

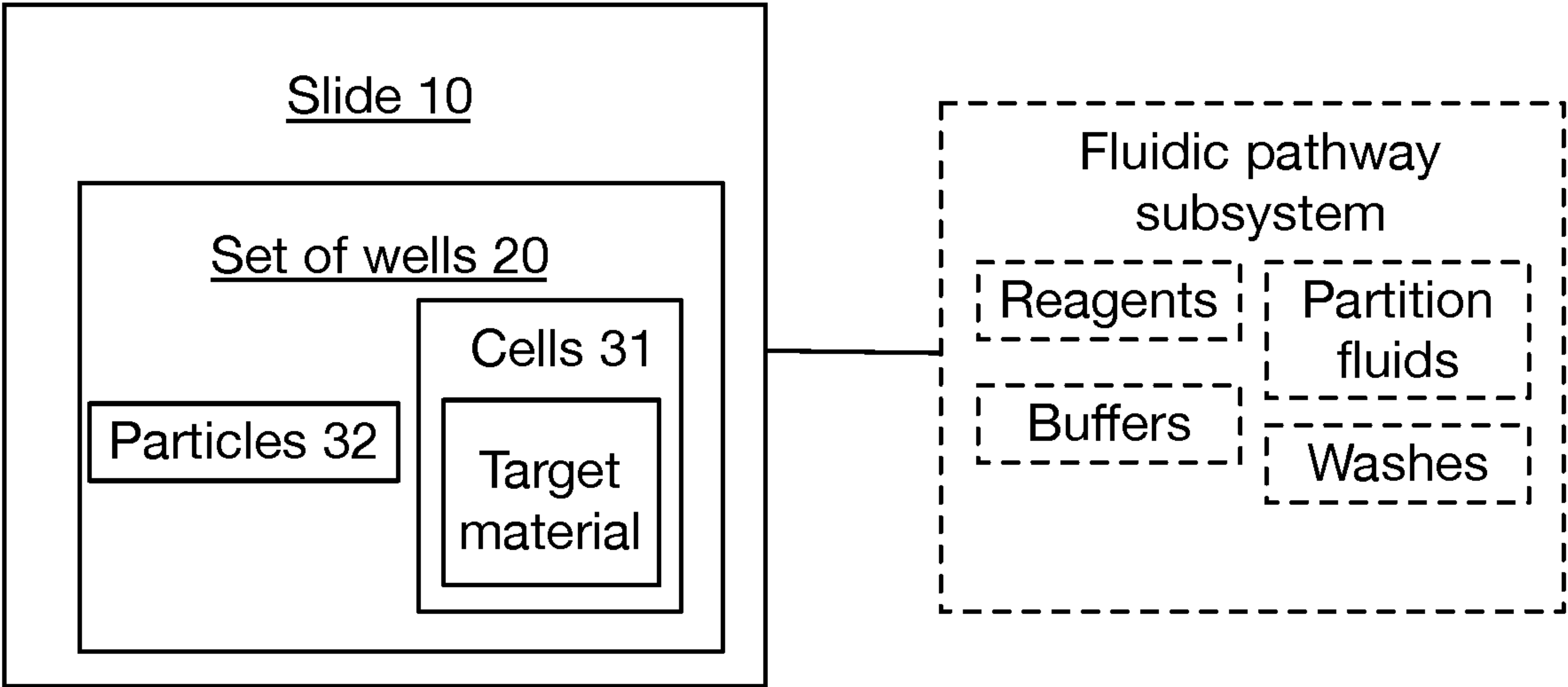


FIGURE 1A

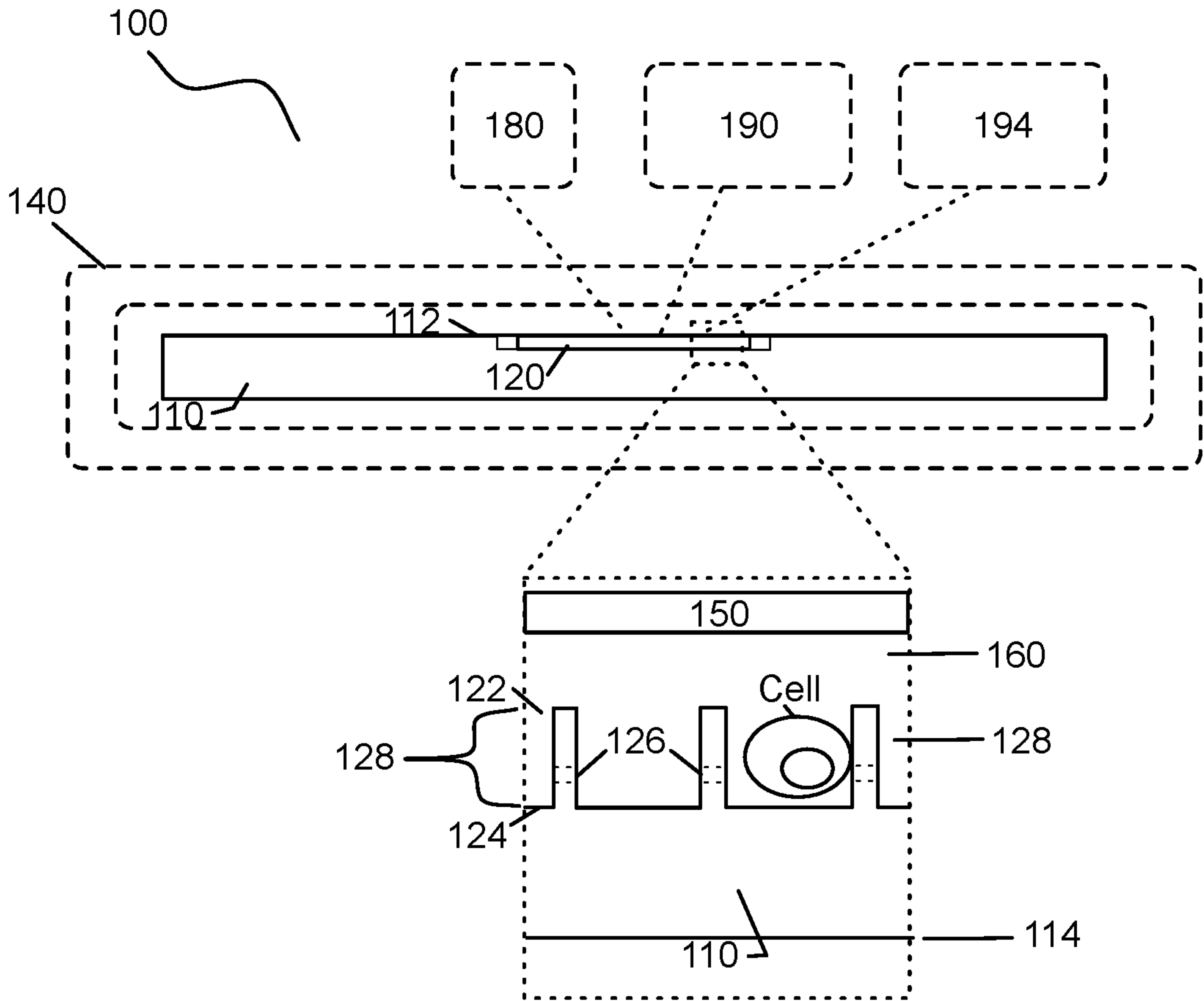


FIGURE 1B

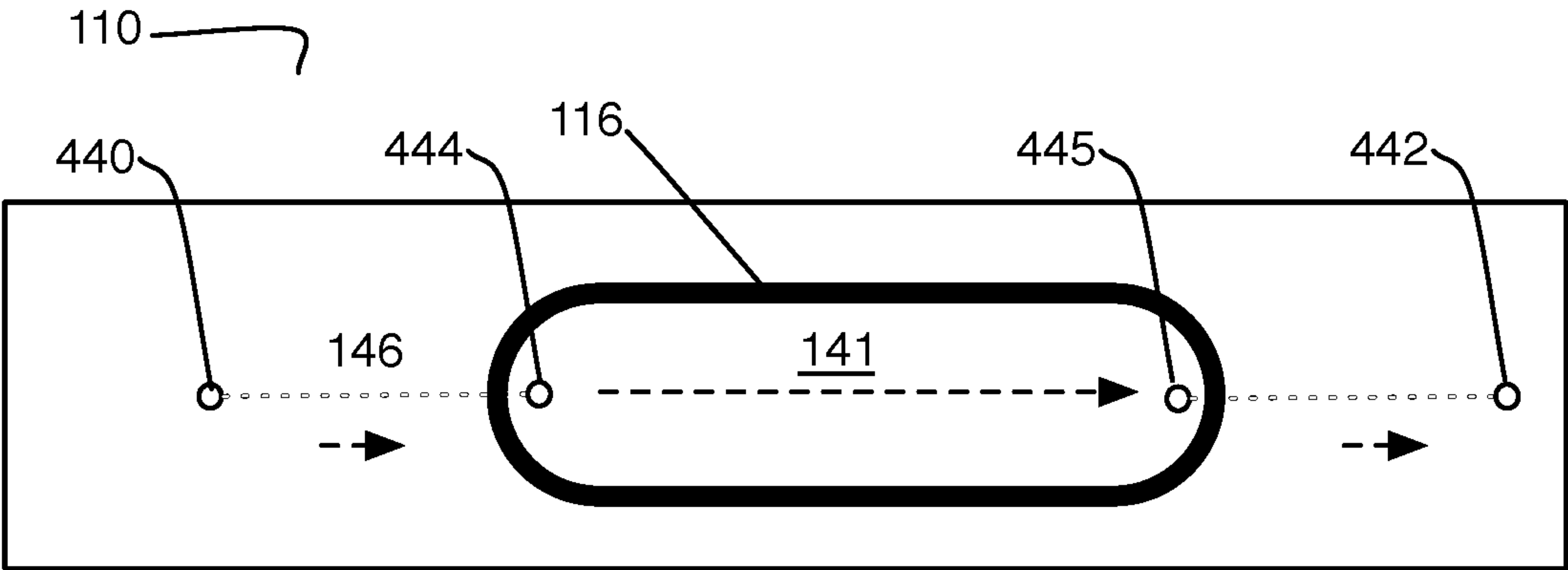


FIGURE 2A

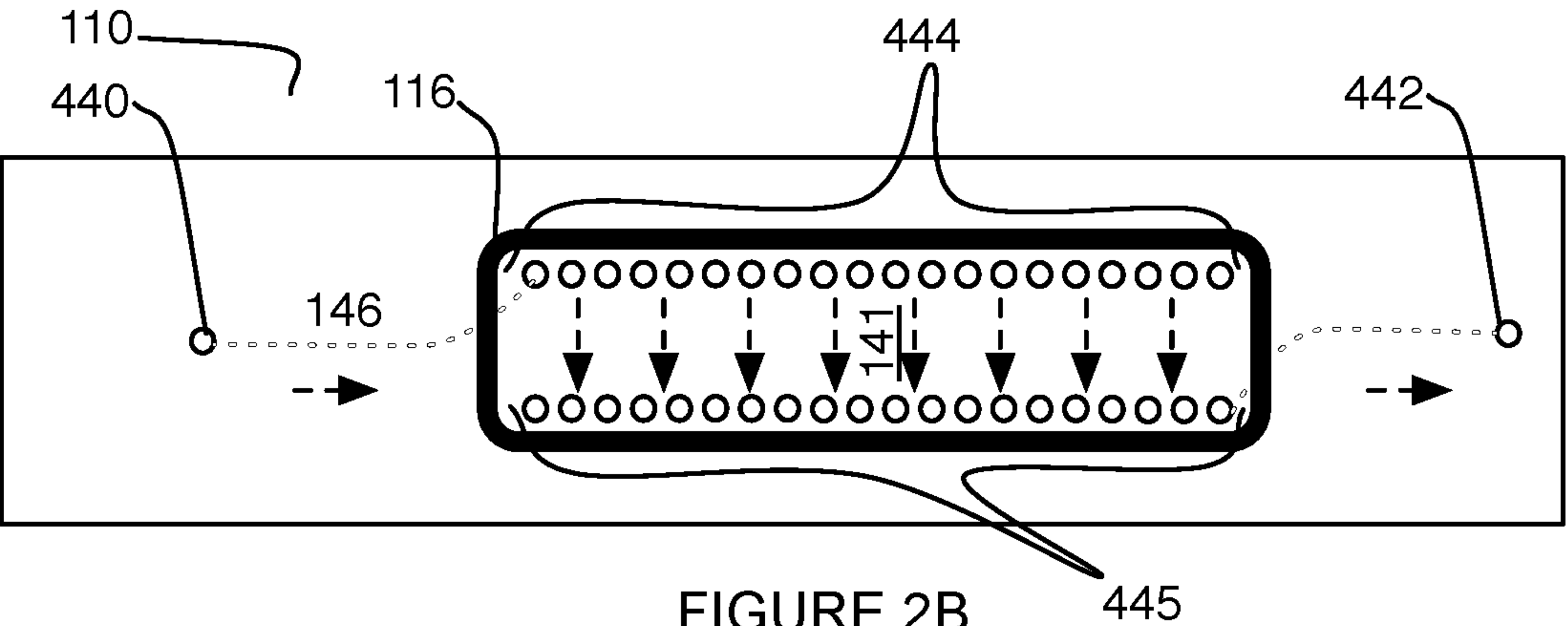


