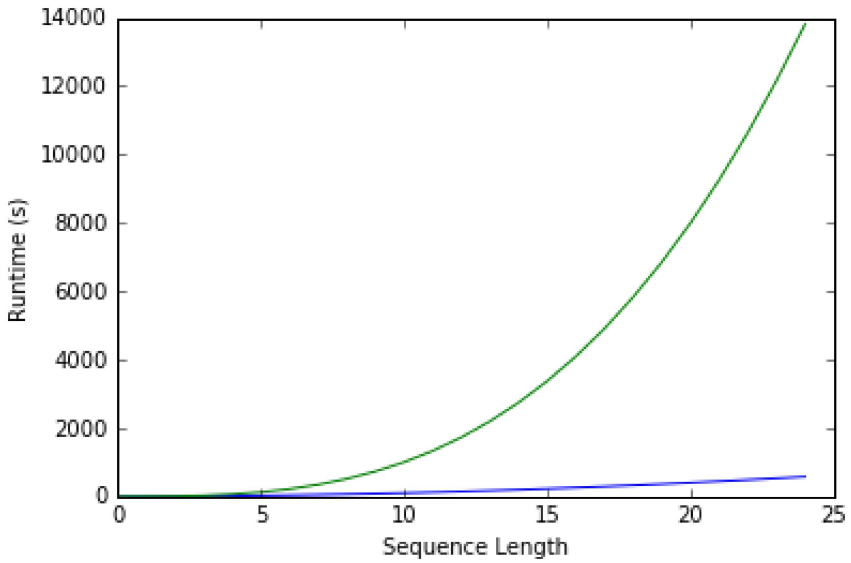
That curve looks steeper than the curve for pairwise alignment, and the values on the y-axis are bigger, but it's not really clear how much of a problem this is until we plot runtime for three sequences in the context of the run times for pairwise alignment.

In [4]:

```
plt.plot(range(25), s2_times)
plt.plot(range(25), s3_times)
plt.xlabel('Sequence Length')
plt.ylabel('Runtime (s)')
```

Out[4]:

```
<matplotlib.text.Text at 0x7f0516a10e48>
```



And for four sequences:

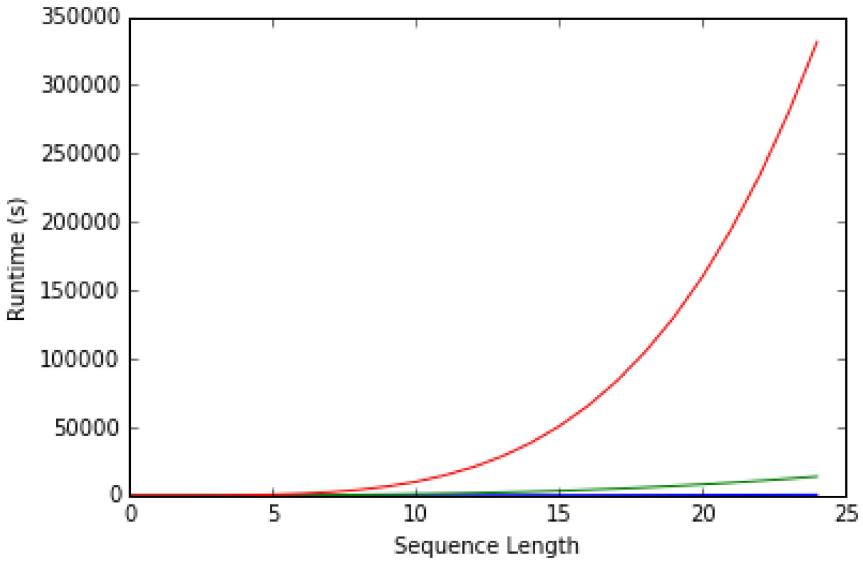
In [5]:

```
s4_times = [t ** 4 for t in range(25)]

plt.plot(range(25), s2_times)
plt.plot(range(25), s3_times)
plt.plot(range(25), s4_times)
plt.xlabel('Sequence Length')
plt.ylabel('Runtime (s)')
```

Out[5]:

```
<matplotlib.text.Text at 0x7f0516907c88>
```



We clearly have a problem here, and that is that the runtime for multiple sequence alignment using full dynamic programming algorithms grows exponentially with the number of sequences to be aligned. If n is our sequence length, and s is the number of sequences, that means that runtime is proportional to n^s. In pairwise alignment, s is always equal to 2, so the problem is more manangeable. However, for the general case of s sequences, we really can't even consider Smith-Waterman or Needleman-Wunsch for more than just a few sequences. The pattern in the plots above should illustrate why.

As we explored with database searching, we need to figure out how to align fewer sequences. This is where *progressive alignment* comes in.

2.3.1 Progressive alignment

[edit] (https://github.com/gregcaporaso/An-Introduction-to-Applied-Bioinformatics/edit/master/book/fundamentals/multiple-sequence-alignment.md#L98)

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In progressive alignment, the problem of exponential growth of runtime and space is managed by selectively aligning pairs of sequences, and aligning alignments of sequences. What we typically do is identify a pair of closely related sequences, and align those. Then, we identify the next most closely related sequence to that initial pair, and align that sequence to the alignment. This concept of aligning a sequence to an alignment is new, and we'll come back to it in just a few minutes. The other concept of identifying the most closely related sequences, and then the next most closely related sequence, and so on should sound familiar. It effectively means that we're traversing a tree. And herein lies our problem: we need a tree to efficiently align multiple sequences, but we need an alignment to build a good tree.

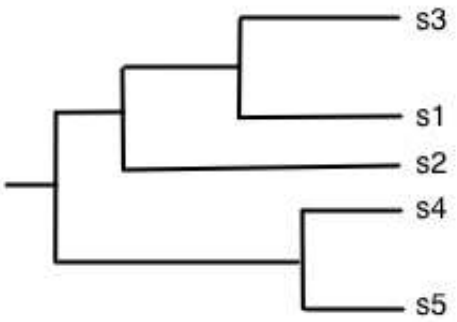
You probably have two burning questions in your mind right now:

1. How do we build a tree to guide the alignment process, if we need an alignment to build a good tree?
2. How do we align a sequence to an alignment, or an alignment to an alignment?

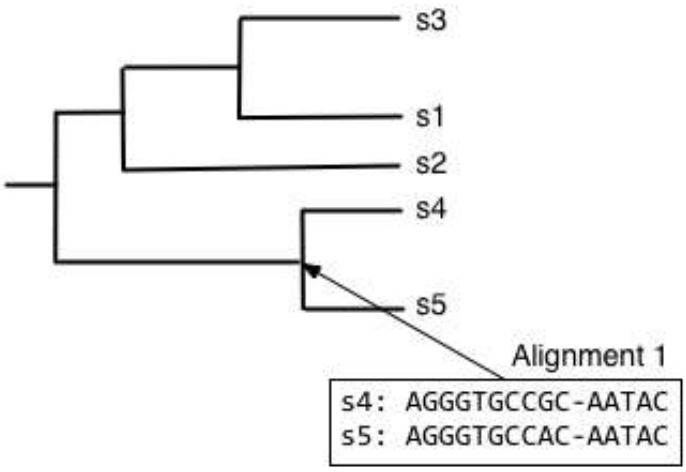
We'll explore both of those through-out the rest of this notebook. First, let's cover the process of progressive multiple sequence alignment, just assuming for a moment that we know how to do both of those things.

The process of progressive multiple sequence alignment could look like the following. First, we start with some sequences and a tree representing the relationship between those sequences. We'll call this our guide tree, because it's going to guide us through the process of multiple sequence alignment. In progressive multiple sequence alignment, we build a multiple sequence alignment for each internal node of the tree, where the alignment at a given internal node contains all of the sequences in the clade defined by that node.

