MACA: Marker-based automatic cell-type annotation for single cell expression data

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Abstract

Summary: Accurately identifying cell-types is a critical step in single-cell sequencing analyses. Here, we present marker-based automatic cell-type annotation (MACA), a new tool for annotating single-cell transcriptomics datasets. We developed MACA by testing 4 cell-type scoring methods with 2 public cell-marker databases as reference in 6 single-cell studies. MACA compares favorably to 4 existing marker-based cell-type annotation methods in terms of accuracy and speed. We show that MACA can annotate a large single-nuclei RNA-seq study in minutes on human hearts with ~290k cells. MACA scales easily to large datasets and can broadly help experts to annotate cell types in single-cell transcriptomics datasets, and we envision MACA provides a new opportunity for integration and standardization of cell-type annotation across multiple datasets.

Availability and implementation: MACA is written in python and released under GNU General Public License v3.0. The source code is available at https://github.com/ImXman/MACA.

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1 Introduction

Identifying constituent cell-types in a single-cell dataset is fundamental to understand the underlying biology of the system. Many computational methods have been proposed to automatically label cells, and a benchmark study shows that a standard Support Vector Machine (SVM) classifier outperforms most other sophisticated supervised methods and can achieve high accuracy in cell-type assignment (Abdelaal, et al., 2019). However, due to lack of ground-truth in most single cell studies, supervised classification approaches are not feasible and may not be generalized for new single cell studies with different experimental designs. Therefore, unsupervised clustering approaches are still the predominant options for single-cell data analysis (Lähnemann, et al., 2020). Unsupervised approaches usually require human assistance in both defining clustering resolution and manual annotation of cell-types. This results in cell-type annotation being time-consuming and less reproducible due to human inference. As more single cell studies are available, summarizing markers identified in these studies to construct a marker database becomes an alternative approach for automatic cell-type annotation. For example, PanglaoDB (Franzén, et al., 2019) and CellMarker (Zhang, et al., 2019) are two marker databases that summarize markers found in numerous single cell studies and cover a broad range of major cell-types in human and mouse. Also, NeuroExpresso (Mancarci, et al., 2017) is a specialized database for brain cell-types. Taking advantage of those databases for robust cell-type identification, we present MACA, a **m**arker-based **a**utomatic **c**ell-type **a**nnotation method and show how MACA automatically annotates cell-types with high speed and accuracy. We envision MACA as an aid for cell-type annotation to be used by both experts and non-experts.

2 MACA implementation

MACA takes as input expression profiles measured by single cell or nuclei RNA-seq experiments. MACA calculates two cell-type labels for each cell based on 1) an individual cell expression profile and 2) a collective clustering profile. From these, a final cell-type label is generated according to a normalized confusion matrix (Figure 1a). MACA first computes cell-type scores for each cell, using a scoring method based on a marker database or user-defined marker lists. The scoring method uses the raw gene count to calculate a cell-type score for each cell, according to gene markers of this cell-type. This results in converting a gene expression matrix to cell-type score matrix. Then, MACA generates a label (Label 1) for each cell by identifying the cell-type associated with the highest score. Independently, using the matrix of cell-type scores as input, the Louvain community detection algorithm (Blondel, et al., 2008) is applied to generate Label 2, which is a clustering label to which a cell belongs. Since the number of cell types is usually unknown, MACA tries clustering at greater resolution to over-cluster cells into many small but homogeneous groups.

Both Label 1 and Label 2 serve complimentary functions. Label 1 is assigned on a per-cell basis which may result in incorrectly annotating many cells due to noisiness in the maximum cell-type score for each cell. This may occur when the putative cell-type feature is covered up by ambient RNAs from dominant cell-types (Pliner, et al., 2019). On the other hand, Label 2 is likely to suffer from a common problem in single cell RNA-seq clustering analysis, where cells may share the same dominant features, even though they have been clustered into different groups because of subtle differences. Additionally, results from a clustering analysis can often vary since clustering

is non-deterministic. Due to its dependence on user's decisions, mostly the choices of clustering resolution and neighborhood size.

