

Claims

1. A process for genotyping single cells having a phenotype of interest comprising:
 - providing a plurality of reservoirs, and
 - a) for a plurality of reservoirs, providing, for each reservoir, at least one barcode sequence and at least one dye, wherein the at least one barcode sequence is associated with the color and the concentration of said at least one dye, and taking an image of the array thereby mapping the color and the intensity of the at least one dye for each reservoir; and
 - b) for a plurality of reservoirs, providing, for each reservoir, at least one single cell and performing for each reservoir a phenotypic assay on said at least one single cell, and taking an image thereby mapping the phenotype of said at least one single cell for each reservoir;
 - wherein step a) is performed before step b) or step b) is performed before step a); then
 - c) for a plurality of reservoirs, linking, for each reservoir, the phenotype of a single cell obtained in step b) to the color and the intensity of the at least one dye obtained in step a); and
 - for a plurality of reservoirs, reverse transcribing, for each reservoir, nucleic acids of said at least one single cell, to obtain single cell cDNA barcoded with said at least one barcode sequence which is associated with the color and concentration of the at least one dye; and
 - linking the genotype with the phenotype of said at least one single cell.
2. The microfluidic process according to claims 1, wherein said method further comprises at least one step selected from the group consisting of:
 - at least one purging step,
 - for a plurality of reservoirs, providing, for each reservoir, at least one oligonucleotide, wherein said at least one oligonucleotide comprises a primer sequence,
 - for a plurality of reservoirs, attaching, for each reservoir, the at least one barcode sequence to the at least one oligonucleotide,
 - for a plurality of reservoirs, providing, for each reservoir, a lysis composition,
 - for a plurality of reservoirs, lysing, for each reservoir, the at least one single cell,
 - for a plurality of reservoirs, providing, for each reservoir, a reverse transcriptase and a reverse transcriptase composition,

- for a plurality of reservoirs, hybridizing, for each reservoir, the at least some of the single cell nucleic acids with the at least one barcoded oligonucleotide,
 - recovering the barcoded single cell cDNA,
 - removing unincorporated oligonucleotides from said barcoded cDNA,
 - amplifying the barcoded single cell cDNA,
 - sequencing the barcoded single cell cDNA.
3. The microfluidic process according to claim 1 or 2, wherein the process is a microfluidic process comprising a step of providing a microfluidic device comprising a chip comprising at least one microfluidic channel and a plurality of reservoirs.
4. The microfluidic process according to claim 3, wherein, for each reservoir, the at least one single cell is provided in a single cell droplet.
5. The microfluidic process according to claim 3 or 4, wherein, for each reservoir, the reverse transcriptase, the reverse transcriptase composition and the at least one oligonucleotide are provided in a RT droplet.
6. The microfluidic process according to any one of claims 1 to 5, wherein, for a plurality of reservoirs, the at least one barcode sequence is linked to the surface of each reservoir of the plurality of reservoirs.
7. The microfluidic process according to claim 6, wherein the at least one barcode sequence is released prior to transcription.
8. A microfluidic process for barcoding single cell nucleic acids, said method comprising:
- providing a microfluidic device comprising a chip comprising at least one microfluidic channel and a plurality of reservoirs,
 - injecting into the inlet of the microfluidic channel a carrier fluid comprising a plurality of droplets of a first type dispersed in the carrier fluid, wherein the droplets of the first type are either single cell droplets or RT droplets, wherein at least some of the RT droplets comprise a reverse transcriptase and at least one oligonucleotide, and wherein at least some of the single cell droplets comprise one single cell, wherein said single cell comprises single cell nucleic acids,

- for a plurality of reservoirs, a first migration step, wherein at least one droplet of the first type among the plurality of droplets is moved into one reservoir of said plurality of reservoirs by buoyancy,
- injecting into the inlet of the microfluidic channel, a carrier fluid comprising a plurality of droplets of a second type dispersed in the carrier fluid, wherein the droplets of the second type are either single cell droplets or RT droplets, and wherein the droplets of the second type are RT droplets when the droplets of the first type are single cell droplets or the droplets of the second type are single cell droplets when the droplets of the first type are RT droplets,
- for a plurality of reservoirs, a second migration step, wherein, at least one part of at least one droplet of the second type enters into one reservoir of said plurality of reservoirs,
- for a plurality of reservoirs, fusing, in or at the edge of each reservoir, said at least one droplet of the first type with said at least one droplet of the second type, thereby resulting in a fused droplet,
- and further comprising the steps of:
 - a) hybridizing, for each fused droplet, the at least some of the single cell nucleic acids with the at least one oligonucleotide in said fused droplet,
 - b) reverse transcribing, in each fused droplet, at least some of the single cell nucleic acids present in said fused droplet, thereby resulting in single cell cDNA, and
 - c) attaching at least one barcode sequence to the single cell cDNA obtained in step b), wherein said at least one barcode sequence encodes the identity of said single cell,

or

- further comprising the steps of:
 - a) hybridizing, for each fused droplet, at least some of single cell nucleic acids from at least one cell with the at least one oligonucleotide in said fused droplet, wherein said at least one oligonucleotide comprises at least one barcode sequence,
 - b) reverse transcribing, in each fused droplet, at least some of the single cell nucleic acids present in said fused droplet, thereby resulting in barcoded single cell cDNA, wherein said at least one barcode sequence encodes the identity of said single cell.

9. The microfluidic process according to claim 8, wherein the RT-droplets further comprise at least one particle of a first type to which the at least one oligonucleotide is bound.

10. The microfluidic process according to claim 8 or 9, wherein the RT-droplets further comprise a polymer.

11. The microfluidic process according to any one of claims 8 to 10, wherein the plurality of droplets of the first type have an average volume from 1pL to 3000pL.
12. The microfluidic process according to any one of claims 8 to 11, wherein the plurality of droplets of the second type have an average volume from 10pL to 5000pL.
13. The microfluidic process according to any one of claims 8 to 12, wherein the first migration step of at least one droplet of the first type results in an occupancy of 90% to 100% of the total number of reservoirs and/or results in a capturing efficiency of 80% to 100% of the injected droplets of the first type.
14. The microfluidic process according to any one of claims 8 to 13, wherein the at least one part of the at least one droplet of the second type refers to 90% to 100% of the total volume of the at least one droplet of the second type or 10% to 30% of the total volume of the at least one droplet of the second type.
15. The microfluidic process according to any one of claims 8 to 14, wherein the fusion step results in a fusion efficiency of 90% to 100% between the droplets of the first type and the droplets of the second type.