FIGURE 2B

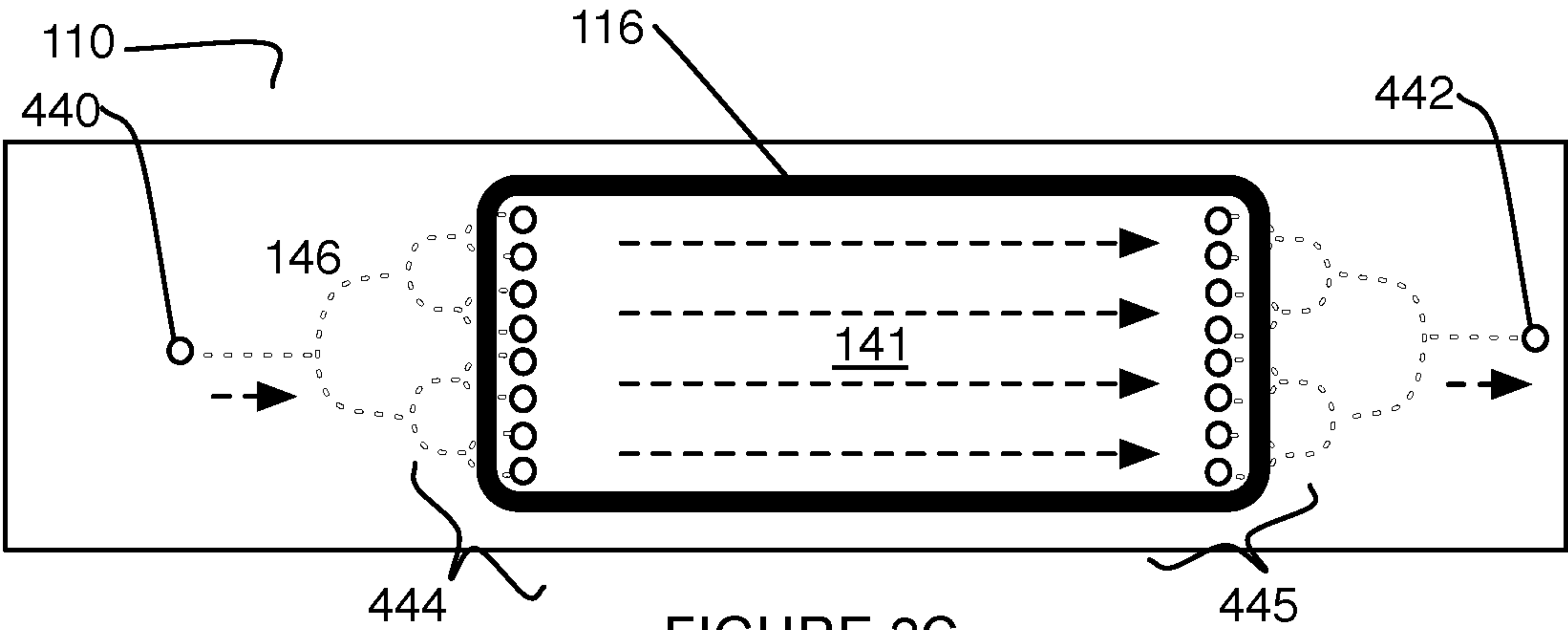


FIGURE 2C

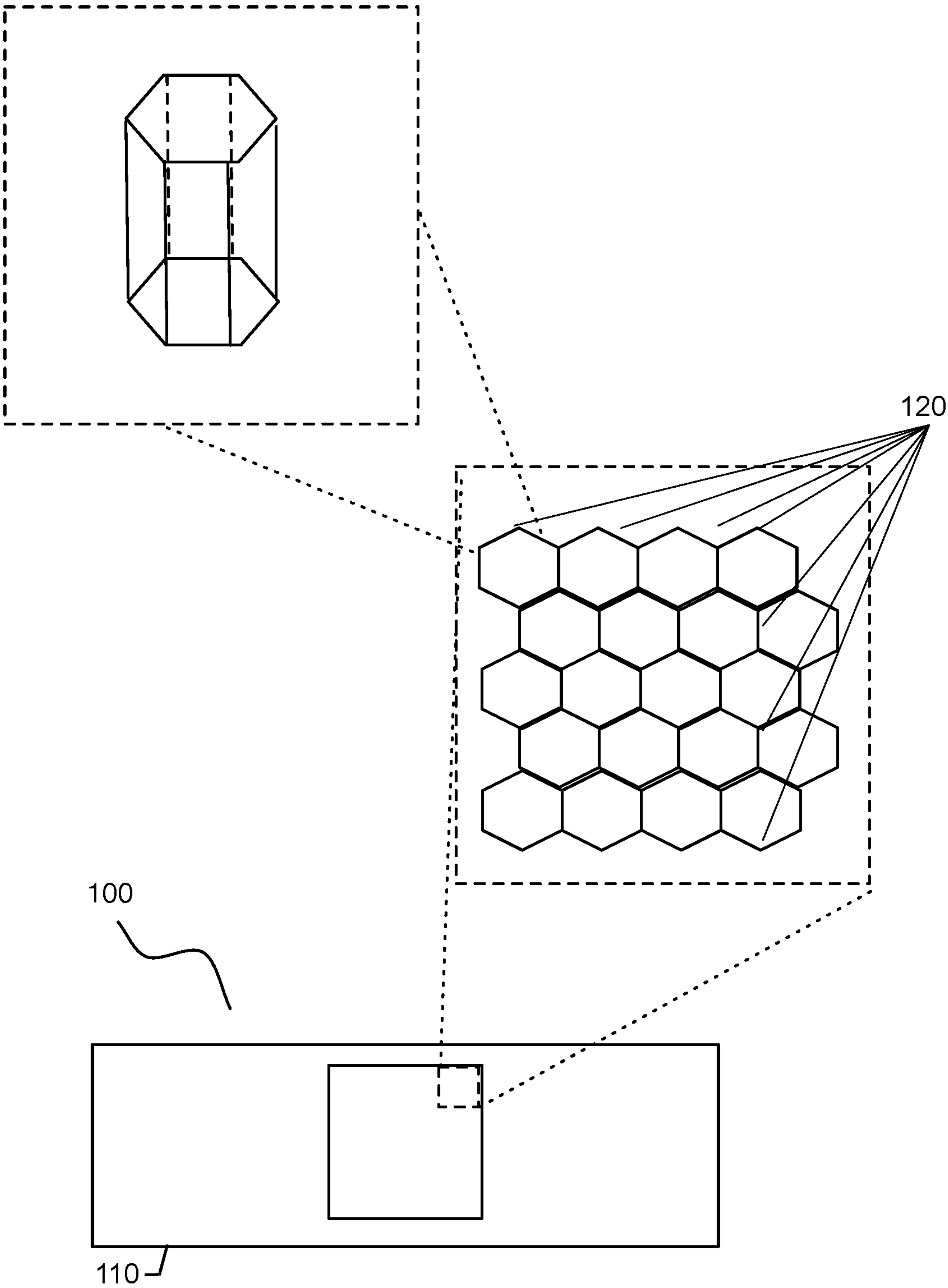


FIGURE 3

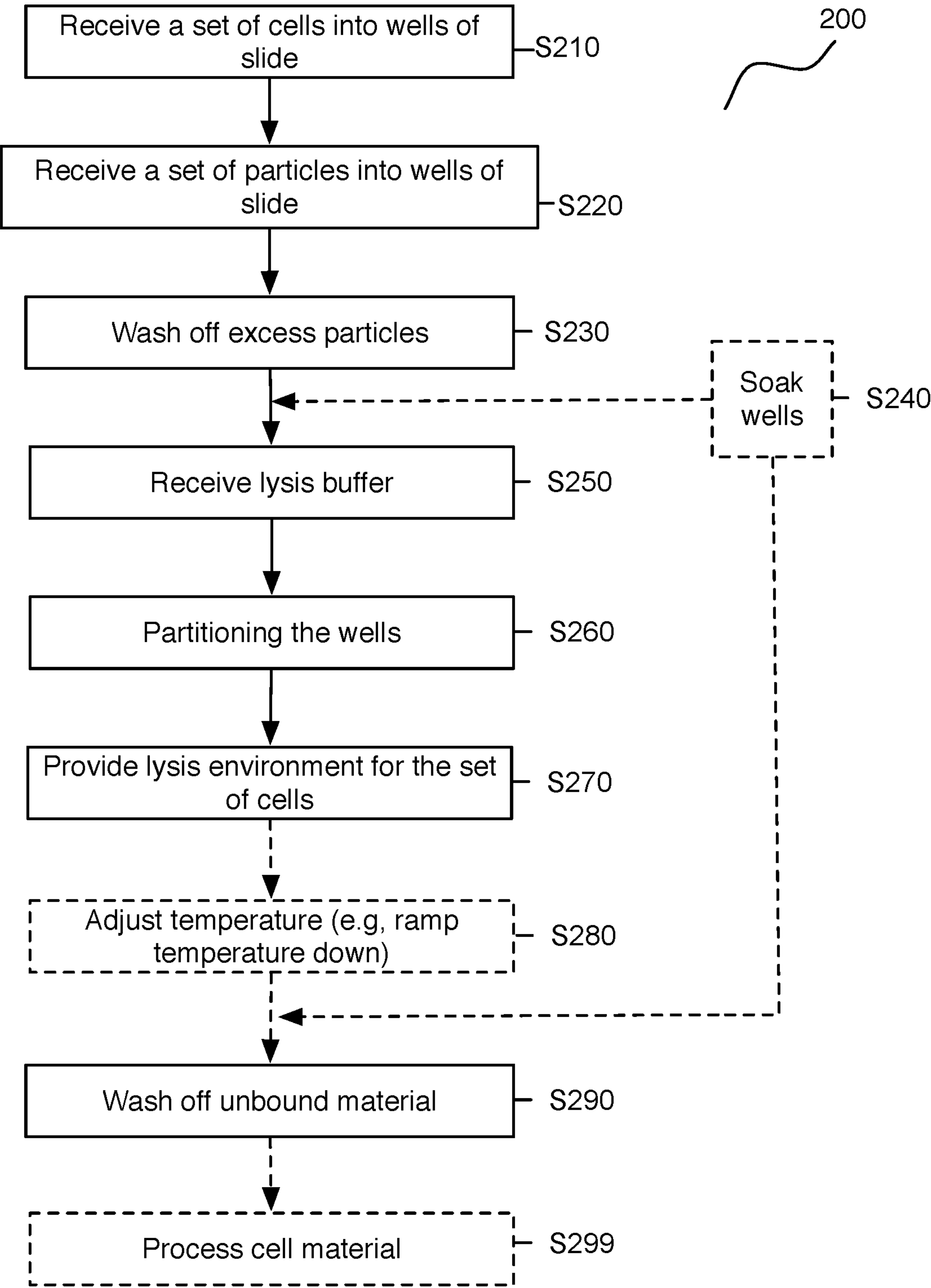


FIGURE 4

