Calculating Percent Passing Filter for Patterned and Nonpatterned Flow Cells

A comparison of methods for calculating percent passing filter on Illumina flow cells.

Introduction

The latest innovation in flow cell technology is the development of the patterned flow cell. Five Illumina sequencing platforms currently take advantage of this advanced technology: the NovaSeq™ 6000 System, the NovaSeq 5000 System, the HiSeq X® System, the HiSeq® 4000 System, and the HiSeq 3000 System. Patterned flow cells consist of a nanowell substrate with billions of ordered wells (Figure 1A). Compared to nonpatterned flow cells (Figure 1B), the uniform cluster sizes enable optimal spacing and increased cluster density.

Although the ordered spacing of patterned flow cells enables significantly higher cluster density,[2-4] patterned flow cells also report comparatively lower percent passing filter (%PF)* values due to differences in the %PF calculation method. This technical note outlines these differences and describes how they lead to lower %PF metrics for patterned flow cells.

Percent Passing Filter with Nonpatterned Flow Cells

In brief, Real-Time Analysis software proceeds through several stages including image analysis, template generation, base calling, passing filter calculations, and quality scoring. Template generation occurs during cycles 1–5 of Read 1 and defines the position of each cluster in a tile. The template is used as a reference for registration and intensity extraction during subsequent sequencing cycles.

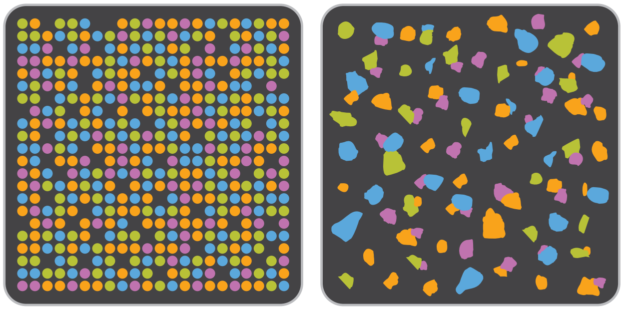

Figure 1: Patterned vs Nonpatterned Flow Cell Cluster Organization. A) Patterned flow cells have clusters with defined sizes, defined shapes, and ordered spacing. B) Nonpatterned flow cells have clusters with varied sizes, undefined shapes, and irregular spacing.

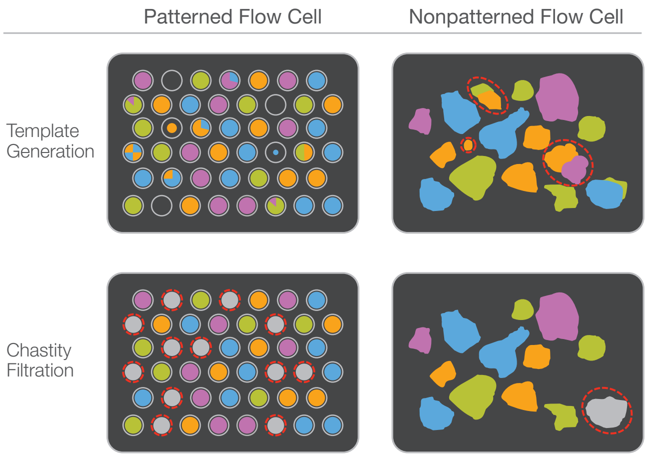

Figure 2: Patterned Flow Cells and %PF. With nonpatterned flow cells, poor quality or dim clusters are filtered during template generation. With patterned flow cells, empty wells and suboptimal clusters are not filtered during template generation—they are filtered during the later stage of chastity analysis, which leads to a lower %PF metric.

* The %PF calculations involve the application of a chastity filter to each cluster. Chastity is defined as the ratio of the brightest base intensity divided by the sum of the brightest and second brightest base intensities. Clusters "pass filter" if no more than 1 base call has a chastity value below 0.6 in the first 25 cycles.[1] This filtration process removes the least reliable clusters from the image analysis results.

With nonpatterned flow cells, dim or low-quality clusters are removed from the raw cluster count during template generation (Figure 2). This effectively acts as a prefiltration step, removing clusters unlikely to pass filter in the first 25 sequencing cycles and yielding relatively high %PF values.

Percent Passing Filter with Patterned Flow Cells

With patterned flow cells, the calculation of %PF is different because *there is no template generation step*—fixed cluster locations eliminate the need for template generation. Because template generation and the associated preliminary filtration steps are not applied, empty wells or clusters that may be dim, low quality, or polyclonal are included in the raw cluster count, which leads to lower %PF values. Although the percent passing filter metric will be much lower with patterned flow cells, it will not affect performance or data quality.[2]

Summary

Due to the differences in the %PF calculation methods, patterned flow cells have artificially lower %PF metrics compared to nonpatterned flow cells. With patterned flow cells, there is no template generation or preliminary filtration step during image analysis, which results in lower %PF values. Although the %PF values are lower, the uniform feature sizes and optimal spacing enable significantly increased cluster density for patterned flow cells.

References

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