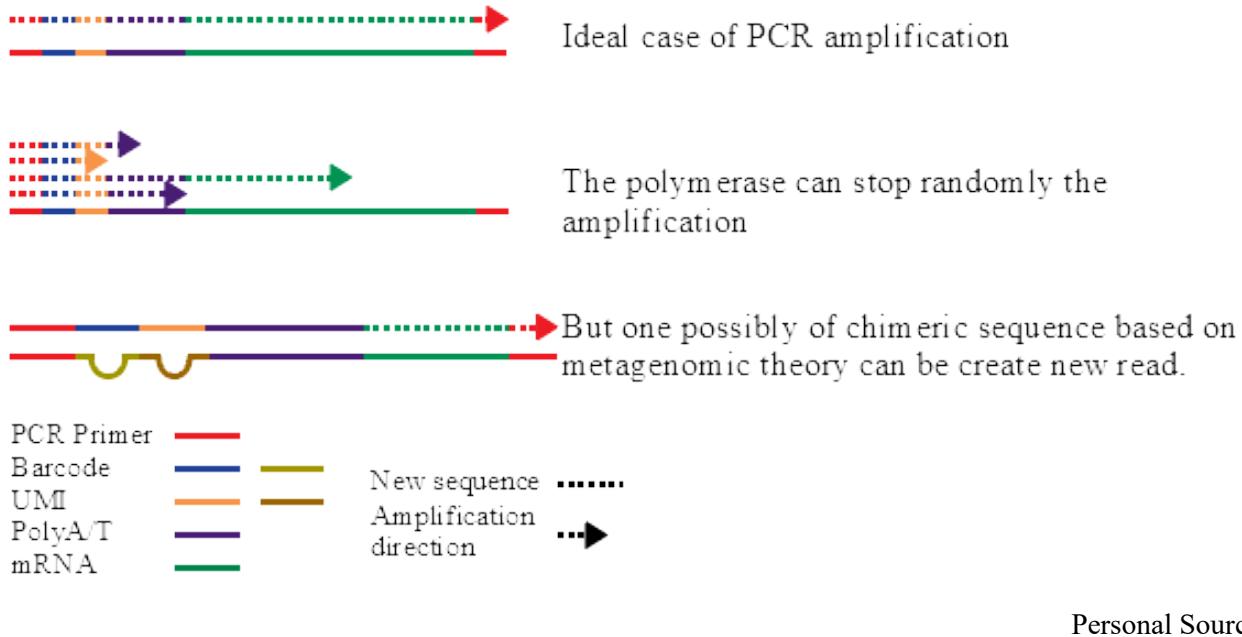


Personal project in single cell

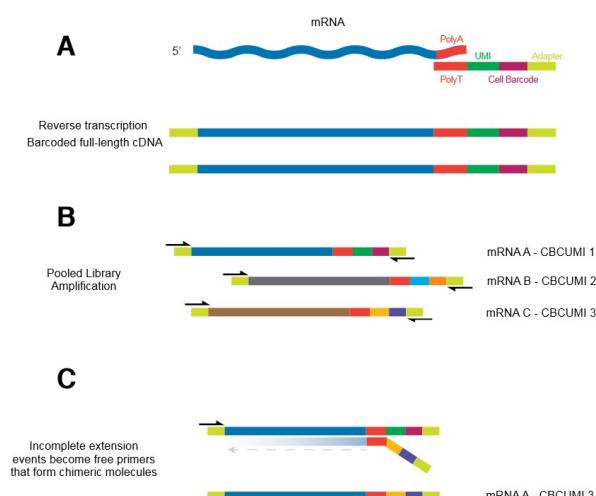
RNA-seq

RNA-seq gave lot of opportunities of scientist to identify different cells types and alternatives transcripts isoforms¹. To identify cell types many tools was created. You can extract specifics genes to identify cells types² by exploiting the differential expression or exploit the data to explain the datasets expression by network³. But RNA-seq take a part of a tissue composed of multiple cells type. For a better resolution single cell approach was developed⁴. Genomics, Epigenomics⁵, Transcriptomics, Proteomics and Metabolomics are now study by single-cell⁶. This new techniques of study need new software to extract information in the data. In example, new algorithm for the genome assembly was created⁷. And in the same time, G&T-seq, a techniques who combine genomic and transcriptomic data extraction from the same cell, was created⁸. Now, they up to 3 data for the same cell⁹. With the correlation of data we can understood more the interaction and improve the data validation by the cross validation between the data. But, we will speak more about the scRNA-seq. In 2015, the first experience of the Drop-seq technique published¹⁰. This technique are one of the cheaper, adaptable in an environment Illumina, and you can sequence the mRNA of 10,000 cells⁶. In the success of this experience, a new way in transcriptomic analyse was create. Other techniques are developed using different approach to separate cells and obtain their mRNA. CEL-Seq2¹¹, STRT¹², sci-RNA¹³, Seq-Well¹⁴, all this techniques use Barcode to identify cells and PCR for the DNA amplification. The result are the same, you obtain a dataset of your scRNA-seq experiment. You have different way to analyse it¹⁵: lineage tracing, the algorithm find the genes for combine cell and obtain the dynamic in the development of cell from the dataset¹⁶⁻¹⁸, network construction, this technique use the genes network to reconstruct the different group of cell^{19,20}, or simply using a tSNE for study the complexity between cells^{21,22}. With the explosion in the difference software, scRNA-tools, a database was created²³. You have many way to analyse your data, and this database can gave you the right tools. But before the analyse, you have to purify your dataset. You can have lot of error, and generate fake informations. How know if your cell are not a subset of one cell? You will take combine cells with same low genes in the biggest? But, how