Yclon: Ultrafast clustering of B cell clones from high-throughput immunoglobulin repertoire sequencing data

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Abstract

Motivation: The next-generation sequencing technologies have transformed our understanding of immunoglobulin (Ig) profiles in various immune states. Clonotyping, which groups Ig sequences into B cell clones, is crucial in investigating the diversity of repertoires and changes in antigen exposure. Despite its importance, there is no widely accepted method for clonotyping, and existing methods are computationally intensive for large sequencing datasets.

Results: To address this challenge, we introduce YClon, a fast and efficient approach for clonotyping Ig repertoire data. YClon uses a hierarchical clustering approach, similar to other methods, to group Ig sequences into B cell clones in a highly sensitive and specific manner. However, our approach is over 130 times faster than existing methods and can process up to 2 million Ig sequences on a laptop computer. This enables in-depth analysis of large and numerous antibody repertoires.

Availability and implementation: YClon is implemented in Python3 and is freely available on GitHub

(https://github.com/jao321/YClon.git)

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1. Introduction

The use of next-generation sequencing in Adaptive Immune Receptor Repertoire (AIRR-Seq) studies provides only a snapshot of the antibody repertoire diversity that is composed of up to 10^{18} distinct antibodies (Briney *et* al., 2019). Besides that, those advanced techniques help to better understand the immune system as it allows the study of millions to billions of distinct antibody sequences (DeWitt *et* al., 2016; Briney *et* al., 2019).

However, identifying antibody sequences that belong to the same B-cell clone in these data remains a challenge (Gupta *et* al., 2018). A B-cell clone or clonotype is defined as a group of antibody sequences derived from a common progenitor B cell (Hershberg et al., 2015) that often bind to the same epitope. Clone size reflects on repertoire diversity, which is related to age (Davydov *et* al., 2018), response to vaccination or infection (Khavrutskii *et* al., 2017), allergy (Wu *et* al., 2014), cancer (Zhang *et* al., 2019), among several other conditions.

B-cell clone identification is crucial, and different approaches attempt to do it. Some researchers consider the same clone as antibody sequences sharing the same V and J genes while having likeness in the third complementary-determining region (CDR3) or junction sequence, both in length and similarity, with thresholds normally ranging from 80% to 100% as the case of IMGT/HighV-QUEST (Li *et* al., 2013) and several other methods (Teng and Papavasiliou, 2007; Elhanati *et* al., 2015; Hershberg and Luning Prak, 2015; Gupta *et* al., 2017). In general, there is a consensus that the V and J genes have to be shared by antibodies to consider them as part of the same clonotype. The main difference in the clonotyping methods is the way and the cut-offs used to group the antibodies CDR3. Some approaches like the one containing in Change-O, and SCOPer assigns clones based on the hamming distance of CDR3 or junction, then performs a clustering method (Gupta *et* al., 2015, Nouri & Kleinstein, 2018) among other strategies (Chen *et* al., 2010; Gupta *et al.*, 2015; Nouri & Kleinstein, 2020; Aouinti *et* al., 2015). One problem with most of the above approaches is the time to process high-throughput data, with negatively impacts downstream analyses, especially in limited resources laboratories. Lindenbaum et al., 2020 proposed a method to group Ig sequences into clonotypes, that does not require gene assignments and is not restricted to a fixed junction length. What it does is vectorize a sequence of 150 nucleotides starting from the latter part of the V gene to the 3’ end into k-mers of size 5, which should be fast. However, they do not take into account if the sequences are annotated as

sharing the same V and J genes. Therefore, the clones assigned by Lindenbaum et al, 2020 do not fit the definition of clone hereby established.

It is worth mentioning that two important repertoire databases, OAS and iReceptor, gather 2,426,208,946 (organized in 15,148 distinct files) and 5,230,217,389 (organized in 9,346 distinct files) human IGH sequences, respectively, on January 13th, 2023 (Corrie *et* al., 2018; Kovaltsuk *et* al., 2018; Olsen *et* al., 2021). From those repertoires that have more than a million sequences, OAS has 575 repertoires, and iReceptor has 257. This shows the need for a more scalable tool, able to clonotype hundreds of antibody repertoire datasets, including large repertoires, quickly and efficiently while respecting the definition of clone.

