Factoring local sequence composition in motif significance analysis

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Motif Finding

- DNA Motif – over-represented nucleotide **pattern** that has biological significance
- Why are we interested in finding motifs?
  - Transcription factor binding sites
- Over 100 motif-finders
  - MEME (Bailey *et al.* 1994)
  - Weeder (Pavesi *et al.* 2004)
  - YMF (Sinha *et al.* 2000)
  - BioProspector (Liu *et al.* 2001)

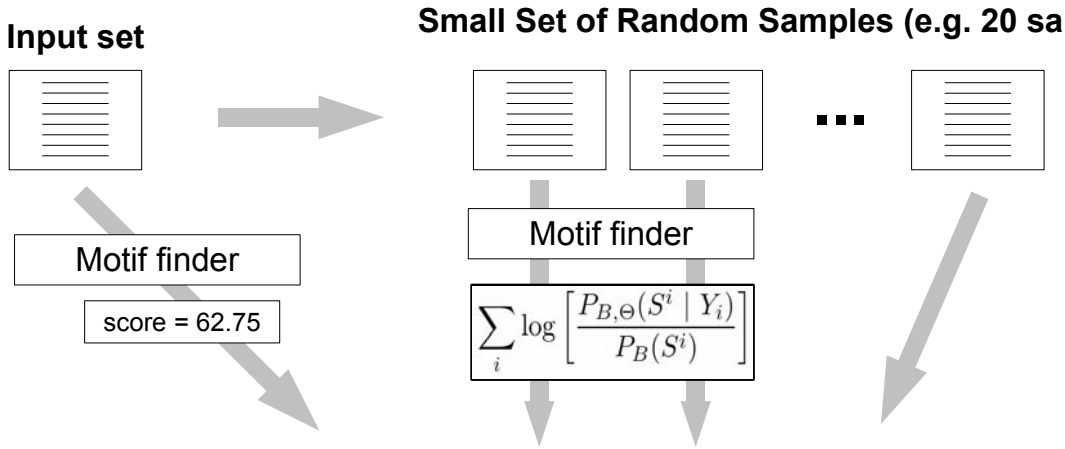
Motif Statistical Significance

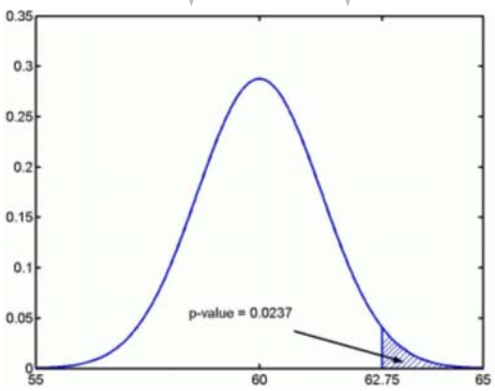
- Does the motif I found have **biological** significance?
  - **Hard problem**
  - Require biological wet-lab experiment (e.g. knockout)
- Is the motif I found **statistically** significant? Is it special comparing to a motif I found in a random set of sequences?
  - **Easier problem**
  - Time and cost efficient
  - Ensemble algorithms
    - Combine results of different motif-finders
    - Combine results over different parameters of a finder

Issues and Challenges

- Motif-finders are **slow** (~30 sec. to 5 min.)
  - Naive Monte-Carlo estimate of *p*-value is intractable
  - e.g. In multiple testing, we may need *p*-value < 0.001
    - 30 seconds * 10,000 random sets ≈ 3 days
- How does one define a **better** motif?
  - Different motif-finders use different scoring metrics
  - Likelihood ratio, Enrichment score, etc.
- What is a **random** set of DNA sequences?
  - Input data has different composition than a random chosen sequence (e.g. GC-content, dinucleotide frequency)