To address these issues, MACA combines Label 1 and Label 2 to get a comprehensive cell-type annotation by mapping Label 2 to Label 1 through a normalized confusion matrix. In the confusion matrix C, $c_{i,j}$ represents the number of cells that were clustered as the ith cluster in Label 2 and labeled as the jth cell-type in Label 1. The basic assumption of mapping Label 2 to Label 1 through a confusion matrix is that cells with the same clustering label (Label 2) should have the same cell-type label (Label 1). Ideally, if cells were identified to be in the same cluster, they should all share the same cell-type, and this cell-type has the highest score for cells in that cluster. However, in real data, this is rarely the case, as we argued above. Therefore, using a confusion matrix, we look for consensus between Label 1 and Label 2, by searching for the highest cell-type score in each cluster. Here, we compute the normalized confusion matrix C_n through dividing confusion matrix C by the size of the cluster: $c_{i,j} = \frac{c_{i,j}}{\sum_{j=1}^{N} c_{i,j}}$, and we search for column number with the largest value for each row (Figure 1b). If $max_j(c_{i,j}) \geq 0.5$, the ith cluster would be assigned as the jth cell-type, as more than 50% of cells in the ith cluster are labeled as the jth cell-type (Case 1). For cases where $max_j(c_{i,j}) < 0.5$, it is likely that cell identities of some cells were covered up by ambient RNAs from dominant cell-types (Case 2). Therefore, MACA records significant or at least the top-3 cell-types for each cell in the ith cluster based on cell-type scores. To find significant cell-types for each cell, we get a distribution of scores of all cell-types for each cell and define those cell-types as significant if their z-scores > 3. If the number of significant cell-types is less than 3, we would keep the top-3 cell-types. Doing this can retrieve more potential cell-type labels for this cluster, and each cell will contribute at least 3 candidates into a pool of candidate cell-types for this cluster.

Then, MACA calculates frequency of each candidate cell-type in this pool and assigns the ith cluster as the cell-type with the highest frequency if the frequency exceeds half the size of the cluster ($max_j(f_{i,j}) \geq 0.5$) (Case 2a). Otherwise, the ith cluster would be labeled as "unassigned" ($max_j(f_{i,j}) < 0.5$) (Case 2b), which is the case that cells in this cluster do not have an agreement on which cell-types they belong to. For the choice of 0.5, we will show our examination in the next Results section. As we mentioned before, clustering-based cell-type identification largely depends on user's choice, for example the choices of clustering resolution and neighborhood size. Therefore, the outcome may vary among different users. To have a more reproducible outcome, we cluster cells with different clustering parameters to get multiple clustering assignments (Label 2s). Repeating the procedure of mapping Label 2 to Label 1 will enable us to get an ensemble annotation through voting, and this ensemble annotation is less influenced by a single clustering choice (Figure 1c). Using ensemble approach also offers a naïve way of scoring MACA-based cell-type predictions. Users can set up a threshold to filter cells whose annotations are less consistent in outcomes of different clustering trials, and we also provide examinations in the next section to help users choose a reasonable threshold for annotation with quality. In this study, we generated clusters using Louvain method with 3 different resolutions and 3 different numbers of neighborhood, which results in 9 different clustering labels (Label 2s). After mapping these 9 Label 2s to Label 1, we generated 9 cell-type annotations. Then, we used a voting approach to get the final annotations (the highest votes from the 9 annotations). Users can also increase the number of clustering trials to have a larger voting pool for annotation ensemble or decrease the number to save computation time.

Back to converting gene expression matrix to cell-type score matrix, we collected 4 different scoring methods that were proposed to do the conversion. These scoring methods are either named

112 by authors, or we named them after the last name of the first author. PlinerScore was a part of
113 Garnett that was designed to annotate cell-types through supervised classification (Pliner, et al.,
114 2019). The uniqueness of PlinerScore is the use of TF-IDF transformation to deal with specificity
115 of a gene marker and a cutoff to deal with issue of free mRNA in single-cell RNA-seq data. AUCell
116 comes from SENIC, which uses gene sets to quantify regulon activities of single-cell expression
117 data (Aibar, et al., 2017). In this study, AUCell quantifies the enrichment of every cell-type as an
118 area under the recovery curve (AUC) across the ranking of all gene markers in a particular cell.
119 This assessment is cell-wise and is different from PlinerScore that requires transformation of the
120 whole dataset. Both CIM and DingScore simply use the total expression of all gene markers of a
121 particular cell-type as the cell-type score (Ding, et al., 2020; Efroni, et al., 2015). CIM normalizes
122 the total expression by multiplying a weight that is defined as the number of expressed gene
123 markers divided by the number of all gene markers of this cell-type. DingScore, on the other hand,
124 normalizes the total expression of one cell-type by dividing total expression of all genes. Since
125 some cell-types have a longer list of marker genes than others, cell-types with more marker genes
126 in the database would have larger cell-type scores. Normalization in CIM was considered to
127 address this issue. However, PlinerScore and DingScore were not intentionally designed to cope
128 with unbalanced marker lists. To deal with this issue, we did a similar processing to normalization
129 in CIM, which is dividing the score of each cell type by the number of expressed markers in that
130 cell type. However, AUCell is a completely different approach from the other 3 scoring methods,
131 which does not simply sum up values of marker genes for a given cell-type. So, we ran AUCell
132 without extra processing for returned values. Meanwhile, we show that the number of expressed
133 marker genes in both PanglaoDB and CellMarker across 6 single cell datasets tested in this study,
134 and we found that most cell-types in PanglaoDB have expressed marker genes within 0~60, while