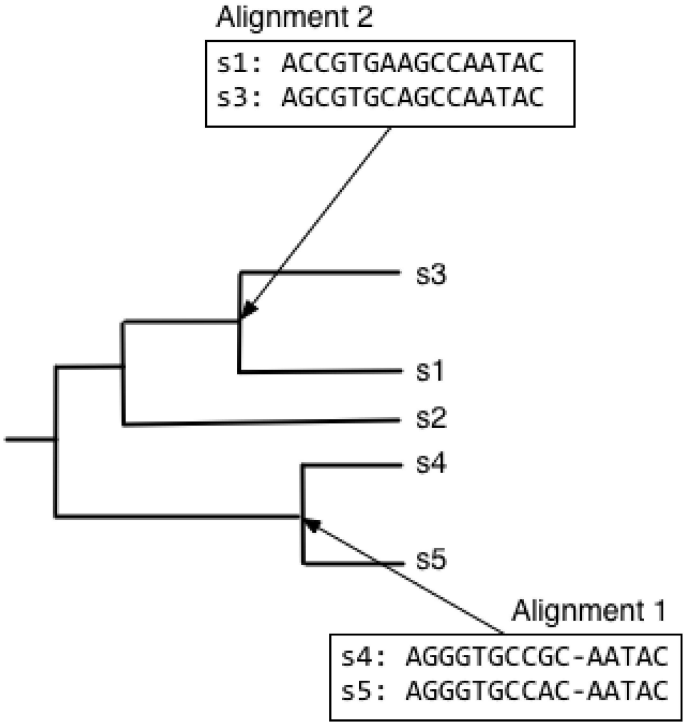
```
s1: ACCGTGAAGCCAATAC
s2: ACGTGCAACCATTAC
s3: AGCGTGCAGCCAATAC
s4: AGGGTGCCGCAATAC
s5: AGGGTGCCACAATAC
```



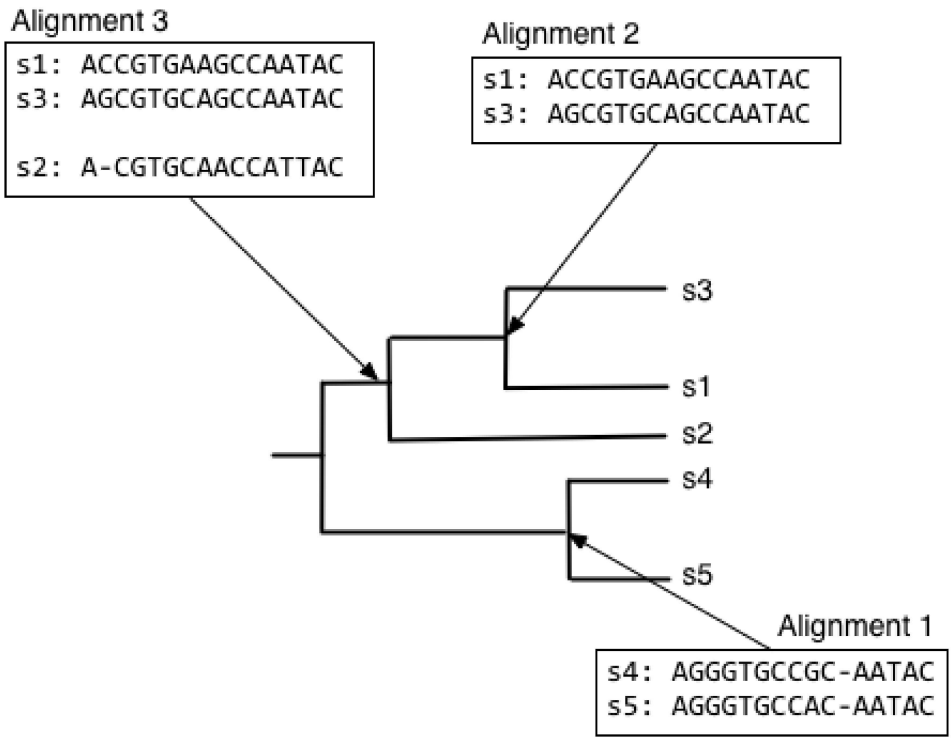
Starting from the root node, descend the bottom branch of the tree until you get to the an internal node. If an alignment hasn't been constructed for that node yet, continue descending the tree until to get to a pair of nodes. In this case, we follow the two branches to the tips. We then align the sequences at that pair of tips (usually with Needleman-Wunsch, for multiple sequence alignment), and assign that alignment to the node connecting those tips.



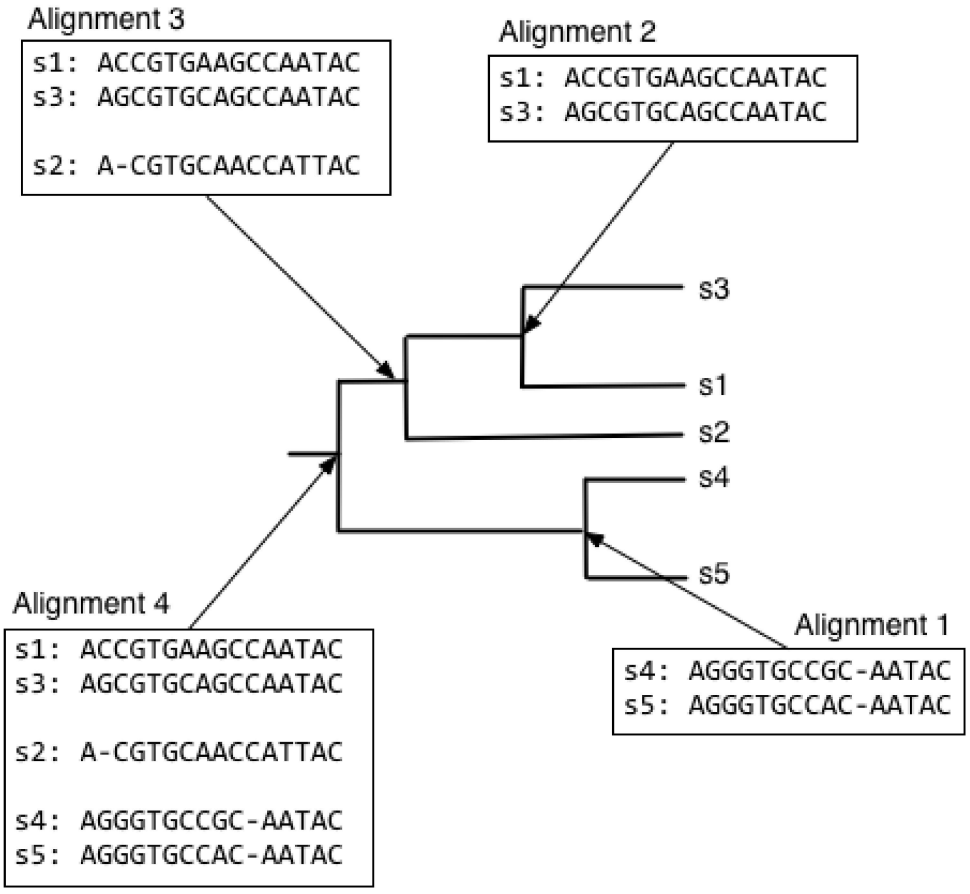
Next, we want to find what to align the resulting alignment to, so start from the root node and descend the top branch of the tree. When you get to the next node, determine if an alignment has already been created for that node. If not, our job is to build that alignment so we have something to align against. In this case, that means that we need to align `s1`, `s2`, and `s3`. We can achieve this by aligning `s1` and `s3` first, to get the alignment at the internal node connecting them.



We can next align the alignment of `s1` and `s3` with `s2`, to get the alignment at the internal node connecting those clades.



And finally, we can compute the alignment at the root node of the tree, by aligning the alignment of `s1`, `s2`, and `s3` with the alignment of `s4` and `s5`.



The alignment at the root node is our multiple sequence alignment.

2.3.1.1 Building the guide tree
[edit] (https://github.com/gregcaporaso/An-Introduction-to-Applied-Bioinformatics/edit/master/book/fundamentals/multiple-sequence-alignment.md#L131)

Let's address the first of our outstanding questions. I mentioned above that *we need an alignment to build a good tree*. The key word here is *good*. We can build a very rough tree - one that we would never want to present as representing the actual relationships between the sequences in question - without first aligning the sequences. Remember that building a UPGMA tree requires only a distance matrix, so if we can find a non-alignment-dependent way to compute distances between the sequences, we can build a rough UPGMA tree from them.

Let's compute distances between the sequences based on their *word* composition. We'll define a *word* here as k adjacent characters in the sequence. We can then define a function that will return all of the words in a sequence as follows. These words can be defined as being overlapping, or non-overlapping. We'll go with overlapping for this example, as the more words we have, the better our guide tree should be.

In [6]:

```
from skbio import DNA
%psource DNA.iter_kmers
```

```
    @stable(as_of="0.4.0")
    def iter_kmers(self, k, overlap=True):
        """Generate kmers of length `k` from this sequence.

        Parameters
        ----------
        k : int
            The kmer length.
        overlap : bool, optional
            Defines whether the kmers should be overlapping or not.

        Yields
        ------
        Sequence
            kmer of length `k` contained in this sequence.

        Raises
        ------
        ValueError
            If `k` is less than 1.

        Examples
        --------
        >>> from skbio import Sequence
        >>> s = Sequence('ACACGACGTT')
        >>> for kmer in s.iter_kmers(4, overlap=False):
        ...     str(kmer)
        'ACAC'
        'GACG'
        >>> for kmer in s.iter_kmers(3, overlap=True):
        ...     str(kmer)
        'ACA'
        'CAC'
        'ACG'
        'CGA'
        'GAC'
        'ACG'
        'CGT'
        'GTT'

        """
        if k < 1:
            raise ValueError("k must be greater than 0.")

        if overlap:
            step = 1
            count = len(self) - k + 1
        else:
            step = k
            count = len(self) // k

        if self.has_positional_metadata():
            for i in range(0, len(self) - k + 1, step):
                yield self[i:i+k]
        # Optimized path when no positional metadata
        else:
            kmers = np.lib.stride_tricks.as_strided(
```

```
                self._bytes, shape=(k, count), strides=(1, step)).T
            for s in kmers:
                yield self._to(sequence=s)
```

In [7]:

```
for e in DNA("ACCGGTGACCAGTTGACCAGTA").iter_kmers(3):
    print(e)
```

```
ACC
CCG
CGG
GGT
GTG
TGA
GAC
ACC
CCA
CAG
AGT
GTT
TTG
TGA
GAC
ACC
CCA
CAG
AGT
GTA
```

In [8]:

```
for e in DNA("ACCGGTGACCAGTTGACCAGTA").iter_kmers(7):
    print(e)
```

```
ACCGGTG
CCGGTGA
CGGTGAC
GGTGACC
GTGACCA
TGACCAG
GACCAGT
ACCAGTT
CCAGTTG
CAGTTGA
AGTTGAC
GTTGACC
TTGACCA
TGACCAG
GACCAGT
ACCAGTA
```

In [9]:

```
for e in DNA("ACCGGTGACCAGTTGACCAGTA").iter_kmers(3, overlap=False):
    print(e)
```

```
ACC
GGT
GAC
CAG
TTG
ACC
AGT
```

If we then have two sequences, we can compute the word counts for each and define a distance between the sequences as the fraction of words that are unique to either sequence.