A Parametric Motif Significance





Harbison *et al.* 2004

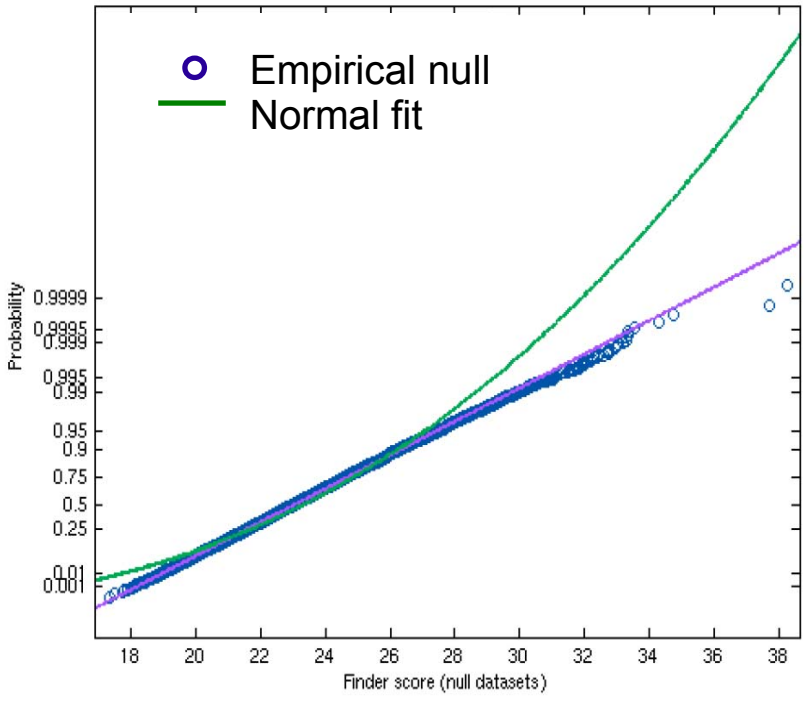
Liu *et al.* 2001

MacIsaac *et al.* 2006

Narlikar *et al.* 2007

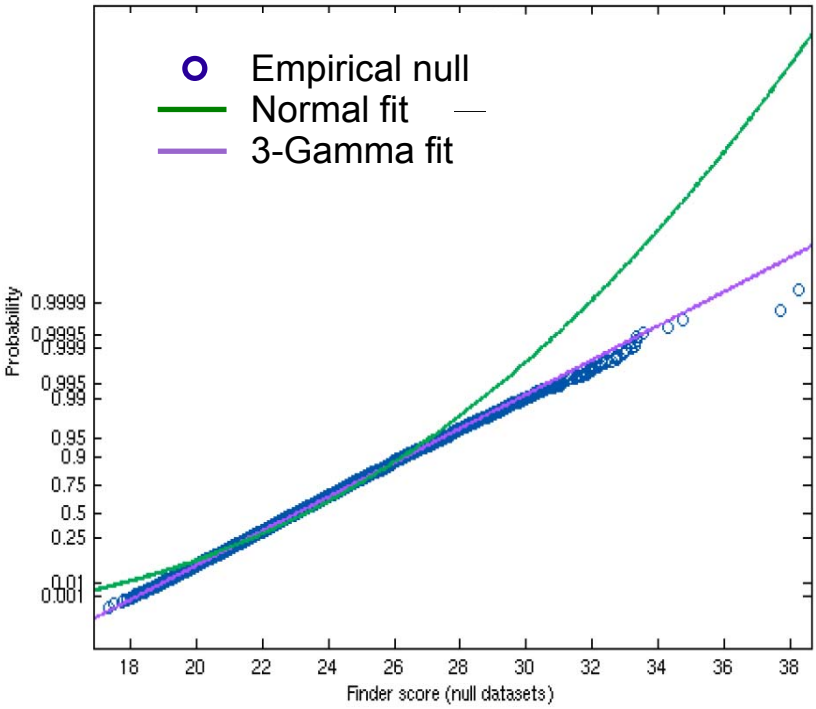
Assume the null distribution of finder's score is normally distributed

Is the Normal distribution a good approximation?



Empirical null is generated by applying GIMSAN (Gibbs sampler) to 10,000 random sequence-sets from *S. cerevisiae* intergenic region. GIMSAN evaluates motif based on log likelihood ratio (LLR) score.

3-Gamma vs Normal



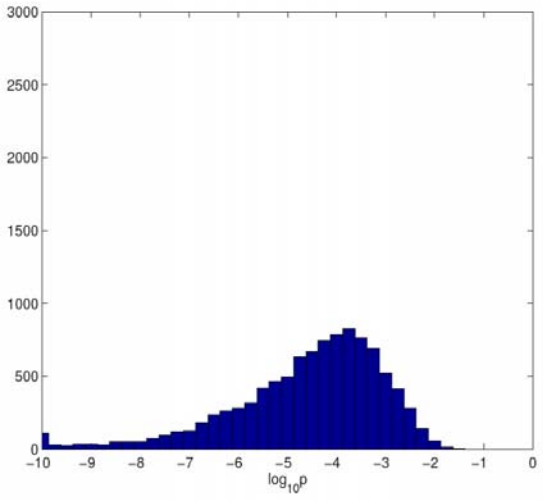
3-Gamma distribution is a good fit
while the normal distribution is not

Normal approximation overestimates the significance

Histograms of 10,000 evaluations of the point estimator for *p*-value = 0.001.