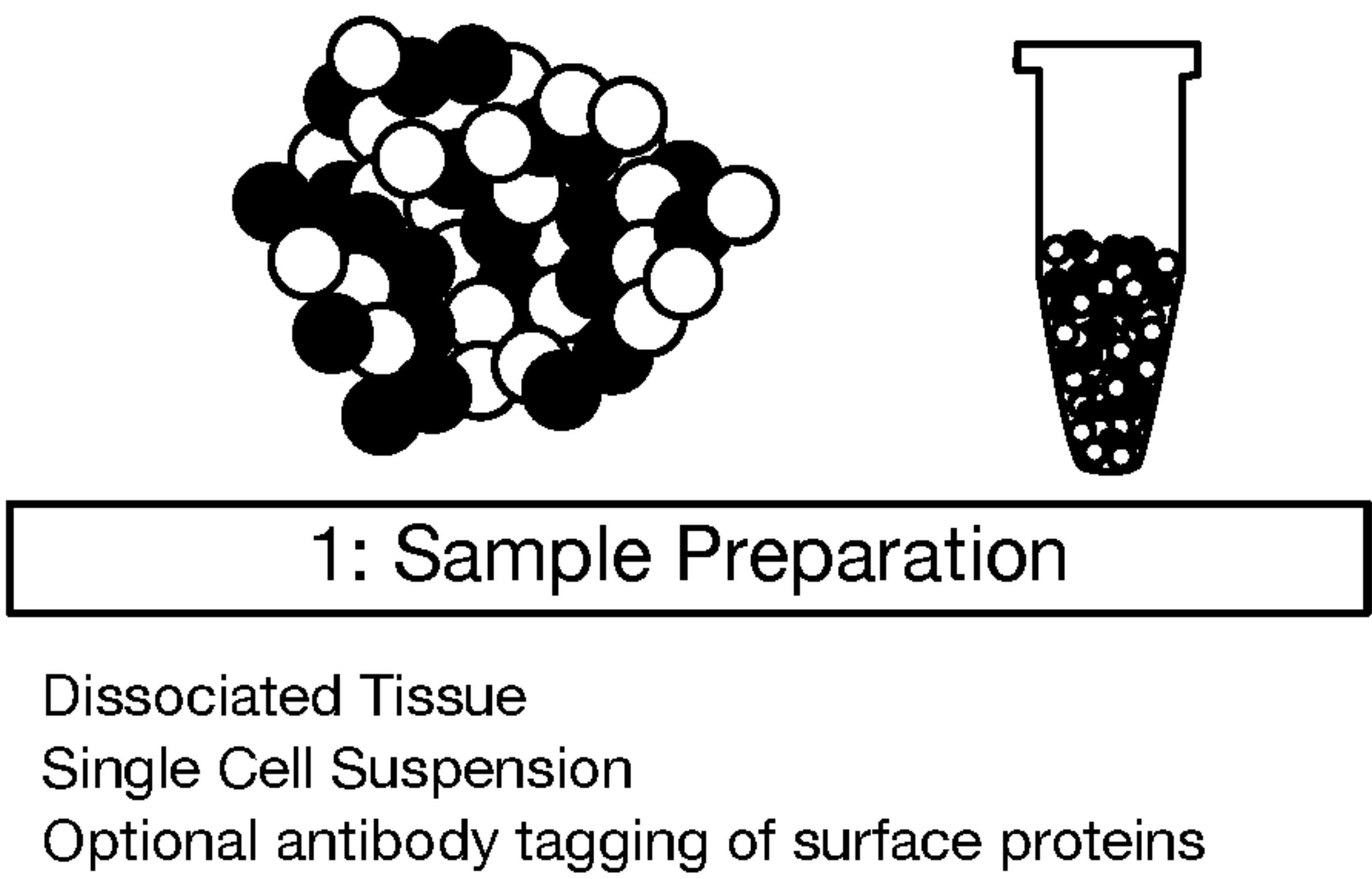


FIGURE 5A

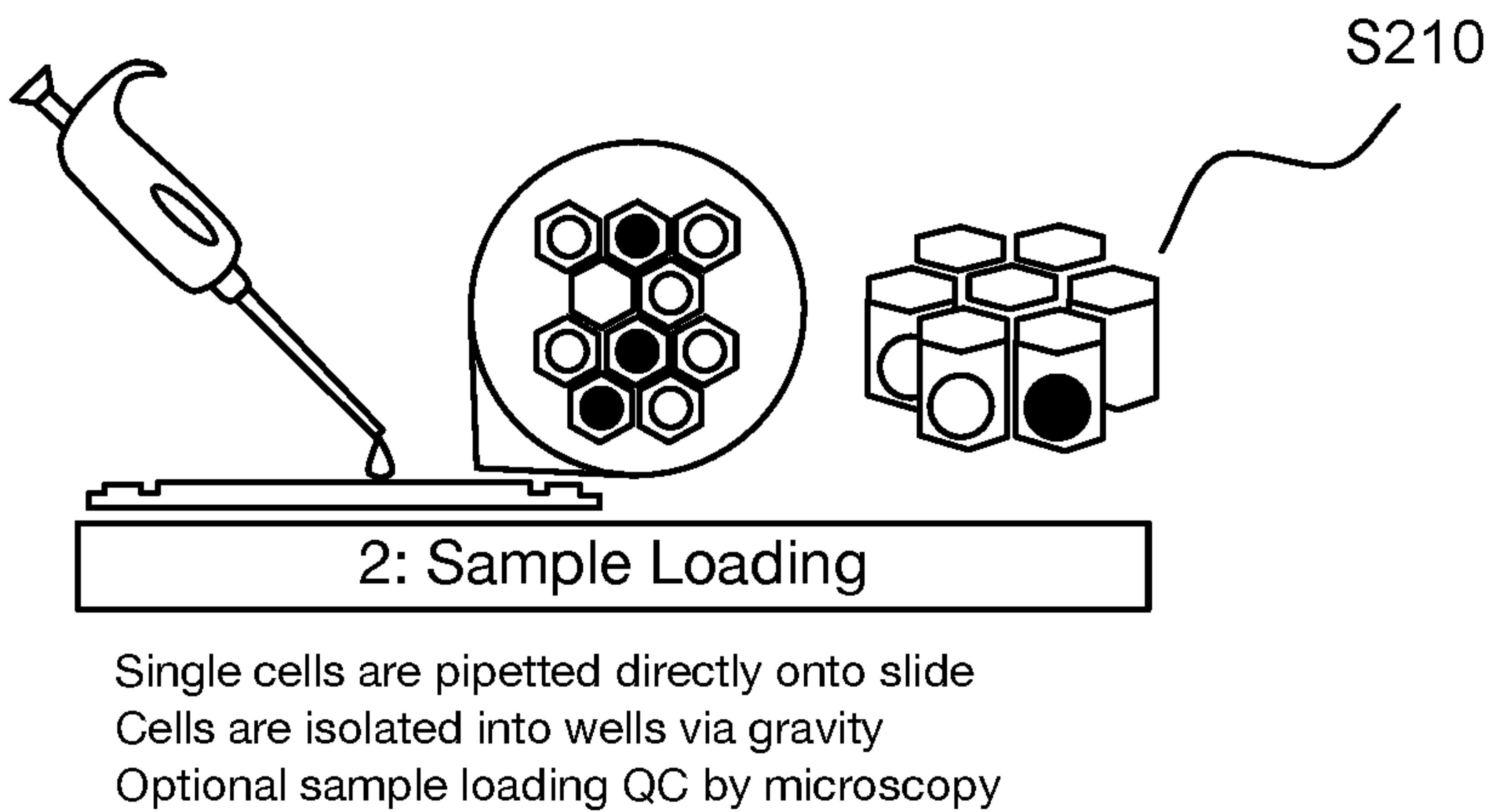


FIGURE 5B

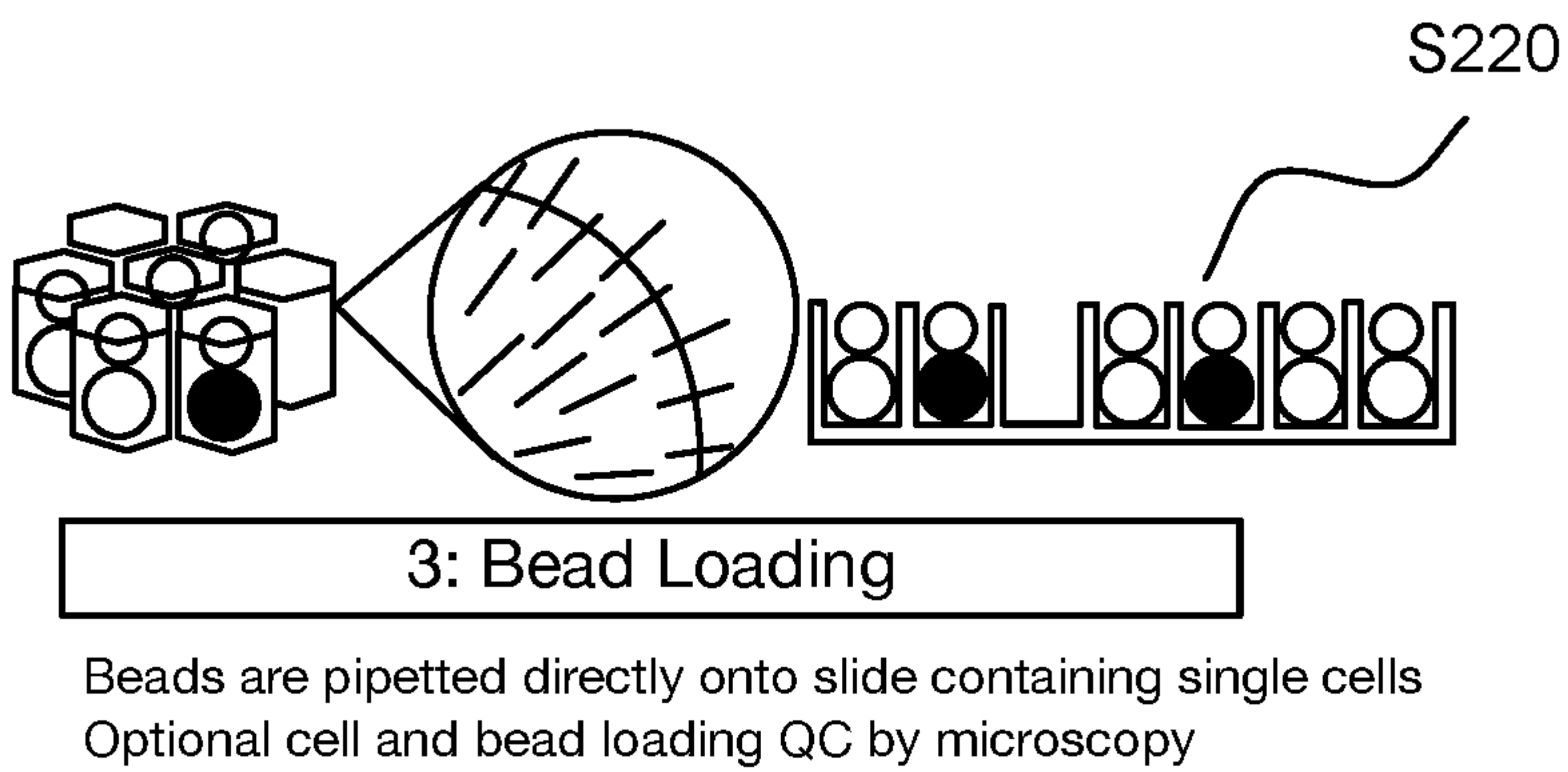
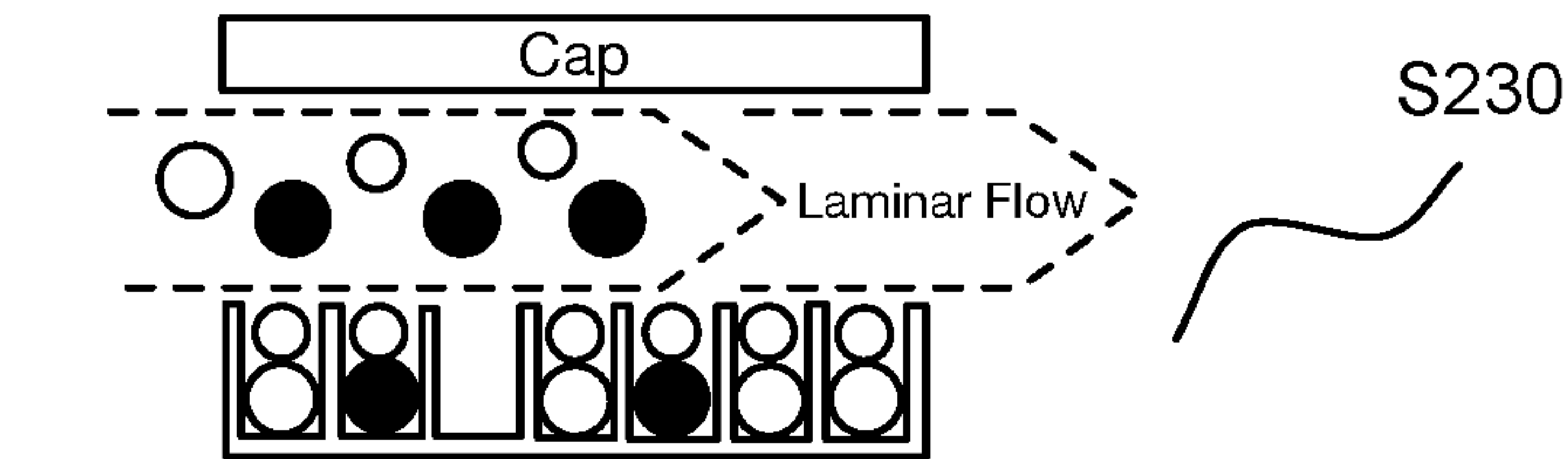