In [10]:

```
from iab.algorithms import kmer_distance
%psource kmer_distance
```

```
    def kmer_distance(sequence1, sequence2, k=3, overlap=True):
        """Compute the kmer distance between a pair of sequences

        Parameters
        ----------
        sequence1 : skbio.Sequence
        sequence2 : skbio.Sequence
        k : int, optional
            The word length.
        overlapping : bool, optional
            Defines whether the k-words should be overlapping or not
            overlapping.

        Returns
        -------
        float
            Fraction of the set of k-mers from both sequence1 and
            sequence2 that are unique to either sequence1 or
            sequence2.

        Raises
        ------
        ValueError
            If k < 1.

        Notes
        -----
        k-mer counts are not incorporated in this distance metric.

        """
        sequence1_kmers = set(map(str, sequence1.iter_kmers(k, overlap)))
        sequence2_kmers = set(map(str, sequence2.iter_kmers(k, overlap)))
        all_kmers = sequence1_kmers | sequence2_kmers
        shared_kmers = sequence1_kmers & sequence2_kmers
        number_unique = len(all_kmers) - len(shared_kmers)
        fraction_unique = number_unique / len(all_kmers)
        return fraction_unique
```

We can then use this as a distance function...

In [11]:

```
s1 = DNA("ACCGGTGACCAGTTGACCAGT")
s2 = DNA("ATCGGTACCGGTAGAAGT")
s3 = DNA("GGTACCAAATAGAA")

print(s1.distance(s2, kmer_distance))
print(s1.distance(s3, kmer_distance))
```

```
0.75
0.8571428571428571
```

If we wanted to override the default to create (for example) a 5-mer distance function, we could use `functools.partial`.

In [12]:

```
fivemer_distance = partial(kmer_distance, k=5)

s1 = DNA("ACCGGTGACCAGTTGACCAGT")
s2 = DNA("ATCGGTACCGGTAGAAGT")
s3 = DNA("GGTACCAAATAGAA")

print(s1.distance(s2, fivemer_distance))
print(s1.distance(s3, fivemer_distance))
```

```
0.9166666666666666
1.0
```

We can now apply one of these functions to build a distance matrix for a set of sequences that we want to align.

In [13]:

```
query_sequences = [DNA("ACCGGTGACCAGTTGACCAGT", {"id": "s1"}),
                   DNA("ATCGGTACCGGTAGAAGT", {"id": "s2"}),
                   DNA("GGTACCAAATAGAA", {"id": "s3"}),
                   DNA("GGCACCAAACAGAA", {"id": "s4"}),
                   DNA("GGCCCACTGAT", {"id": "s5"})]
```

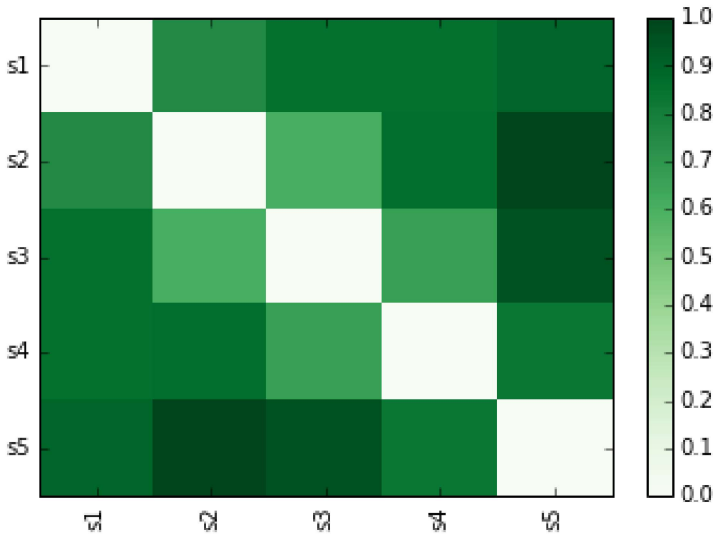
In [14]:

```
from skbio import DistanceMatrix

guide_dm = DistanceMatrix.from_iterable(query_sequences, metric=kmer_distance, key
='id')
```

scikit-bio also has some basic visualization functionality for these objects. For example, we can easily visualize this object as a heatmap.

In [15]:

```
fig = guide_dm.plot(cmap='Greens')
```

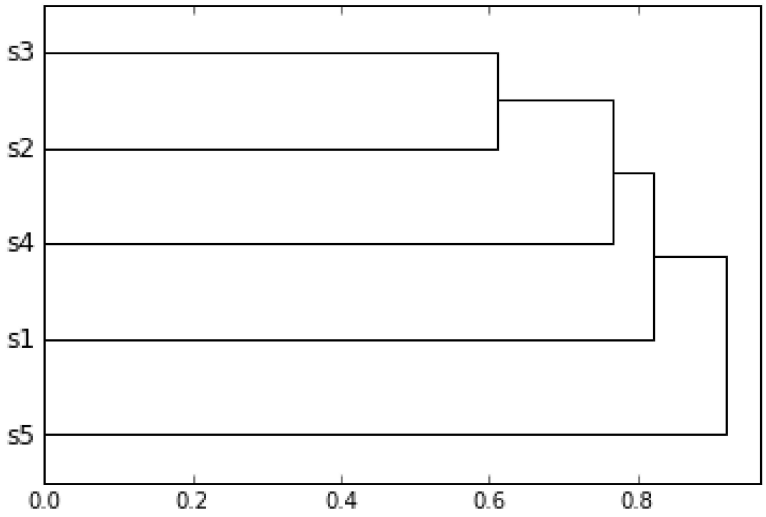


We can next use some functionality from SciPy to cluster the sequences with UPGMA, and print out a dendrogram.

In [16]:

```
from scipy.cluster.hierarchy import average, dendrogram, to_tree

for q in query_sequences:
    print(q)

guide_lm = average(guide_dm.condensed_form())
guide_d = dendrogram(guide_lm, labels=guide_dm.ids, orientation='right',
                     link_color_func=lambda x: 'black')
guide_tree = to_tree(guide_lm)
```

```
ACCGGTGACCAGTTGACCAGT
ATCGGTACCGGTAGAAGT
GGTACCAAATAGAA
GGCACCAAACAGAA
GGCCCACTGAT
```



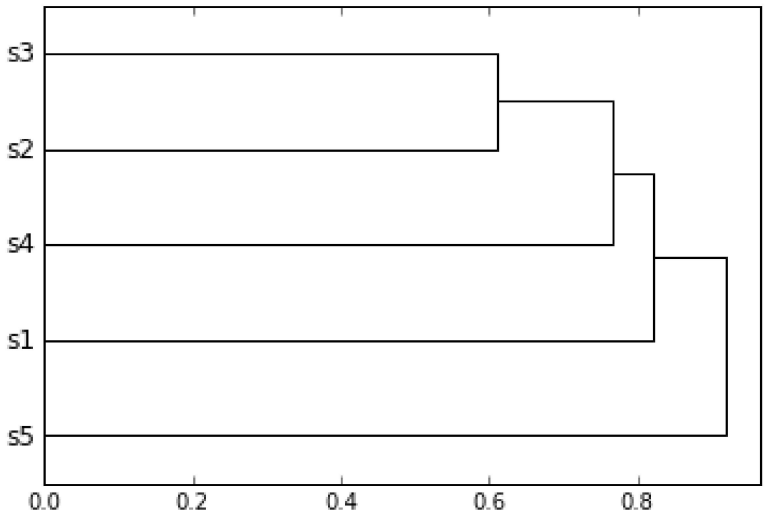
In [17]:

```
from iab.algorithms import guide_tree_from_sequences
%psource guide_tree_from_sequences
```

```
def guide_tree_from_sequences(sequences,
                              metric=kmer_distance,
                              display_tree = False):
    """ Build a UPGMA tree by applying metric to sequences

    Parameters
    ----------
    sequences : list of skbio.Sequence objects (or subclasses)
      The sequences to be represented in the resulting guide tree.
    metric : function
      Function that returns a single distance value when given a pair
of
      skbio.Sequence objects.
    display_tree : bool, optional
      Print the tree before returning.

    Returns
    -------
    skbio.TreeNode

    """
    guide_dm = DistanceMatrix.from_iterable(
                    sequences, metric=metric, key='id')
    guide_lm = sp.cluster.hierarchy.average(guide_dm.condensed_form
())
    guide_tree = to_tree(guide_lm)
    if display_tree:
        guide_d = sp.cluster.hierarchy.dendrogram(guide_lm, labels=gu
ide_dm.ids, orientation='right',
                link_color_func=lambda x: 'black')
    return guide_tree
```

In [18]:

```
t = guide_tree_from_sequences(query_sequences, display_tree=True)
```



We now have a guide tree, so we can move on to the next step of progressive alignment.