most cell-types have less than 10 marker genes expressed in CellMarker (Supplementary Figure S1). For both PanglaoDB and CellMarker, we can conclude that cell-types with over 100 expressed marker genes are a minority.

In practice, we build MACA in the analysis pipeline of Scanpy, and MACA takes data in the format of "anndata" in Python (Wolf, et al., 2018). Expression data are preprocessed through cell and gene filtering, and transformed by LogNormlization method, the common practice in single cell analysis. Then, the user provides marker information in the form of Python dictionary, and MACA transforms gene expression matrix to cell-type score matrix. Next, annotation by MACA can be summarized into 4 steps as shown in Figure 1: 1) Louvain clustering to generate Label 2; 2) Generating Label 1 via max function; 3) Mapping Label 2 to Label 1 through normalized confusion matrix; 4) Repeating step 1 to 3 to have ensembled annotation.

3 Results

The key component for optimal performance of MACA is constructing cell-type scores from the gene expression matrix. We investigated 4 scoring methods that have been proposed to transform gene expression matrix to cell-type score matrix (Aibar, et al., 2017; Ding, et al., 2020; Efroni, et al., 2015; Pliner, et al., 2019), and we tested these methods with 2 public marker databases (Franzén, et al., 2019; Zhang, et al., 2019) in 6 single cell studies comprised of 3000 to 20000 cells (Baron, et al., 2016; Cui, et al., 2019; Tian, et al., 2019; Vieira Braga, et al., 2019; Wang, et al., 2020; Zheng, et al., 2017), which include 3 benchmark datasets (Supplementary Table S1) (Abdelaal, et al., 2019). To evaluate these annotation outcomes, we used Adjusted Rand Index (ARI) and Normalized Mutual Information (NMI). Both ARI and NMI are calculated by

157 measuring similarity or agreement between our annotations and authors' annotations. For the 3
158 benchmark datasets, authors' annotations would be the ground truth label, while authors'
159 annotations in the other 3 datasets are at least created under careful investigation. Therefore, use
160 of ARI and NMI, in this case, is to show how well we can reproduce authors' outcomes. We found
161 annotations using PlinerScore with markers in PanglaoDB have the largest agreement with authors'
162 annotations for all 6 datasets, in terms of both ARI and NMI (Table 1). Therefore, MACA uses
163 PanglaoDB with PlinerScore as the main marker database and scoring method, respectively. When
164 we define if Label 2 agrees with Label 1, we selected 0.5 as the threshold. It is out of a simple
165 reasoning of whether the half agrees. However, it is possible to set up a less or more stringent
166 threshold to define the consensus between Label 1 and 2. Thus, we further tested how different
167 thresholds will affect MACA's performance. We changed the threshold from 0.2 to 0.9 and
168 performed our test in these 6 datasets. We expect annotations would vary, but surprisingly,
169 MACA's performance is quite robust to the choice of this parameter, except that we observed
170 drops of ARI and NMI in two datasets when using 0.9 as threshold (Supplementary Table S2).