Because of this, we propose YClon, a tool that rapidly processes repertoires, including the ones with more than 2 million sequences in less than one hour, considering identical V and J genes and similarity in the CDR3.

2. Material and Methods

2.1. YClon Implementation

YClon is a script written in Python3 and receives as input, on its default setting, ". tsv" files formatted in the MiAIRR data standard, annotated with IgBlast. The file must contain at least four columns, and they should be assigned as "sequence_id" with a unique identifier for the sequence, "v_call" with the V gene annotation, "j_call" with the J gene annotation, and "cdr3" containing the sequence of the CDR3. In order to clonotype the sequences, YClon goes through 5 steps:

I. Subsetting: The first step is to subset the data sharing the same V and J gene segments and CDR3 length.

This is achieved using the following steps:

a) We recover v_call, j_call, and cdr3 columns indexes and their associated values in the subsequent rows of the .tsv file.

b) We calculate the length of the cdr3 sequence and the v_call and j_call column's gene names (without allele information). The combination of these attributes will be the key to a dictionary. For each key, there is a list of tuples containing the sequence_id and the cdr3 sequence.

c) For each new key, a new list is appended to a dictionary. If the key is already in the dictionary another tuple is appended to the list.

II. Vectorizing CDR3 nucleotide sequence: CDR3 sequences of the data subsetted were deconstructed in n-grams of size 3 with a sliding window of 1 nucleotide as reviewed by Vinga & Almeida, 2003 and determined using different n-gram sizes as described below (section 2.2).

Yclon can use two different vectorizing methods.

The default method counts the number of times a specific n-gram is detected along the sequence using a sliding window of one nucleotide (Figure 1A) using the CountVectorizer function of the scikit-learn library (Pedregosa *et* al., 2011).

The user can also choose to use the frequency-inverse document frequency (tf-idf) (Figure 1B) with the TfidfVectorizer function of the scikit-learn library (Pedregosa *et* al., 2011).

The tf-idf is calculated in a similar way as the one described in Lindenbaum *et* al, 2020. These methods ignore the locations of particular n-grams within each sequence.

III. Comparing vectors: Afterwards, a similarity matrix is built by comparing every vector, representing the CDR3 sequences, using cosine similarity (Sidorov *et* al., 2014).

This is achieved using the cosine_similarity function from the scikit-learn library (Pedregosa *et* al., 2011).

IV. Clustering

In the Yclon default method, the resulting square distance matrix is the input for a hierarchical agglomerative method using an AgglomerativeClustering method from the scikit-learn library, with a default threshold distance of 0.09, which was determined as described below (section 2.2) (Pedregosa *et* al., 2011).

In addition to the default method, it is also possible to remove redundant sequences and compare only the unique ones, instead of every sequence from that particular subset. For this, the function drop_duplicates from Phyton3 was used for each key. After the clustering, those collapsed unique sequences are re-expanded using the data of the original key.

V. Writing output file.

The last step consists of writing an output file containing all assigned clone sequences into the “clone_id” column. If the file already has a “clone_id” column, the new column is called “clone_id_yclon”. The output file will be named by adding “Yclon_clonotyped” to the input file name.

