Comparison of sequence alignment methods

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Outline

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Why sequence alignment is needed?

Sequence alignment plays an important role in bioinformatics, which could be the fundamental tasks in bioinformatics. The purpose is to align sequences in such a way to reflect the biological relationship between the input sequences. Through arranging the sequences of DNA, RNA or protein, scientists expect to identify similar regions between these sequences, which might indicate functional, structual or evolutionary relationships.

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- Constructing sequence profiles
- An important prerequisite for the construction of phylogenetic trees, which could be a predictor of evolutionary relationships

[Bodenhofer et al., 2015]

Basic Idea of Sequence Alignment

- Given N sequences and a scoring scheme for determining the best matches of the letters, find the optimal pairing of letters between the sequences.
- The scoring scheme used by pairwise sequence alignment (PSA) defines the best alignment of two sequences as the sum of score matrix for each pair of letters minus gap penalties.
- Multiple sequence alignment (MSA) is an extension of PSA. The most widely-used algorithms are progressive alignments, which aligning a set of N sequences by performing N-1 pairwise alignments to create a distance matrix.

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- Global alignment methods fulfill global optimization, which spans the entire length of the input sequences, i.e., aligning the every residue of the sequences.
- Local alignment identifies similar region within a long sequence, i.e., regions of aligned sub-sequences might be surrounded by sequences that are completely unrelated.

Dynamic Programming

Dynamic programming is widely applied to the sequence alignment problem. However, its high computational costs in time and memory might cause problem when aligning extremely long sequences or large number of sequences.

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Main Algorithms

- the Needleman-Wunsch algorithm is applied to produce global alignment
- the Smith-Waterman algorithm is applied to produce local alignment

Dynamic Programming Cont.

Dynamic Programming Cont.

Scoring Scheme

- Protein alignments use a substitution matrix to assign scores to amino-acid matches or mismatches, and a gap penalty for matching an amino acid in one sequence to a gap in the other.
- DNA and RNA alignments use a scoring matrix to paired nucleic acids, such as assigning a positive match score, a negative mismatch score and a negative gap penalty.

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Gap Penalty

The values assigned to gap openings and gap extensions, which could be either same or different. The values can also be either fixed or adjusted.

Needleman-Wunsch Algorithm

To fill the matrix F,

- Introduce gap penalty: the top row and left column are filled with $i \times d, i = 1, ...m$ and $j \times d, j = 1, ..., n$, where m and n are the length of two sequences respectively.
- $F(0, 0) = 0$ is the start position, and $F(n, m)$ is the start position of tracking back.
- For each cell, do the following calculation to get the score value, where $S(X_i, Y_i)$ is the pair-score obtained from score matrix,

$$F(i, j) = \max \begin{cases} F(i-1, j-1) + S(X_i, Y_i) \\ F(i-1, j) - d \\ F(i, j-1) - d \end{cases}$$

- Track back from the right-bottom position and end with $F(0, 0)$ to find the best path.

[Durbin et al., 1998]

Smith-Waterman Algorithm

To fill the score matrix F,

- Begin with filling zeros in the top row and left column.
- For each cell, do the following calculation to get the score value

$$F(i,j) = \max \begin{cases} 0 \\ F(i-1,j-1) + S(X_i, Y_i) \\ F(i-1,j) - d \\ F(i,j-1) - d \end{cases}$$

- Once $F(i,j) < 0$, assign zero to that cell, which allows us to restart alignment at this position
- Find the cell with highest score value and track back from this position until the first time touch the cell with score of zero to find the optimal path

[Durbin et al., 1998]

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User input:

- Input data : FASTA file, two GeneBank identifiers to retrieve protein sequences, or two protein sequences
- Substitution matrix file name
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- Gap-opening and gap-extension penalties

Output: a text file

- Alignment score
- Aligned sequences with gaps, could be the entire length of sequences for global alignment or sub-sequences for local alignment.

