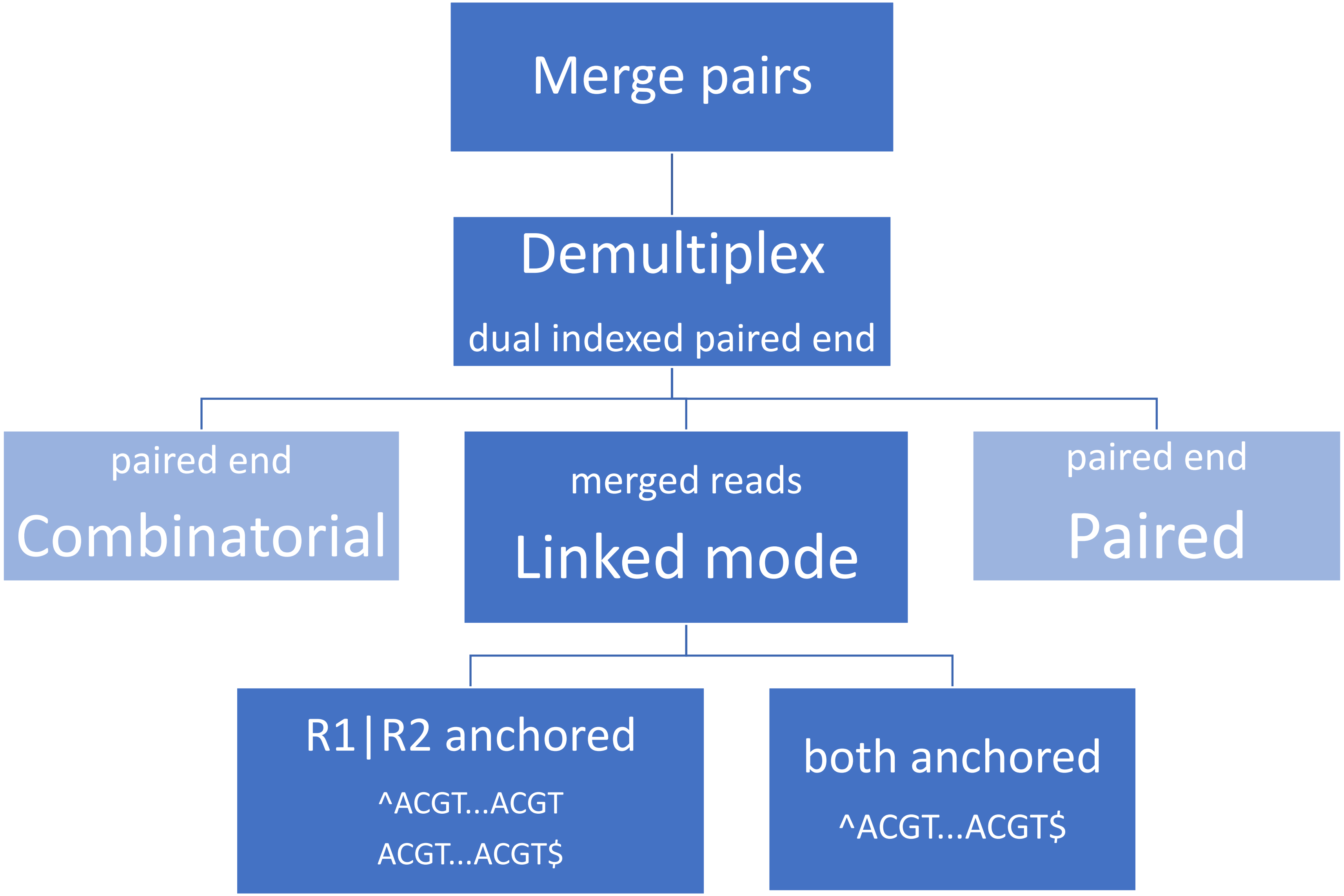


Merge pairs

Demultiplex

dual indexed paired end

paired end

Combinatorial

merged reads

Linked mode

paired end

Paired

R1|R2 anchored

^ACGT...ACGT

ACGT...ACGT$

both anchored

^ACGT...ACGT$

If you adoted the following primer design:

A1 F1-R1
A2 F2-R1
A3 F3-R1
...
A4 F1-R2
A5 F2-R2
A6 F3-R2
...
A7 F1-R3
A8 F2-R3
A9 F3-R3

Your choice was the **combinatorial** strategy

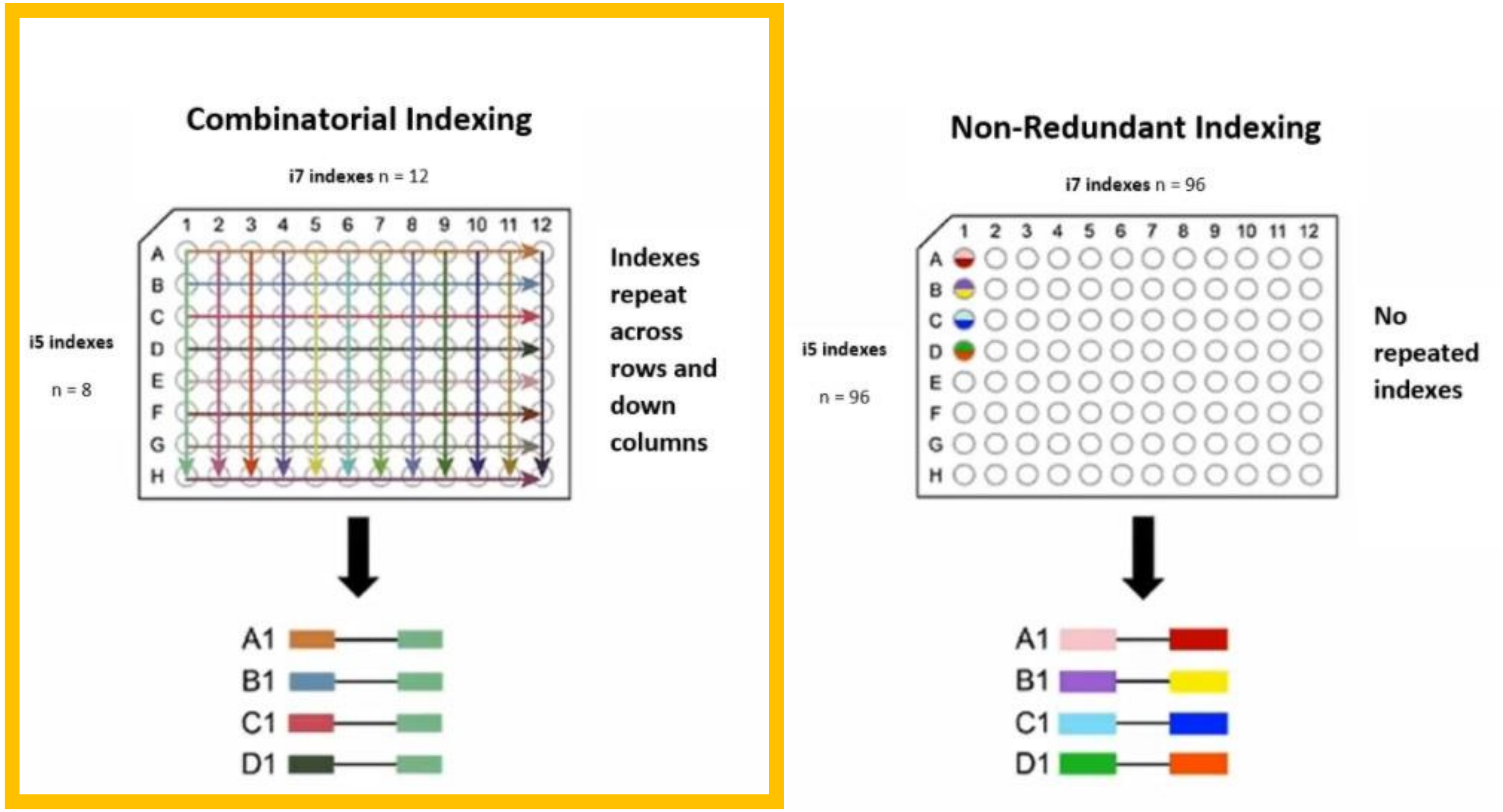


Figure 1: With combinatorial dual indexing, every i5 and i7 is shared among other samples on the same plate with indexes repeating across rows and down columns; the combinations are unique though the individual indexes are not. In contrast to combinatorial dual indexing, unique dual indexing has completely unique indexes (eg, 96 unique i5s and 96 unique i7s per 96-well plate).