detect barcode with multiple cell? With the total level of expression. But it is right? We create error before the sequencing step by the catch step, and the PCR. We take a little proportion of mRNA during the catch step⁶. This is random between cells, and we can't control that. During PCR, the gene with lot of expression will be more dominant and hide the little express gene. In the same time, PCR create difference between cell, in other word cell with certain barcode are more amplified from the other²⁴. But we can use T7 polymerase, but if we increase the error rate²⁵⁻²⁷ on all sequence, we increase the barcode and UMI errors. In addition we know in metagenomics, the PCR create chimeric sequence^{28,29} with closes sequences. "Chimeras are artificial recombinants between two or more parental sequences, and they are normally formed when prematurely terminated fragments reanneal to other template DNA during PCR amplification"³⁰. You can have 5% of error rate³¹ in your dataset. To identify them, they develop lot of tools to find a way to identify them, but this operation take time and calculation resources. What we know in scRNA-seq? We have the same problem³².

Why a chimeric sequence are a problem in our data?



In this case we can simply detect chimeric product. The sequence have the same UMI from another sequence. But, the problem will be the quantity? A chimeric product can appear more than one time. And we can remove the real sequence if we delete the product. In the same time, A. Dixit speak about one possibility in chimeric sequence creation:



In the concept, only polyA sequence are the problem for the creation in chimeric sequence. Can it false? And if we have shift between sequence?



You can't just base your correction with UMI or cell barcode sequences.

It is why I developed a new approach.

Results

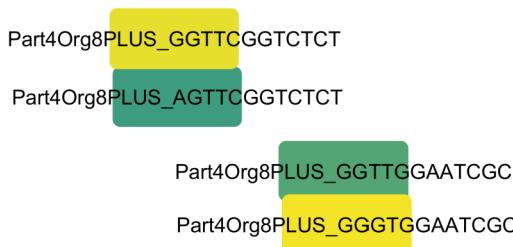
I test the software on data from Quadrato & al.²². This data have always the barcode in the cell name, and they have lot of informations. I use Cytoscape for the visualisation. A file with the result can be download on: <https://github.com/studyfranco/STUDER/blob/master/Internal.cys>

But if you have a dataset to suggest, I can test it and add in the result. Send me a link where I can download the expression matrix file.

The first goal are: I want find all same cells informations with different barcode. If you prefers: PCR + Chimeric sequence can create error in the barcode, and if we want find the identical cell in the dataset the work are complicate.