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- The locally aligned sequences will always start with a "match"

Example of NW and SW algorithm

Using BLOSUM50 and using equally gap penalty "8" for gap opening and gap extension
sequence 1:"HEAGAWGHEE" and sequence 2:"PAWHEAE"

```
> output
$path
        [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8]
X.align "*"  "H"  "E"  "A"  "G"  "A"  "W"  "G"
Y.align "*"  "_"  "_"  "P"  "-"  "A"  "W"  "-"
        [,9] [,10] [,11] [,12] [,13]
X.align "H"  "E"   "_"   "E"   "*"
Y.align "H"  "E"   "A"   "E"   "*"

$fMatrix
        H   E   A   G   A   W   G   H   E   E
    0  -8 -16 -24 -32 -40 -48 -56 -64 -72 -80
P  -8  -2  -9 -17 -25 -33 -41 -49 -58 -65 -73
A -16 -10  -3  -4 -12 -20 -36 -41 -49 -57 -66
W -24 -18 -11  -6  -7 -15  -5 -13 -21 -37 -60
H -32 -14 -18 -13  -8  -9 -13  -7  -3 -11 -19
E -40 -22  -8 -16 -16  -9 -12 -15  -7   3  -5
A -48 -30 -16  -3 -11 -11 -12 -12 -15  -5   2
E -56 -46 -24 -11  -6 -12 -14 -15 -12  -9   1
```

```
$path.list
$path.list[[1]]
        [,1] [,2] [,3] [,4] [,5]
X.align "A"  "W"  "G"  "H"  "E"
Y.align "A"  "W"  "-"  "H"  "E"


$bestScore
[1] 28

$fMatrix
     H  E  A  G  A  W  G  H  E  E
  0  0  0  0  0  0  0  0  0  0  0
P 0  0  0  0  0  0  0  0  0  0  0
A 0  0  0  5  0  5  0  0  0  0  0
W 0  0  0  0  2  0 20 12  4  0  0
H 0 10  2  0  0  0 12 18 22 14  6
E 0  2 16  8  0  0  4 10 18 28 20
A 0  0  8 21 13  5  0  4 10 20 27
E 0  0  6 13 18 12  4  0  4 16 26
```

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Clustal W

The "W" in the name stands for "weighting", which indicates assigning different weights to sequences and parameters at different positions in alignment.

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Basic multiple alignment consists of three main stages:

- Calculate all pairwise sequence similarity to construct a distance matrix, giving the divergence of each of sequences via full dynamic programming
- Construct a guide tree from the distance matrix via neighbor-joining algorithm and derives sequence weights
- Do progressive alignment [Hogeweg and Hesper, 1984] according to the branching order in the guide tree

[Thompson et al., 1994]

Clustal W Highlights

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- Assign individual weights to each sequence in a partial alignment to down-weight near-duplicate sequences and up-weight the most divergent ones
- Do dynamic calculation of sequence- and position-specific gap penalties as the alignment proceeds

[Thompson et al., 1994, Higgins et al., 1996]

MUSCLE

- MUSCLE (multiple sequence comparison by log-expectation) was introduced in 2004
- Matrix-based algorithm, start with a guide tree construction, the essential step is pairwise alignment
- Two distinguish features:
  - Using both the k-mer distance for an unaligned pair and the Kimura distance for an aligned pair to calculate all pairwise sequence distance
  - At the completion of any stage of the algorithm, a MSA is available and the algorithm can be terminated

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Stage 1: draft progressive

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Stage 2:improved progressive

compute Kimura distance matrix and re-estimating the guide tree via UPGMA allgorithm, and do progressive alignment to produce second MSA. Compare the guide tree from stage 1 and 2, identifying a set of nodes for which the branching order is different. Build new MSA if the order has changed, or keep the first MSA

MUSCLE Main Stages Cont.

Stage 3

refinement: delete an edge of the guide tree from stage 2, which divides the tree into two sub-trees and calculate the profile of multiple alignment for each sub-tree. Re-align the profiles from the two sub-tree to produce a new MSA. Keep the new MSA only if the new sum-of-pairs score is improved. Iterate this process until convergence or hitting the user-defined limit.

