

Smashing single cells into k -mer sketches

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Abstract

Single-cell RNA-sequencing is a powerful technology for identifying novel and known cell types, however its power is limited to organisms with well-annotated genomes. We demonstrate the utility of using annotation-agnostic methods which quantify cell-cell similarity using k -mer profiles. We benchmark a few methods and demonstrate the utility of converting cell types from mouse to human and back, and compare to using purely 1:1 mapped orthologous genes.

Introduction

There are a predicted 8.7 million Eukaryotic species on earth [1], yet only 14% (1,233,500) have been catalogued and 0.001% (9,449) have genomes deposited in the National Center for Biotechnology Information Genome Assembly [2]. And yet, the genome sequence is not enough. To truly understand the diversity of life on this planet, we need to determine not just the DNA blueprints of life, but understand the instantiation of the DNA, the cell types of the species. While sequencing DNA gives a quantitative measure of the nucleotide differences, it does not inform the functional strategies that change with DNA sequence. As new species can be defined by a new cell type. For example, the existence of a single cell type, the Cnidocyte [3], a stinging cell of a single-celled biological weapon, defines the phylum Cnidaria. Thus, entire clades, not only species, can be defined by the introduction of an additional cell type or state.

Novel organizations of existing cell states can also define cell types. For example, the development of genitalia in amniotes, while using similar cell types, ultimately uses a different physical organization of cell types to generate genitalia in mammals compared to reptiles [4]

Determining common gene ancestry (“orthology”) is a difficult problem. Many approaches exist [5,6].

Determining common ancestry of cell types (“orthologous cell types”) [7,8] is an additional difficult problem. Comparative transcriptomics begins with finding a common feature set for embedding molecular profiles across divergent species into a common space. Many researchers take the approach of using one-to-one orthologous genes [Cite: brawand2011, CCA, LIGER, Scanorama, basically all the single cell “alignment” packages], others use clusters of orthologous groups [9], others map reads onto a common genome derived from whole-genome alignment [cite: recent primate brain paper from Barbara Treutlein], or map onto native genomes [10] and re-annotate using a tool such as Comparative Annotation Toolkit [11].

k-mers have been proposed for comparing single cells [12] as they are a fast, simple way to create cell-cell similarities. However, the work so far has focused on using annotated organisms and not cross-species analyses.

Methods

Methods go here.

Experimental

Primate brain organoid protocols

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Single-cell capture of primate brain organoids

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Long read library prep

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Short read library prep

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Sequencing

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Computational

k-mer comparison of orthologous genes

We used ENSEMBL version 97. We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Extraction of putative coding reads from RNA-seq

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

bam2fasta conversion

We did things. One sentence per line. Prefer DOI for references, but for Biorxiv use the URL. DOI example: [5]. Biorxiv example: [6]. Multiple citations per line example: [5,6].

Results

To determine whether short segments of sequences could detect gene orthologues, we *k*-merized orthologous genes derived from the ENSEMBL version 97 [13] COMPARA database [14] (Figure [1]). We compared human protein sequences to orthologous chimpanzee, mouse, (orangutan, bonobo, gorilla, macaque, opossum, platypus, chicken) protein sequences, as these are species used in [15]. As a background, we randomly chose 10 non-orthologous genes relative to the human gene. In addition to *k*-merizing the protein-coding sequence, we also re-encoded the protein-coding sequence into Dayhoff [16] and hydrophobic-polar encodings [17], show in Table [1].

Table 1: Dayhoff and hydrophobic-polar encodings are a reduced amino acid alphabet allowing for permissive cross-species sequence comparisons. For example, the amino acid sequence SASHAFIERCE would be Dayhoff-encoded to bbbdbfecdac , and HP-encoded to phpphhpppp .