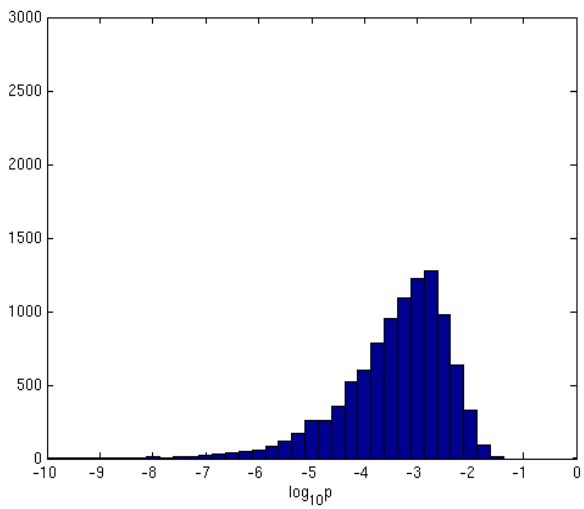
The data was resampled from the HSA1 intergenic region.

Normal



$\hat{p}_n$ overestimates the significance

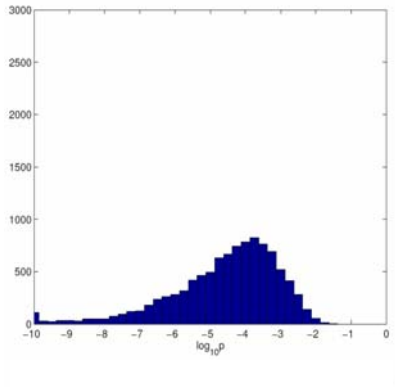
3-Gamma



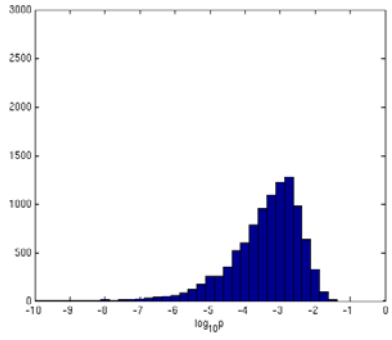
An ideal estimator *p(s)* should have all the mass on the point log(*p*) = -3

Can we do better?

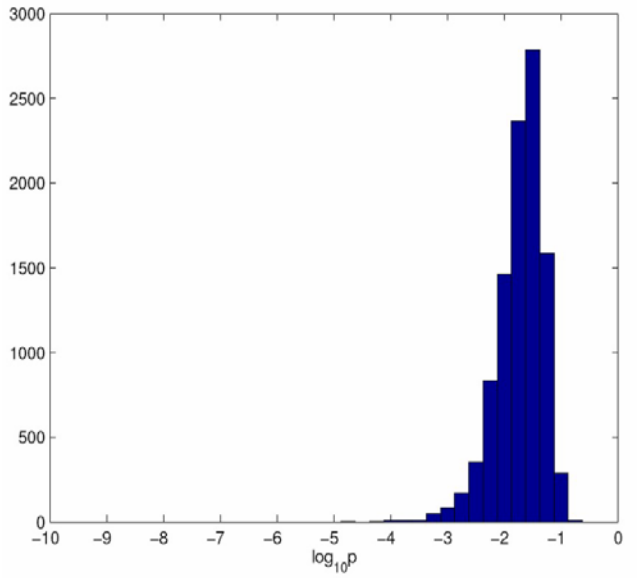
Normal *p*-value point estimator



3-Gamma *p*-value point estimator



3-Gamma "Confidence *p*-value"



(b) $\hat{p}_c(s, X)$ is mostly conservative

An ideal estimator *p(s)* should have all the mass on the point *log*(*p*) = -3

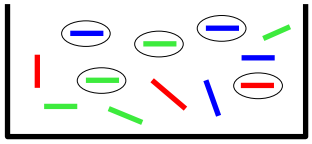
"Random" set of DNA sequences

- Is the predicted motif from my input data special comparing to a motif I found in a **random** set of sequences?
- What is a "random" set of DNA sequences?
  - Uniformly sampled from a genome (widely used technique)
    - For example, from *S. cerevisiae* intergenic region
    - Harbison *et al.* 2004, Liu *et al.* 2001, Narlikar *et al.* 2007
  - High-order Markov Model
  - Shuffling