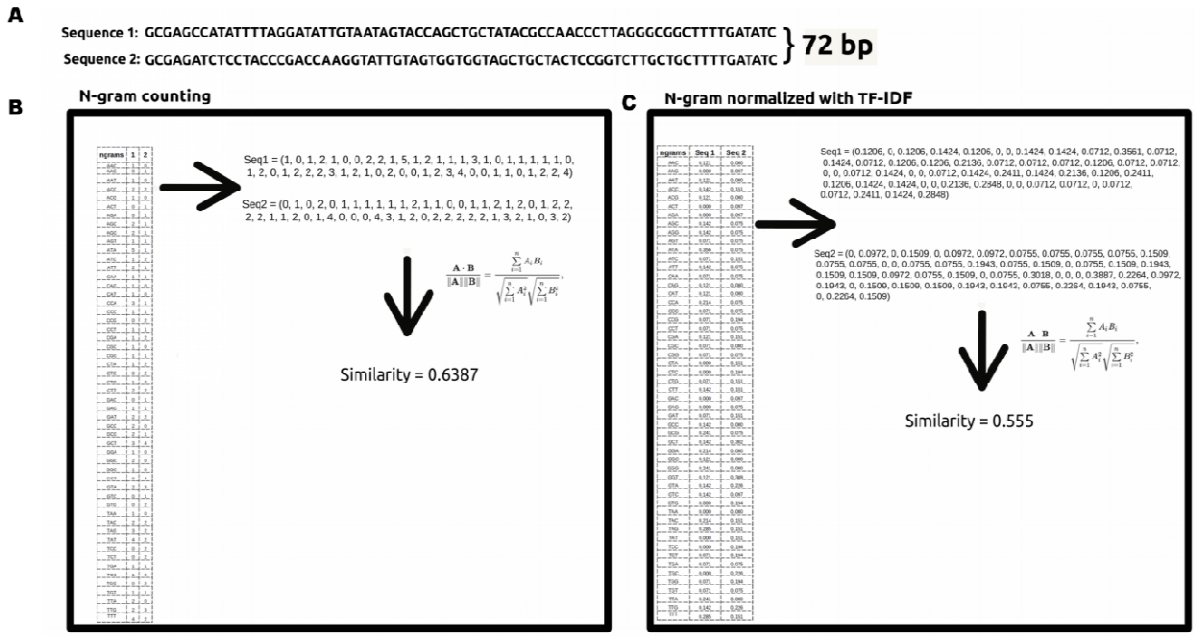

Figure 1: YClon Vectorization Methods: An Overview.
This figure illustrates the two vectorization methods used in YClon. (A) Two sequences of equal length, 72 bp, are compared using two different methods described in (B) and (C). (B) The sequences are decomposed into n-grams of size 3 (as determined in this study using simulated repertoires). A vector is formed by counting the n-grams present in the sequence. The cosine similarity between the vectors is calculated to determine the similarity of the sequences. The formula shows how the cosine similarity is calculated between vectors A and B. (C) The sequences are again decomposed into n-grams of size 3. The n-gram frequency is then normalized using TF-IDF (term frequency-inverse document frequency) weighting. This weighting takes into account both the frequency of the n-gram in the sequence and the inverse presence of the n-gram in all sequences being compared. The resulting vector is calculated by dividing the n-gram frequency in the sequence by the total number of n-grams in the sequence (TF) and then multiplying this TF by the inverse frequency (IDF) of the n-gram in all sequences being compared. The IDF is calculated by dividing the number of sequences being compared by the number of sequences containing the n-gram. The cosine similarity between the normalized vectors is calculated to determine the similarity of the sequences.

2.2. Testing different n-gram sizes and clustering thresholds

We evaluate different n-gram sizes to vectorize CDR3 nucleotide sequences using 74 simulated repertoires data from Lindenbaum *et* al., 2020, by calculating the values of sensitivity, specificity, and PPV. We created the metric SSP by multiplying sensitivity, specificity, and PPV to compare the different thresholds. The SSP values closer to 1 mean better YClon performance for clonotyping. We ran Yclon with an n-gram size ranging from 1 to 9.

We also tested 100 different values to decide the threshold for agglomerative clustering, from

0.00 to 0.99.

To obtain the average CDRH3 (junction) length, we accessed iReceptor and filtered their data using the following parameters: Organism as Homo sapiens (NCBITaxon:9096), PCR target as IGH, Cell subset as B cell, germinal center B cell, immature B cell, naive B cell, peripheral blood mononuclear cell, plasmablast. The filtered data were analyzed by measuring the length of every CDR3, multiplying that number by the number of times that junction appeared, and dividing by the total number of sequences.