[Edgar, 2004]

Clustal Omega

- Completely rewritten and revised version of Clustal series of programs for MSA
- Retains the basic progressive alignment where the order of alignment is determined by a guide-tree
- Apply the mBed algorithm [Blackshields et al., 2010] for calculating guide trees, which allows it can deal with very large numbers of DNA/RNA or protein sequences
- Apply the HHalign method for aligning profile hidden Markov models, which considerably improves the accuracy

[Sievers and Higgins, 2014]

Clustal Omega Highlights

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mBed algorithm:

It calculates the pairwise distance of all N sequences with respect to $logN$ randomly chosen seed sequences only, which reduces the time and memory complexity for guide tree calculation from $O(N^2)$ to $O(NlogN)$)

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HHalign method:

HHalign is entirely based on Hidden-Markov Models(HMMs). Sequences and intermediary profiles are converted into HMMs, which are aligned in turn. There are two HMM alignment algorithm: Maximum Accuracy (MAC) algorithm and Viterbi algorithm. MAC is the default

[Sievers and Higgins, 2014, Sievers et al., 2011]

Outline

Simulation Data Generation

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User Input

- lenth – lenth of sequences, can be scalar or vector
- sampleNum – number of sequences need to be generated
- type – specify the type of sequences
- dir – set up the working directory
- out.file – output file's name

Simulation Data Generation Cont.

Simulation Data Generation Cont.

Output

- generated sequences
- true alignment
- fasta file: if user input the file name, then it would be saved under the user-defined directory or current working directory

Compare Running Time

Data: sequence length is 500, number of sequences is 9

Clustal W

Parameters: gapOpening=10, gapExtension=0.2, substitution matrix=BLOSUM

$$t = 0.64, 0.66, 0.71, 0.66, 0.66, 0.64, 0.67, 0.67, 0.64, 0.72$$

Summary: min=0.64, mean=0.667, max=0.72

Compare Running Time Cont.