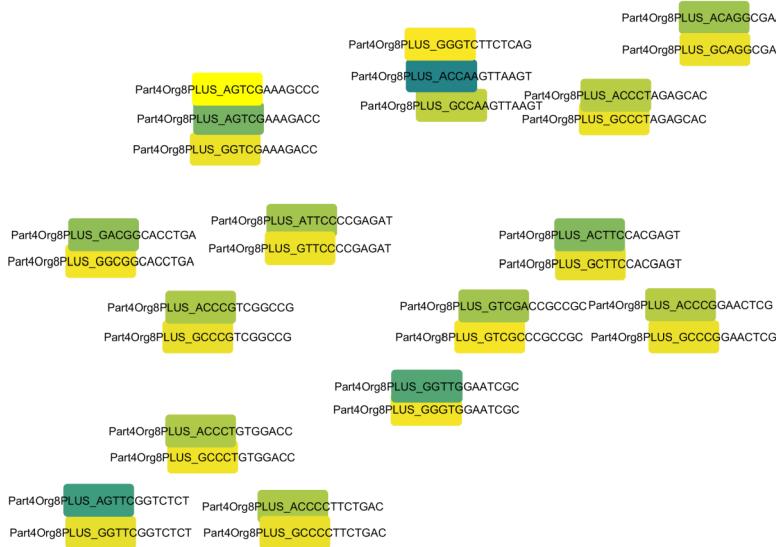
In the ideal case:

If we use only the basic gene information we obtain for the Organoid4H:



We detect four cell with the possibility to merge the informations. We regroup the cell by two, and we can see 1 base different in the barcode. The yellow cell have less genes express than the green.

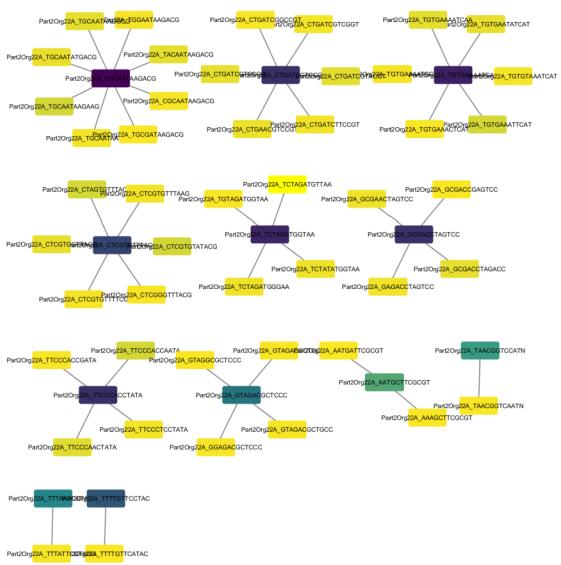
With my software, for the same organoïd:



We identify more barcode error. 16 cells are just a subset of one other cell. 16 cells are 0,5% of our total dataset. This proportion are not so important in our dataset. But possibly have an impact in the algorithm for the analyse.