2.3.1.2 Generalization of Needleman-Wunsch (with affine gap scoring) for progressive multiple sequence alignment

[edit] (https://github.com/gregcaporaso/An-Introduction-to-Applied-Bioinformatics/edit/master/book/fundamentals/multiple-sequence-alignment.md#L235)

Next, we'll address our second burning question: aligning alignments. As illustrated above, there are basically three different types of pairwise alignment we need to support for progressive multiple sequenence alignment with Needleman-Wunsch. These are:

1. Alignment of a pair of sequences.
2. Alignment of a sequence and an alignment.
3. Alignment of a pair of alignments.

Standard Needleman-Wunsch supports the first, and it is very easy to generalize it to support the latter two. The only change that is necessary is in how the alignment of two non-gap characters is scored. Recall that we previously scored an alignment of two characters by looking up the score of subsitution from one to the other in a substitution matrix. To adapt this for aligning a sequence to an alignment, or for aligning an alignment to an alignment, we compute this subsitution as the average score of aligning the pairs of characters.

For example, if we want to align the alignment column from $aln1$:

```
A
C
```

to the alignment column from $aln2$:

```
T
G
```

we could compute the subsitution score using the matrix m as:

$$s = \frac{m[A][T] + m[A][G] + m[C][T] + m[C][G]}{aln1_{length} \times aln2_{length}}$$

The following code adapts our implementation of Needleman-Wunsh to support aligning a sequence to an alignment, or aligning an alignment to an alignment.

In [19]:

```
from iab.algorithms import format_dynamic_programming_matrix, format_traceback_mat
rix
from skbio.alignment._pairwise import _compute_score_and_traceback_matrices

%psource _compute_score_and_traceback_matrices
```

```
    def _compute_score_and_traceback_matrices(
            aln1, aln2, gap_open_penalty, gap_extend_penalty, substitutio
    n_matrix,
            new_alignment_score=-np.inf, init_matrices_f=_init_matrices_n
    w,
            penalize_terminal_gaps=True, gap_substitution_score=0):
        """Return dynamic programming (score) and traceback matrices.

        A note on the ``penalize_terminal_gaps`` parameter. When this val
    ue is
        ``False``, this function is no longer true Smith-Waterman/Needlem
    an-Wunsch
        scoring, but when ``True`` it can result in biologically irreleva
    nt
        artifacts in Needleman-Wunsch (global) alignments. Specifically,
    if one
        sequence is longer than the other (e.g., if aligning a primer seq
    uence to
        an amplification product, or searching for a gene in a genome) th
    e shorter
        sequence will have a long gap inserted. The parameter is ``True``
    by
        default (so that this function computes the score and traceback m
    atrices as
        described by the original authors) but the global alignment wrapp
    ers pass
        ``False`` by default, so that the global alignment API returns th
    e result
        that users are most likely to be looking for.

        """
        aln1_length = aln1.shape.position
        aln2_length = aln2.shape.position
        # cache some values for quicker/simpler access
        aend = _traceback_encoding['alignment-end']
        match = _traceback_encoding['match']
        vgap = _traceback_encoding['vertical-gap']
        hgap = _traceback_encoding['horizontal-gap']

        new_alignment_score = (new_alignment_score, aend)

        # Initialize a matrix to use for scoring the alignment and for tr
    acing
        # back the best alignment
        score_matrix, traceback_matrix = init_matrices_f(
            aln1, aln2, gap_open_penalty, gap_extend_penalty)

        # Iterate over the characters in aln2 (which corresponds to the v
    ertical
        # sequence in the matrix)
        for aln2_pos, aln2_chars in enumerate(aln2.iter_positions(), 1):
            aln2_chars = str(aln2_chars)

            # Iterate over the characters in aln1 (which corresponds to t
    he
            # horizontal sequence in the matrix)
            for aln1_pos, aln1_chars in enumerate(aln1.iter_positions(),
```

```
1):
            aln1_chars = str(aln1_chars)

            # compute the score for a match/mismatch
            substitution_score = _compute_substitution_score(
                aln1_chars, aln2_chars, substitution_matrix,
                gap_substitution_score, aln1.dtype.gap_chars)

            diag_score = \
                (score_matrix[aln2_pos-1, aln1_pos-1] + substitution_
score,
                 match)

            # compute the score for adding a gap in aln2 (vertical)
            if not penalize_terminal_gaps and (aln1_pos == aln1_lengt
h):
                # we've reached the end of aln1, so adding vertical g
aps
                # (which become gaps in aln1) should no longer
                # be penalized (if penalize_terminal_gaps == False)
                up_score = (score_matrix[aln2_pos-1, aln1_pos], vgap)
            elif traceback_matrix[aln2_pos-1, aln1_pos] == vgap:
                # gap extend, because the cell above was also a gap
                up_score = \
                    (score_matrix[aln2_pos-1, aln1_pos] - gap_extend_
penalty,
                     vgap)
            else:
                # gap open, because the cell above was not a gap
                up_score = \
                    (score_matrix[aln2_pos-1, aln1_pos] - gap_open_pe
nalty,
                     vgap)

            # compute the score for adding a gap in aln1 (horizontal)
            if not penalize_terminal_gaps and (aln2_pos == aln2_lengt
h):
                # we've reached the end of aln2, so adding horizontal
gaps
                # (which become gaps in aln2) should no longer
                # be penalized (if penalize_terminal_gaps == False)
                left_score = (score_matrix[aln2_pos, aln1_pos-1], hga
p)
            elif traceback_matrix[aln2_pos, aln1_pos-1] == hgap:
                # gap extend, because the cell to the left was also a
gap
                left_score = \
                    (score_matrix[aln2_pos, aln1_pos-1] - gap_extend_
penalty,
                     hgap)
            else:
                # gap open, because the cell to the left was not a ga
p
                left_score = \
                    (score_matrix[aln2_pos, aln1_pos-1] - gap_open_pe
nalty,
                     hgap)
```

```
            # identify the largest score, and use that information to
populate
            # the score and traceback matrices
            best_score = _first_largest([new_alignment_score, left_sc
ore,
                                         diag_score, up_score])
            score_matrix[aln2_pos, aln1_pos] = best_score[0]
            traceback_matrix[aln2_pos, aln1_pos] = best_score[1]

    return score_matrix, traceback_matrix
```

In [20]:

```
from skbio.alignment._pairwise import _traceback
%psource _traceback
```

```
    def _traceback(traceback_matrix, score_matrix, aln1, aln2, start_row,
                   start_col):
        # cache some values for simpler reference
        aend = _traceback_encoding['alignment-end']
        match = _traceback_encoding['match']
        vgap = _traceback_encoding['vertical-gap']
        hgap = _traceback_encoding['horizontal-gap']
        gap_character = aln1.dtype.default_gap_char

        # initialize the result alignments
        aln1_sequence_count = aln1.shape.sequence
        aligned_seqs1 = [[] for e in range(aln1_sequence_count)]

        aln2_sequence_count = aln2.shape.sequence
        aligned_seqs2 = [[] for e in range(aln2_sequence_count)]

        current_row = start_row
        current_col = start_col

        best_score = score_matrix[current_row, current_col]
        current_value = None

        while current_value != aend:
            current_value = traceback_matrix[current_row, current_col]

            if current_value == match:
                for aligned_seq, input_seq in zip(aligned_seqs1, aln1):
                    aligned_seq.append(str(input_seq[current_col-1]))
                for aligned_seq, input_seq in zip(aligned_seqs2, aln2):
                    aligned_seq.append(str(input_seq[current_row-1]))
                current_row -= 1
                current_col -= 1
            elif current_value == vgap:
                for aligned_seq in aligned_seqs1:
                    aligned_seq.append(gap_character)
                for aligned_seq, input_seq in zip(aligned_seqs2, aln2):
                    aligned_seq.append(str(input_seq[current_row-1]))
                current_row -= 1
            elif current_value == hgap:
                for aligned_seq, input_seq in zip(aligned_seqs1, aln1):
                    aligned_seq.append(str(input_seq[current_col-1]))
                for aligned_seq in aligned_seqs2:
                    aligned_seq.append(gap_character)
                current_col -= 1
            elif current_value == aend:
                continue
            else:
                raise ValueError(
                    "Invalid value in traceback matrix: %s" % current_val
    ue)

        for i in range(aln1_sequence_count):
            aligned_seq = ''.join(aligned_seqs1[i][::-1])
            constructor = aln1.dtype
            aligned_seqs1[i] = constructor(aligned_seq)

        for i in range(aln2_sequence_count):
```

```
            aligned_seq = ''.join(aligned_seqs2[i][::-1])
            constructor = aln2.dtype
            aligned_seqs2[i] = constructor(aligned_seq)

        return aligned_seqs1, aligned_seqs2, best_score, current_col, cur
    rent_row
```