2.3. YClon Performance

We tested YClon against grounded true data using 74 simulated repertoires from Lindenbaum et al., 2020. For this analysis, we also run different clonotyping methods to compare with YClon: Change-O, using thresholds ranging from 0.12 to 0.16 determined using SHazaM (Gupta et al., 2015), SCOPer (Nouri & Kleinstein, 2018) and Lindenbaum's method (Lindenbaum et al., 2020). To evaluate Yclon performance and compare it with other methods, we calculated specificity [1] for each of the methods by dividing the number of sequences that were assigned as not being in a clone and truly not being in it (TN) by the sum of TN with the number of sequences assigned as being in that clone but in fact not being in it (FP). The calculation of sensitivity [2] was conducted by dividing the number of sequences assigned to a clone and truly being in it (TP) by the sum of TP with the number of sequences assigned to a different clone, but actually being part of the analyzed clone (FN).

Finally, to calculate the positive predictive value (PPV) [3] we divided TP by the sum of TP with FP, adjusting the weight of the PPV in the whole repertoire.

The mean values of specificity, sensitivity, and PPV were calculated for each clone and used to assess YClon's performance.

$$Specificity_{i} = TN/(TN + FP)$$

[1]

$$Specificity_{whole\ repertoire} = \frac{\sum_{i=1}^{number\ of\ clones} specificity_{i}}{number\ of\ clones}$$

$$Sensitivity_{i} = [TP/(TP + FN)] \times N_{clones}$$

[2]

$$Sensitivity_{whole\ repertoire} = \frac{\sum_{i=1}^{number\ of\ clones} sensitivity_i}{number\ of\ sequences\ in\ the\ repertoire}$$

$$PPV_i = TP/(TP + FP) \times N_{clones}$$

[3]

$$PPV_{whole\ repertoire} = \frac{\sum_{i=1}^{number\ of\ clones} PPV_i}{number\ of\ sequences\ in\ the\ repertoire}$$

To evaluate the tool's functionality, we ran YClon with 3 antibody repertoire datasets of different sizes (Table 1) in a Desktop with an Intel(R) Core(TM) i5-4590 CPU @ 3.30GHz, with 16 Gb of RAM, running ubuntu 20.04 and compared the time each software took to complete the task. The smaller repertoire (Ghraichy et al., 2020) was re-annotated using IMGT/HighV-Quest (Aouinti et al., 2015), while the two others were downloaded already annotated (Chang et al., 2016; Briney et al., 2019) from iReceptor (Corrie et al. 2018) and OAS (Olsen et al., 2021) respectively. We also clonotyped the same repertoires using Change-O, using the threshold of 0.12 determined using SHazaM (Gupta et al., 2015), the hierarchical clustering function of SCOPer (Nouri & Kleinstein, 2018) and the method presented by Lindenbaum et al., 2020 to compare the results with YClon.

We also evaluate the upper limit of sequences YClon can handle for each V, J, and CDR3 length combination. For that, we chose a set of 463 sequences that shared the same V, J gene annotations and CDR3 length of a simulated repertoire and run YClon. This procedure was repeated, adding in each cycle 463 more sequences until it reaches the maximum memory capacity and the script stops.

Table 1: Details of the datasets of different sizes antibody repertoires used in this study

	Dataset 1	**Dataset 2**	**Dataset 3**
Number of sequences	82,966	365,370	1,741,413
Database cointaing Sequences	OAS	iReceptor	iReceptor
Study ID in iReceptor	-	PRJNA628125	PRJEB9332
Subject	C.037.1	7450	Family 4 Carrier
Sample	-	M369-S001	F4Cpost
Study	Ghraichy *et* al., 2020	Nielsen *et* al., 2020	Chang *et* al., 2016
Sex	Male	Female	Female
Age	4	73	3
Condition	Healthy	Covid-19	hepatitis vaccination, 1 day after

2.4. CDR3 intraclonal similarity

To assess the intraclonal similarity of CDR3 sequences generated by YClon, Change-O, SCOPer, and Lindenbaum's methods, we used the hamming distance to compare the sequences within each clone. We applied this calculation to the 800 largest clones from the 74 repertoires described in Lindenbaum et al., 2020. We used the python library "distance" and the function "hamming()" to compute the proportion of differences between each pair of sequences, assuming they were aligned due to equal length. The average similarity was calculated for each clone.