171 Next, we seek to compare MACA with other existing marker-based annotation tools. CellAssign
172 and SCINA are two computational methods that have been proposed for automatic cell-type
173 assignment (Zhang, et al., 2019; Zhang, et al., 2019). Both methods rely on statistical interference
174 to compute the probabilities of cell types, which are time- and computation- intensive. Recently,
175 Cell-ID was released for extraction of gene signature as well as cell-type annotation (Cortal, et al.,
176 2021). We also noticed scCATCH and SCSA, which are both cluster-based annotation tools (Cao,
177 et al., 2020; Shao, et al., 2020). Both scCATCH and SCSA require identifying differential marker
178 genes for each cluster via a statistical test implemented in Seurat and then matching identified
179 cluster markers to marker database (Butler, et al., 2018). Here, we compared MACA with

180 CellAssign, SCINA, Cell-ID, and scCATCH using these 6 single cell studies and cell markers in
181 PanglaoDB. We tested MACA, CellAssign, SCINA, Cell-ID, and scCATCH on a workstation with
182 16-core CPU and 64GB memory. MACA can finish annotation within 1 minute (cells around 3,000)
183 and less than 2 minutes for a relatively large dataset (cells up to 20,000 cells). On the datasets used
184 and on our computational resources, scCATCH and Cell-ID took longer than MACA to compute
185 annotations and ranks as the second and third fastest. In our hands, SCINA took around 20-minute
186 time to finish annotation for a large dataset, and CellAssign took the longest time to complete cell-
187 type assignment and failed to annotate data with > 20,000 cells due to lack of memory
188 (Supplementary Table S3). Because annotation by scCATCH needs clustering first and differential
189 marker identification is highly affected by clustering outcome, the investigator will need to do a
190 thorough investigation to make sure that clustering is not overdone or underestimated. In this study,
191 we reported the highest and the averaged outcomes of scCATCH in each dataset. Comparing these
192 results with manual annotations from the authors, we found 1) MACA labels cells had a higher
193 consensus than CellAssign, SCINA, Cell-ID, and scCATCH, in terms of both ARI and NMI, and
194 2) MACA and scCATCH identify similar numbers of cell-types to author's annotations, while the
195 other 3 methods, especially Cell-ID, report overall more different cell-types (Table 1). The low
196 ARIs and NMIs of CellAssign and Cell-ID can be counted as results of 1) many "unassigned" cells
197 and 2) exceeding numbers of different cell-types over the numbers reported by authors. It is
198 important to note that other methods compared here were run on their default parameters. In future,
199 parameter tuning of those methods on a computer with higher memory should be carried out for a
200 comprehensive benchmarking on many datasets. Finally, to better evaluate annotations, we used a
201 machine learning approach to assess cell-type assignment. Training classifiers was recently
202 proposed by (Miao, et al., 2020) to assist in finding a good clustering resolution, and we adopt this

203 idea to evaluate our annotations. Basically, if the annotation is good enough, we can train a
204 classifier to predict cell type using gene-expression values with high accuracy. Conversely, if there
205 are many wrong labels, it would be hard for a classifier to make the right decision. We performed
206 5-fold cross-validated training, where we split one dataset into 4-fold training set and 1-fold testing
207 set and trained a SVM classifier on the training sets and applied the classifier to predict labels for
208 the testing set. This procedure repeats 5 times to get a mean accuracy. Instead of treating authors'
209 annotations as ground truth, this machine-learning evaluation provides an independent angle to
210 judge annotation quality. Indeed, MACA achieves high concordance with authors' reported
211 annotations and higher mean of accuracies than other methods (Supplementary Table S4). Of note,
212 high accuracy of SVM classifier is not equal to correctness of annotation. Meanwhile, ARI and
213 NMI reports similarity between two annotations but cannot reflect the difference of annotation
214 resolution. For example, MACA may return less cell-types than authors. Moreover, annotation
215 resolution of MACA highly depends on the number of cell-types in the marker database, and it is
216 likely that MACA cannot annotate some rare subtypes that do not show up in the marker database.
217 Here, we used confusion matrix to show how cell-type labels by MACA are against cell-type labels
218 by authors (Supplementary Figure S2). Take annotation of human pancreas as an example, cells
219 annotated by MACA as "Pancreatic stellate cells" fall into 3 groups that were annotated by author
220 as "activated stellate cells", "quiescent stellate cells", and "Schwann cells", respectively. Since
221 MACA may have a different annotation resolution from the author's, we performed a test to show
222 how different annotation resolutions can affect calculations of ARI and NMI. We included the
223 human kidney (CD10-) data, which has 3 different annotation resolutions by the authors, from 5
224 major cell-types to 29 intermediate cell-types, and to 50 fine cell-types (Kuppe, et al., 2021). We
225 used MACA to annotate this data and compared MACA's annotation with these 3 annotations. We

226 found NMI is more robust to change of annotation resolution than ARI. It also suggests that a
227 higher ARI reflects similar resolution between MACA and author. (Supplementary Figure S3).