Local Sequence Composition

S. cerevisiae genome
> 13 million bp

Divide into overlapping windows of 50bp



a sequence from input set
< 2000 bp

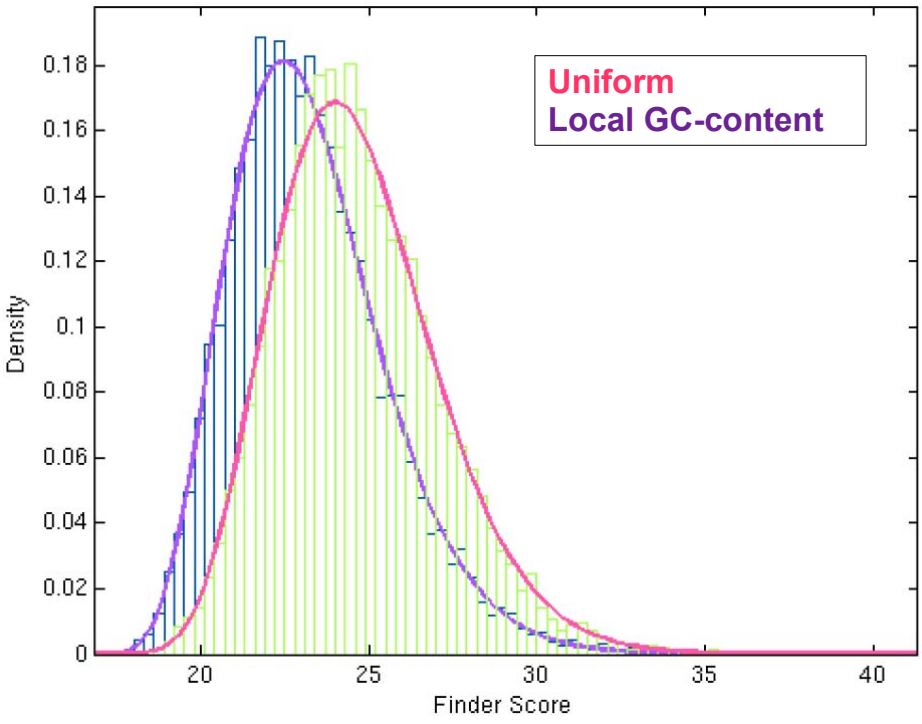
AATGGTTGGTCAAGGATGCGCAACCCAATGATTCTTTGTTCCTTCATTATTCTGGACA

Divide into non-overlapping windows of 50bp

Replace each original window with a randomly drawn window from a bin with similar local composition (e.g. GC-content)

GAGAACGCGTGAGACAGATCAAATTCTCAGCAGCAGATGTTGTTATGTTATC

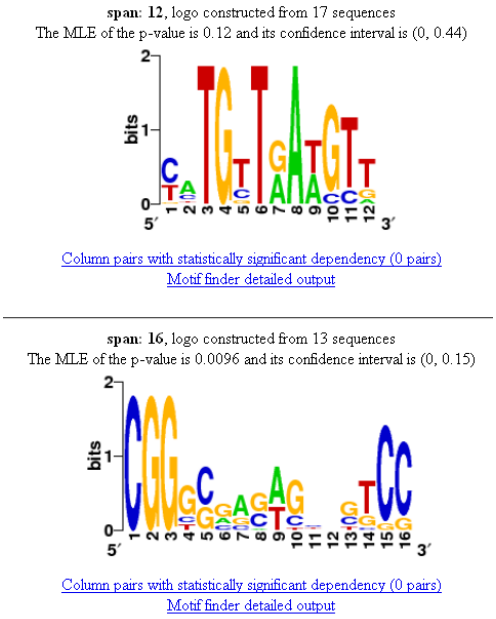
Does preserving local GC-content make a difference?



Comparing empirical null distributions of log likelihood ratio (LLR) score from GIMSAN
Data from *S. cerevisiae* intergenic region

GIMSAN (Web Application)