3. Results and Discussion

In this work, the best n-gram size and cut-off to vectorize and cluster sequences were determined using 74 simulated antibody repertoires. It was found that n-gram size of 3 (Figure 2A) and a threshold value of 0.09 (Figure 2B) resulted in the best SSP score, and were therefore used as the

default settings in YClon . However, the user can change these parameters to fit their specific data.

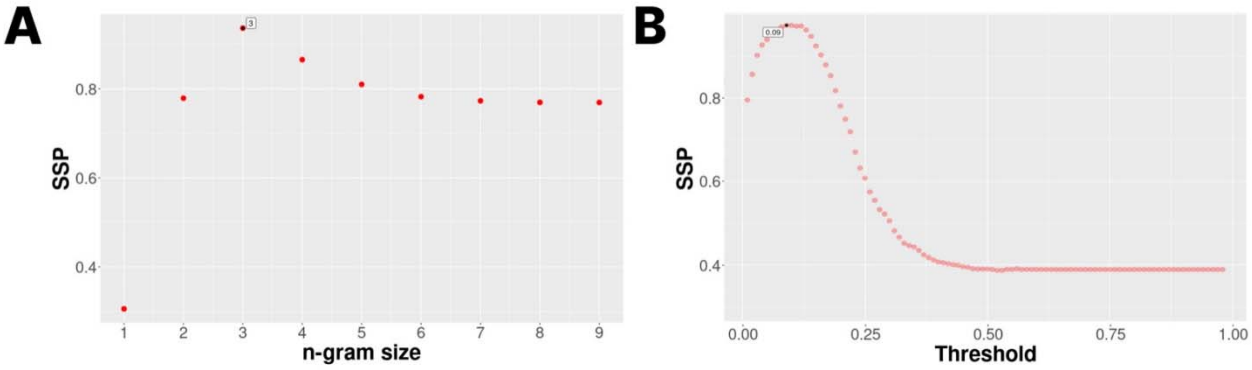

Figure 2: Evaluation of YClon using Different N-gram Sizes and Clustering Thresholds.
To verify the best Yclon n-gram size and threshold we clonotyped 74 simulated repertoire and calculated the values of sensitivity, specificity, and positive predictive value (PPV). We combined these values into a single metric, SSP (sensitivity x specificity x PPV), which ranges from 0 to 1, with a higher value indicating better clonotyping results.
(A) Evaluation of YClon using n-gram sizes ranging from 1 to 9 bases. (B) YClon using threshold values ranging from 0 to 1, with increments of 0.01.

Differently, Lindenbaum et al., 2020 used an n-gram size of 5 when comparing sequences of 150 nucleotides, which covers the entire J segment and the adjacent CDR3/junction region. Our method prioritizes sensitivity, specificity, and PPV over just sensitivity, and only considers sequences with the same CDR3 length, which is smaller than Lindenbaum's region. The average CDR3 length in the iReceptor data (122,229,351 sequences) was 47.52 nucleotides (15.90 amino acids), which is less than a third of the sequence length used by Lindenbaum et al. That may be a reason to why YClon got better results with smaller n-gram.

YClon uses a fixed threshold of 0.09 to group sequences into clones chosen based on the 74 simulated antibody repertoire. This is in contrast to other clonotyping methods such as SCOPer, which adjusts the threshold value while clonotyping, Change-O, which advises users to use SHazaM to determine the optimal threshold for each sample, and Lindenbaum’s method, which uses a negation table to define the threshold.

When checking intraclonal CDR3 similarity among the clonotyping methods, we found that YClon, Change-O, SCOPer, and Lindenbaum's methods group most sequences within clones with over 95% similarity (Figure 3). However, there were a few outliers with lower sequence

similarity in each method, ranging from 45.2% to 94.2% identity among sequences in the same clone. These outliers were present in 7.88% of the sequences for SCOper, 3.29% of the sequences for Change-O, 5.36% of the sequences for YClon, and 3.83% of the sequences for Lindenbaum's method (Figure 3).