MUSCLE

Parameter: gapOpening=10, gapExtension=0.2, cluster=UPGMAMAX

$$t = 0.44, 0.46, 0.46, 0.46, 0.46, 0.44, 0.43, 0.46, 0.47, 0.46$$

Summary: min=0.43, mean=0.454, max=0.47

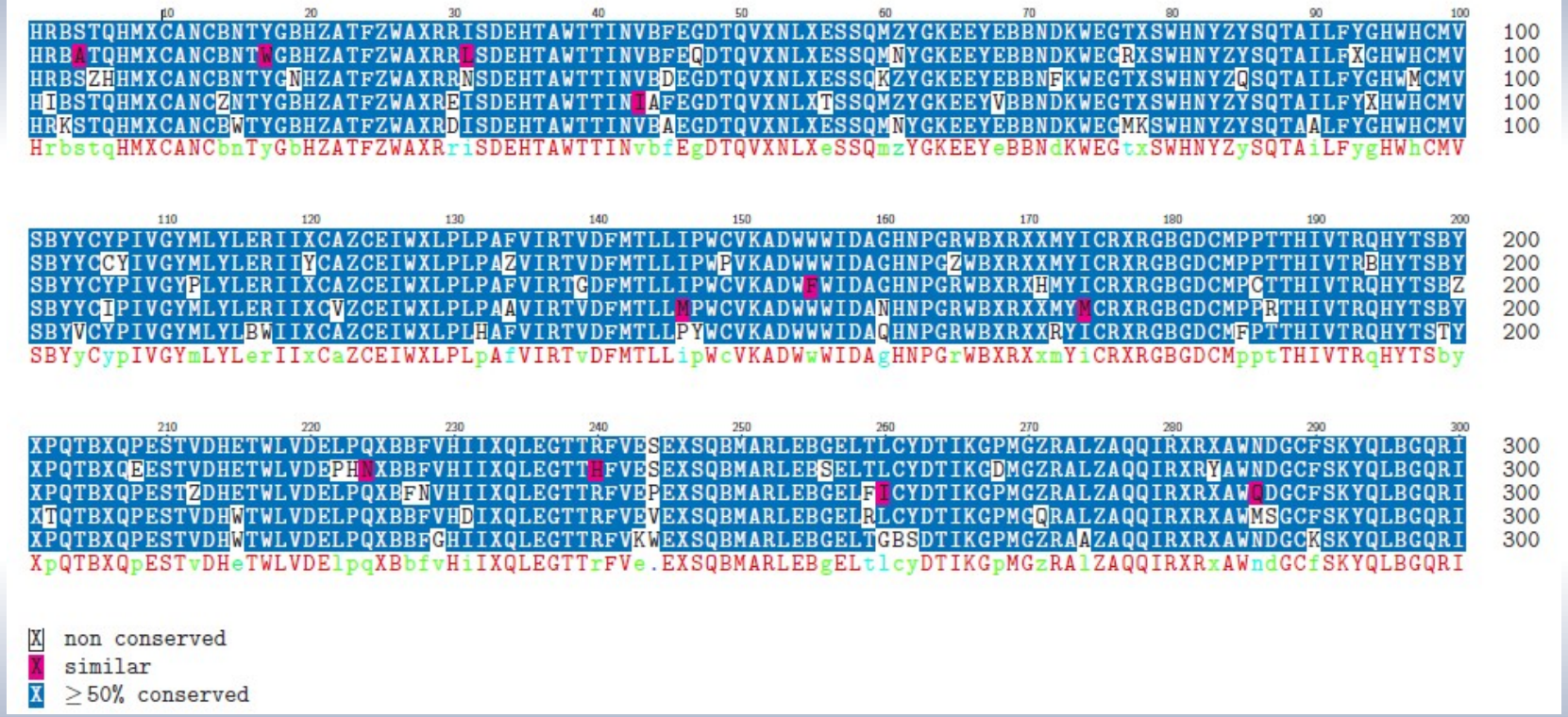
Clustal Omega

Parameter: cluster=mBed, cluster.size=100

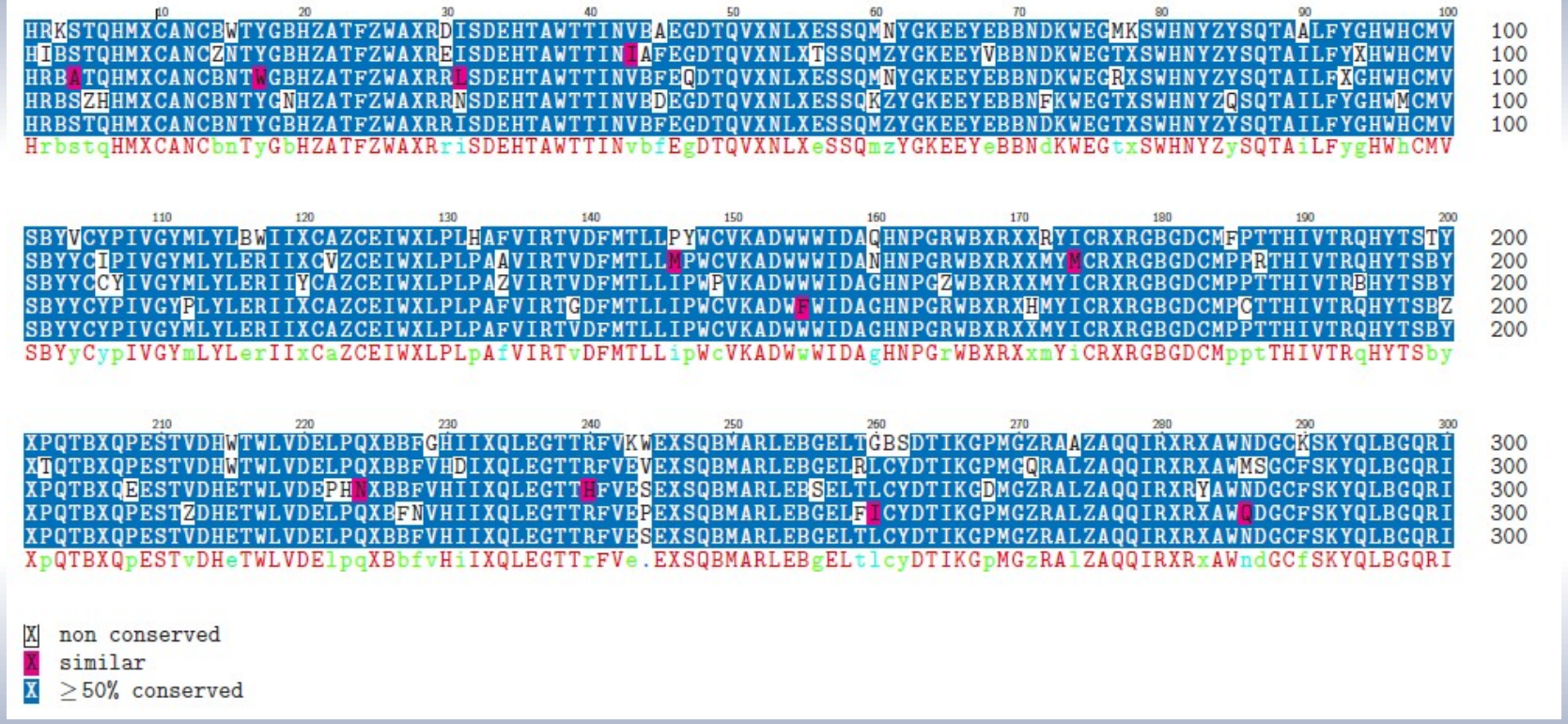
$$t = 0.56, 0.51, 0.5, 0.5, 0.51, 0.5, 0.57, 0.47, 0.5, 0.5$$