228 As we mentioned above, using ensemble approach also offers user an option to filter cells whose
229 annotations are less consistent in outcomes of different clustering trials. However, it also causes
230 loss of cells for downstream analysis, like cellular composition analysis. To find a good balance
231 between having higher annotation quality and keeping most cells for downstream analysis, we
232 tested threshold of voting from 1/9 to 9/9, where the numerator means the minimum number of
233 votes required to keep the cell-annotation. With 1/9, all cells will be kept, with 2/9, cells with
234 annotations with at least 2 votes will be kept, while only cells that have the same annotation across
235 9 clustering trials will be considered if threshold is set up as 9/9. We reported the results across 10
236 datasets in Supplementary Table S5, and it may provide a reference for user to choose a threshold
237 that serves user's need. Of note, we kept all cells in other evaluations. Particularly, all cells were
238 used in benchmark with other methods. Here, we suggest setting up the threshold as 7/9. Next, we
239 expect to show that annotation by MACA is applicable for most single cell RNA-seq platforms.
240 We re-annotated PBMC data from a new study by (Ding, et al., 2020). This data consists of two
241 biological samples from 9 platforms. We found that 1) both PBMC samples have the same major
242 cell-types, and these 9 platforms can successfully profile them (Supplementary Figure S4a), and
243 2) annotation by MACA shows that all platforms profile similar cellular components for these two
244 PBMC samples, except CEL-Seq2 (Supplementary Figure S4b). These results are largely
245 consistent to the original report (Ding, et al., 2020). However, this PBMC data didn't come with a
246 ground-truth annotation, we further added the human pancreas data, which consists of 5
247 independent studies profiled by 4 different single-cell RNA-seq platforms (Baron, et al., 2016;
248 Grün, et al., 2016; Lawlor, et al., 2017; Muraro, et al., 2016; Segerstolpe, et al., 2016). Annotation

249 by MACA has 0.929 ARI and 0.908 NMI against author-reported annotation, and we also observed
250 all major cell-types were revealed across all 4 platforms (Supplementary Figure S4c).

251 Finally, we applied MACA to a single-nuclei RNA-seq dataset from all 4 chambers of the human
252 heart, comprised of ~290k nuclei (Tucker, et al., 2020). MACA could annotate each of the 4
253 chambers comprising of ~80K cells each in < 6 mins. Annotations by MACA have major
254 agreement with author's reported annotations with an average ARI and NMI of 0.63 and 0.76,
255 respectively (Supplementary Table S6). However, we also found some disagreements exist in
256 annotation of cells in from left and right atria. Therefore, we investigated disagreement between
257 MACA's and author's annotations, and found the biggest difference stems from disagreement in
258 assignments for neuronal cells and lymphocytes, which are both small-population cell types in this
259 dataset (1702 neuronal cells and 1503 lymphocytes out of ~290k). We found neuronal cells weren't
260 revealed and author-reported lymphocytes were reported as memory T cells in MACA's
261 annotation (Supplementary Table S7a and b).

262 By default, MACA works with the list marker genes and cell-types present in PanglaoDB, but
263 users can also input their own gene-lists. A major limitation of MACA is that it can only annotate
264 cell-types that are pre-defined in the marker reference, but with more marker gene-sets becoming
265 available with single-cell sequencing studies, we believe that MACA will be useful to annotate
266 heterogeneous single-cell datasets. This points us two future directions to improve MACA. First,
267 with more atlas studies that profile all sorts of biological systems, more refined markers for small
268 cell populations can be defined, and MACA could reach finer annotation resolution by integrating
269 markers from these new atlas studies. Second, weights of markers should be incorporated into the
270 scoring method of MACA, for example marker specificity and expression strength. However, at
271 the current stage, all markers have equal weights when they contribute to cell-type scores, and we

272 believe that incorporating marker weights will be beneficial for accurate annotation. With a more
273 refined marker database and cell-type scoring method, MACA would rapidly perform integrated
274 annotation across multiple datasets, and this is very critical for downstream analyses like cellular
275 component analysis across datasets under different conditions. In fact, we noticed that combining
276 PlinerScore and PanglaoDB to generate new features has the advantages of correcting batch effects
277 for integrated annotation across datasets, and we aim to extend the use of MACA to standardization
278 of cell-type annotation across datasets in the future (see application in integrated annotation on
279 GitHub of MACA). Finally, we conclude that MACA is a suitable tool for automatic cell-type
280 annotation that can aid both experts and non-experts in rapid annotation of their single-cell datasets.

