Supporting Information

Improvement of LATE-PCR to allow single-cell analysis by pyrosequencing

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Electronic Supplementary Material (ESI) for Analyst

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Table S-1. Primers for the comparison of imLATE-PCR and LATE-PCR

Name	Sequence (5′-3′)*	Tm (°C)	Concentration (μM)	Amplicon length (bp)	Amplicon Tm (°C)
	imLATE-PCR Primer				
BRCA1-1 (PX)	GCGGTCACCCACAGTCGGGAAACAAGCAT	68.8	1		
BRCA1-2 (PL1)	CGAGCAGATGACTGTCGCTTTGAAAC	59.1	0.1	107	71.8
BRCA1-3 (PL2)	TAAGGACCGAGAGTGGGCAGAGAAT	60.0	0.1	171	74.3
BRCA1-4 (PL3)	CGTTCTGACCAACCACAGGAAAGCC	61.0	0.1	301	74.5
BRCA1-5 (PL4)	CGTCGGAGTAAAGAGTCCAGTTTCGTTG	59.6	0.1	402	74.8
BRCA1-6 (PL5)	GCGAATGACTCGATTGGAAAAAGTGGTG	59.2	0.1	464	74.7
	LATE-PCR Primer				
BRCA1-1 (PL)	GCGGTCACCCACAGTCGGGAAACAAGCAT	66.5	0.1		
BRCA1-2 (PX1)	CGAGCAGATGACTGTCGCTTTGAAAC	61.5	1	107	71.8
BRCA1-3 (PX2)	TAAGGACCGAGAGTGGGCAGAGAAT	62.7	1	171	74.3
BRCA1-4 (PX3)	CGTTCTGACCAACCACAGGAAAGCC	63.6	1	301	74.5
BRCA1-5 (PX4)	CGTCGGAGTAAAGAGTCCAGTTTCGTTG	61.9	1	402	74.8
BRCA1-6 (PX5)	GCGAATGACTCGATTGGAAAAAGTGGTG	61.5	1	464	74.7

*Bases underlined mean the artificially mismatched bases. Primer and amplicon Tm values were estimated by the nearest neighbor formula and a salt adjustment using input concentrations of 1 μM for the excess primer and amplicon, 0.1μM for the limiting primer, and 50 mM for monovalent cations (OligoAnalyzer 3.0, a Web-based program to compute these values, is available at http://scitools.idtdna.com/analyzer/).

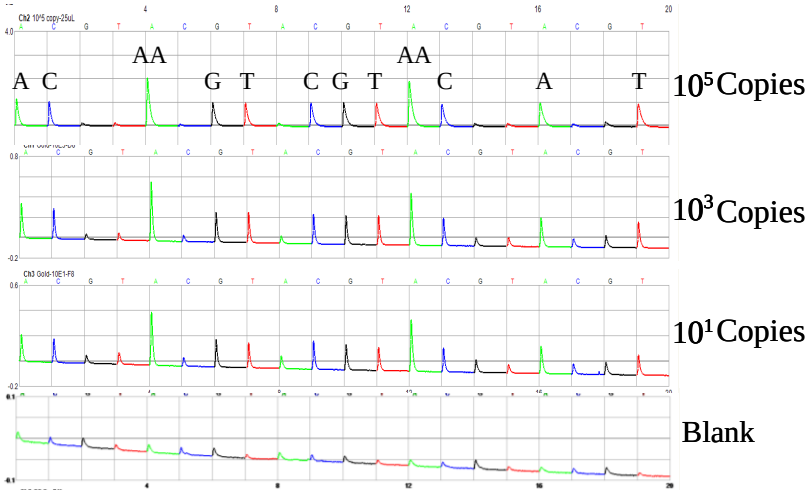

Fig. S-1. Pyrograms of different amounts templates (10^5, 10^3, 10^1 copies, and blank) by 1 μL of imLATE-PCR products on slide. The expected sequence was indicated above peak.

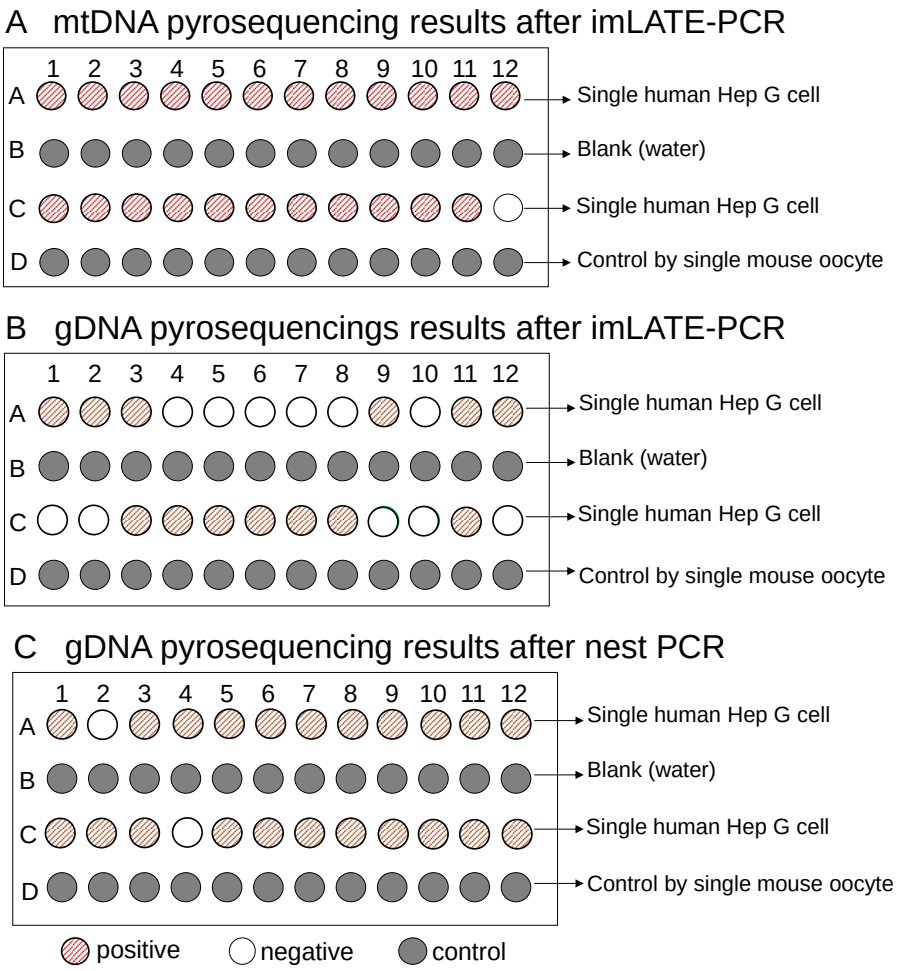

Fig. S-2. Tests for evaluating cross-contamination between wells on the slide. The positioning pattern of samples is single cancer cells (Hep G) at lanes A and C for positive control, water at lane B for blank control, and single mouse oocytes at lane D for negative control. (A) Pyrosequencing of the products from imLATE-PCR of the 1555A>G on mtDNA. About 95.8% (23/24) wells were positively detected. (B) Pyrosequencing of the products from imLATE-PCR of the the BRCA1 gene on gDNA. About 54.2% (13/24) wells were positively detected. (C) Pyrosequencing of the products from imLATE-PCR of the the BRCA1 gene on gDNA by coupling a nest PCR before imLATE-PCR. About 91.7% (22/24) wells were positively detected. No cross-talk was found for all tests.