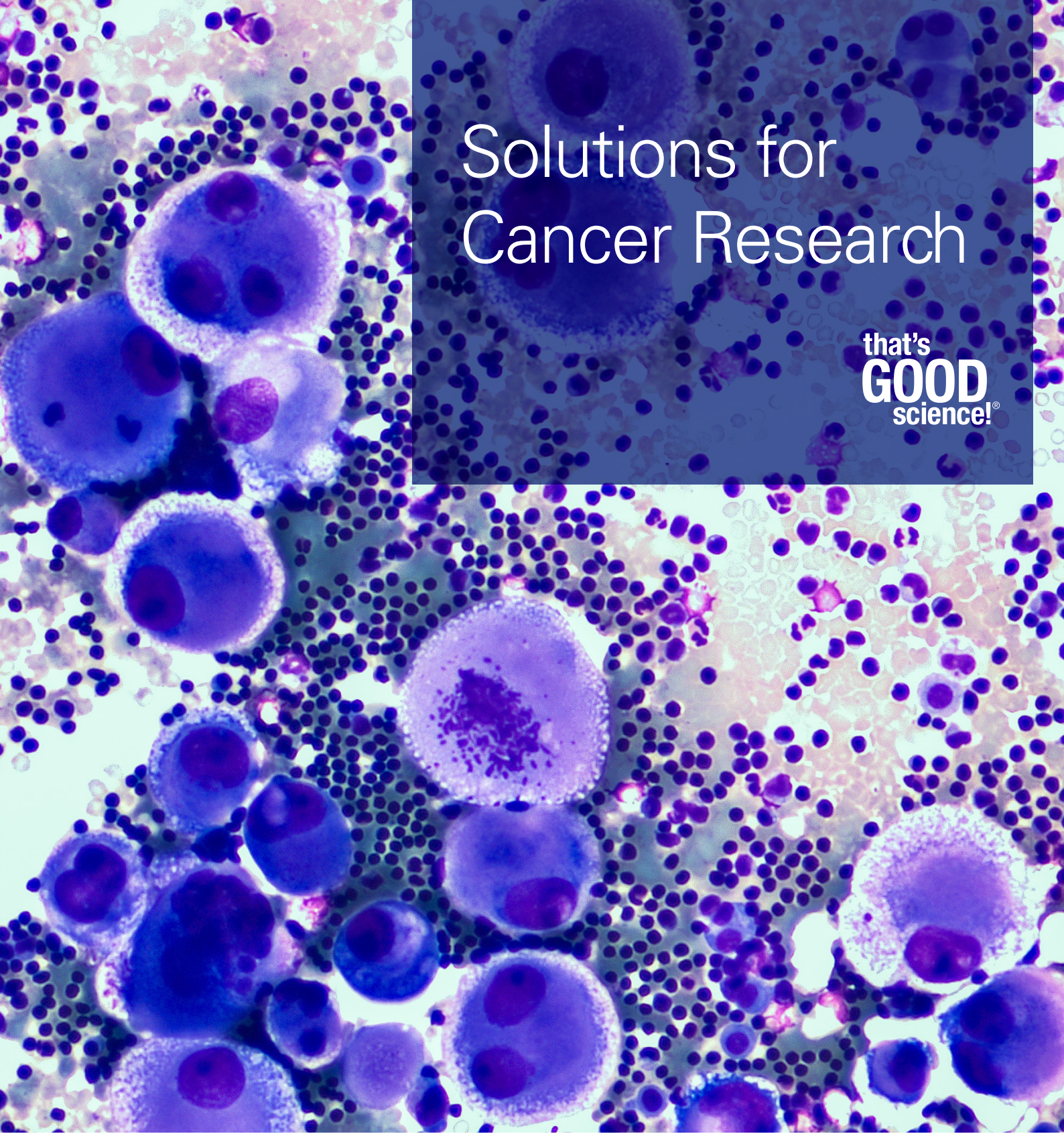

Solutions for Cancer Research

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Introduction

Cancer is a multifaceted disease that arises from numerous modifications to the cellular genome including gene mutations, copy number variations, and structural changes such as chromosomal translocations and deletions. These genome alterations in parallel with dysregulations within the epigenetic landscape are hallmarks of cancer and account for the complexity of the disease.

The fight against cancer remains an uphill battle and the efficacies of traditional treatments for cancer such as radiotherapy and chemotherapy are often limited by the occurrence of severe toxicities, resulting in numerous side effects being experienced by cancer patients. However, ongoing advances in cancer immunotherapy offer a great hope in the fight against cancer as it utilizes components of a patient's own immune system to selectively target and kill cancer cells, thus mitigating many of the side effects associated with traditional treatment options.

Takara Bio offers a broad range of innovative technologies to accelerate your cancer research discovery and therapeutic workflows spanning from tools for cancer biomarker discovery, single cancer cell analysis, cancer epigenomic analysis, HLA typing, T-cell therapy and profiling, antibody therapeutics, and CRISPR/Cas9 gene editing.