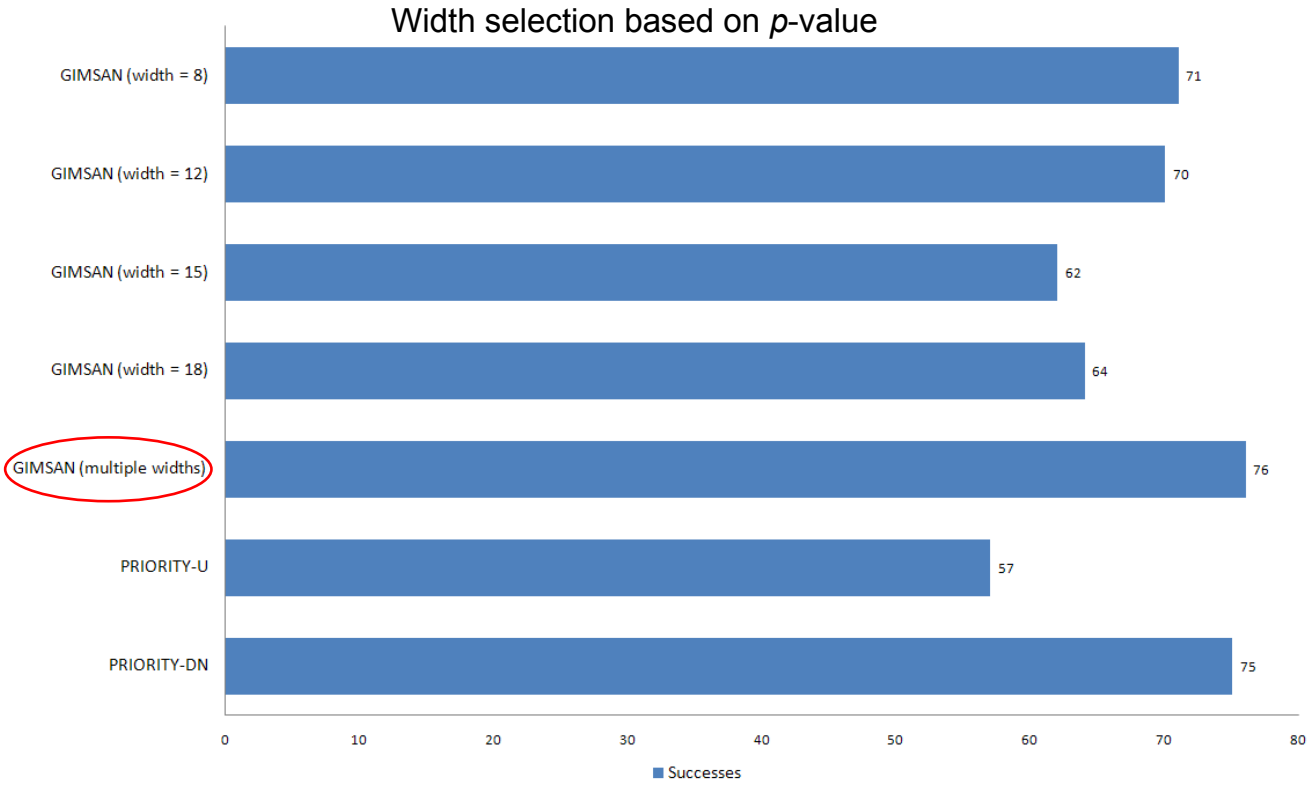
- GIMSAN (GIbbsMarkov with Significance ANalysis) is a novel web application
  - Gibbs sampler for motif finding
  - Biologically realistic and reliable statistical significance analysis



Results on Biological Data

- ChIP-chip data of transcription factors for *S. cerevisiae* from Harbison *et al.* 2004
  - Benchmark from Narlikar *et al.* 2007
    - 156 sequence-sets from 80 TFs
    - PRIORITY, AlignACE, MEME, MDscan, CONVERGE, conservation-based method of Kellis *et al.*
- How are successes defined?
  - Average mean-square-error

Using *p*-values to improve performance



Competitive with PRIORITY-DN (best finder from Narlikar 2007)

How well calibrated are our *p*-values?

- How well calibrated are our 3-Gamma confidence *p*-values on biological data?
- Perform hypothesis testing with a *p*-value threshold
- Is the observed rate FP / (FP + TN) consistent with the theoretical rate?
  - ChIP-chip data of *S. cerevisiae* TFs from Narlikar *et al.*
    - At a 5% *p*-value threshold, observed rate of our *p*-value is 11% while the rate of its normal approximation counterpart is 74% (6-fold)
  - False-positive correction: motifs that have matches in database but does not match literature
    - At a 5% *p*-value threshold, observed rate is 8.3%
    - At a 10% *p*-value threshold, observed rate is 13.9%

Summary & Future Research

- 3-Gamma approximation
  - Much better fit than normal approximation for motif significance evaluation
- Local sequence composition
  - Framework for factoring biologically realistic information
  - Can be extended to factor other features
  - Use HMM model rather than our window-based method
- GIMSAN available as a web application
  - Competitive to other motif-finders that use additional information (e.g. conservation, nucleosome positioning)
  - Our *p*-values are well calibrated on biological data

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