In [21]:

```
from skbio.alignment import global_pairwise_align_nucleotide
%psource global_pairwise_align_nucleotide
```

```
@experimental(as_of="0.4.0")
def global_pairwise_align_nucleotide(seq1, seq2, gap_open_penalty=5,
                                     gap_extend_penalty=2,
                                     match_score=1, mismatch_score=-
2,
                                     substitution_matrix=None,
                                     penalize_terminal_gaps=False):
    """Globally align nucleotide seqs or alignments with Needleman-Wu
nsch

    Parameters
    ----------
    seq1 : DNA, RNA, or TabularMSA[DNA|RNA]
        The first unaligned sequence(s).
    seq2 : DNA, RNA, or TabularMSA[DNA|RNA]
        The second unaligned sequence(s).
    gap_open_penalty : int or float, optional
        Penalty for opening a gap (this is substracted from previous
best
        alignment score, so is typically positive).
    gap_extend_penalty : int or float, optional
        Penalty for extending a gap (this is substracted from previou
s best
        alignment score, so is typically positive).
    match_score : int or float, optional
        The score to add for a match between a pair of bases (this is
added
        to the previous best alignment score, so is typically positiv
e).
    mismatch_score : int or float, optional
        The score to add for a mismatch between a pair of bases (this
is
        added to the previous best alignment score, so is typically
        negative).
    substitution_matrix: 2D dict (or similar)
        Lookup for substitution scores (these values are added to the
        previous best alignment score). If provided, this overrides
        ``match_score`` and ``mismatch_score``.
    penalize_terminal_gaps: bool, optional
        If True, will continue to penalize gaps even after one sequen
ce has
        been aligned through its end. This behavior is true Needleman
-Wunsch
        alignment, but results in (biologically irrelevant) artifacts
when
        the sequences being aligned are of different length. This is
``False``
        by default, which is very likely to be the behavior you want
in all or
        nearly all cases.

    Returns
    -------
    tuple
        ``TabularMSA`` object containing the aligned sequences, align
ment score
        (float), and start/end positions of each input sequence (iter
```

```
able
        of two-item tuples). Note that start/end positions are indexe
s into the
        unaligned sequences.

    See Also
    --------
    local_pairwise_align
    local_pairwise_align_protein
    local_pairwise_align_nucleotide
    skbio.alignment.local_pairwise_align_ssw
    global_pairwise_align
    global_pairwise_align_protein

    Notes
    -----
    Default ``match_score``, ``mismatch_score``, ``gap_open_penalty``
and
    ``gap_extend_penalty`` parameters are derived from the NCBI BLAST
    Server [1]_.

    This function can be use to align either a pair of sequences, a p
air of
    alignments, or a sequence and an alignment.

    References
    ----------
    .. [1] http://blast.ncbi.nlm.nih.gov/Blast.cgi

    """
    for seq in seq1, seq2:
        if not isinstance(seq, (DNA, RNA, TabularMSA)):
            raise TypeError(
                "`seq1` and `seq2` must be DNA, RNA, or TabularMSA, n
ot type "
                "%r" % type(seq).__name__)
        if isinstance(seq, TabularMSA) and not issubclass(seq.dtype,
                                                          (DNA, RN
A)):
            raise TypeError(
                "`seq1` and `seq2` must be TabularMSA with DNA or RNA
dtype, "
                "not dtype %r" % seq.dtype.__name__)

    # use the substitution matrix provided by the user, or compute fr
om
    # match_score and mismatch_score if a substitution matrix was not
provided
    if substitution_matrix is None:
        substitution_matrix = \
            make_identity_substitution_matrix(match_score, mismatch_s
core)

    return global_pairwise_align(seq1, seq2, gap_open_penalty,
                                 gap_extend_penalty, substitution_mat
rix,
                                 penalize_terminal_gaps=penalize_term
```

```
inal_gaps)
```

For the sake of the examples below, I'm going to override one of the `global_pairwise_align_nucleotide` defaults to penalize terminal gaps. This effectively tells the algorithm that we know we have a collection of sequences that are homologous from beginning to end.

In [22]:

```
global_pairwise_align_nucleotide = partial(global_pairwise_align_nucleotide, penal
ize_terminal_gaps=True)
```

For example, we can still use this code to align pairs of sequences (but note that we now need to pass those sequences in as a pair of one-item lists):

In [23]:

```
aln1, _, _ = global_pairwise_align_nucleotide(query_sequences[0], query_sequences
[1])
print(aln1)
```

```
/home/iab/.conda/envs/iab/lib/python3.5/site-packages/skbio/alignmen
t/_pairwise.py:601: EfficiencyWarning: You're using skbio's python im
plementation of Needleman-Wunsch alignment. This is known to be very
slow (e.g., thousands of times slower than a native C implementatio
n). We'll be adding a faster version soon (see https://github.com/bio
core/scikit-bio/issues/254 to track progress on this).
  "to track progress on this).", EfficiencyWarning)
```

```
TabularMSA[DNA]
----------------------
Stats:
    sequence count: 2
    position count: 21
----------------------
ACCGGTGACCAGTTGACCAGT
ATCGGT-ACCGGTAGA--AGT
```

We can align that alignment to one of our other sequences.

In [24]:

```
aln1, _, _ = global_pairwise_align_nucleotide(aln1, query_sequences[2])
print(aln1)
```

```
/home/iab/.conda/envs/iab/lib/python3.5/site-packages/skbio/alignmen
t/_pairwise.py:601: EfficiencyWarning: You're using skbio's python im
plementation of Needleman-Wunsch alignment. This is known to be very
slow (e.g., thousands of times slower than a native C implementatio
n). We'll be adding a faster version soon (see https://github.com/bio
core/scikit-bio/issues/254 to track progress on this).
  "to track progress on this).", EfficiencyWarning)



TabularMSA[DNA]
----------------------
Stats:
    sequence count: 3
    position count: 21
----------------------
ACCGGTGACCAGTTGACCAGT
ATCGGT-ACCGGTAGA--AGT
---GGTACCAAATAGA--A--
```

Alternatively, we can align another pair of sequences:

In [25]:

```
aln2, _, _ = global_pairwise_align_nucleotide(query_sequences[2], query_sequences
[3])
print(aln2)
```

```
/home/iab/.conda/envs/iab/lib/python3.5/site-packages/skbio/alignmen
t/_pairwise.py:601: EfficiencyWarning: You're using skbio's python im
plementation of Needleman-Wunsch alignment. This is known to be very
slow (e.g., thousands of times slower than a native C implementatio
n). We'll be adding a faster version soon (see https://github.com/bio
core/scikit-bio/issues/254 to track progress on this).
  "to track progress on this).", EfficiencyWarning)

TabularMSA[DNA]
----------------------
Stats:
    sequence count: 2
    position count: 14
----------------------
GGTACCAAATAGAA
GGCACCAAACAGAA
```

And then align that alignment against our previous alignment:

In [26]:

```
aln3, _, _  = global_pairwise_align_nucleotide(aln1, aln2)
print(aln3)
```

```
/home/iab/.conda/envs/iab/lib/python3.5/site-packages/skbio/alignmen
t/_pairwise.py:601: EfficiencyWarning: You're using skbio's python im
plementation of Needleman-Wunsch alignment. This is known to be very
slow (e.g., thousands of times slower than a native C implementatio
n). We'll be adding a faster version soon (see https://github.com/bio
core/scikit-bio/issues/254 to track progress on this).
  "to track progress on this).", EfficiencyWarning)
```

```
TabularMSA[DNA]
----------------------
Stats:
    sequence count: 5
    position count: 21
----------------------
ACCGGTGACCAGTTGACCAGT
ATCGGT-ACCGGTAGA--AGT
---GGTACCAAATAGA--A--
---GGTACCAAATAGA----A
---GGCACCAAACAGA----A
```

2.3.1.3 Putting it all together: progressive multiple sequence alignment [edit] (https://github.com/gregcaporaso/An-Introduction-to-Applied-Bioinformatics/edit/master/book/fundamentals/multiple-sequence-alignment.md#L318)

We can now combine all of these steps to take a set of query sequences, build a guide tree, perform progressive multiple sequence alignment, and return the guide tree (as a SciPy linkage matrix) and the alignment.