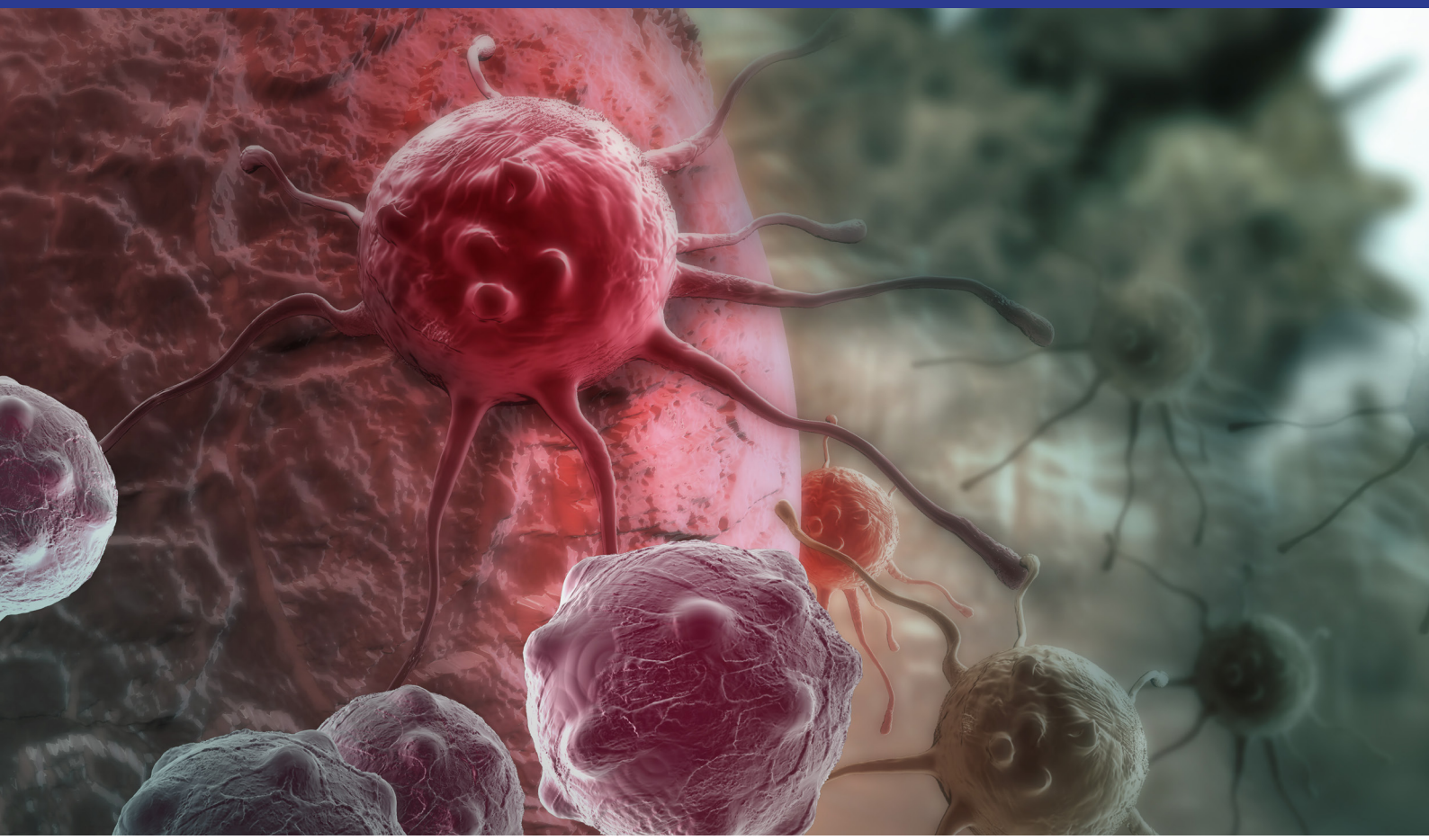

Table of Contents

Cancer Biomarker Discovery

Cancer consists of a multigene disorder that arises from gene mutations as well as changes in transcriptional, epigenetic, and proteomic profiles. These changes can often serve as valuable biomarkers for both early detection/diagnosis and for the development of individualized therapy. Mutations in several known oncogenes (e.g., EGFR, HER2, KRAS) and tumor suppressor genes (e.g., TP53, PTEN, PI3K) are already being used as biomarkers to guide therapy in breast cancer, ovarian cancer, lung cancer, and prostate cancer, among others.

Cancer biomarker discovery has been greatly facilitated by Next-Generation Sequencing (NGS) analysis of circulating nucleic acids within blood, urine, saliva, pleural effusions, and cerebrospinal fluid (*i.e.* non-invasive liquid biopsies) which can contain tumour-derived genetic information (1). Indeed, NGS is a high-throughput genome sequencing technology that enables sequencing of entire genomes or thousands of mutations simultaneously in a cost-effective manner and hence can serve as a very powerful tool in biomarker detection and discovery. The molecular profiles gathered from circulating tumoral (ct) DNA via NGS can be further complemented with those obtained through analysis of circulating tumour cells (CTCs), as well as RNA, proteins, and lipids contained within cell derived vesicles, such as exosomes.

Recent advances in cancer biology have highlighted the importance of exosomes as carriers of genetic and biological messages between cancer cells and their immediate and/or distant environments. Cancer cells secrete exosomes containing diverse molecules that can be transferred to recipient cells and/or *vice versa* to induce a plethora of biological processes, including angiogenesis, metastasis formation, and therapeutic resistance. Therefore, the molecular cargo of exosomes represent a rich source for novel cancer biomarker discovery (2).

1. Siravegna G. *et al.* (2017). Integrating liquid biopsies into the management of cancer. Nature Reviews Clinical Oncology 14: 531-548
2. Sundararajan V. *et al.* (2018). The versatile role of exosomes in cancer progression: diagnostic and therapeutic implications. Cellular Oncology 41 (3): 223-252

Highlighted products

Takara Bio provides a number of innovative technologies to speed up your cancer biomarker discovery workflow, including our **SMARTer® ThruPLEX® Plasma-seq** kit (Figure 1), designed to construct NGS libraries from DNA present within body fluids and liquid biopsies such as cell free DNA (cfDNA) and circulating tumoral DNA (ctDNA). Our chemistry has been optimized to work efficiently with precious cancer liquid biopsy samples and is compatible with leading target enrichment platforms for whole exome-sequencing or specific gene panels. Moreover, our **SMARTer ThruPLEX** technology has been successfully employed and cited by a number of groups (Table 1), demonstrating the applicability of non-invasive monitoring of tumor chemo-resistance by sequencing ctDNA from liquid biopsies in various types of cancers.

Tools to accelerate cancer biomarker discovery from exosome molecular cargo such as microRNAs, include our **Capturem™ Exosome Isolation Kit** that allows fast (< 30 minutes) and easy isolation of exosomes from cell culture media, and our **SMARTer smRNA-Seq Kit** for Illumina® which provides a streamlined and fast NGS workflow for the global analysis of small RNAs (smRNAs) from picogram-amount of RNA extracted from exosomes plasma, serum and liquid biopsies. Indeed, Guelfi *et al.* successfully utilized our **SMARTer smRNA-Seq Kit** to carry out an NGS-based miRNA profiling for non-invasive biomarker discovery in the diagnosis of prostate cancer (Table 2).

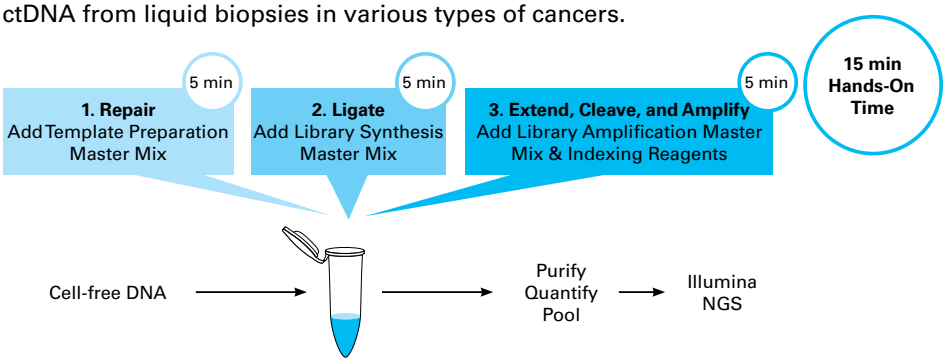

Figure 1: SMARTer ThruPLEX Plasma-seq Kit Single-Tube Workflow. Starting with 1 to 30 ng of cell-free DNA, ThruPLEX Plasma-seq Kit creates indexed libraries in 3 simple steps: end repair, adapter ligation, and high-fidelity library amplification. No purification or sample transfer steps are required. The streamlined workflow is performed in 2 hours in a single tube or well, preventing sample loss and enhancing positive sample identification.

Table 1: Selected publications citing the use of SMARTer ThruPLEX-Plasma Seq Kit for non-invasive monitoring of tumor chemo-resistance

1. Murtaza M. *et al.* (2013). Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 497: 108-112
2. Xia Y. *et al.* (2016). Copy number variations in urine cell free DNA as biomarkers in advanced prostate cancer. Oncotarget 7: 35818-35831
3. Patel K.M. *et al.* (2017). Association Of Plasma And Urinary Mutant DNA With Clinical Outcomes In Muscle Invasive Bladder Cancer. Scientific Reports 7: 5554

Table 2: Selected publications citing the use of SMARTer smRNA-Seq Kit for non-invasive miRNA profiling in prostate cancer diagnosis

1. Guelfi G. *et al.* (2018). Next Generation Sequencing of urine exfoliated cells: an approach of prostate cancer microRNAs research. Scientific Reports 8: 7111

Single Cancer Cell Analysis

Key genomic alteration of cancer cells can be structured including deletions, translocations, or amplification of genes/portions of the genome, or can affect the DNA sequence itself, *i.e.* mutations. Cancer progression can be caused by clonal expansion and selection of these driver mutations resulting in a plethora of malignant alterations characterizing the tumoral DNA.

Recent advances in NGS have now made it possible to profile the genomes of single tumoral cells. This allows the systematic documentation of cancer cells' mutational DNA make-up, the tracking of clonal and sub-clonal heterogeneity/generation/phylogeny, as well as monitoring the effect of anticancer therapies at a single cell level (1). The complexity and heterogeneity of tumoral cells also translates at the transcriptomic level where genomic heterogeneity is mirrored by single-cell variations within the transcriptome of cancer cells, cancer persister cells and CTCs.

Studying single cells from precious samples such as cancer persister cells and CTCs requires extraordinarily sensitive and reproducible methodologies. Therefore, the accurate capture and quantification of RNA transcript variations from single tumoral cells remains a major challenge, but would allow researchers to gain insights into tumor complexity, and ultimately help in the development of tailored anti-cancer therapies (2).