Summary: min=0.47, mean=0.512, max=0.57

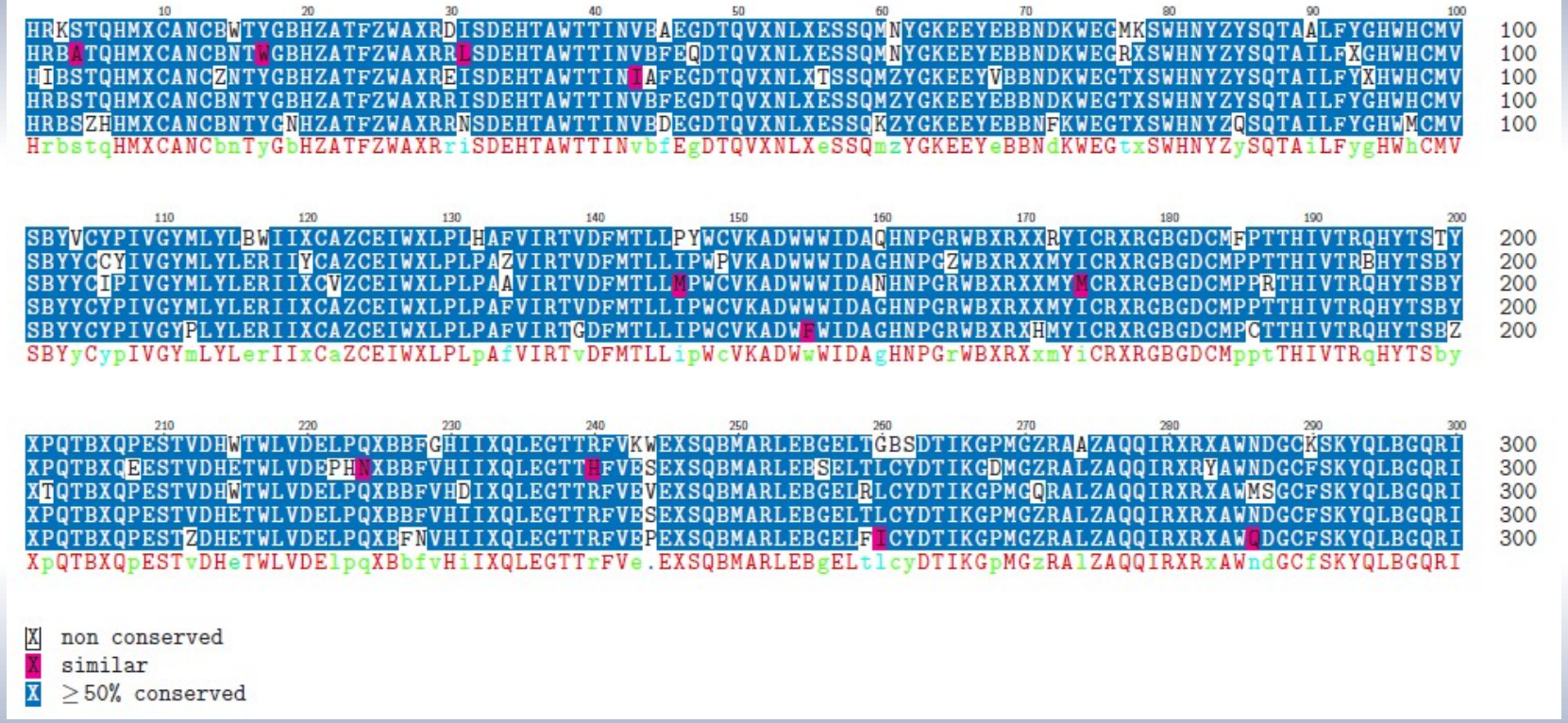
Compare Accuracy



Compare Accuracy Cont.