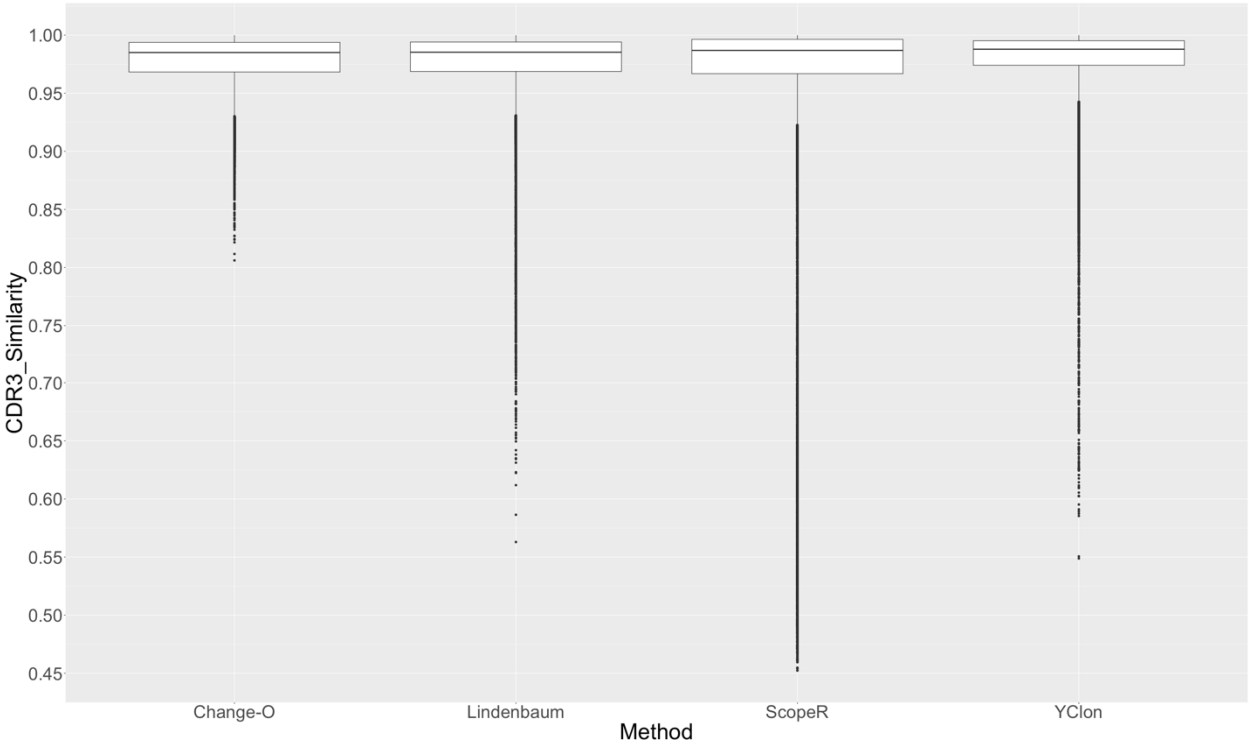

Figure 3: Sequence similarity of the CDR3 sequences within each clone.
We applied this calculation to the 800 largest clones from the 74 repertoires described in Lindenbaum et al., 2020. The box plots enclose the 25th-100th percentiles of the data, with whiskers extending to the 25th percentiles and individual outliers represented by circles. Outliers were defined as data points beyond 1.5 times the interquartile range from the lower and upper hinges of the box plot.

We also evaluated the upper limit of sequences YClon can handle for each V, J, and CDR3 length combination, and found that the largest set of sequences that YClon can compare at once is 93,526 sequences.

The performance of YClon was evaluated using a dataset of 74 simulated antibody repertoires, whose correct clonal assignments were known. This allowed the calculation of sensitivity, specificity, and positive predictive values (PPV) of YClon in comparison to other clonotyping methods, including Change-O, SCOPer, and Lindenbaum's method.