281

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285

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289

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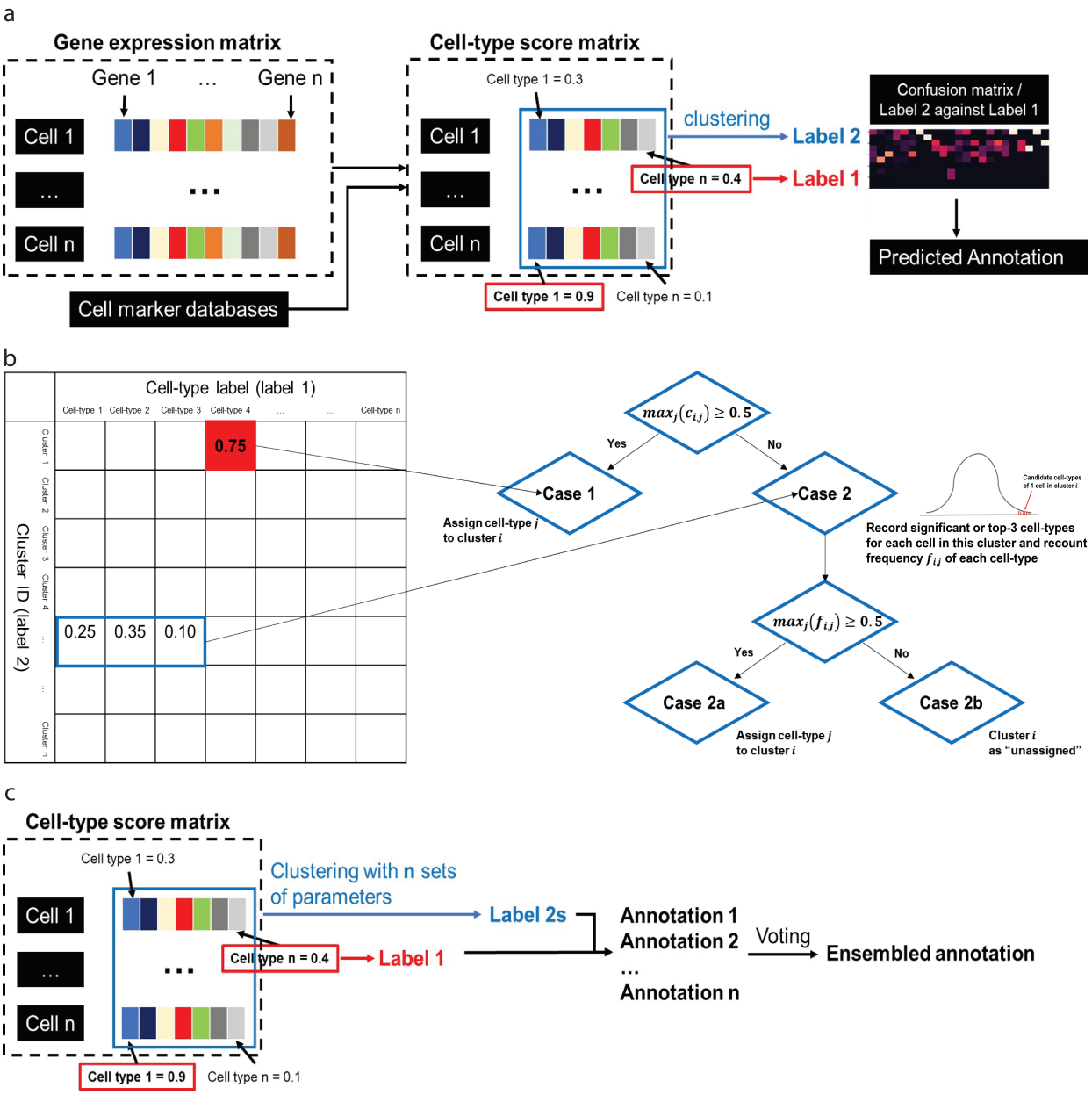
351

352 **Figure 1**



353

354 **Figure 1.** Schematic workflow of MACA. a, MACA converts gene expression matrix into cell-type score
355 matrix based on cell marker database. MACA generates Label 1 by using max function and Label 2 by over-
356 clustering all cells into small groups. MACA finally maps Label 2 to Label 1 via confusion matrix. b, Use
357 of confusion matrix for cell-type annotation. c, In practical implementation, *n* sets of clustering parameters
358 are used to generate *n* Label 2s. Mapping all Label 2s to Label 1 returns multiple annotations, and MACA
359 ensembles these annotations by voting to generate the final cell-type prediction.