Compare Accuracy Cont.



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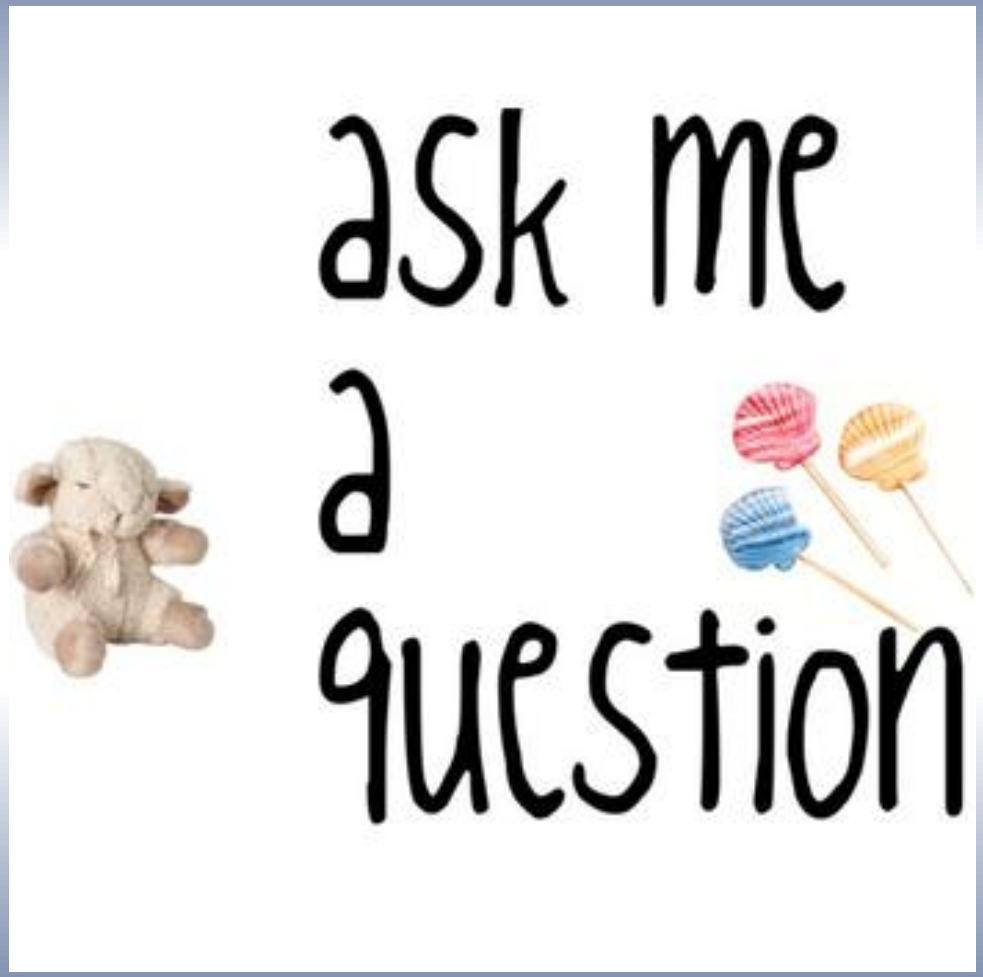
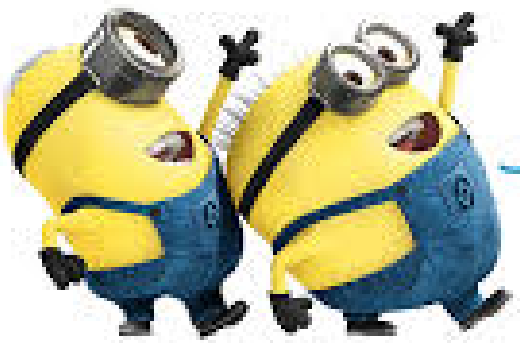
Questions





thank you!