1. Van Loo P. and Voet T. (2014). Single cell analysis of cancer genomes. Current Opinion in Genetics & Development 24: 82-91.
2. Zhu S. *et al.* (2017). Advances in single-cell RNA sequencing and its applications in cancer research. Oncotarget 16: 8(32): 53763-53779.

Highlighted products

Single-cell Genome Sequencing

SMARTer PicoPLEX WGA/DNA-seq NGS kits are based on Takara Bio's patented **SMARTer PicoPLEX** technology for single-cell whole genome amplification, which uses multiple cycles of quasi-random priming for reproducible library construction, suitable for sequencing on Illumina platforms. Indeed, our **SMARTer PicoPLEX** technology allows the precise and impartial analysis of the genome for many applications in cancer research, including the study of chromosomal aneuploidies, copy number variations (CNV), and the detection of insertions/deletions. Many publications have cited the use of the **SMARTer PicoPLEX** technology for high performance CNV analysis, and the genomic profiling of single cells from FFPE tumor tissues and CTCs (Table 3). Moreover, our **SMARTer PicoPLEX** technology is a key component of the recently developed single-cell Genome & Transcriptome-seq approach (G&T-seq; Table 3).

Table 3: Selected publications citing the use of PicoPLEX technology for high performance CNV analysis and the genomic profiling of single cells from FFPE tumor tissues and circulating tumor cells, and G&T-seq

1. Lieselot D. *et al.* (2017). Performance of four modern whole genome amplification methods for copy number variant detection in single cells. Scientific Reports 7: 3422
2. Babayan A. *et al.* (2017). Comparative study of whole genome amplification and next generation sequencing performance of single cancer cells. Oncotarget 8: 56066-56080
3. Williamson S.C. *et al.* (2016). Vasculogenic mimicry in small cell lung cancer. Nature Communications 7: 13322
4. Morrow C. J. *et al.* (2016). Tumourigenic non-small-cell lung cancer mesenchymal circulating tumour cells: a clinical case study. Annals of Oncology 27 (6): 1155–1160
5. Premasekharan G. *et al.* (2016). An improved CTC isolation scheme for pairing with downstream genomics: Demonstrating clinical utility in metastatic prostate, lung and pancreatic cancer. Cancer Letters 380 (1): 144 – 152
6. Cayrefourcq L. *et al.* (2015). Establishment and Characterization of a Cell Line from Human Circulating Colon Cancer Cells. Cancer Research 75 (5): 892-901
7. Macaulay I.C. *et al.* (2015). G&T-seq: parallel sequencing of single-cell genomes and transcriptomes. Nature Methods 12: 519–522

Single-cell Transcriptome Sequencing

Takara Bio has always been at the forefront of single-cell mRNA-Seq research, by leveraging our patented **SMART-Seq®** technology, to provide NGS kits with the capability to obtain full-length mRNA sequence information, including splice junction/alternative transcript information, from single cells. Takara Bio's 4th generation **SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing** and the **SMART-Seq HT (High Throughput) Kit** represent Takara Bio's most sensitive mRNA-seq solutions for single cells, a few cells, and ultra-low inputs of RNA.

These kits rely on oligo(dT) priming and proprietary SMART (Switching Mechanism at 5' end of RNA Template) technology to ensure full-length, unbiased mRNA coverage. Intact cells can be used directly as input for these kits, guaranteeing high-quality input RNA and full-length cDNA coverage. Our SMART-Seq v4 technology is now also fully integrated into the **SMARTer Apollo™ NGS** library prep system and the **SMARTer ICELL8™ Single-Cell system**, our advanced automation platform for high-throughput single-cell RNA-seq.

Moreover, many publications have cited the use of our SMART-Seq solutions for single-cell RNA-seq in various different cancer applications (Table 4), including tumor cell and tumor infiltrating immune cell profiling, the analysis of cancer stem cell heterogeneity, identifying tumor cell clones resistant to therapy, and simultaneous genome and transcriptome sequencing of cancer cells.

Finally, the newly launched **SMART-Seq Stranded Kit** enables the generation of stranded Illumina-compatible sequencing-ready libraries at the single-cell level. This kit combines features of our **SMART-Seq v4** technology with the unique features of our referenced **SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian Kit** suitable for library preparation from picogram input amounts of tumoral total RNA (Table 4) including very degraded FFPE samples from tumor biopsies.

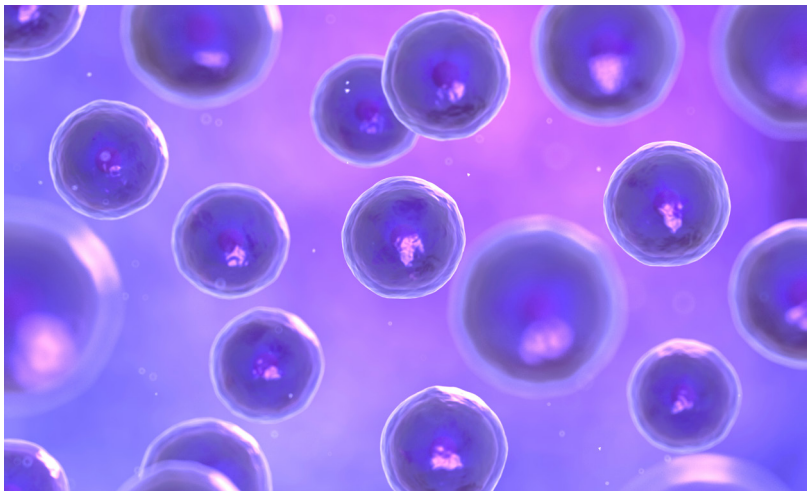

Table 4: Selected publications citing the use of SMART-Seq solutions for single-cell RNA-seq in various different cancer applications

1. Chung W. *et al.* (2017). Single-cell RNA-seq enables comprehensive tumour and immune cell profiling in primary breast cancer. Nature Communications 8: 15081
2. Zheng H. *et al.* (2018). Single-cell analysis reveals cancer stem cell heterogeneity in hepatocellular carcinoma. Hepatology doi: 10.1002/hep.29778. [Epub ahead of print]
3. Kim K.T. *et al.* (2015). Single-cell mRNA sequencing identifies subclonal heterogeneity in anti-cancer drug responses of lung adenocarcinoma cells. Genome Biology 16: 127
4. Han K.Y. *et al.* (2018). SIDR: simultaneous isolation and parallel sequencing of genomic DNA and total RNA from single cells. Genome Research 28: 75–87
5. Chiu H.S. *et al.* (2018). Pan-Cancer Analysis of lncRNA Regulation Supports Their Targeting of Cancer Genes in Each Tumor Context. Cell Reports 23(1): 297–312

Cancer Genomics and Epigenomics

Genome alterations such as mutations, copy number variations and structural variations in parallel with dysregulations within the epigenetic landscape (*i.e.* DNA methylation status) are hallmarks of cancer. Whole genome sequencing and targeted-sequencing approaches allow cancer researchers to obtain a comprehensive picture of genomic alterations, mainly focused on the detection of somatic variants and CNVs.

Targeted approaches such as exome or panel sequencing further helps to focus on specific regions/genes of interest and allow deeper sequencing for increased sensitivity in variant detection with respect to whole genome sequencing. Epigenetic approaches on the other hand have proven useful in describing cancer specific DNA-binding proteins, histone modifications, and the DNA methylation make-up of cancer cells. Understanding how these epigenetic changes act in concert with genomic alterations in tumor onset, progression and in tumor resistance to therapy is of crucial importance to improve cancer care.

Highlighted products

Takara Bio provides a number of innovative technologies to speed up your whole genome and targeted sequencing workflow for somatic variant and CNV discovery, including our patented **SMARTer ThruPLEX** technology. All of our SMARTer ThruPLEX kits feature unparalleled ease of use, to reduce user error, sample loss and contamination, with a single-tube, 2-hour, 3-step workflow (Figure 2). The very sensitive **SMARTer ThruPLEX DNA-seq Kit** allows the construction of NGS libraries from picogram amounts of DNA, including from FFPE samples and ChIP DNA. The **SMARTer ThruPLEX Plasma-seq Kit** has been specifically designed for use with cell free DNA (cfDNA) and circulating tumoral DNA (ctDNA) present in precious cancer liquid biopsy samples.