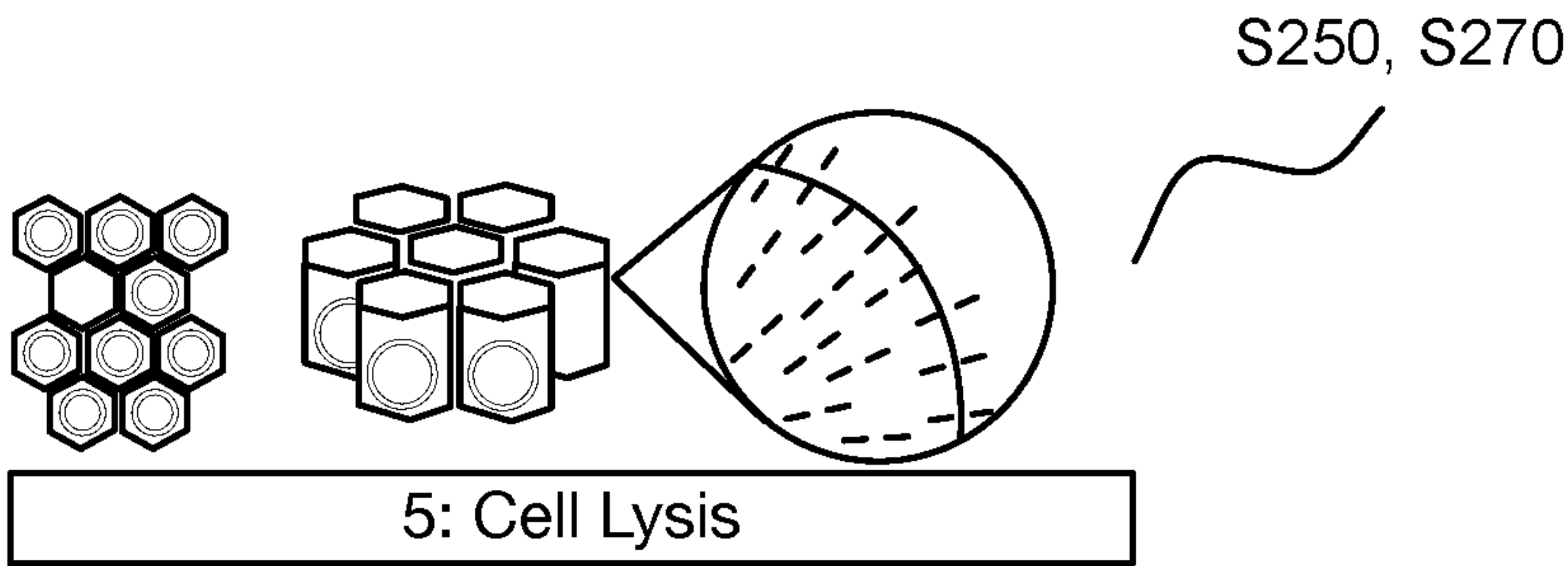
FIGURE 5C



4: Slide Station Assembly

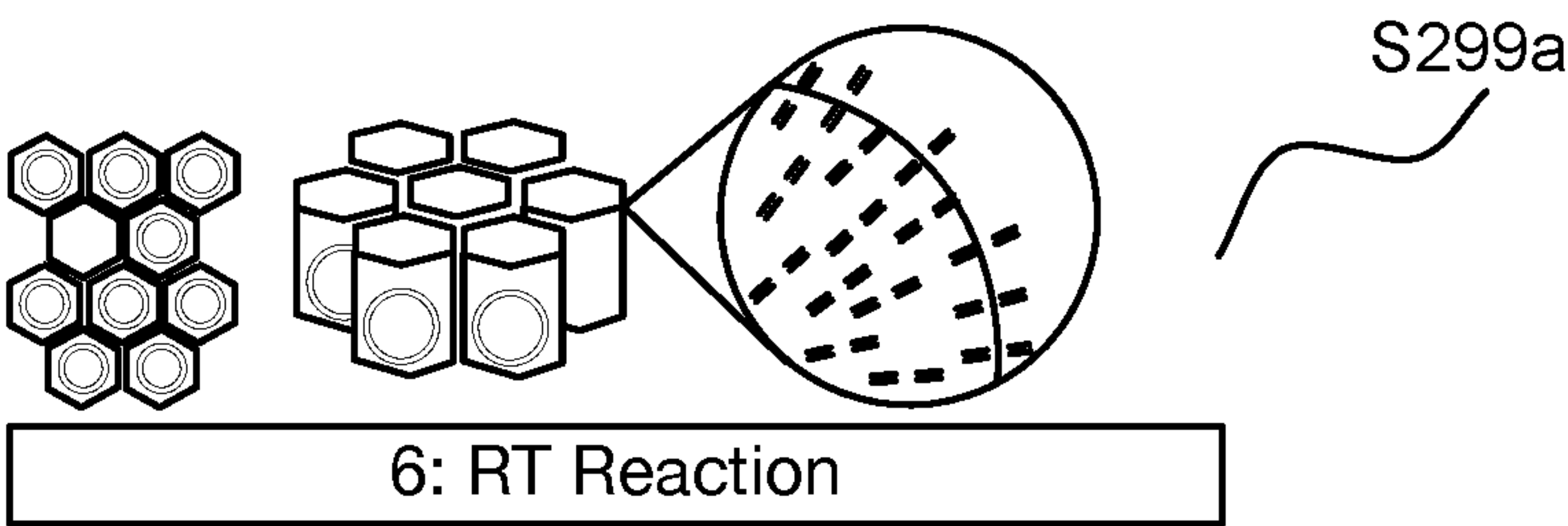
Slides are capped and inserted into slide station
Excess beads are washed away
Slide is ready for on-instrument lysis and RT

FIGURE 5D



Cells are lysed and mRNA hybridizes to beads

FIGURE 5E



Each single cell is uniquely tagged with a cellular
Barcode and unique molecular indices