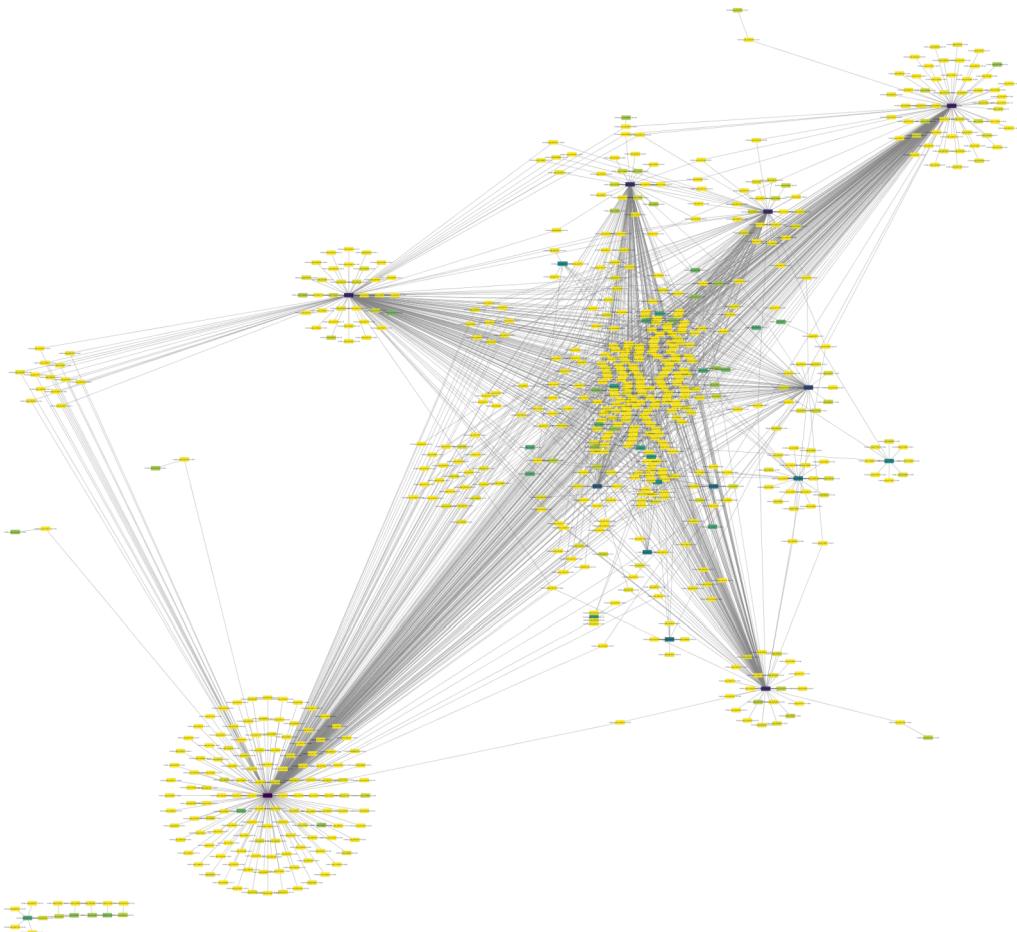
Here, the dataset are good.

Orga1A have more problem. When we want detect barcode error with only the raw gene expression, we obtain:



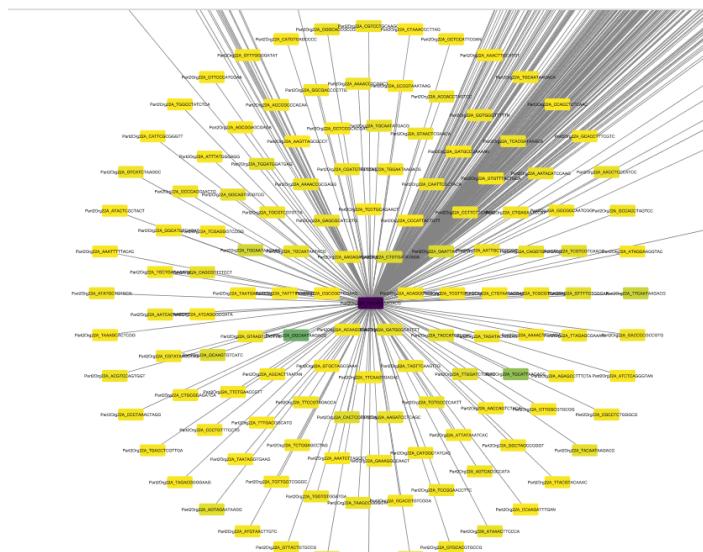
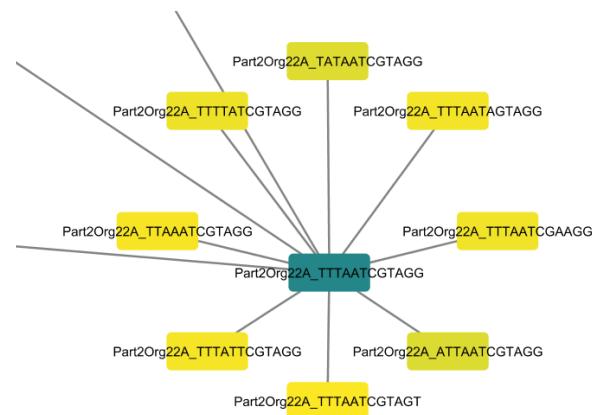
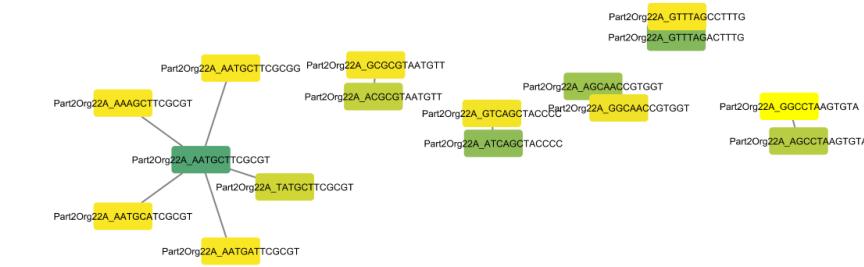
We detect 47 cells, can be a subset in one cell. 1,3% of all cell are not good.