Lastly, the **SMARTer ThruPLEX Tag-seq Kit** contains more than 16 million unique sequences, used to tag individual DNA fragments prior to amplification, allowing the tracking of fragments through the library preparation, target enrichment, and data analysis processes to detect low-frequency alleles or to count individual fragments. **SMARTer ThuPLEX** technology is also compatible and has been validated for use with major target enrichment platforms such as Agilent SureSelect, Roche Nimblegen SeqCap EZ, and IDT xGen Lockdown probes. Moreover, the **SMARTer ThruPLEX** technology has been successfully utilized and cited for whole genome sequencing, targeted sequencing, CNV analysis and ChIP-seq studies in various types of cancers (Table 5).

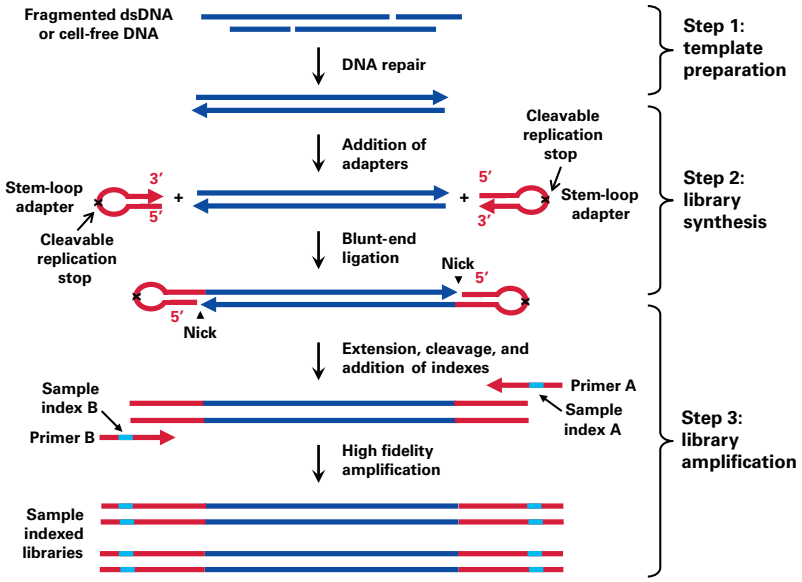

Figure 2: Workflow for SMARTer ThruPLEX technology

Table 5: Selected publications citing the use of SMARTer ThruPLEX technology for whole genome sequencing, targeted sequencing, CNV analysis and ChIP-seq studies in various types of cancers

1. McNair C. *et al.* (2018). Differential impact of RB status on E2F1 reprogramming in human cancer. Journal of Clinical Investigation 128(1): 341–358
2. Jeselsohn R. *et al.* (2018). Allele-Specific Chromatin Recruitment and Therapeutic Vulnerabilities of ESR1 Activating Mutations. Cancer Cell 33(2): 173-186
3. Cato L. *et al.* (2017). Development of Bag-1L as a therapeutic target in androgen receptor-dependent prostate cancer. eLife 6: e27159
4. Jin X. *et al.* (2017). Targeting glioma stem cells through combined BMI1 and EZH2 inhibition. Nature Medicine 23(11): 1352-1361
5. Wang X. *et al.* (2017). Purine synthesis promotes maintenance of brain tumor initiating cells in glioma. Nature Neuroscience 20: 661–673
6. Markus H. *et al.* (2018). Evaluation of pre-analytical factors affecting plasma DNA analysis. Scientific Reports 8: 7375
7. Patel K.M. *et al.* (2017). Association Of Plasma And Urinary Mutant DNA With Clinical Outcomes In Muscle Invasive Bladder Cancer. Scientific Reports 7: 5554
8. Weiss G.J. *et al.* (2017). Tumor Cell-Free DNA Copy Number Instability Predicts Therapeutic Response to Immunotherapy. Clinical Cancer Research 23(17): 5074-5081
9. Klevebring D. *et al.* (2014). Evaluation of exome sequencing to estimate tumor burden in plasma. PLoS One 18;9(8): e104417
10. Murtaza M. *et al.* (2013). Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 497: 108-112

HLA typing for cancer

Human Leukocyte Antigen (HLA) is a highly polymorphic region composed of several genes involved in immune regulation. HLA typing is the characterization of this set of genes, and is a valuable tool to target recurrent mutations and hotspot sites implicated in cancer pathogenesis. This method is also used to match donor and patient before solid organ or allogenic stem cell transplants, often used to treat cancers such as leukemia, lymphoma, multiple myeloma and neuroblastoma. Next-Generation Sequencing (NGS) is the latest technology used to perform HLA typing offering better precision at a lower cost than traditional techniques such as LD PCR (1). NGS in the HLA typing context requires specificity, fidelity and robustness to work with a wide range of complex DNA templates. In this context, Takara Bio offers high quality and high performance tools for HLA Typing including high fidelity polymerases for targeted sequencing and NGS library preparation kits.

1. Hosomichi K. *et al.* (2015). The impact of next-generation sequencing technologies on HLA research. Journal of Human Genetics 60(11): 665-673

Highlighted products

Takara Bio's **PrimeSTAR™ GXL** and **TaKaRa LA Taq™ DNA Polymerase** are ideal enzymes for HLA typing via targeted sequencing (NGS and Sanger) due to their high fidelity, ability to robustly amplify long fragments and GC-rich template tolerance features. Indeed, a number of publications have cited the use of **PrimeSTAR GXL** and/or **LA Taq** enzymes for HLA Typing (Table 6); both Anthony Nolan and NHSBT (London, United Kingdom) are routinely using these enzymes for this application.

Takara Bio's **SMARTer PicoPLEX WGA** and **SMARTer PicoPLEX DNA-seq** kits allow robust whole genome amplification (downstream applications: HLA typing via NGS, Sanger or array). Moreover, **SMARTer PicoPLEX** technology allows robust and reproducible whole genome amplification with a simple and streamlined workflow from single cells. **SMARTer ThruPLEX DNA-seq kits** can also be used for NGS library preparation. Table 7 shows a selected list of publications that have cited the use of **SMARTer PicoPLEX** kits for HLA Typing.

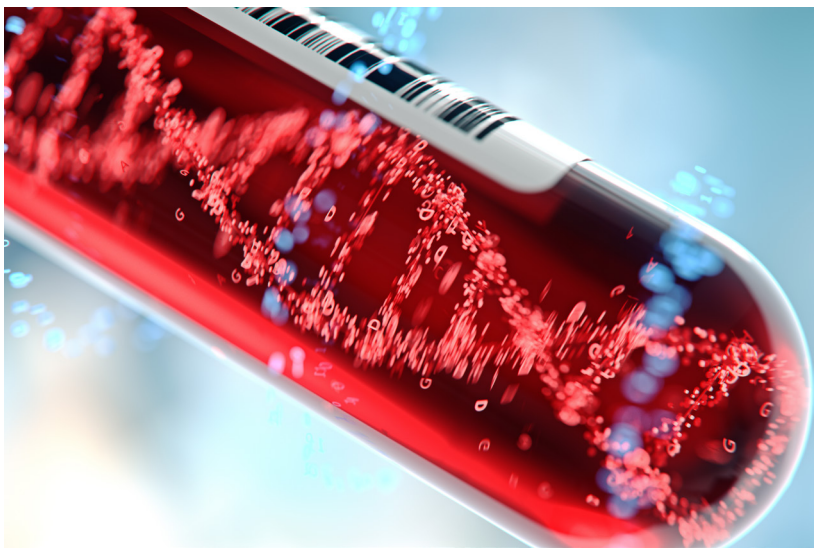

Table 6: Selected publications citing the use of PrimeSTAR GXL and/or TaKaRa LA Taq enzymes for HLA Typing