FIGURE 5F

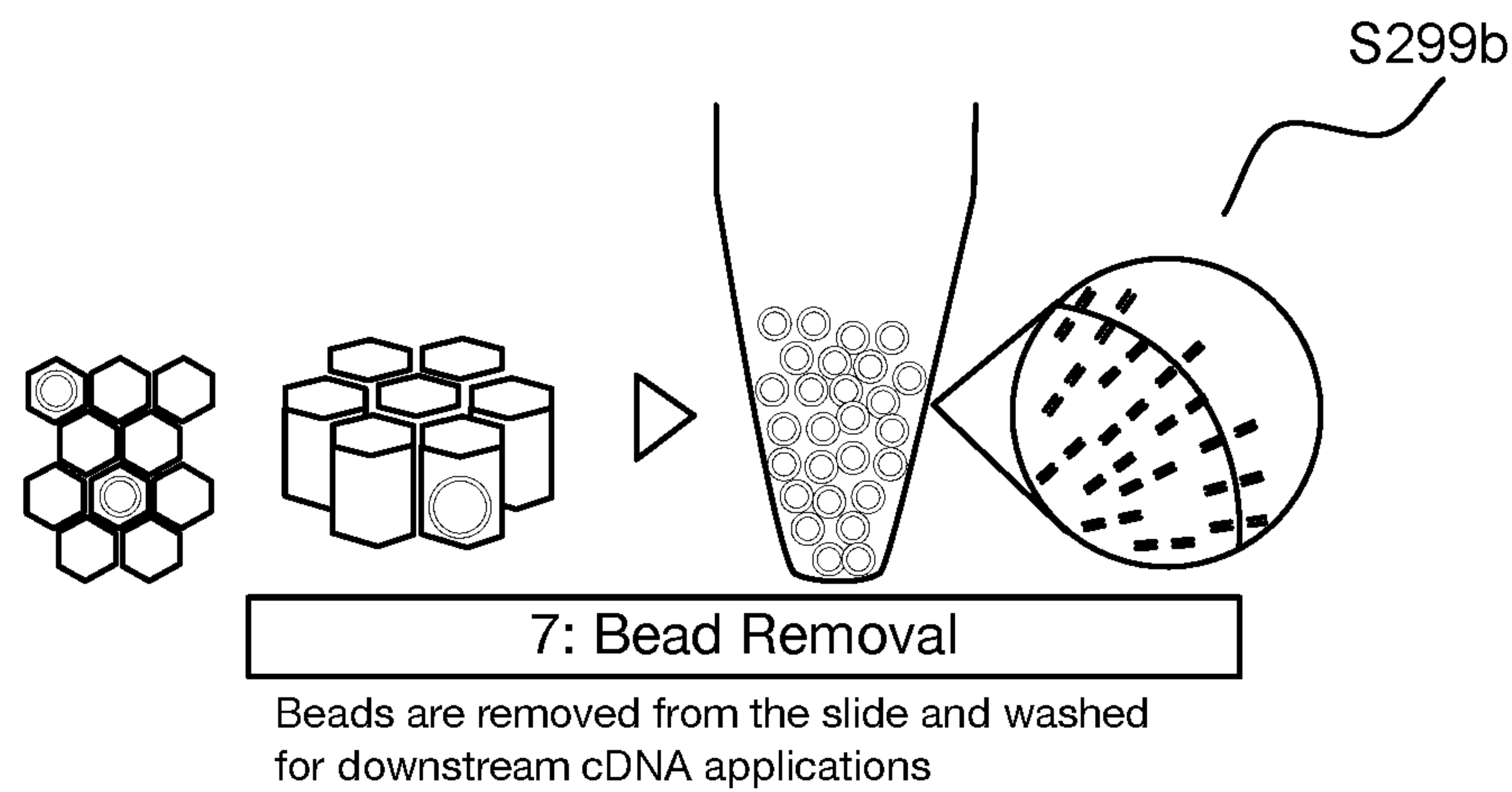


FIGURE 5G

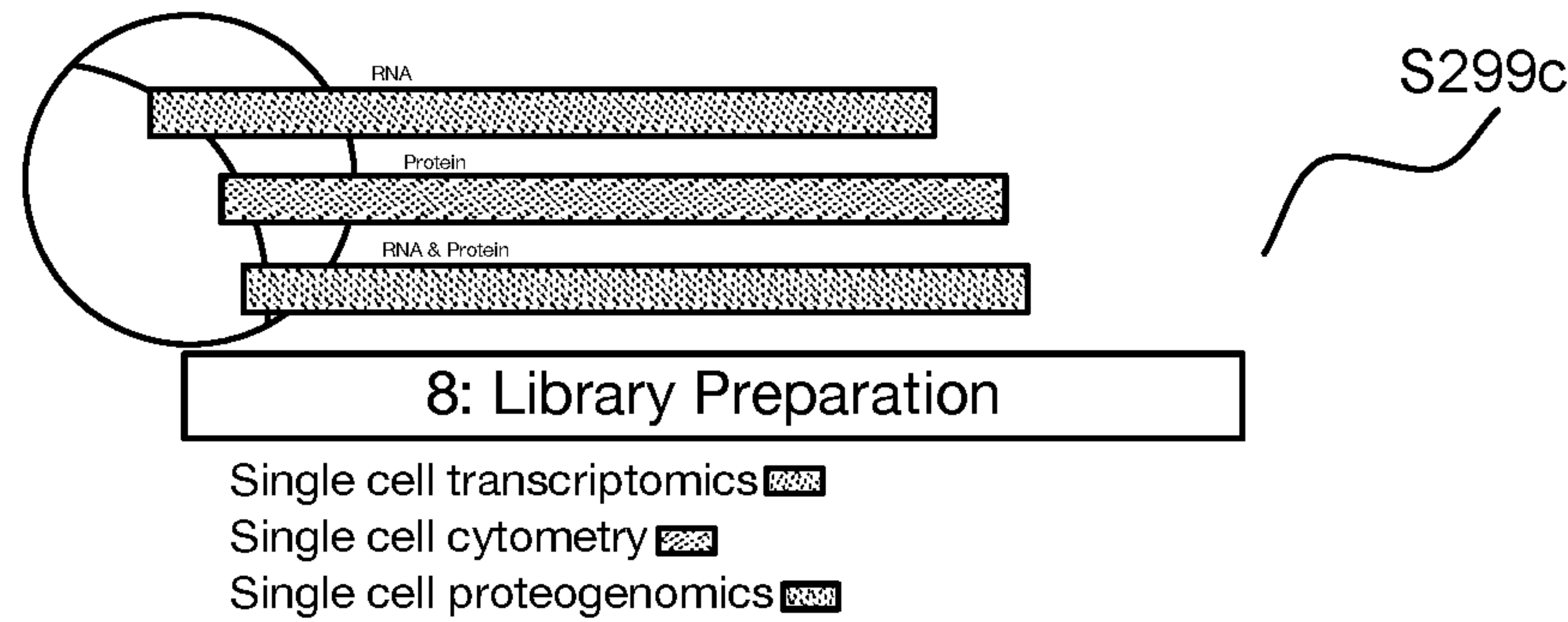


FIGURE 5H

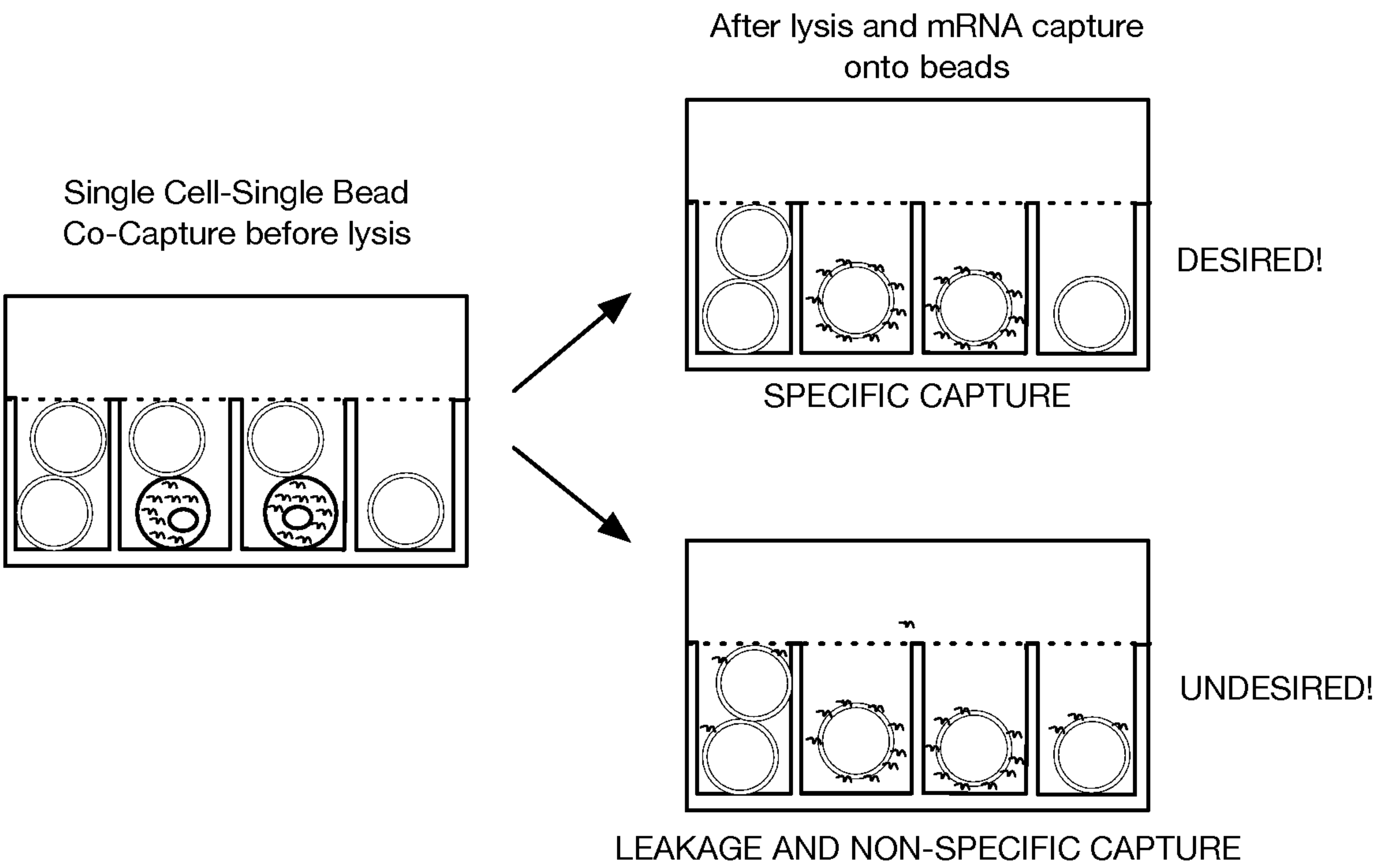


