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Denaturation of Sub-nanomolar DNA Libraries Protocol

Materials Required:

Low-bind centrifuge tubes
0.2 M NaOH (freshly prepared)
0.1 M HCl (freshly prepared)
Hybridization Buffer: 0.75 M NaCl, 0.075 M $Na_3C_6H_5O_7$ (Sodium Citrate), 0.1% Tween-20. Filter sterilize.

Illumina compatible DNA libraries that are less than 1 nM require a few additional preparation steps prior to flow cell loading. When working with DNA libraries at a concentration less than 1 nM, low-bind centrifuge tubes are recommended as DNA can adhere to the sides of regular tubes, decreasing the concentration over time. Sub-nanomolar libraries should only be diluted when you are ready to load onto a flow cell and should undergo minimal freeze thaw cycles.

1) Make a volume of 0.2 M NaOH equal to your sample volume. For example, if your sample volume is 15 µL, make 15 µL of 0.2 M NaOH.
2) Make a volume of 0.1 M HCl twice that of your 0.2 M NaOH volume. For example, if your NaOH volume is 15 µL make 30 µL of 0.1 M HCl.
3) Add your HCl to your hybridization buffer. Use a sufficient hybridization buffer volume to dilute your DNA library to the desired final concentration (in most cases this ranges from 5-8 pM). Mix well and put on ice.
4) Take the chosen volume of library and add an equal volume of 0.2M NaOH, mix well, spin down and let sit at room temperature for 5 minutes.
5) Add the acidified hybridization buffer to the denatured library.
6) Spot 10 µL on indicator paper to ensure the library is of neutral pH.
7) Load library directly onto a flow cell.

For example, if your DNA library sample volume is 15 µL with a concentration of 450 pM and your desired final flow cell loading concentration is 8 pM, add 30 µL of 0.1M HCl to 783.8 µL hybridization buffer. Add 15 µL of NaOH to your DNA library sample and incubate for 5 minutes at room temperature. Add your acidified hybridization buffer to the denatured library. After assuring that the pH is neutral, your sample is now ready for loading onto the flow cell.