1. Liu C. *et al.* (2018). Accurate Typing of Human Leukocyte Antigen Class I Genes by Oxford Nanopore Sequencing. Journal of Molecular Diagnostics 2: 006
2. Xu Y.-P. *et al.* (2017). A novel HLA-E allele, HLA-E*01:01:01:06, identified in a Chinese Leukemia patient. HLA 89: 260-262
3. Yin Y. *et al.* (2016). Application of High-Throughput Next-Generation Sequencing for HLA Typing on Buccal Extracted DNA: Results from over 10,000 Donor Recruitment Samples. PLOS ONE 11(10): e0165810.
4. Mayor N.P. *et al.* (2015). HLA Typing for the Next Generation. PLOS ONE 10 (5): e0127153.
5. Lan, J. H. *et al.* (2015). Impact of Three Illumina Library Construction Methods on GC Bias and HLA Genotype Calling. Human Immunology 76(2-3), 166–175
6. Ozaki Y. *et al.* (2015). Cost-efficient multiplex PCR for routine genotyping of up to nine classical HLA loci in a single analytical run of multiple samples by next generation sequencing. BMC Genomics 16:318
7. Ozaki Y. *et al.* (2013). HLA-DRB1, -DRB3, -DRB4 and -DRB5 genotyping at a super-high resolution level by long range PCR and high-throughput sequencing. Tissue Antigens 83: 10-16

Table 7: Selected publications citing the use of SMARTer PicoPLEX technology for HLA Typing

1. Murphy N.M. *et al.* (2016). Haplotyping the human leukocyte antigen system from single chromosomes. Scientific Reports 6: 30381
2. Png E. *et al.* (2011) A genome-wide association study of hepatitis B vaccine response in an Indonesian population reveals multiple independent risk variants in the HLA region. Human Molecular Genetics 20 (19): 3893–3898

Cancer Immunotherapy

Traditional cancer treatments, including surgery, chemotherapy and radiation therapy, have demonstrated very limited efficacy for patients with late-stage disease. In addition, chemotherapy and radiotherapy often cause considerable side effects. Therefore, innovative and effective cancer treatments are urgently needed for cancer patients with late-stage and refractory disease. To this end, cancer immunotherapy has emerged as a promising approach for cancer treatment and holds several key advantages over traditional therapies including high specificity, little or no side effects, and a good safety profile. The key point of immunotherapy is to use the patient's own immune system to control and destroy cancer cells. Immunotherapies that have gained traction in recent years include adoptive T-cell therapy and the use of monoclonal and bispecific antibodies as therapeutic molecules against cancer cells (1).

1. Koury J. *et al.* (2018). Immunotherapies: Exploiting the Immune System for Cancer Treatment. Journal of Immunology Research: 9585614

T-Cell Therapy

The use of genetically modified T cells to target cancer is a very promising approach, especially for cancers which are difficult to treat using traditional methods. The two most common approaches revolve around genetically engineering T cells to introduce either a new T-cell receptor (TCR) or a chimeric antigen receptor (CAR) (1). The actual T-cell therapy involves removing a patient's own T cells, modifying them *ex-vivo*, and then re-infusing the modified cells back into the same patient (Figure 3). In addition to this so-called autologous approach, serveral companies worldwide are working on developing therapies that can be produced from a single donor and then used to treat thousands of patients (allogeneic approach). Both of these approaches have in common that the modification of the T cells is taking place outside of the patient's body (*ex-vivo* gene therapy).

1. Humphries, C. (2013). Adoptive cell therapy: Honing that killer instinct. Nature 504: S13–S15

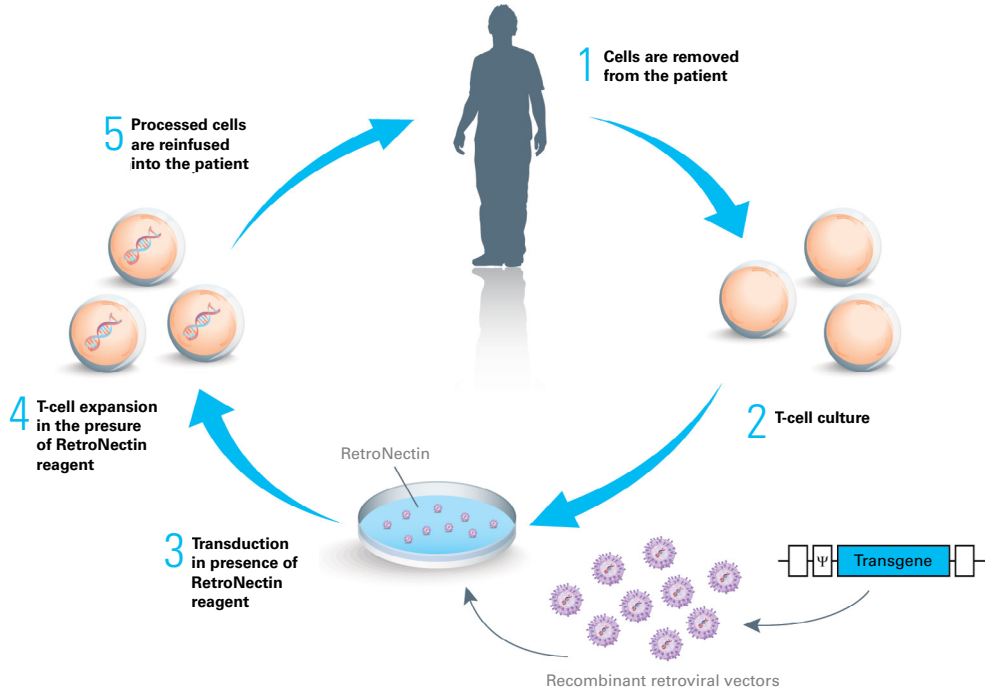

Figure 3: Autologous T-cell therapy

Highlighted products

A high transduction efficiency is essential in T-cell therapies in order to efficiently introduce the TCR/CAR genes into T lymphocytes. In this context, **RetroNectin® GMP grade** reagent can be used to both enhance viral transduction and for T-cell expansion, and is manufactured as a quality-assured product according to guidelines for Good Manufacturing Practice (GMP) for Investigational Products. Indeed, our **RetroNectin GMP grade** reagent has been used in over 68 protocols for gene therapy clinical trials, at 44 institutions worldwide. Takara Bio also supplies **GMP grade Anti-CD3 antibody** for T-cell activation as well as **GT-T551 T-cell Culture medium** which has been optimized for use with **RetroNectin**.

How does RetroNectin work?

RetroNectin reagent is a recombinant human fibronectin fragment that contains three functional domains: the cell-binding domain (C-domain), the heparin-binding domain (H-domain), and the CS-1 sequence. **RetroNectin** reagent enhances lentiviral- and retroviral-mediated gene transduction by aiding the co-localization of target cells and viral particles.

Specifically, virus particles bind RetroNectin reagent via interaction with the H-domain, and target cells bind mainly through the interaction of cell surface integrin receptors VLA-5 and/or VLA-4 with the fibronectin C-domain and CS-1 sites, respectively (Figure 4). By facilitating close physical proximity, RetroNectin reagent can enhance viral-mediated gene transfer to target cells expressing integrin receptors VLA-4 and/or VLA-5.