All methods showed high values of specificity (around 100%) (Figure 4A) meaning that the majority of the methods assigned sequences which truly belong to the same clone.

YClon presented best performance (96.4%) compared to SCOPer (93.6%) and worse than Change-O (99.9%) and Lindenbaum's method (97.18%) in terms of sensitivity (Figure 4B). YClon showed a higher PPV (99.8%) compared to SCOPer and Lindenbaum's method (96.7%) and a similar PPV to Change-O (99.7%) (Figure 4C).

It's worth mentioning that the results from the SCOPer paper (Nouri and Kleinstein, 2018) are similar in terms of specificity and sensitivity compared to our findings, but the node PPV calculated by Lindenbaum and colleagues (2020) is different from what we found (84.7% vs >90%). This discrepancy may be due to variations in the calculation of these parameters. Meanwhile, as the Change-O paper (Gupta et al., 2015) did not present any data on sensitivity, specificity, or PPV, a comparison of results with Change-O paper is not possible.

Overall, Change-O achieved a higher sensitivity-specificity-positive predictive value (SSP) value (99%) compared to YClon (96%), SCOper (90%), and Lindenbaum's (82%) methods (Figure 4D). However, when evaluating the performance of each method using 3 antibody repertoire datasets of different sizes (as seen in Table 1), YClon showed improved performance, with a clonotyping speed that was 130 times faster than Change-O and 53 times faster than SCOPer for the smaller repertoire, and 77 times faster than Change-O and 262 times faster than SCOPer for the medium-sized repertoire. For a large dataset containing over 2 million sequences, YClon was the only method that could run on the computer, taking 15 minutes and 44 seconds.