Amino acid	Property	Dayhoff	Hydrophobic-polar (HP)
C	Sulfur polymerization	a	p
A, G, P, S, T	Small	b	A, G, P: h S,T: p
D, E, N, Q	Acid and amide	c	p
H, K, R	Basic	d	p
I, L, M, V	Hydrophobic	e	h
F, W, Y	Aromatic	f	h

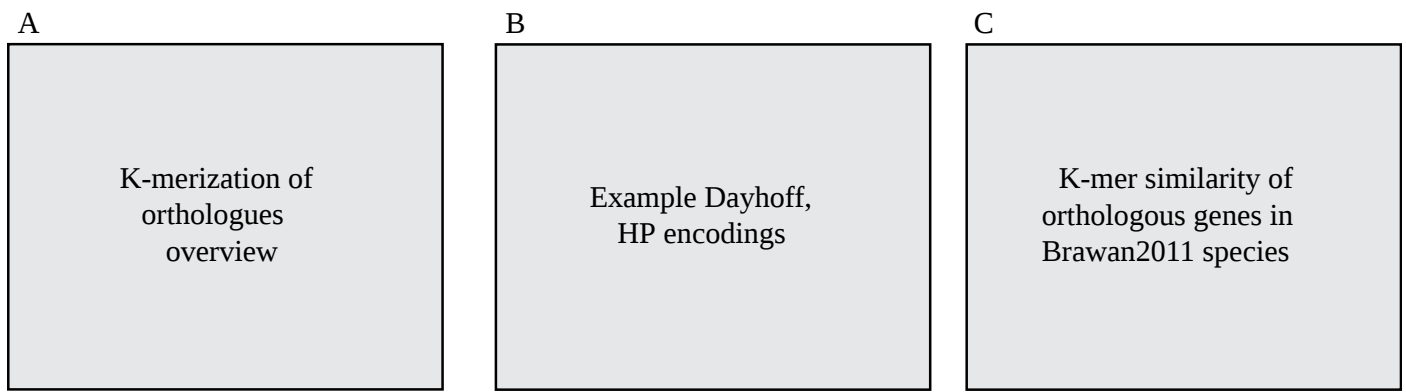


Figure 1: Figure 1.

We found that, consistent with previous knowledge, that 1:1 orthologues had higher k -mer similarities as determined by the Jaccard Index.

Additionally, more recently diverged genes had higher k -mer similarity as well.

A

Extract coding sequence using human proteins,
k-merize coding sequence and minhash, com
pare samples/cells across minhashes

B

kNN graph of
Brawand2011
MinHashes

C

kNN graph of
Brawand2011
Gene expression

D

K-mers driving
similarity in
brawand2011

E

Are the k-mers from
unmapped reads or
unannotated genes?

Figure 2: Figure 2.

Outlines

Figure 1 outline

- Kmers can approximate orthologies
 - Jaccard similarity of orthologues is higher than non-orthologues
 - Benchmarking using <https://orthology.benchmarkservice.org/cgi-bin/gateway.pl>
 - Finding orthologues
 - Gold standard
 - ENSEMBL COMPARA
 - Quest for Orthologs consortium, Altenhoff, A. M., Boeckmann, B., Capella-Gutierrez, S., Dalquen, D. A., DeLuca, T., et al. (2016). Standardized benchmarking in the quest for orthologs. *Nature Methods*, 13(5), 425–430. <http://doi.org/10.1038/nmeth.3830> [5]
 - Orthologous groups/Conserved Domain Database [18]
- Kmers can find correct reading frame of RNA-seq reads
 - Human peptides → human RNAseq
 - Human peptides → chimp RNAseq
 - Human peptides → mouse RNAseq
- Kmers can find only transcription factor reads of TFs from RNA-seq reads
 - Human TFs → human RNAseq
 - Human TFs → chimp RNAseq
 - Human TFs → mouse RNAseq
- Overview of kmermaid pipeline
 - Comparison of tissue across species
 - Partition reads to coding/noncoding bins
 - MinHash the Dayhoff-encoded coding sequences
 - Jaccard similarity on the MinHashes

Figure 2 outline

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No need for 1:1 orthology

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Taxa-restricted genes

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Don't need to normalize gene expression counts since we just have presence/absence

Full transcript analyses

- Brain Organoid – Droplet + PacBio data

Correlated evolution of celltypes?

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Cell type evolution

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Figure 3 – long evolutionary distances with HP encoding?

Metazoan body plan formation

- Early development in Cnidarians/Hydra [29,30]
- sponges and others [30]
- planaria [31,32],
- drosophila [33]
- zebrafish [34,35,36],
- mouse [37]

Figure 4 – What features are k-mers able to pick up that mapping doesn't?

- Which reads are found to have coding features but didn't map to the genome?
- Do these features map to novel genes or gene fusions?

Discussion

Conclusions and future directions go here.

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