FIGURE 6A

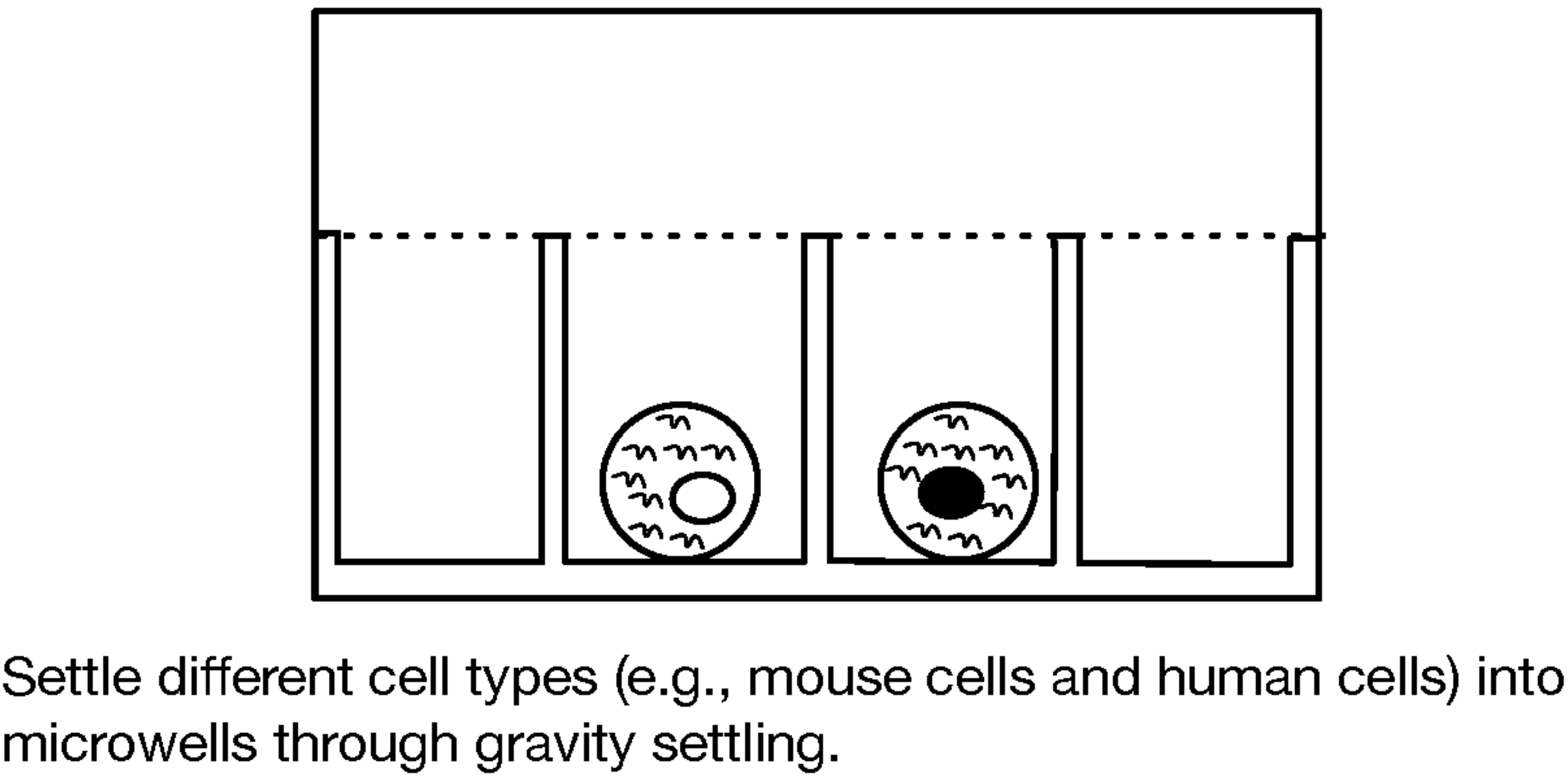
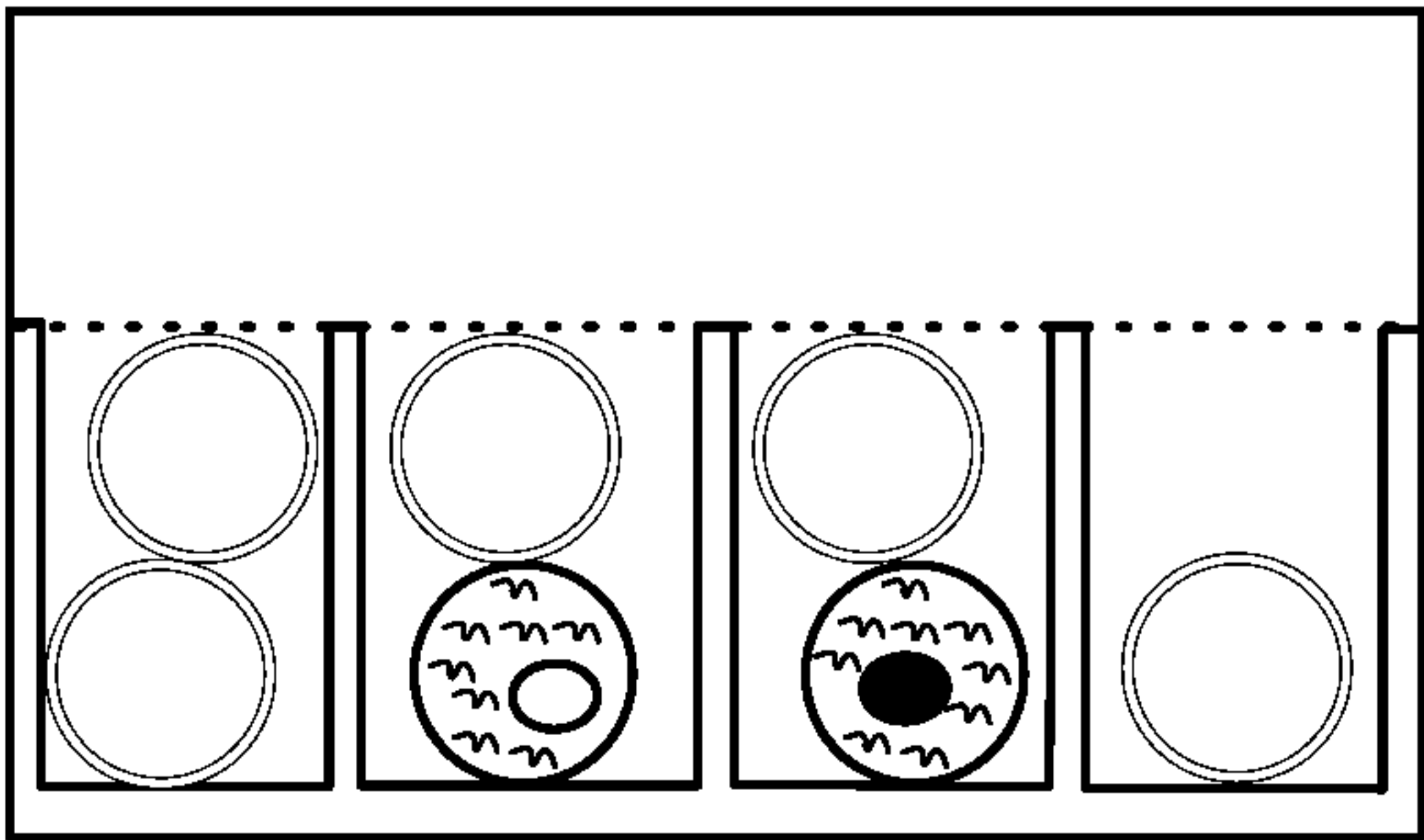
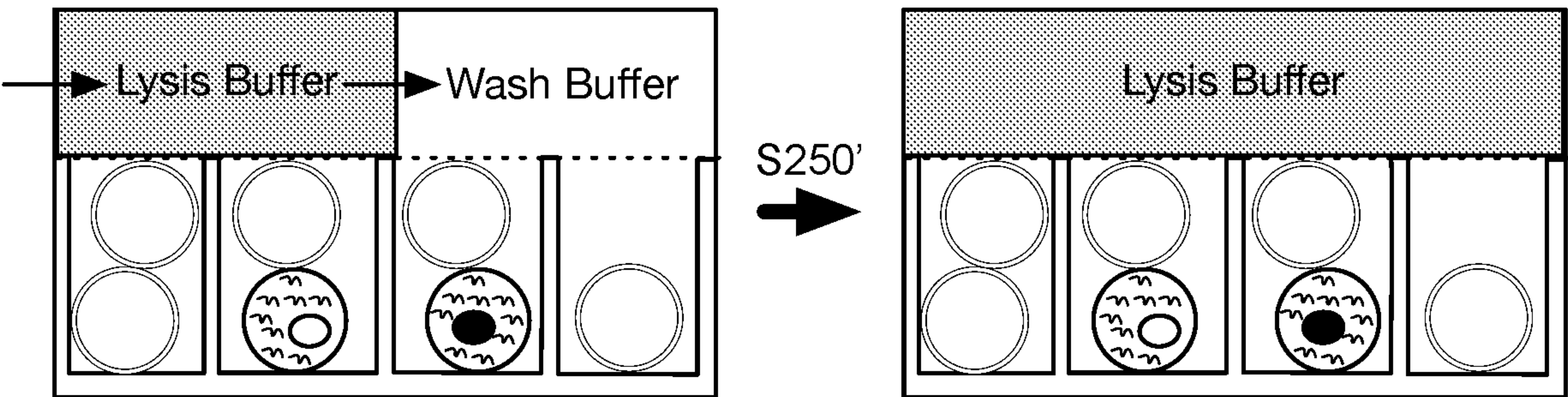