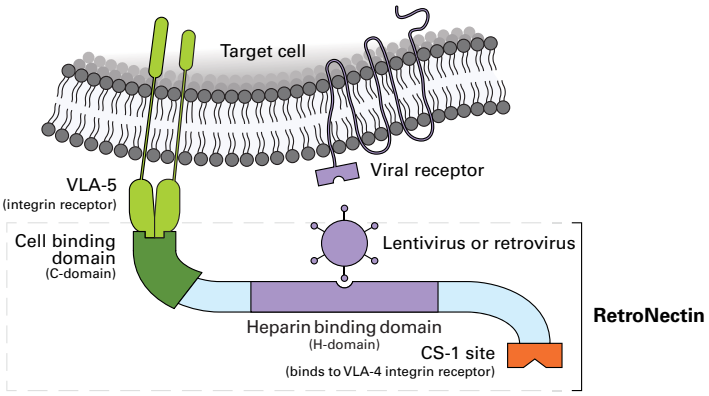

Figure 4: Structure and fuction of RetroNectin

Examples of Clinical Use

Dr. Steven Rosenberg at the National Institutes of Health (US), one of the pioneers of adoptive T-cell therapy, is currently conducting clinical trials of TCR/CAR gene therapy, and his group uses **RetroNectin GMP grade** reagent to transduce patient-derived lymphocytes with TCR/CAR genes that recognize cancer antigens (e.g., MART-1, gp100, or NY-ESO-1) for therapy (1-3, Table 8).

In addition, researchers at Mie University Hospital (Japan), in collaboration with Takara Bio Inc., are conducting clinical research on TCR/CAR gene therapy for oesophageal cancer. Relapsed/refractory acute lymphoblastic leukaemia (R/R ALL) is another cancer with extremely poor prognosis as few therapeutic options are available.

Scientists at Memorial Sloan-Kettering Cancer Center (US) reported an immunotherapy strategy for the treatment of five adult patients with acute lymphoblastic leukemia. Each patient's T cells were isolated, altered by introduction of DNA that would cause the cells to target CD19 and thus attack tumor cells, and infused back into the patient's bloodstream. According to researchers, all patients achieved tumour eradication and complete remission. **RetroNectin GMP grade** reagent was used during T-cell transduction (4, Table 8).

Other selected publications citing RetroNectin GMP grade reagent use in clinical studies are also shown in Table 8.

Table 8: Selected publications citing RetroNectin GMP grade reagent use in TCR/CAR therapies

1. Kochenderfer, J. N., *et al.* (2012) B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor–transduced T cells. Blood 119 (12):2709–2720
2. Robbins, P. F., *et al.* (2011) Tumor Regression in Patients With Metastatic Synovial Cell Sarcoma and Melanoma Using Genetically Engineered Lymphocytes Reactive With NY-ESO-1. J. Clin. Oncol. 29 (7):917–924
3. Zhang, L., *et al.* (2013) Evaluation of γ-retroviral vectors that mediate the inducible expression of IL-12 for clinical application. J. Immunother. 35(5):430–439
4. Brentjens, R., *et al.* (2013) CD19-Targeted T Cells Rapidly Induce Molecular Remissions in Adults with Chemotherapy-Refractory Acute Lymphoblastic Leukemia. Science Translational Medicine 5 (177):177ra38
5. Ramos C. A. *et al.* (2017). Clinical and Immunological Responses after CD30-Specific Chimeric Antigen Receptor-Redirected Lymphocytes. The Journal of Clinical Investigation 127 (9): 3462–71
6. Tang X.Y. *et al.* (2016). Third-Generation CD28/4-1BB Chimeric Antigen Receptor T Cells for Chemotherapy Relapsed or Refractory Acute Lymphoblastic Leukaemia: A Non-Randomised, Open-Label Phase I Trial Protocol. BMJ Open 6 (12)
7. Ali S.A. *et al.* (2016). T Cells Expressing an Anti-B-Cell Maturation Antigen Chimeric Antigen Receptor Cause Remissions of Multiple Myeloma. Blood 128 (13): 1688–1700
8. Stroncek D.F. *et al.* (2016). Myeloid Cells in Peripheral Blood Mononuclear Cell Concentrates Inhibit the Expansion of Chimeric Antigen Receptor T Cells. Cytotherapy 18 (7): 893–901
9. Tomuleasa C. *et al.* (2018). Chimeric Antigen Receptor T-Cells for the Treatment of B-Cell Acute Lymphoblastic Leukemia. Frontiers in Immunology: 19 February

TCR Profiling

Cellular immunity is mediated by T cells (or T lymphocytes), which participate directly in the detection and neutralization of pathogenic threats via T-cell receptors (TCRs). Given the relative specificity of TCR-antigen interactions, a high diversity of TCRs are required to recognize the myriad of pathogenic agents one might encounter. To this end, the adaptive immune system has evolved a system for somatic diversification of TCRs that is unrivaled in all of biology. TCRs are heterodimers composed of two distinct subunit chains, in majority α- and ß-chains, resulting from these somatic rearrangements in a process called V(D)J recombination (Figure 5). A large repertoire of T-cells with diverse TCRs sequences is key for the immune system to recognize mutated proteins and neoantigens from tumoral cells. One challenge of immunogenomics applied to cancer is thus to determine T cell repertoires and to identify tumor-reactive T-cell clones, before and in response to immunotherapy in cancer patients (1).

1. Liu X.S. and Mardis E.R. (2017). Applications of Immunogenomics to Cancer. Cell 168(4): 600-612

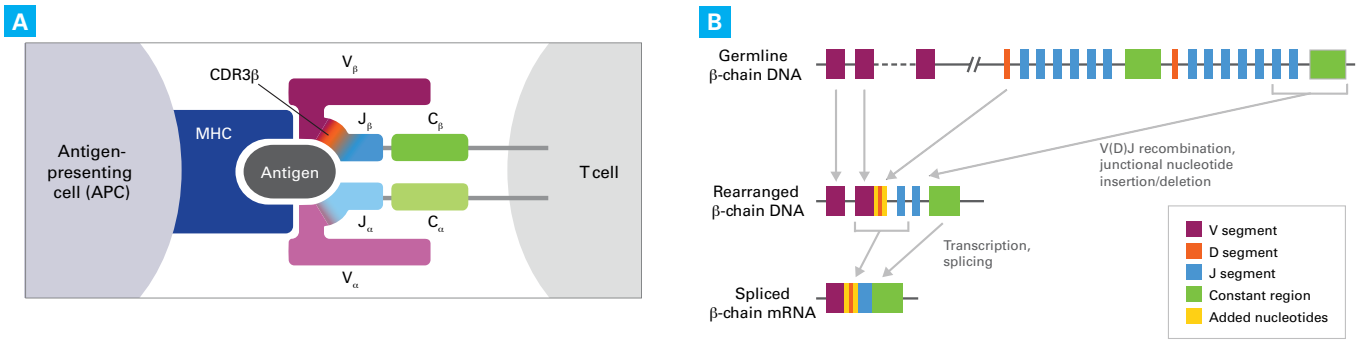

Figure 5: T-cell receptor structure and diversification. Panel A. A functional αß TCR heterodimer consisting of α and ß subunit chains. TCR α subunit chains consist of "variable" (V), "joining" (J), and "constant" (C) segments depicted in magenta, blue, and green, respectively, while TCR ß subunit chains include these and an additional "diversity" (D) segment, depicted in orange. The CDR3 region of the TCR ß subunit is labeled. The TCR is depicted on the T cell surface, bound to an antigen associated with an MHC molecule on the surface of an APC. Panel B. V(D)J recombination and posttranscriptional processing of a TCR ß subunit chain. The TCR ß locus includes over 50 V segments (magenta), 2 D segments (orange), and 13 J segments (blue). During somatic diversification, at least one of each segment type is randomly selected and further variability is introduced through the incorporation and/or deletion of additional nucleotides (yellow). Splicing of TCR mRNA combines a subset of the respective segments (along with a constant region) into a continuous unit. TCR α subunit chains are generated via analogous mechanisms.

Highlighted products

The **SMARTer Immune Profiling kits** leverage the SMART technology and a 5′-RACE-based approach to capture full-length information from V(D)J variable regions of TCRs. These kits streamline the process of sample preparation and provide reproducible results for a wide range of inputs of mouse/human samples (purified cells, spleen, PBMCs, Jurkat cells), and are highly sensitive in detecting low-abundance transcripts.