In [27]:

```
from skbio import TreeNode
guide_tree = TreeNode.from_linkage_matrix(guide_lm, guide_dm.ids)
```

We can view the guide tree in newick format (http://scikit-bio.org/docs/latest/generated/skbio.io.newick.html) as follows:

In [28]:

```
print(guide_tree)
```

```
(s5:0.45975877193,(s1:0.410714285714,(s4:0.382575757576,(s2:0.3055555
55556,s3:0.305555555556):0.0770202020202):0.0281385281385):0.04904448
62155);
```

In [29]:

```
from iab.algorithms import progressive_msa
%psource progressive_msa
```

```
def progressive_msa(sequences, pairwise_aligner, guide_tree=None,
                    metric=kmer_distance):
    """ Perform progressive msa of sequences

    Parameters
    ----------
    sequences : skbio.SequenceCollection
        The sequences to be aligned.
    metric : function, optional
      Function that returns a single distance value when given a pair
of
      skbio.Sequence objects. This will be used to build a guide tree
if one
      is not provided.
    guide_tree : skbio.TreeNode, optional
        The tree that should be used to guide the alignment process.
    pairwise_aligner : function
        Function that should be used to perform the pairwise alignmen
ts,
        for example skbio.alignment.global_pairwise_align_nucleotide.
Must
        support skbio.Sequence objects or skbio.TabularMSA objects
        as input.

    Returns
    -------
    skbio.TabularMSA

    """

    if guide_tree is None:
        guide_dm = DistanceMatrix.from_iterable(
                        sequences, metric=metric, key='id')
        guide_lm = sp.cluster.hierarchy.average(guide_dm.condensed_fo
rm())
        guide_tree = TreeNode.from_linkage_matrix(guide_lm, guide_dm.
ids)

    seq_lookup = {s.metadata['id']: s for i, s in enumerate(sequence
s)}
    c1, c2 = guide_tree.children
    if c1.is_tip():
        c1_aln = seq_lookup[c1.name]
    else:
        c1_aln = progressive_msa(sequences, pairwise_aligner, c1)

    if c2.is_tip():
        c2_aln = seq_lookup[c2.name]
    else:
        c2_aln = progressive_msa(sequences, pairwise_aligner, c2)

    alignment, _, _ = pairwise_aligner(c1_aln, c2_aln)
    # this is a temporary hack as the aligners in skbio 0.4.1 are dro
pping
    # metadata - this makes sure that the right metadata is associate
d with
    # the sequence after alignment
```

```
    if isinstance(c1_aln, Sequence):
        alignment[0].metadata = c1_aln.metadata
        len_c1_aln = 1
    else:
        for i in range(len(c1_aln)):
            alignment[i].metadata = c1_aln[i].metadata
        len_c1_aln = len(c1_aln)
    if isinstance(c2_aln, Sequence):
        alignment[1].metadata = c2_aln.metadata
    else:
        for i in range(len(c2_aln)):
            alignment[len_c1_aln + i].metadata = c2_aln[i].metadata

    return alignment
```

In [30]:

```
msa = progressive_msa(query_sequences, pairwise_aligner=global_pairwise_align_nucl
eotide, guide_tree=guide_tree)
print(msa)
```

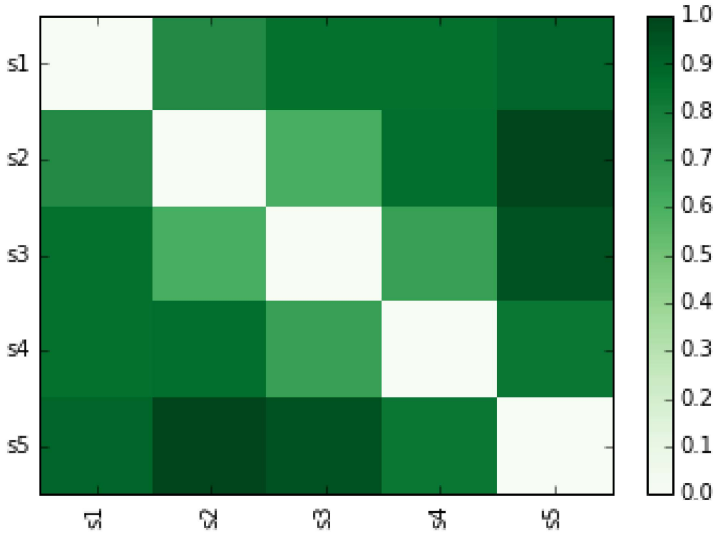
```
/home/iab/.conda/envs/iab/lib/python3.5/site-packages/skbio/alignmen
t/_pairwise.py:601: EfficiencyWarning: You're using skbio's python im
plementation of Needleman-Wunsch alignment. This is known to be very
slow (e.g., thousands of times slower than a native C implementatio
n). We'll be adding a faster version soon (see https://github.com/bio
core/scikit-bio/issues/254 to track progress on this).
  "to track progress on this).", EfficiencyWarning)
```

```
TabularMSA[DNA]
----------------------
Stats:
    sequence count: 5
    position count: 21
----------------------
---GGC--CCA-----CTGAT
ACCGGTGACCAGTTGACCAGT
---GGCACCAAACAGA--A--
ATCGGTACC-GGTAGA--AGT
---GGTACCAAATAGA--A--
```

We can now build a (hopefully) improved tree from our multiple sequence alignment. First we'll look at our original distance matrix again, and then the distance matrix generted from the progressive multiple sequence alignment.

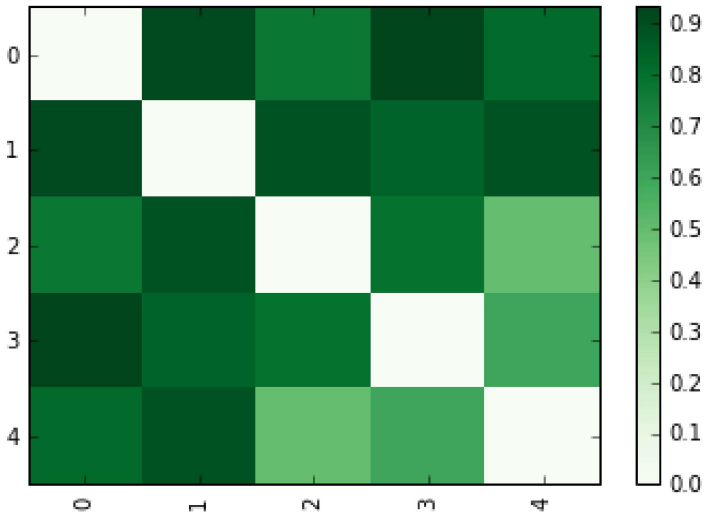
In [31]:

```
fig = guide_dm.plot(cmap='Greens')
```



In [32]:

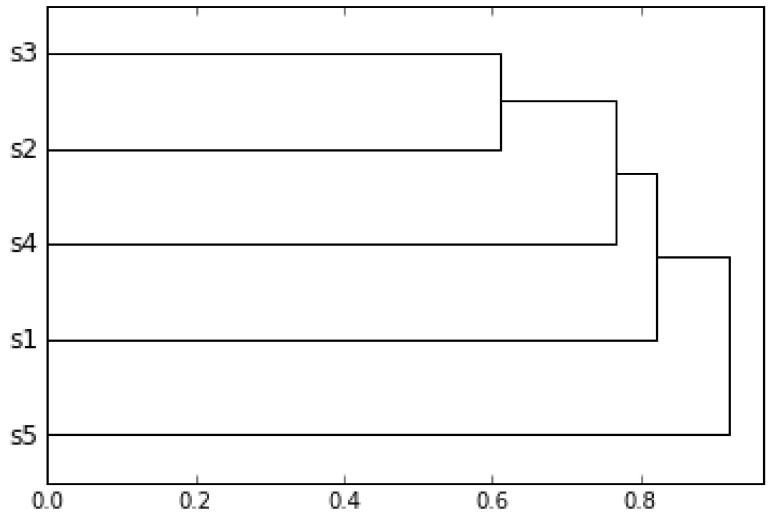
```
msa_dm = DistanceMatrix.from_iterable(msa, metric=kmer_distance)
fig = msa_dm.plot(cmap='Greens')
```



The UPGMA trees that result from these alignments are very different. First we'll look at the guide tree, and then the tree resulting from the progressive multiple sequence alignment.