FIGURE 6B



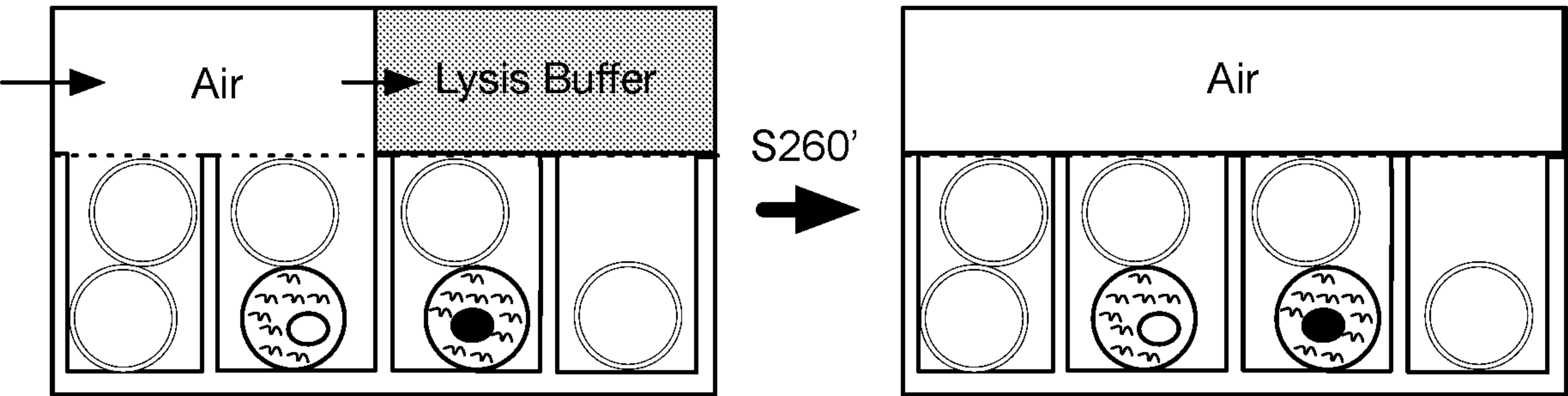
Settle barcoded beads into microcells by gravity

FIGURE 6C



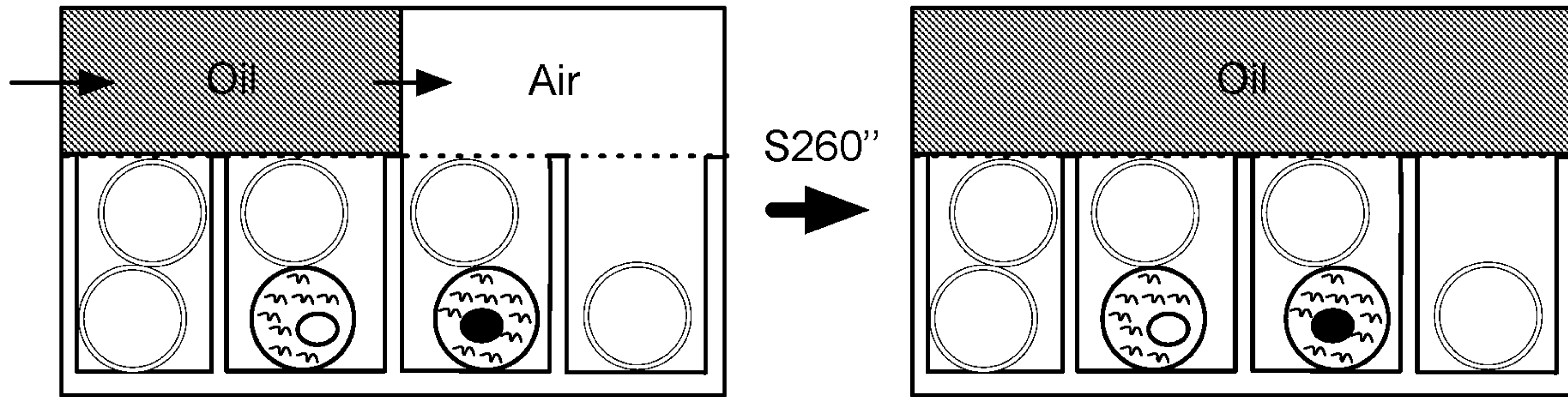
Pump cold (e.g., between 2C and 10C) lysis buffer (e.g., of a predetermined cocktail and concentration) over the microcells for a predetermined residence time (e.g., less than 30 seconds). The residence time and lysis concentration are important. The amount of lysis reagents delivered into the microcells is directly proportional to the concentration of lysis reagents and the residence time. The residence time is optimized such that enough lytic agents are delivered to microcells but not long enough for cells to lyse, release mRNA, and have it diffuse out of the microcells. The diffusion time of mRNA can optionally be slowed down by lowering the temperature and/or deepening the wells.