https://support.illumina.com/bulletins/2018/08/understanding-unique-dual-indexes--udi--and-associated-library-p.html

You can choose if you'd like toi merge the paired ends or to keep them separated, as needed for running DADA, for example, or for any other reason, but the combinatorial strategy is about tre primer design, not "merged or not". Right?

When you choose to **merge** your Illumina pairs **before** demultiplexing (for any reason), this is what happens in the merge step:

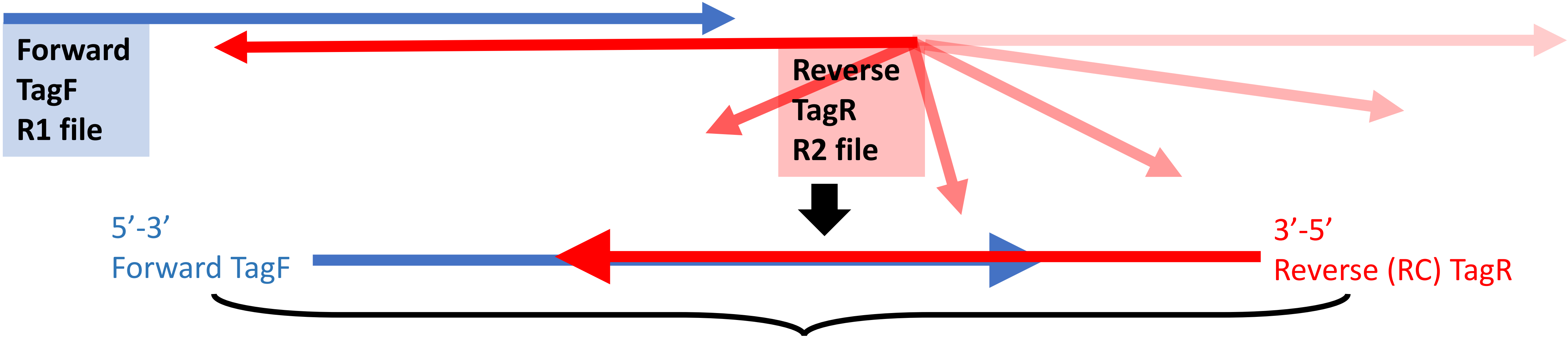
R1 file
5’ end
Forward Tag
R2 file
5’ end
Reverse Tag

@M01334:388:000000000-JBK78:1:1109:19410:16178 1:N:0:CTCTCGTC+TATAACCT
CCTAAACTACGGCCTGACATGGCTTTTCCCCGAATAAATAATATAAGATTTTGGTTATTACCTCCCTCTCT

@M01334:388:000000000-JBK78:1:1109:19410:16178 2:N:0:CTCTCGTC+TATAACCT
CCATCACATAGGTCGGGGTGGCCAAAGAACCAAAATAGATGTTGGTAAAGAATTGGGTCTCCCCCTCCA

Mapping file: barcodes.txt
A1 CCTAAACTACGG CCATCACATAGG → **BOTH FORWARD DIRECTION**

Both pairs are in **forward** direction in the **original** fastq files, as you see above, but when they merge, the **R2 sequences become reverse** (see below, red). Because of this, when demultiplexing with linked mode, the vast majority of the merged reads are **TagF – reverse TagR** or **reverse TagF – TagR**:



Because of this I **recommend** to demultiplex **merged** pairs in **Linked adapter** mode, when both tags are **required** (and optionally **anchored**, in order to **minimize wrong** combinations during library preparation).