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Preparing Combined Indexed Primer Plates (IDT Standard) for the PacBio Sequel2 - Sequel Dual Indices

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ABSTRACT

The preparation of diluted combined (F+R) IDT working primer stocks of PacBio Dual Index primers for use in IMR PCR preps.

MATERIALS

The following materials list contains those consumables used specifically at the IMR to complete the present protocol.

IDT stock primer DWP
Eppendorf (or similar) DWPs
Microplates sealing film Bio-Rad
Tips ClipTip 20
Tips ClipTip 200
Tips ClipTip 300
UltraPure water
Reservoirs

Order Primers

1 Use our Excel template (PacBio-CDI-16bp-customfusionprimers-template.xlsx) to copy existing full-length 16S/18S/ITS primers or to design your own custom gene primers with the proper PacBio indices. No special synthesis type (unlike longer Illumina fusion primers) is needed for these primers as they are close to the length of standard PCR primers. Order the indexed primers at [M] 25 nanomolar (nM) scale in deep-well plates (DWP); one set per 96-well plate, arranged as follows, leaving blank rows in between sets:

bc1001	bc1002	bc1003	bc1004	bc1005	bc1006	bc1007	bc1008	←	Forward Set 1		
bc1017	bc1018	bc1019	bc1020	bc1021	bc1022	bc1023	bc1024	bc1025	bc1026	bc1027	bc1028
								↖	Reverse Set 1		
bc1029	bc1030	bc1031	bc1032	bc1033	bc1034	bc1035	bc1036	bc1037	bc1038	bc1039	bc1040
								↖	Reverse Set 2		

Prepare Archival Stocks

2 1m Once arrived, do a short spin of the plate in case lyophilized material was dislodged, then add 500 µL of PCR-grade water to each well containing the primers in order to reconstitute them at a concentration of [M] 50 micromolar (µM) (1/2th the typical 100 µM working stock concentration for primers). Mix well by pipetting up and down at least 3 times and seal the plate with Bio-Rad film. Alternatively, the plate is sealed with Bio-Rad film and mixed well by vortexing it on a benchtop vortex for 00:00:30 and then doing a short spin at approx. 500 rpm, 00:00:30. We have found that these primers usually need a significant incubation time for the lyophilized pellets to re-suspend well – we typically leave them overnight at 4 °C before continuing.

Note

Note that the above 500 µL volume can be slightly adjusted for the actual final nmols of primer in the IDT yields. Verify in the provided spec sheet what the average nmols were for all primers combined and then adjust the volume accordingly: normally 10X the nmol amount = the volume for each well to make [M] 100 micromolar (µM) stock, but here we are making twice as much volume (= [M] 50 micromolar (µM), since otherwise the volumes would be a bit small for the DWPs), so use **20X the average nmol amount**. For example, if the average for the primers was 22 nmol, then you would add 440 µL to each well above.

Also note that if you take this approach of variable volumes, you may be left over with an uneven final aliquot of these primers as you consume them over time and will have to simply remember to adjust the last aliquot you use to make working stocks accordingly.

Prepare Intermediate Dilution Plate

3 Prepare a [M] 10 micromolar (µM) **Intermediate Dilution** plate of the Archival Stock plate above by pipetting 352 µL of PCR-grade water into each corresponding well of a 96-well DWP from a sterile reservoir. Working by row and changing tips each time, transfer 88 µL of reconstituted primer from above into each well of each corresponding row, mixing well by pipetting (final volume of 440 µL which will be enough use the F1 primer 3 times to make working plates below [R primers will last longer]). The idea is to "stamp/copy" the exact layout of the above Archival Stock plate here into the Intermediate Dilution - this would normally mean, then, only Rows 1 (A1-A8 = F1 primers), 5 (E1-E12 = R1 primers) and 7 (G1-G12 = R2 primers) would be present in this new diluted plate. Seal the Archival Stock plate with PCR film and store at -20 °C.

Note

The choice of [M] 10 micromolar (µM) for the intermediate dilution level is to be consistent with the Illumina MiSeq archival stock primers which are also at [M] 10 micromolar (µM), hence maintaining consistency in subsequent dilution steps to generate working stock plates.

Prepare Combined Working Stocks

4 Prepare the combined [M] 1 micromolar (µM) working stock **F1R1 Primer Plate** by pipetting 216 µL of PCR-grade water into each well of an empty 96-well DWP from a sterile reservoir. Rotate the above Intermediate Dilution primer plate 90° clockwise and align it so that the 8 occupied wells (= 8 different F1 indices) of Row 1 line up with the 8 rows of the new working stock plate. Working by column and keeping the same set of tips, transfer 12 µL of reconstituted primer into each well of each column, mixing well by pipetting.

Now align the deep-well primer plate horizontally (normal orientation) so that the 12 occupied wells (= 12 different R1 indices) of Row 5 line up with the 12 columns of the working stock plate. Working by row and changing tips each time, transfer 12 µL of reconstituted primer into each well of each row, mixing well by pipetting. Once complete, the resulting plate will have enough primer for 30 PCR plates (8 µL combined F+R per rxn × 30 = 240 µL). Seal the plate with PCR film and store at -20 °C.

5 Prepare the combined [M] 1 micromolar (µM) working stock **F1R2 Primer Plate** by repeating Step 4, but using Row 1 (=F1) and Row 7 (=R2) instead.

6 Once all aliquoting is complete, seal the DWPs with PCR film and archive at -20 °C until new aliquots are required (minimized freeze-thaw cycles).