FIGURE 6D



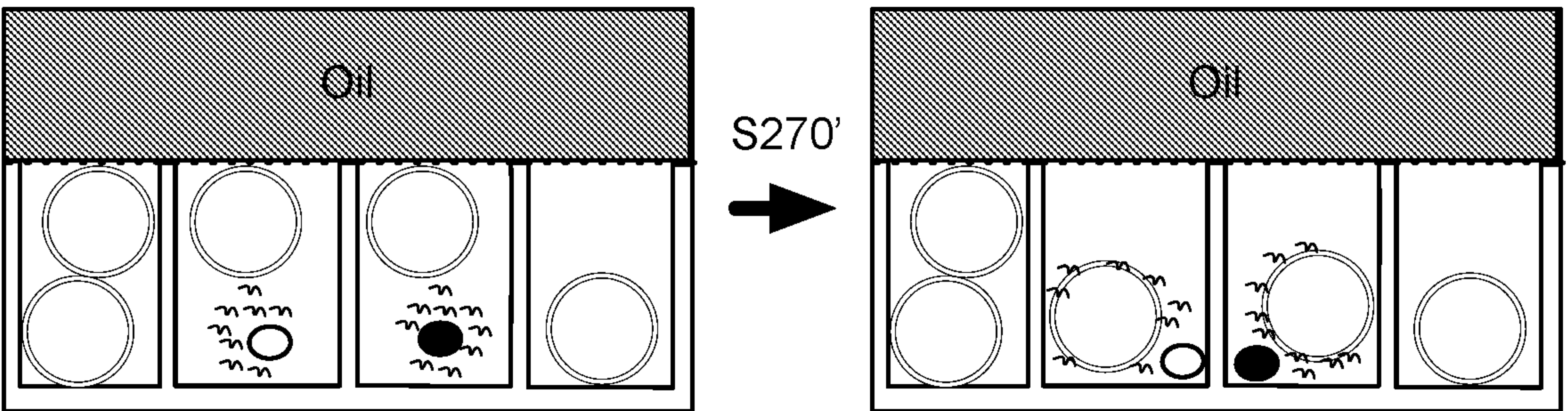
After lysis buffer resides over the microwells for a predetermined amount of time (e.g., between 5 and 60 seconds), air is pumped over the microwells to remove the lysis buffer and partition the microwells from each other.

FIGURE 6E



Oil is pumped over the microwells to partition the microwells from each other. Oil helps prevent evaporation of liquid from the microwells during an incubation time (e.g., long incubation time) described in FIGURE 3G.

FIGURE 6F



The temperature of the microwells is increased (e.g., to 26C) and held (e.g., for between 15 and 30 minutes). The cells lyse, thereby releasing mRNA. The mRNA starts to bind the oligonucleotide fragments of the bead through Poly A (present on mRNA) and Poly T (present on the bead) interactions. The oil partition prevents mRNA from one well escaping to neighboring wells.

FIGURE 6G

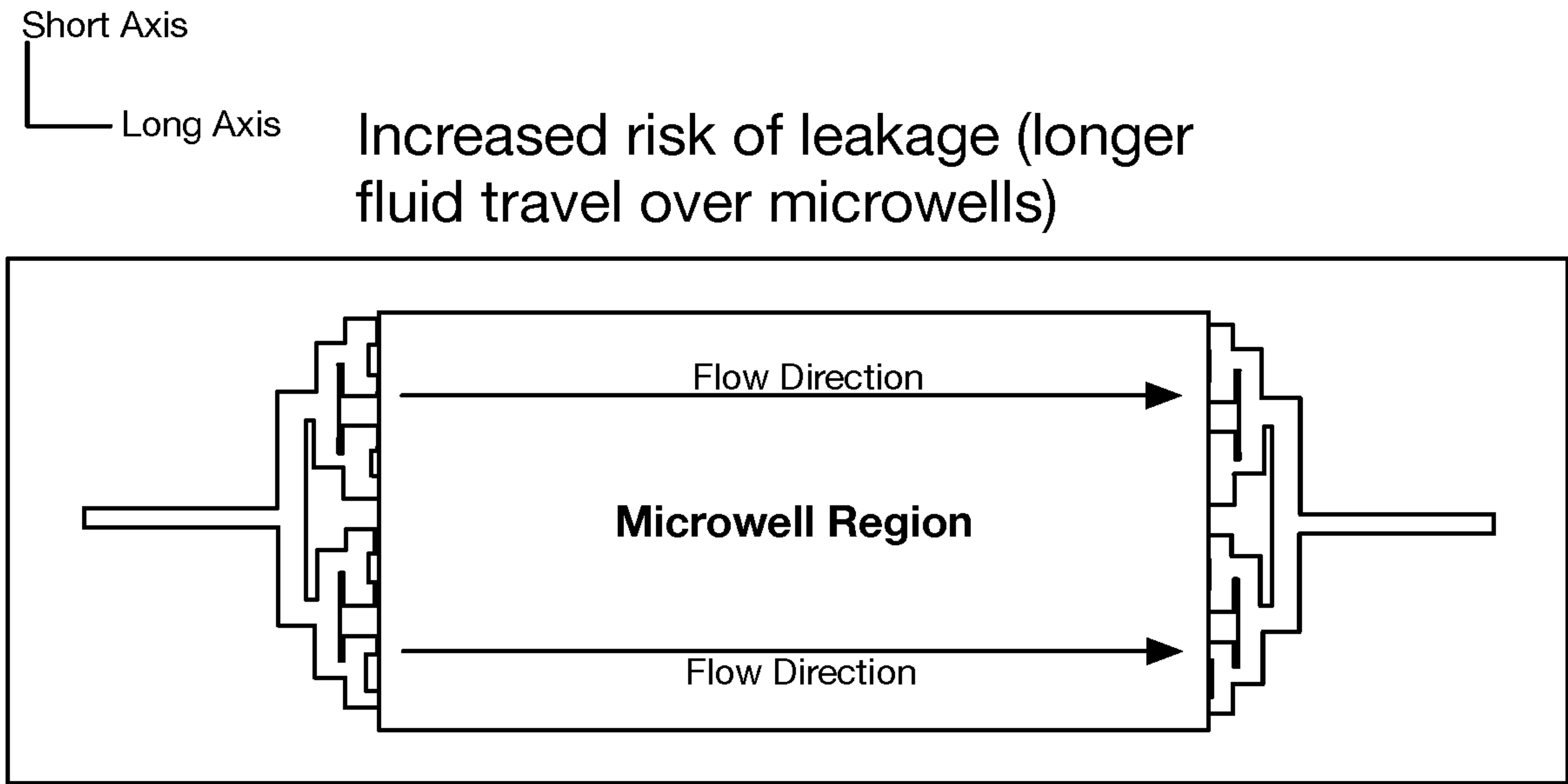


FIGURE 7A

Reduced risk of leakage (shorter fluid travel over microwells)

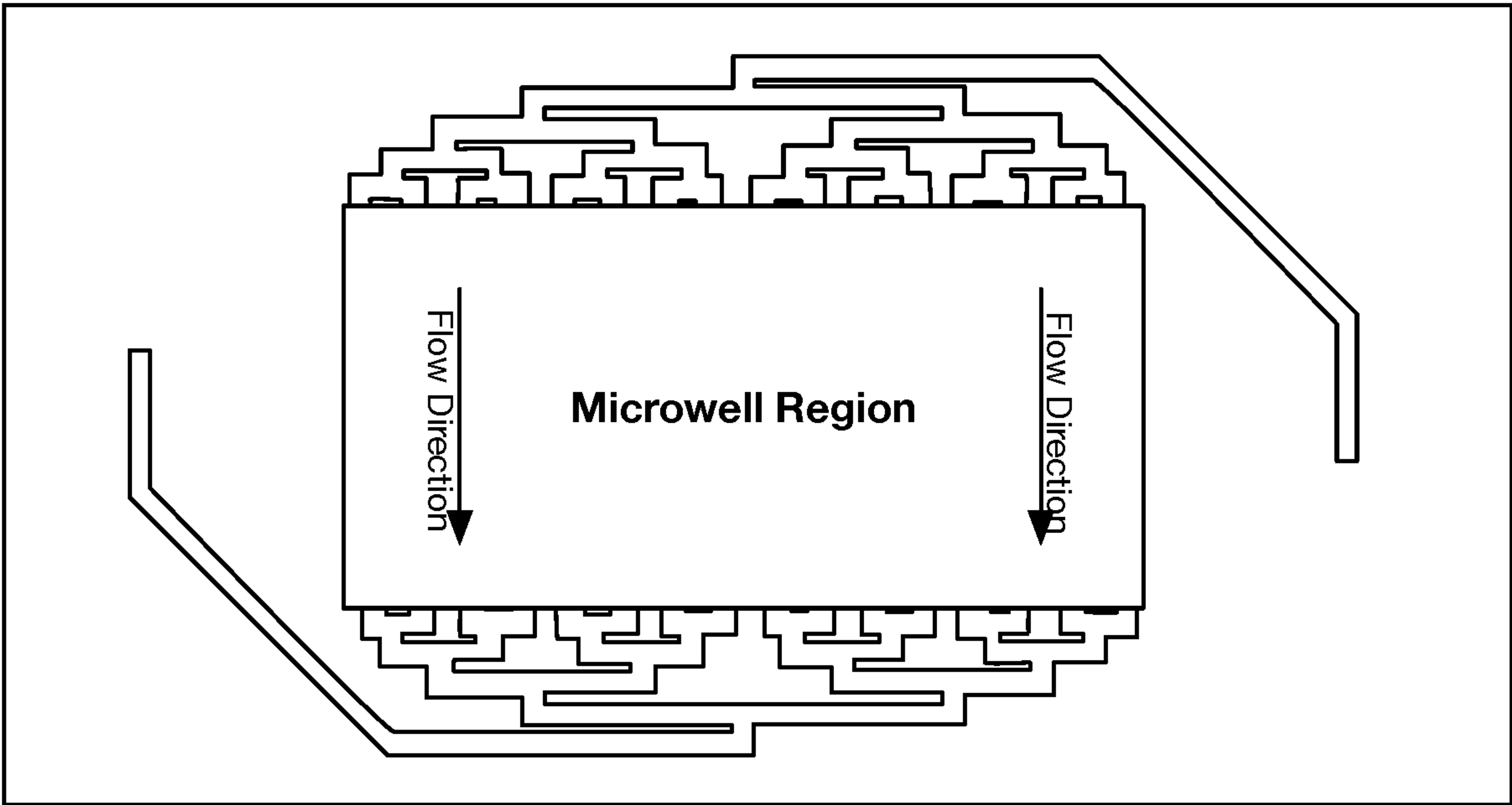


FIGURE 7B