Table 1

ARI	PBMC (Zheng et al., 2017)	CellBench (Tian et al., 2019)	Pancreas (Baron et al., 2016)	Heart (Wang et al., 2020)	Heart (Cui et al., 2019)	Lung (Vieira et al., 2019)
PanglaoDB+PlinerScore	**0.95**	**0.92**	**0.90**	**0.71**	**0.61**	**0.45**
PanglaoDB+AUCell	0.04	0.00	0.78	0.39	0.47	0.29
PanglaoDB+CIM	0.28	**0.65**	**0.90**	0.27	0.30	0.33
PanglaoDB+DingScore	**0.83**	**0.74**	0.69	0.07	0.44	0.20
CellMarker+PlinerScore	0.38	0.43	0.27	**0.57**	0.13	0.21
CellMarker+AUCell	0.29	0.52	0.32	0.34	0.09	0.14
CellMarker+CIM	0.24	0.60	0.54	**0.56**	0.07	0.09
CellMarker+DingScore	0.22	0.55	0.38	0.37	0.19	NA
SCINA	0.46	0.63	**0.89**	0.13	**0.55**	0.31
CellAssign	NA	0.00	**0.89**	0.15	**0.53**	0.26
Cell-ID	0.50	0.17	0.57	0.10	0.49	**0.35**
scCATCH (best)	**0.62**	0.56	0.86	0.04	0.14	**0.60**
scCATCH (average)	0.57	0.40	0.66	0.04	0.05	**0.35**
NMI	PBMC (Zheng et al., 2017)	CellBench (Tian et al., 2019)	Pancreas (Baron et al., 2016)	Heart (Wang et al., 2020)	Heart (Cui et al., 2019)	Lung (Vieira et al., 2019)
PanglaoDB+PlinerScore	**0.89**	**0.92**	**0.88**	**0.59**	**0.62**	**0.59**
PanglaoDB+AUCell	0.09	0.00	0.79	**0.41**	0.50	0.31
PanglaoDB+CIM	0.51	**0.80**	**0.88**	0.30	0.44	0.40
PanglaoDB+DingScore	0.74	**0.85**	0.70	0.10	0.47	0.33
CellMarker+PlinerScore	0.44	0.64	0.57	**0.51**	0.32	0.42
CellMarker+AUCell	0.23	0.67	0.46	0.32	0.33	0.17
CellMarker+CIM	0.49	0.78	0.73	**0.41**	0.31	0.21
CellMarker+DingScore	0.43	0.73	0.60	0.34	0.33	0.08
SCINA	0.54	0.71	0.84	0.07	**0.54**	0.46
CellAssign	NA	0.06	**0.86**	0.08	0.51	0.49
Cell-ID	0.67	0.38	0.74	0.08	**0.55**	0.58
scCATCH (best)	**0.77**	0.70	0.84	0.05	0.30	**0.73**
scCATCH (average)	**0.75**	0.62	0.75	0.04	0.12	**0.63**
# of cell-types	PBMC (Zheng et al., 2017)	CellBench (Tian et al., 2019)	Pancreas (Baron et al., 2016)	Heart (Wang et al., 2020)	Heart (Cui et al., 2019)	Lung (Vieira et al., 2019)
MACA	8	6	11	8	7	13
SCINA	14	14	17	16	23	41
CellAssign	NA	9	17	18	24	31
Cell-ID	33	55	48	35	37	63
scCATCH (best)	9	5	10	3	3	16
Author's annotation	5	5	14	5	9	13

Table 1. Performance of MACA, CellAssign, SCINA, Cell-ID, and scCATACH in 6 scRNA-seq datasets, measured by ARI and NMI. 8 different settings of MACA include using 4 cell-type scoring methods (PlinerScore, AUCell, CIM, and DingScore) with 2 marker databases (PanglaoDB and CellMarker).