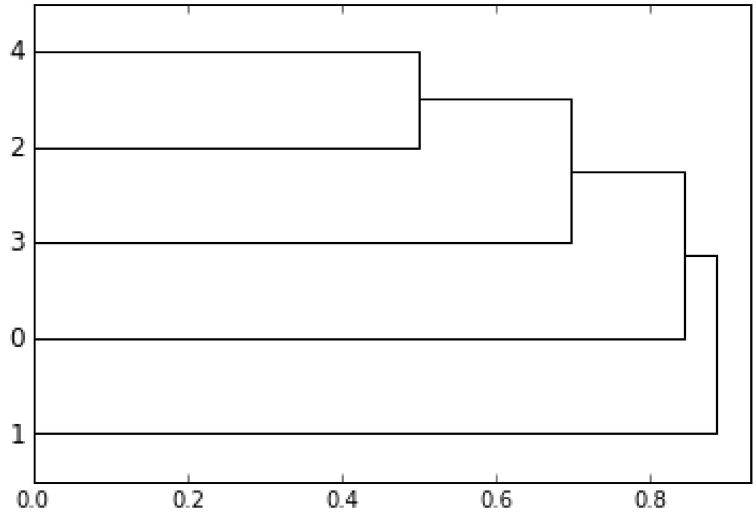
In [33]:

```
d = dendrogram(guide_lm, labels=guide_dm.ids, orientation='right',
               link_color_func=lambda x: 'black')
```



In [34]:

```
msa_lm = average(msa_dm.condensed_form())
d = dendrogram(msa_lm, labels=msa_dm.ids, orientation='right',
               link_color_func=lambda x: 'black')
```



And we can wrap this all up in a single convenience function:

In [35]:

```
from iab.algorithms import progressive_msa_and_tree
%psource progressive_msa_and_tree
```

```
def progressive_msa_and_tree(sequences,
                             pairwise_aligner,
                             metric=kmer_distance,
                             guide_tree=None,
                             display_aln=False,
                             display_tree=False):
    """ Perform progressive msa of sequences and build a UPGMA tree
    Parameters
    ----------
    sequences : skbio.SequenceCollection
        The sequences to be aligned.
    pairwise_aligner : function
        Function that should be used to perform the pairwise alignmen
ts,
        for example skbio.alignment.global_pairwise_align_nucleotide.
Must
        support skbio.Sequence objects or skbio.TabularMSA objects
        as input.
    metric : function, optional
      Function that returns a single distance value when given a pair
of
      skbio.Sequence objects. This will be used to build a guide tree
if one
      is not provided.
    guide_tree : skbio.TreeNode, optional
        The tree that should be used to guide the alignment process.
    display_aln : bool, optional
        Print the alignment before returning.
    display_tree : bool, optional
        Print the tree before returning.

    Returns
    -------
    skbio.alignment
    skbio.TreeNode

    """
    msa = progressive_msa(sequences, pairwise_aligner=pairwise_aligne
r,
                          guide_tree=guide_tree)

    if display_aln:
        print(msa)

    msa_dm = DistanceMatrix.from_iterable(msa, metric=metric, key='i
d')
    msa_lm = sp.cluster.hierarchy.average(msa_dm.condensed_form())
    msa_tree = TreeNode.from_linkage_matrix(msa_lm, msa_dm.ids)
    if display_tree:
        print("\nOutput tree:")
        d = sp.cluster.hierarchy.dendrogram(msa_lm, labels=msa_dm.id
s, orientation='right',
                    link_color_func=lambda x: 'black')
    return msa, msa_tree
```

In [36]:

```
msa = progressive_msa(query_sequences, pairwise_aligner=global_pairwise_align_nucl
eotide, guide_tree=guide_tree)
```

```
/home/iab/.conda/envs/iab/lib/python3.5/site-packages/skbio/alignmen
t/_pairwise.py:601: EfficiencyWarning: You're using skbio's python im
plementation of Needleman-Wunsch alignment. This is known to be very
slow (e.g., thousands of times slower than a native C implementatio
n). We'll be adding a faster version soon (see https://github.com/bio
core/scikit-bio/issues/254 to track progress on this).
  "to track progress on this).", EfficiencyWarning)
```

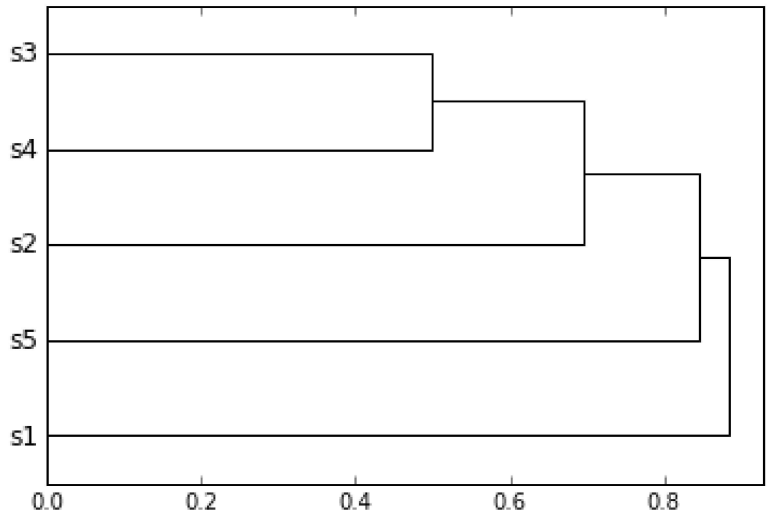
In [37]:

```
msa, tree = progressive_msa_and_tree(query_sequences, pairwise_aligner=global_pair
wise_align_nucleotide,
                                     display_tree=True, display_aln=True)
```

```
/home/iab/.conda/envs/iab/lib/python3.5/site-packages/skbio/alignmen
t/_pairwise.py:601: EfficiencyWarning: You're using skbio's python im
plementation of Needleman-Wunsch alignment. This is known to be very
slow (e.g., thousands of times slower than a native C implementatio
n). We'll be adding a faster version soon (see https://github.com/bio
core/scikit-bio/issues/254 to track progress on this).
  "to track progress on this)."", EfficiencyWarning)
```

```
TabularMSA[DNA]
----------------------
Stats:
    sequence count: 5
    position count: 21
----------------------
---GGC--CCA-----CTGAT
ACCGGTGACCAGTTGACCAGT
---GGCACCAAACAGA--A--
ATCGGTACC-GGTAGA--AGT
---GGTACCAAATAGA--A--
```

Output tree:



2.3.2 Progressive alignment versus iterative alignment

[edit] (https://github.com/gregcaporaso/An-Introduction-to-Applied-Bioinformatics/edit/master/book/fundamentals/multiple-sequence-alignment.md#L383)

In an iterative alignment, the output tree from the above progressive alignment is used as a guide tree, and the full process repeated. This is performed to reduce errors that result from a low-quality guide tree.

In [38]:

```
from iab.algorithms import iterative_msa_and_tree
%psource iterative_msa_and_tree
```

```
   def iterative_msa_and_tree(sequences,
                              num_iterations,
                              pairwise_aligner,
                              metric=kmer_distance,
                              display_aln=False,
                              display_tree=False):
       """ Perform progressive msa of sequences and build a UPGMA tree
       Parameters
       ----------
       sequences : skbio.SequenceCollection
          The sequences to be aligned.
       num_iterations : int
          The number of iterations of progressive multiple sequence alig
   nment to
          perform. Must be greater than zero and less than five.
       pairwise_aligner : function
          Function that should be used to perform the pairwise alignment
   s,
          for example skbio.alignment.global_pairwise_align_nucleotide.
   Must
          support skbio.Sequence objects or skbio.TabularMSA objects
          as input.
       metric : function, optional
         Function that returns a single distance value when given a pair
   of
         skbio.Sequence objects. This will be used to build a guide tree
   if one
         is not provided.
       display_aln : bool, optional
          Print the alignment before returning.
       display_tree : bool, optional
          Print the tree before returning.

       Returns
       -------
       skbio.alignment
       skbio.TreeNode

      """
       if num_iterations > 5:
           raise ValueError("A maximum of five iterations is allowed."
                            "You requested %d." % num_iterations)
       previous_iter_tree = None
       for i in range(num_iterations):
           if i == (num_iterations - 1):
               # only display the last iteration
               display = True
           else:
               display = False
           previous_iter_msa, previous_iter_tree = \
               progressive_msa_and_tree(sequences,
                pairwise_aligner=pairwise_aligner,
                metric=metric,
                guide_tree=previous_iter_tree,
                display_aln=display_aln and display,
                display_tree=display_tree and display)
```

```
    return previous_iter_msa, previous_iter_tree
```