SMARTer Human/Mouse TCR a/b Profiling Kits are the ideal tools for TCR profiling to gain insights into TCR repertoire diversity from bulk samples (total RNA or purified cells).

Since the unique alpha-beta chain pairing of a TCR mediates antigen specificity, obtaining pairing information is crucial to gain insights into antigen recognition, for the efficient design of TCRs for targeted immunotherapy, and to help establish ancestral relationships of T-cell populations.

The **SMARTer Human scTCR a/b Profiling Kit** enables the full capture of TCR-alpha and TCR-beta variable regions, to elucidate TCR a/b pairing information within single T-cells. Furthermore, the **SMARTer ICELL8 Human TCR a/b Profiling** is now available on the high throughput single-cell **SMARTer ICELL8** system to capture complete V(D)J variable regions of TCR transcripts from hundreds of single cells.

Antibody Therapeutics

The use of monoclonal antibodies (mAbs) for cancer therapy has achieved considerable success in recent years due to their high specificity, activity, favourable pharmacokinetics, and the availability of strategies to successfully engineer antibodies into humanized forms. Antibodies are capable of recruiting the immune system to attack cancer cells through complement-dependent cytotoxicity or antibody dependent cellular cytotoxicity (1). Mechanisms of direct tumor cell killing by antibodies include antibody recognition of cell-surface bound enzymes to neutralize enzyme activity and signaling, or induction of receptor agonist or antagonist activity, both resulting in cellular apoptosis. In another approach, antibodies are also being used to deliver drugs to target cancer cells, via antibody drug conjugates (ADCs), thereby causing cancer cell death. ADCs direct cytotoxic compounds to tumor cells, after selective binding to cancer cell surface antigens, internalization, and intracellular drug release (1). Moreover, ADCs are powerful new treatment options for lymphomas and solid tumours, and immunomodulatory antibodies have recently achieved remarkable clinical success. New advances in protein engineering technology have also generated multiple bispecific antibody (BsAb) formats capable of targeting multiple antigens as a single agent and directly targeting immune cells to tumors, thus reducing drug resistance and severe adverse side effects (2).

1. Wold E. D. *et al.* (2016). Antibody Therapeutics in Oncology. Immunotherapy (Los Angeles, Calif.) 2(1): 108
2. Thakur A. *et al.* (2018). Bispecific antibody based therapeutics: Strengths and challenges. Blood Reviews 2: 004

Highlighted products

In-Fusion® HD Cloning Plus technology enhances antibody discovery, engineering and therapeutic workflows by speeding up the generation of antibody expression constructs. **In-Fusion HD Cloning** is fast (15 mins), highly efficient (>95% cloning efficiency), sequence independent (any PCR insert can be cloned into any vector at any locus), seamless (no extra bp or amino acids added to target antibody), directional, and HTP ready. Several publications have utilized the **In-Fusion HD Cloning Plus** technology for HTP antibody fragment cloning due to its high cloning efficiency and accuracy (Table 9).

After expression construct generation and downstream expression of the target antibody, our next-gen **Capturem™ Protein A/G** technology can be utilized for rapid (5-15 mins) and easy resin-free purification of high quality and concentrated monoclonal/polyclonal antibodies. The revolutionary **Capturem Protein A/G** technology, available in miniprep, maxiprep, 96-well/24-well plate formats, consists of spin columns or plates containing high-capacity membranes immobilized with Protein A or G, thus allowing antibody purification directly from complex matrices, such as hybridoma supernatants or serum, within minutes (Figure 6). Indeed, the Capturem Protein A/G 96-well/24-well plate formats are ideal for rapid HTP screening of hybridoma clones.

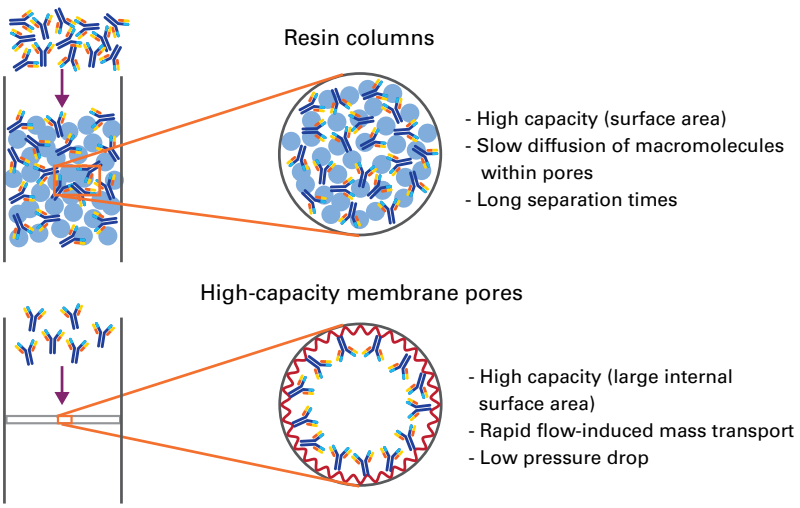

Figure 6: Capturem Protein A/G technology. Capturem Protein A/G mini/maxi spin columns or 96-well/24-well plates contain a high-capacity nylon membrane, containing immobilized Protein A or Protein G, with increased surface area, providing much higher protein binding capabilities per ml of membrane than per ml of resin.

Table 9: Selected publications citing the use of In-Fusion HD Cloning for HTP antibody cloning

1. Spidel J.L. *et al.* (2016). Rapid high-throughput cloning and stable expression of antibodies in HEK293 cells. Journal of Immunological Methods 439: 50-58
2. Chen C.G. *et al.* (2014). One-step zero-background IgG reformatting of phage-displayed antibody fragments enabling rapid and high-throughput lead identification. Nucleic Acids Research 42 (4): e26
3. Meng W *et al.* (2015). Efficient generation of monoclonal antibodies from single rhesus macaque antibody secreting cells. mAbs 7 (4): 707-718

CRISPR/Cas9 Gene Editing for Cancer Therapy & Drug Discovery

CRISPR/Cas9 gene editing has become a powerful method to edit to the genomes of many different organisms. First discovered in bacteria as part of an adaptive immune system, CRISPR/Cas9 and modified versions are now broadly used to engineer genomes and to activate or to repress the expression of specific genes. Furthermore, CRISPR/Cas9 gene editing promises to accelerate cancer research by providing an efficient technology to dissect mechanisms of tumorigenesis, identify targets for drug development, and possibly arm cells for cell-based therapies (1).

1. Moses C. *et al.* (2018). Hallmarks of cancer: The CRISPR generation. European Journal of Cancer 93: 10-18

Highlighted products

Cancer Therapy

Genome editing approaches have enormous potential for targeted, locus-specific cancer treatments. The human papilloma virus (HPV) genes E6 and E7 contribute to the hallmark of resisting cell death by disrupting normal cell cycle and tumor suppressor function. Cas9-mediated HPV E7 oncogene disruption leads to significant inhibition of HPV-induced cancerous activity both *in vitro* and *in vivo*, as described by Lao YH et al., 2018. The authors used Takara Bio's **Guide-it™ Mutation Detection Kit** and **Guide-it Indel Identification Kit** to check for efficient gene editing (1, Table 10).

Genome editing approaches have also shown promising results in cancer immunotherapy, to oppose the cancer hallmark of evading immune destruction. Modified chimeric antigen receptor (CAR) T cells have been generated for improved cancer targeting and destruction. Knock-in genome modifications in T cells have also been generated with Cas9-sgRNA ribonucleoprotein (RNP) complexes.

Kagoya *et al.*, 2018 (2, Table 10), report that inhibiting DOT1L, a histone H3-lysine 79 methyltransferase, alleviates allogeneic T-cell responses. The authors used the **Guide-it sgRNA *In Vitro* Transcription Kit** and **Guide-it Recombinant Cas9 (Electroporation-Ready)** for CRISPR-mediated TCR ablation in CAR-T cells. Using electroporation of RNP complexes, they could achieve ~30% TCR knockout efficiency in CAR-T cells (2, Table 10).