With my software:



We have a new pattern.

We have one part with the expected results.



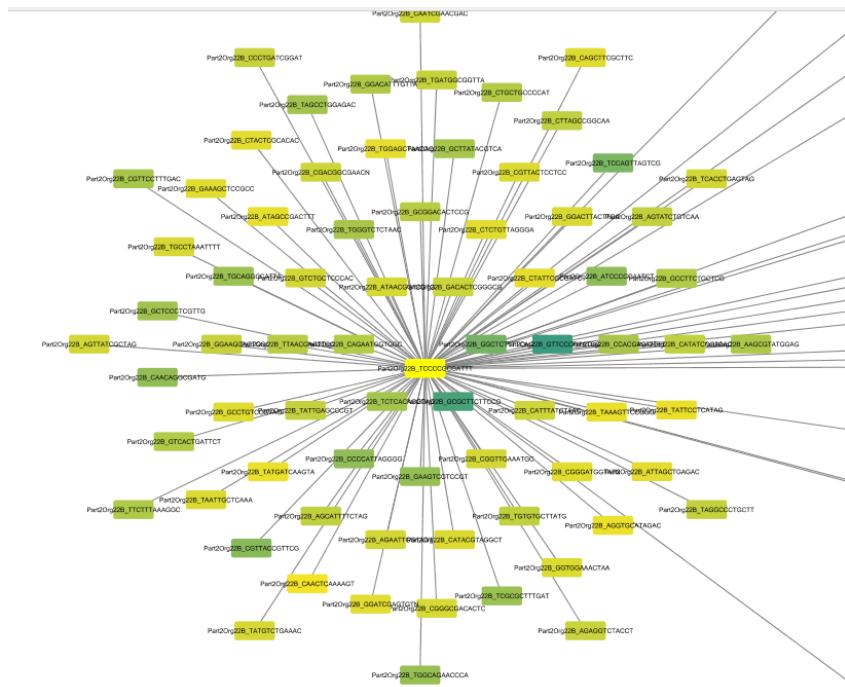
More you are purple, more your cell contain genes in the initial.

But if we analyse the different nodes, we can found a pattern.

The purple node have lot of gene express and lot of total expression. It can be a drop-let with more than 1 cells.

This is the second goal of my software: Detect barcode who contain the expression information from more than 1 cell.

The third goal of my software are in the same time, detect cell with low expression. For this example I will take Orga1B.



We can see one cell, with 409 genes express in the raw dataset. It validate only 93 genes for this cell. And after treatment we detect this cell internal in 92 other cell.

I need 10-30 min for create this Cytoscape representation and clean the dataset.

Conclusion

The software can detect more barcode error, multiple cell in the same barcode, and low coverage cell expression. We can define the threshold for select what is a bad cell, and correct the dataset. In bad dataset, we can correct rapidly the errors. But this software are one more hide utility. It clean fake raw gene expression from chimeric sequence.

A clean dataset, are the insurance of a good analyse.

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