Tohoku Univ. Technology

Target specific and non-biased gene amplification

Gene expression analysis is possible without changing copy number ratio in a sample!

Introduction

In comprehensive gene expression analysis such as T cell receptor (TCR) repertoire analysis, it is important that target specific and non-biased gene amplification products are obtained. Conventional methods often ligate adapters with restriction enzyme site to synthesized cDNA ends and treat adapter-ligated cDNAs with said restriction enzyme before specific amplification. In such methods, however, unused and untreated adapters often inhibit target specific and non-biased amplification.

This invention performs target specific, non-biased gene amplification by combination of uracil-containing adapter and uracil DNA glycosylase (UNG) treatment (Fig 1).

Effect

TCR sequencing by NGS was more successfully performed with sufficient read length and overlaps among reads in TCR repertoire analysis using amplified products by this invention, when compared with that using products by conventional adaptor ligation method (Fig 2).

This invention performs gene amplification without changes of copy number ratio in a sample, showing usefulness for various applications other than TCR analysis, such as RNA-seq, intestinal flora analysis and environmental (cell free) DNA analysis. The kit to do this invention is simply available by adding uracil-containing adapter and UNG to conventional PCR kit.

IP Data

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Fig. 1. Illustration of this invention when used in TCR repertoire analysis

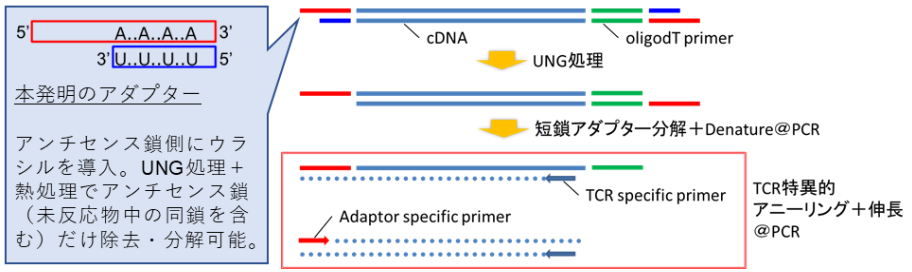

Fig. 2. Comparison of performance as viewed from TCR sequencing success

		overlap			TCR			
		総リード数	マージ数	成功率(%)	解析リード数	成功数	失敗数	成功率(%)
東北大学	sample21	142955	126106	88.2	10000	9838	162	98.4
	sample61	219646	193596	88.1	10000	9575	425	95.8
	Average	181301	159851	88.2	10000	9707	293	97.1
X社	T11	164995	16370	9.9	10000	8966	1034	89.7
	T12	416040	24999	6.0	10000	6023	3977	60.2
	Average	290518	20685	8.0	10000	7495	2505	75.0

東北大学：**85.6%** ＞＞＞＞＞＞ X社：**6.0%**

Contact

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