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Cross-platform compatibility of Hi-Plex, a streamlined approach for targeted massively parallel sequencing

Tú Nguyen-Dumont[a], **Bernard J. Pope**[b,c], **Fleur Hammet**[a], **Maryam Mahmoodi**[a], **Helen Tsimiklis**[a], **Melissa C. Southey**[a], and **Daniel J. Park**[a,*]

[a]Genetic Epidemiology Laboratory, Department of Pathology, University of Melbourne, Melbourne, Victoria 3010, Australia

[b]Victorian Life Sciences Computation Initiative, University of Melbourne, Melbourne, Victoria 3010, Australia

[c]Department of Computing and Information Systems, University of Melbourne, Melbourne, Victoria 3010, Australia

Abstract

Although per-base sequencing costs have decreased during recent years, library preparation for targeted massively parallel sequencing remains constrained by high reagent cost, limited design flexibility, and protocol complexity. To address these limitations, we previously developed Hi-Plex, a polymerase chain reaction (PCR) massively parallel sequencing strategy for screening panels of genomic target regions. Here, we demonstrate that Hi-Plex applied with hybrid adapters can generate a library suitable for sequencing with both the Ion Torrent and the TruSeq chemistries and that adjusting primer concentrations improves coverage uniformity. These results expand Hi-Plex capabilities as an accurate, affordable, flexible, and rapid approach for various genetic screening applications.

Keywords

Massively parallel sequencing; Targeted sequencing; High-Plex; Disease gene screening; Molecular diagnostics

High-Plex polymerase chain reaction (PCR),[1] as a sequence enrichment method for massively parallel sequencing (MPS), has been made available recently through a range of commercial solutions. Such technology has great potential in numerous settings, including diagnostics, disease risk predictive screening, and gene discovery screening. However, protocol complexity, expense, and limited design flexibility reduce their suitability in settings involving modest target size or requiring low cost and high throughput. In addition, these commercial enrichment kits are platform specific. For instance, although Haloplex is available for both Ion Torrent and Illumina chemistries, different kits need to be purchased to allow sequencing.

We previously reported Hi-Plex, a novel, streamlined, highly multiplexed PCR approach for targeted MPS [1]. The Hi-Plex system is complemented by simple, automated primer design

*Corresponding author. Fax: +61 3 8344 4004. djp@unimelb.edu.au.

[1]*Abbreviations used:* PCR, polymerase chain reaction; MPS, massively parallel sequencing; PGM, Personal Genome Machine; LCL, lymphoblastoid cell line.

software that allows insert size definition and complete overlap of read pairs following paired-end sequencing, an important feature to facilitate stringent filtering of sequencing errors. Library building using Hi-Plex consists of a single-tube, single-PCR amplification, followed by size selection. In our initial report, we showed that, in a 60-plex setting using primers targeting the breast cancer predisposition genes *PALB2* and *XRCC2* [2–4], Hi-Plex enables efficient, cost-effective, and rapid sequencing on the Ion Torrent PGM (Personal Genome Machine) instrument (Life Technologies).

Here, we demonstrate that Hi-Plex can be used to generate a single library that is suitable for sequencing using both the Ion Torrent (PGM and Proton instruments, Life Technologies) and TruSeq (MiSeq and HiSeq instruments, Illumina) chemistries. A library was prepared using a lymphoblastoid cell line (LCL)-derived DNA using the protocol and oligos described in Ref. [1] except that hybrid adapters, containing 5′-TruSeq compatible and 3′-Ion Torrent compatible sequences, were used (Table 1). The library design also included Illumina Nextera dual indexing. The library was subsequently sequenced on both the Ion Torrent PGM and MiSeq instruments using the PGM 200-bp sequencing kit and a 314 chip and the MiSeq Reagent Kit version 2, respectively. Sequencing primers were spiked in the latter assay (Table 1). When mapped to the entire human genome, we observed that 95.22% and 98.12% of the reads were on-target, respectively. For the PGM run, assessment of the coverage distribution showed that 83.33% (50/60), 93.33% (56/60), 98.33% (59/60), and 100% of targeted amplicons were represented within 5-, 10-, 15-, and 25-fold of the median on-target coverage (4950 reads), respectively. Following the MiSeq run, the relevant dual-indexed library yielded 86.67% (52/60), 96.67% (58/60), 98.33% (59/60), and 100% representation of the targeted amplicons within 5-, 10-, 15-, and 20-fold of the median on-target coverage (813 reads), respectively. The MiSeq sequencing run included a panel of libraries not associated with our study, which explains the lower-than-maximum possible yields for the instrument. These results demonstrate a flexibility that has not been described previously.