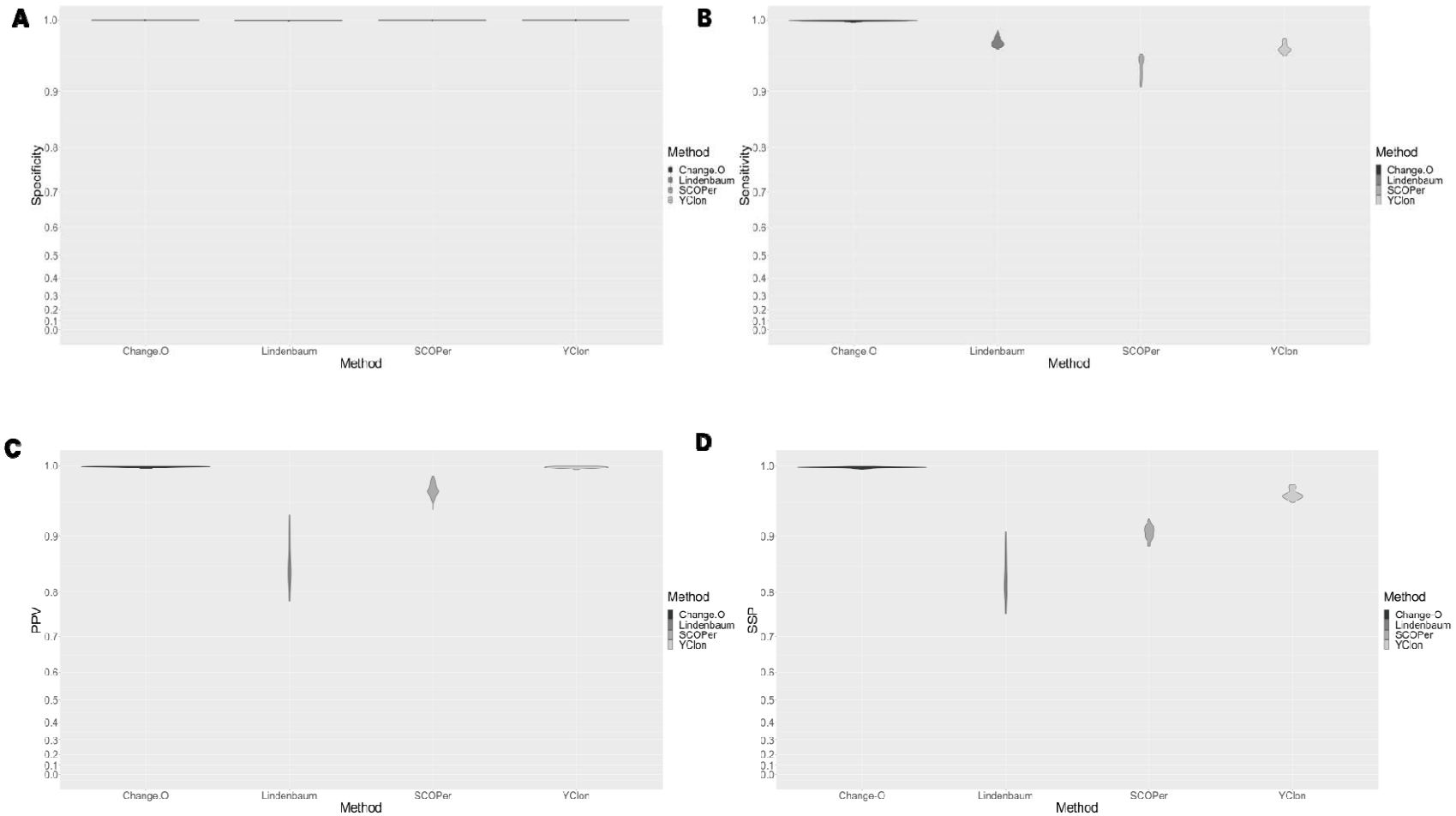

Figure 4: Simulated repertoire clone identification metrics. Violin plot displaying the values for (A) specificity, (B) sensitivity, (C) positive predictive value (PPV), and (D) SSP (sensitivity x specificity x PPV) in clone identification of 74 simulated repertoires from Lindenbaum et al. (2020). The specificity (A) indicates the ability to identify sequences that are not part of a clone, while sensitivity (B) indicates the ability to identify sequences that are part of a clone. PPV (C) represents the probability that sequences in a clone are indeed part of it. The analysis was performed using four clonotyping methods: YClon, the method presented here, Change-O, SCOPer, and Lindenbaum's method.

Table 2 : Performance Comparison of YClon, Lindenbaum's Method, SCOPer and Change-O for Clonotype Identification across Different Repertoire Sizes.

	Dataset 1				Dataset 2				Dataset 3 *
	YClon	Lindenbaum	SCOPer	Change-O	YClon	Lindenbaum	SCOPer	Change-O	Yclon
Number of sequences	82,928				365,707				2,081,722
Total number of clones	24,127	978	22,680	24,031	73,741	27,921	60,561	72,446	1,610,373
Clones with more than 1 sequence	15,833	673	14,477	15,648	35,537	9,783	15,095	35,474	203,766
Benchmark time	**7s**	30m54s	6min13s	15m12s	**50s**	16h19m	4h51m	1h11m 20s	**15min44s**
Number of clones that represents the most abundant 5% sequences	35	1	23	33	2	1	2	2	946
Number of clones that represents the most abundant 15% sequences	376	1	162	327	22	2	15	19	32,836
Number of clones that represents the most abundant 25% sequences	1,180	1	719	1,190	305	3	94	276	126,468

* For the dataset 3 it was only possible to run YClon in the computer used for clonotyping

Conclusion

In recent years, the demand for the analysis of large antibody repertoire datasets, containing millions of sequences, has been on the rise. The iReceptor Gateway integrates over 8,919 antibody repertoires, with 856 of them having more than 1 million sequences, and OAS has 15,148 repertoires with 631 of them having more than 1 million unique sequences. YClon provides a solution to this challenge by making clonal assignment faster, more reliable, and possible on an ordinary computer. This step is critical in gaining insights from antibody repertoire data.

Authors Contribution

JG designed the study, performed analysis, generated the data and wrote the paper. AF analyzed and generated data. LFF designed the study, discussed the results and wrote the paper.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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