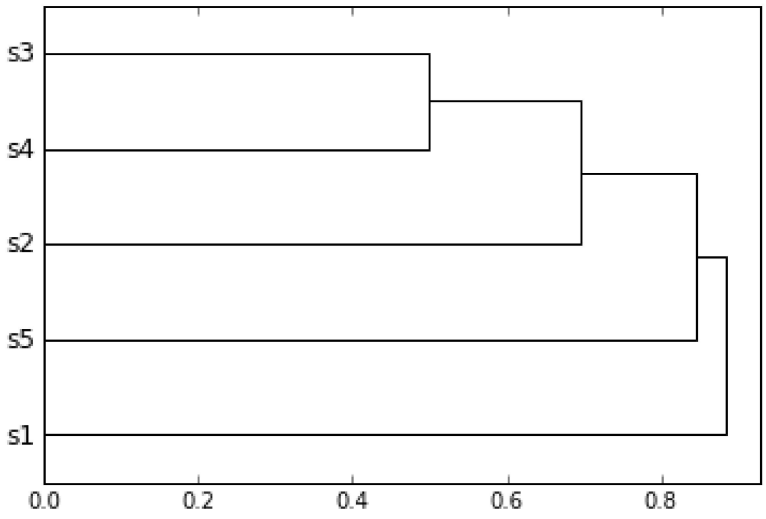
In [39]:

```
msa, tree = iterative_msa_and_tree(query_sequences, pairwise_aligner=global_pairwise_align_nucleotide, num_iterations=1, display_aln=True, display_tree=True)
```

```
/home/iab/.conda/envs/iab/lib/python3.5/site-packages/skbio/alignment/_pairwise.py:601: EfficiencyWarning: You're using skbio's python implementation of Needleman-Wunsch alignment. This is known to be very slow (e.g., thousands of times slower than a native C implementation). We'll be adding a faster version soon (see https://github.com/biocore/scikit-bio/issues/254 to track progress on this).
  "to track progress on this).", EfficiencyWarning)
```

```
TabularMSA[DNA]
----------------------
Stats:
    sequence count: 5
    position count: 21
----------------------
---GGC--CCA-----CTGAT
ACCGGTGACCAGTTGACCAGT
---GGCACCAAACAGA--A--
ATCGGTACC-GGTAGA--AGT
---GGTACCAAATAGA--A--
```

Output tree:



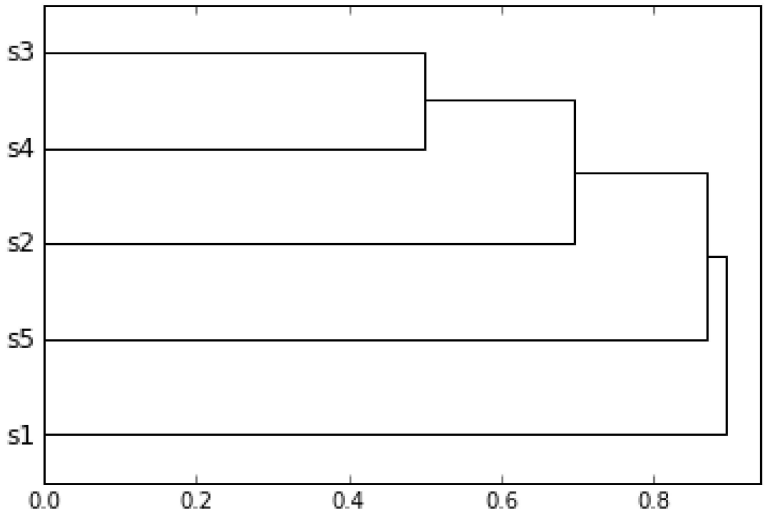
In [40]:

```
msa, tree = iterative_msa_and_tree(query_sequences, pairwise_aligner=global_pairwise_align_nucleotide, num_iterations=2, display_aln=True, display_tree=True)
```

```
/home/iab/.conda/envs/iab/lib/python3.5/site-packages/skbio/alignment/_pairwise.py:601: EfficiencyWarning: You're using skbio's python implementation of Needleman-Wunsch alignment. This is known to be very slow (e.g., thousands of times slower than a native C implementation). We'll be adding a faster version soon (see https://github.com/biocore/scikit-bio/issues/254 to track progress on this).
  "to track progress on this)", EfficiencyWarning)
```

```
TabularMSA[DNA]
----------------------
Stats:
    sequence count: 5
    position count: 21
----------------------
ACCGGTGACCAGTTGACCAGT
---GGC--CCACT-----GAT
ATCGGTACC-GGTAGA--AGT
---GGCACCAAACAGA--A--
---GGTACCAAATAGA--A--
```

Output tree:



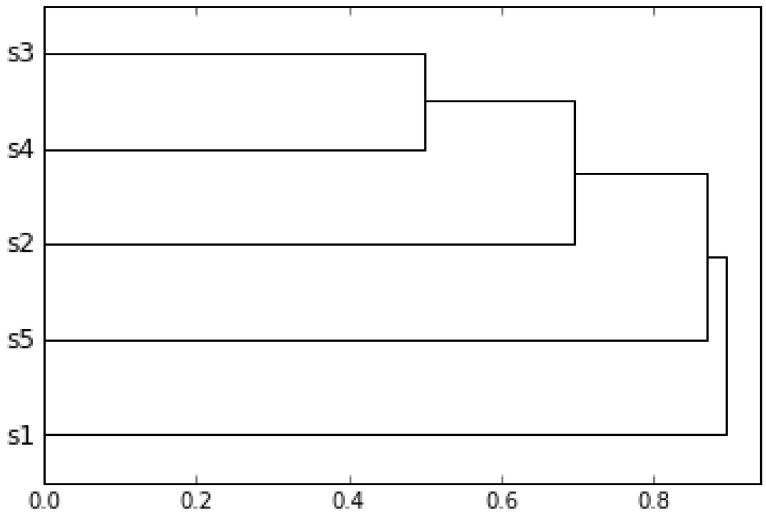
In [41]:

```
msa, tree = iterative_msa_and_tree(query_sequences, pairwise_aligner=global_pairwise_align_nucleotide, num_iterations=3, display_aln=True, display_tree=True)
```

```
/home/iab/.conda/envs/iab/lib/python3.5/site-packages/skbio/alignment/_pairwise.py:601: EfficiencyWarning: You're using skbio's python implementation of Needleman-Wunsch alignment. This is known to be very slow (e.g., thousands of times slower than a native C implementation). We'll be adding a faster version soon (see https://github.com/biocore/scikit-bio/issues/254 to track progress on this).
  "to track progress on this)", EfficiencyWarning)
```

```
TabularMSA[DNA]
----------------------
Stats:
    sequence count: 5
    position count: 21
----------------------
ACCGGTGACCAGTTGACCAGT
---GGC--CCACT-----GAT
ATCGGTACC-GGTAGA--AGT
---GGCACCAAACAGA--A--
---GGTACCAAATAGA--A--
```

Output tree:



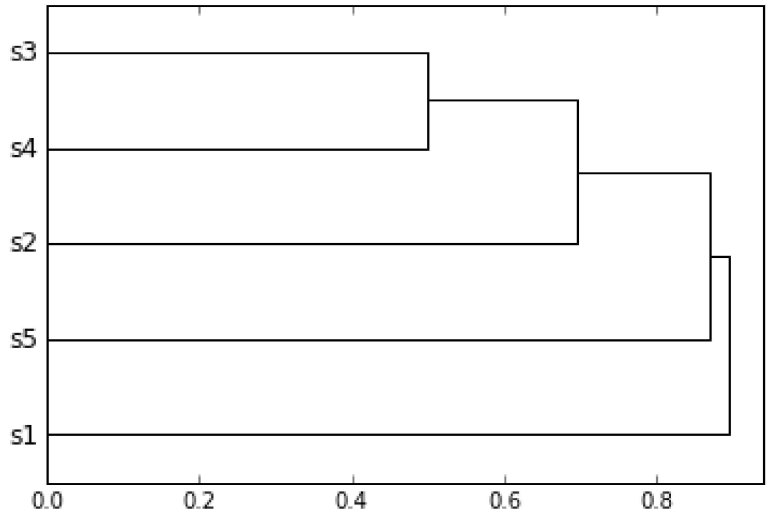
In [42]:

```
msa, tree = iterative_msa_and_tree(query_sequences, pairwise_aligner=global_pairwise_align_nucleotide, num_iterations=5, display_aln=True, display_tree=True)
```

```
/home/iab/.conda/envs/iab/lib/python3.5/site-packages/skbio/alignment/_pairwise.py:601: EfficiencyWarning: You're using skbio's python implementation of Needleman-Wunsch alignment. This is known to be very slow (e.g., thousands of times slower than a native C implementation). We'll be adding a faster version soon (see https://github.com/biocore/scikit-bio/issues/254 to track progress on this).
  "to track progress on this)", EfficiencyWarning)
```

```
TabularMSA[DNA]
----------------------
Stats:
    sequence count: 5
    position count: 21
----------------------
ACCGGTGACCAGTTGACCAGT
---GGC--CCACT-----GAT
ATCGGTACC-GGTAGA--AGT
---GGCACCAAACAGA--A--
---GGTACCAAATAGA--A--
```

Output tree:



Some references that I used in assembling these notes include 1 (http://statweb.stanford.edu/~nzhang/345_web/sequence_slides3.pdf), 2 (http://math.mit.edu/classes/18.417/Slides/alignment.pdf), 3 (http://www.sciencedirect.com/science/article/pii/0378111988903307), 4 (http://bioinformatics.oxfordjournals.org/content/23/21/2947.full), and 5 (http://nar.oxfordjournals.org/content/32/5/1792.full).