In performing the earlier reported experiments, primer sequences were not altered from those produced by the initial automated primer software, and all gene-specific primers were present in equimolar amounts. Here, in an additional experiment, we sought further optimization by performing Hi-Plex with adjusted gene-specific primer concentrations for “overachieving” amplified fragments, that is, any amplicons for which the coverage relative to the median across all on-target amplicons was greater than 1.5-fold. Gene-specific primer concentrations were decreased by a factor calculated as √(coverage median coverage across all amplicons (Table 2). A second library was prepared from another LCL-derived DNA using the adjusted gene-specific primer pool and sequenced on the MiSeq instrument (version 2 flow cell), as described above. For the relevant dual-indexed library, we observed that 95.33% of the mapped reads were on-target, with a median coverage across all exons of 243 reads. The coverage difference between the highest and lowest covered amplicons decreased from 197-fold without adjustment to 37-fold following concentration adjustment of 18 primer pairs. In the latter case, coverage ranged from 12.15-fold lower to 3.11-fold higher than the median. As a result, 93.33% (56/60), 98.33% (59/60), and 100% of the targeted amplicons were represented within 5-, 10-, and 12.5-fold of the median to demonstrate that Hi-Plex is readily optimized, if required, without the need to redesign the constituent primers.

The results reported here further establish the capabilities of Hi-Plex as an accurate, affordable, flexible, and rapid approach to high-throughput disease gene characterization with promise in a range of genetic screening applications.

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References

[1]. Nguyen-Dumont T, Pope BJ, Hammet F, Southey MC, Park DJ. A High-Plex PCR approach for massively parallel sequencing. BioTechniques. 2013; 55:69–74. [PubMed: 23931594]

[2]. Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T, Jayatilake H, McGuffog L, Hanks S, Evans DG, Eccles D, Easton DF, Stratton MR. *PALB2*, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat. Genet. 2007; 39:165–167. [PubMed: 17200668]

[3]. Southey MC, Teo ZL, Dowty JG, Odefrey FA, Park DJ, Tischkowitz M, Sabbaghian N, Apicella C, Byrnes GB, Winship I, Baglietto L, Giles GG, Goldgar DE, Foulkes WD, Hopper JL. A *PALB2* mutation associated with high risk of breast cancer. Breast Cancer Res. 2010; 12:R109. [PubMed: 21182766]

[4]. Park DJ, Lesueur F, Nguyen-Dumont T, Pertesi M, Odefrey F, Hammet F, Neuhausen SL, John EM, Andrulis IL, Terry MB, Daly M, Buys S, Le Calvez-Kelm F, Lonie A, Pope BJ, Tsimiklis H, Voegele C, Hilbers FM, Hoogerbrugge N, Barroso A, Osorio A, Giles GG, Devilee P, Benitez J, Hopper JL, Tavtigian SV, Goldgar DE, Southey MC. Rare mutations in *XRCC2* increase the risk of breast cancer. Am. J. Hum. Genet. 2012; 90:734–739. [PubMed: 22464251]

Table 1

Hybrid adapter and sequencing primer sequences used by Hi-Plex to generate a library compatible with both Ion Torrent and TruSeq sequencing chemistries.

Name	5′ to 3′ Sequence
N501_TSIT_A[a]	AATGATACGGCGACCACCGAGATCTACACTAGATCGCccatctcatccctgcgtgtctccgactcag
N701_TSIT_P[a]	CAAGCAGAAGACGGCATACGAGATTCGCCTTActccgctttcctctctatgggcagtcggtgat
TSIT_Read1[b]	ccatctcatccctgcgtgtctccgactcag
TSIT_Read2[b]	ctccgctttcctctctatgggcagtcggtgatT
TSIT_i7_read[b]	aatcaccgactgcccatagagaggaaagcggag

[a]Adapter indexing design was based on Nextera dual indexing. TruSeq-compatible sequence is indicated in uppercase, index sequence is indicated in uppercase and underlined, and Ion Torrent-compatible sequence is indicated in lowercase.

[b]Here, 3.4 μl of 100 μM sequencing primers was added to the respective read1, read2, and i7 primer reservoirs in the reagent cartridge prior to the MiSeq run.

Table 2

Adjustment factor and reaction concentration of “overachieving” gene-specific primers.