Table 10: Selected publications citing the use of various Guide-it CRISPR/Cas9 kits for different cancer applications

1. Lao Y.H. *et al.* (2018). HPV Oncogene Manipulation Using Nonvirally Delivered CRISPR/Cas9 or Natronobacterium gregoryi Argonaute. Advanced Science: 1700540
2. Kagoya Y. *et al.* (2018). DOT1L inhibition attenuates graft-versus-host disease by allogeneic T cells in adoptive immunotherapy models. Nature Communications 9: 1915

Drug Discovery

Genome-wide knockout screens are a powerful functional genomics tool to discover novel drug targets for cancer therapy. For pooled knockout screens with CRISPR/Cas9, a cell population with a diversity of gene knockouts needs to be generated. Lentiviral particles encoding a sgRNA library are used to infect Cas9-expressing cells at a low multiplicity of infection, so that every cell potentially carries a distinct sgRNA cassette and specific gene knockout. Subsequently, this pool of knockout cells is exposed to selected pertubations, followed by NGS analysis compared to a reference control cell population. By this means, it is possible to monitor the phenotypic effect of specific gene knockouts within the cell population.

The **Guide-it CRISPR Genome-Wide sgRNA Library System** is a pooled lentiviral sgRNA library targeting the whole human genome for knockout screens, and thus serves as an ideal tool to discover novel drug targets for cancer therapy. The library contains sgRNAs from the Brunello library, based on a recent algorithm for optimized guide sequences for each gene (1, 2):

- 4 guides per gene
- 76610 guides in total (includes 172 negative controls)
- 19114 genes targeted

1. Doench J.G. *et al.* (2016). Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nature Biotechnology 34 (2): 184-191
2. Doench J.G. *et al.* (2018). Am I ready for CRISPR? A user's guide to genetic screens. Nature Reviews Genetics 19 (2): 67-80

Solutions for Cancer Research

Cancer Biomarker Discovery

- SMARTer ThruPLEX-Plasma Seq kit
- Capturem Exosome Isolation Kit
- SMARTer smRNA-Seq Kit

Single Cancer Cell Analysis

- SMARTer PicoPLEX WGA/DNA-seq Kits
- SMART-Seq v4 Ultra Low Input RNA Kit
- SMART-Seq HT (High Throughput) Kit
- SMARTer Apollo NGS library prep system
- SMARTer ICELL8 Single-Cell system
- SMARTer-Seq Stranded Kit

Cancer Genomics and Epigenomics

- SMARTer ThruPLEX DNA-seq Kit
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- SMARTer ThruPLEX Plasma-seq Kit

HLA typing for cancer

- PrimeSTAR GXL
- TaKaRa LA Taq DNA Polymerase
- SMARTer PicoPLEX WGA/DNA-seq Kits
- SMARTer ThruPLEX DNA-seq Kit

Cancer Immunotherapy

T-Cell Therapy

- RetroNectin GMP grade reagent
- Anti-CD3 mAb GMP grade
- GT-T551 T-cell culture medium

TCR Profiling

- SMARTer Human/Mouse TCR a/b Profiling Kits
- SMARTer Human scTCR a/b Profiling Kit
- SMARTer ICELL8 Human TCR a/b Profiling

Antibody Therapeutics

- In-Fusion HD Cloning Plus System
- Capturem™ Protein A/G Kits

CRISPR/Cas9 Gene Editing for Cancer Therapy/Drug Discovery

- Guide-it Mutation Detection Kit
- Guide-it Indel Identification Kit
- Guide-it sgRNA *In Vitro* Transcription Kit
- Guide-it Recombinant Cas9
- Guide-it CRISPR Genome-Wide sgRNA Library System

About Takara Bio

Takara Bio provides kits, reagents, instruments, and services that enable life sciences researchers to achieve their experimental objectives. The Takara Bio Group holds a leadership position in the global market and is committed to improving the human condition through biotechnology. Going forward, Takara Bio will continue to develop high-quality, innovative tools and services that accelerate scientific discovery.

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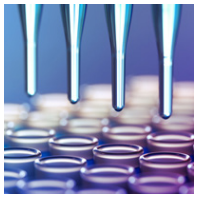

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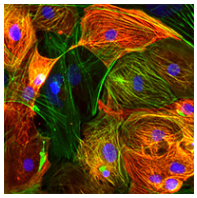

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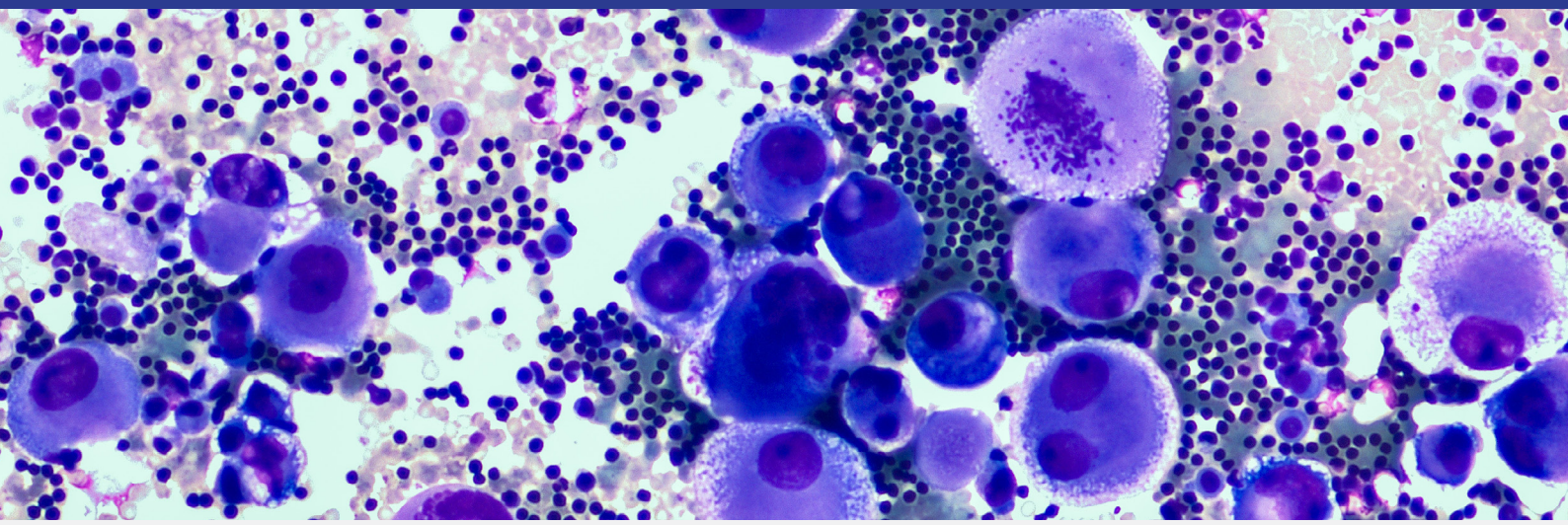

Worldwide Offices

Europe
Takara Bio Europe, SAS
2 Avenue du President Kennedy
78100 Saint-Germain-en-Laye, France

United States & Canada
Takara Bio USA, Inc
1290 Terra Bella Avenue
Mountain View, CA 94043 USA

China
Takara Biomedical Technology (Beijing) Co., Ltd.
Life Science Park
22 KeXueYuan Road
Changping District
Beijing 102206, China

India/Bangladesh/Sri Lanka
DSS Takara Bio India Pvt. Ltd.
A-5 Mohan Co-op Industrial Estate
New Delhi 110044, India

Japan
Takara Bio Inc.
Nojihigashi 7-4-38
Kusatsu, Shiga 525-0058, Japan

Korea
Takara Korea Biomedical Inc.
601, New T Castle
108, Gasan Digital 2-ro, Geumcheon-Gu
Seoul 08506, South Korea

www.takarabio.com

Takara Bio Europe
infoEU@takarabio.com • +33 (0)1 3904 6880

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