Protocol for tiling amplicon generation for MinION sequencing

Description:

This protocol is for tiling two-step RT-PCR: i) random hexamer priming for reverse transcription and, ii) multiplex specific primers for PCR. You will need Protoscript II for RT and Q5 high-fidelity DNA polymerase for PCR.

Reverse transcription:

Mix the following in a PCR tube

7 ul RNA
1 ul Random hexamers (50 ng/µl)

Mix by inversion
Heat in the thermocycler at 65°C for 5 minutes then place on ice
Add the following to each tube

10 ul ProtoScript II Reaction Mix (2X)
2 ul ProtoScript II Enzyme Mix (10X)

Place on the thermocycler and start the following program

25°C for 5 minutes
48°C for 15 minutes
80°C for 5 minutes

PCR:

Make the following mastermix in a 1.5 ml Eppendorf tube

	1x	2.5x
5X Q5 Reaction Buffer	5 ul	12.5 ul
10 mM dNTPs	0.5 ul	1.25 ul
Q5 DNA Polymerase	0.25 ul	0.625 ul
Nuclease-free water	16.75 ul	41.875 ul
cDNA	2.5 ul	6.25 ul

Mix by inversion and spin down
Label 2 0.2 ml PCR tubes
Pipette 24 ul master mix into the corresponding tube
Add 1 ul of the respective primer pool (~0.01 uM per primer)

PCR program:

Step 1
95°C 30 seconds
Step 2 (35 cycles)
95°C 15 seconds
65°C 15 minutes

Primer pools:

Pool 1

400_1_out_L	GACAGTTCGAGTTTGAAGCGAAAG	400_1_out_R	AGTATGCACTCCCACGTCTAGT
400_3_out_L	AGATGACGTCGATTGTTGGTGC	400_3_out_R	TACGGTGACACAACCTCCATGT
400_5_out_L	AGAACGTTAGTGGACAGAGGCT	400_5_out_R	TGTGCGTCCTTGAACTCTACCA
400_7_out_L	TGAAGGGCGTGTCATACTCCTT	400_7_out_R	CGCCTCCAACTGATCCAAAGTC
400_9_out_L	GCCTTAGGGGGAGTGTTGATCT	400_9_out_R	GAGTGGGCATTCCTTCAGTGTG
400_11_out_L	CAGCCGTTATTGGAACAGCTGT	400_11_out_R	CCTGGGCCTTATCTCCATTCCA
400_13_out_L	TGGCAGTGCTGGTAGCTATGAT	400_13_out_R	AGAGAGAGGAGCATAAACCCCC
400_15_out_L	CCCTAGCGAAGTACTCACAGCT	400_15_out_R	TACACTCCATCTGTGGTCTCCC
400_17_out_L	GTGGTCCATGGAAGCTAGATGC	400_17_out_R	CCTCTAAGGGCCTCCTCCATTT
400_19_out_L	TATGGATGAGGCCCACTTCACA	400_19_out_R	GCCATCAAGTATGACCGGCTTT
400_21_out_L	AGAGACTGACGAAGACCATGCA	400_21_out_R	CTCCAAAAGCCGCTCCTCTTTT
400_23_out_L	CGTCTTGATGAGGAACAAGGGC	400_23_out_R	AAGTGGTCACTGCATGTTGGAC
400_25_out_L	CCCTGACCCTAATAGTGGCCAT	400_25_out_R	CCTTCCATTTCTCTCCCAGGGT
400_27_out_L	AGTGCAAAGCTGAGATGGTTGG	400_27_out_R	ATGTGTAGAGTTGCGGGAGAGT
400_29_out_L	AGGATGTGAATCTCGGCTCTGG	400_29_out_R	ATGCTGCATTGCTACGAACCTT
400_31_out_L	ACAAGGGGAATTTGGAAAGGCC	400_31_out_R	CGTAAGTGACAACTTGTCCGCT
400_33_out_L	CAAACGAATGGCAGTCAGTGGA	400_33_out_R	ATCCACACTCTGTTCCACACCA
400_35_out_L	ACCACCTGGGCTGAGAACATTA	400_35_out_R	ACCACTAGTCCCTCTTCTGGAG

Pool 2

400_2_out_L	AAGAAAGATCTGGCTGCCATGC	400_2_out_R	TGATTCCAACCAGGTTTGCGAC
400_4_out_L	TCAGGTGCATAGGAGTCAGCAA	400_4_out_R	GGAGCCATGAACTGACAGCATT
400_6_out_L	TTGATTGTGAACCGAGGACAGG	400_6_out_R	CCATCTGTCCCTGCGTACTGTA
400_8_out_L	GGGAGAAGAAGATCACCCACCA	400_8_out_R	TTGACTGCTGCTGCCAATCTAC
400_10_out_L	ACGGTCGTTGTGGGATCTGTAA	400_10_out_R	GTGGGACTTTGGCCATTCACAT
400_12_out_L	CACTAAGGTCCACGTGGAGGAA	400_12_out_R	TATCAGCGCCAGATGAGCTACA
400_14_out_L	CAATGGTTTTGCTTTGGCCTGG	400_14_out_R	TTTCCCATGTGATGTCACCTGC
400_16_out_L	GTGGCATGAACCCAATAGCCAT	400_16_out_R	GCTCCAATGTCCCCATCCTTTG
400_18_out_L	CTGTTGAGTGCTTCGAGCCTTC	400_18_out_R	TGGTGAGTTGGAGTCCGGAAAT
400_20_out_L	GGCTGGAAAACGGGTCATACAG	400_20_out_R	CCTTTGCTCCGTCCTAAGCTTG
400_22_out_L	TGGACCAGACACGGAGAGAAAA	400_22_out_R	ATTCTGGCTGGCTCAATTTCCG
400_24_out_L	TAATGGGAAGGAGAGAGGAGGG	400_24_out_R	TCTCCACTTGGGGGTCAATTGT
400_26_out_L	ACTGGAACTCCTCTACAGCCAC	400_26_out_R	ACCAGGGCCTCCTTTTGTGTAT
400_28_out_L	GGTGGGGGATTGGCTTGAAAAA	400_28_out_R	GGGCCTCATAGCTTCCATGGTA
400_30_out_L	AAAAGTGGACACTAGGGTGCCA	400_30_out_R	TAATCCCAGCCCTTCAACACCA
400_32_out_L	AAATGGAAAAAGGGCACAGGGC	400_32_out_R	TGTCCCATCCAGTTGAGGGTTT
400_34_out_L	ATTTCCACAGAAGGGACCTCCG	400_34_out_R	TGACTAGCAGGCCTGACAACAT