Primer name	5′ to 3′ Sequence	Adjustment factor[a]	Final reaction concentration[b] (nM)
itPALB2_X3_F1	ctctctatgggcagtcggtgattAAATGAATAATAAAGCAGGCATAAG	1.64	2.4
itPALB2_X3_R1	ctgcgtgtctccgactcagCGTGCCCAAAGAGCTGAAAAG		
itPALB2_X4_F1	ctctctatgggcagtcggtgattATCATCATCATCATCATCATCAAA	2.51	1.6
itPALB2_X4_R1	ctgcgtgtctccgactcagATTGTGAACCACTTTTGCCAACT		
itPALB2_X4_F6	ctctctatgggcagtcggtgattTCTGCAGGAAACAGAAGGCCT	2.28	1.8
itPALB2_X4_R6	ctgcgtgtctccgactcagACACTCTTGATGGCAGGAATG		
itPALB2_X4_F9	ctctctatgggcagtcggtgattGCTTTATTTACAAGGAGGTTATCT	1.48	2.7
itPALB2_X4_R9	ctgcgtgtctccgactcagAGCACCTTGAACACATTCCTC		
itPALB2_X4_F10	ctctctatgggcagtcggtgattAGTAAGTTCACTGCTACCTTTAG	2.48	1.6
itPALB2_X4_R10	ctgcgtgtctccgactcagTGCCCAACCAGAAAAAGGTGTT		
itPALB2_X5_F1	ctctctatgggcagtcggtgattACTAAGGCATTTCATTCCTTCAG	1.54	2.6
itPALB2_X5_R1	ctgcgtgtctccgactcagAACACCTCCACCCATTGAGTC		
itPALB2_X5_F3	ctctctatgggcagtcggtgattGGGTGGTATGTGGTTTTGCTG	1.27	3.1
itPALB2_X5_R3	ctgcgtgtctccgactcagGCTCCTATGAAAAAGCATCTACA		
itPALB2_X5_F4	ctctctatgggcagtcggtgattGCAAGTTCGTCCAGCAACTTC	2.02	2.0
itPALB2_X5_R4	ctgcgtgtctccgactcagTAAATACGGTTGCGCCTGATGA		
itPALB2_X5_F6	ctctctatgggcagtcggtgattAGTTTGGCCTTTTGGGATGTG	1.32	3.0
itPALB2_X5_R6	ctgcgtgtctccgactcagAAAATGTTTGGAGAGAGACATCTT		
itPALB2_X6_F1	ctctctatgggcagtcggtgattACACGAGACACTGGAAGAGAAT	2.09	1.9
itPALB2_X6_R1	ctgcgtgtctccgactcagTACATAAAGTGTAGACTAATGATGT		
itPALB2_X7_F1	ctctctatgggcagtcggtgattATTATCAGGCAAATGGCTGCAAA	1.31	3.1
itPALB2_X7_R1	ctgcgtgtctccgactcagTGTATCATAACTGCTTGCGAAGA		
itPALB2_X7_F2	ctctctatgggcagtcggtgattGAGCTTTCCAAAGAGAAACTACA	1.78	2.2
itPALB2_X7_R2	ctgcgtgtctccgactcagCACTTTAACAGAACTGTTGCCAT		
itPALB2_X10_F2	ctctctatgggcagtcggtgattCTTCTTGCATCCCTTGGACCT	1.66	2.4
itPALB2_X10_R2	ctgcgtgtctccgactcagGAGAAGGGCTACCTAGAGACT		
itPALB2_X12_F1	ctctctatgggcagtcggtgattTGCACAGTGCCTTTCAGAATGT	1.29	3.1
itPALB2_X12_R1	ctgcgtgtctccgactcagCCTGTGTTTCAGCTCATTGTGA		
itXRCC2_X1_F1	ctctctatgggcagtcggtgattGCCTTGTTCCCATCTCCCTCA	1.95	2.1
itXRCC2_X1_R1	ctgcgtgtctccgactcagTTGGTGAATGGCGTTGGTGGC		
itXRCC2_X3_F3	ctctctatgggcagtcggtgattTGCCATGCCTTACAGAGATAAG	1.66	2.4
itXRCC2_X3_R3	ctgcgtgtctccgactcagTAAATGACTATCGCCTGGTTCTT		
itXRCC2_X3_F5	ctctctatgggcagtcggtgattCTTTCTCCTCCATTGACGCGG	3.33	1.2
itXRCC2_X3_R5	ctgcgtgtctccgactcagTGTACTGCAGTAGTAGCACCC		
itXRCC2_X3_F8	ctctctatgggcagtcggtgattTACATCGTGCTGTTAGGTGATAA	1.68	2.4
itXRCC2_X3_R8	ctgcgtgtctccgactcagTGCAGACTTTGCATTTTGTAACC		

[a] Calculated as √(coverage median coverage across all amplicons). Coverage based on the Ion Torrent PGM experiment is described in this report. Forward and reverse primers were decreased by the same factor.

[b] All other gene-specific primers were used at 4 nM each.