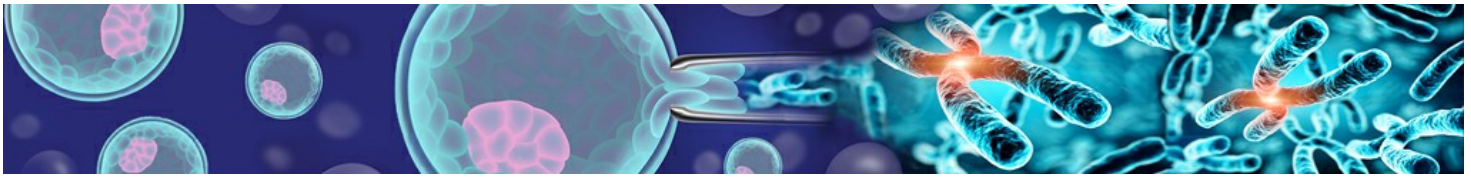
Preimplantation genetic testing powered by PicoPLEX technology



Assisted reproductive technologies provide tremendous hope for couples who are trying to start or grow their families. These couples often struggle with infertility or have a family history of a serious disease and worry about passing it on to their offspring. *In vitro* fertilization (IVF) is one procedure used to treat infertility, and a couple may opt to select healthy embryos before implantation to help ensure a successful pregnancy. In this case, preimplantation genetic testing (PGT) is performed to screen embryos for chromosomal abnormalities (PGT for aneuploidy [PGT-A]) or known/suspected genetic conditions (PGT for monogenic diseases [PGT-M]). Advances in PGT techniques and technologies have made a real difference in increasing the chances of pregnancy and reducing the risk of miscarriage and congenital disabilities due to genetic disorders. PGT can also shorten the time to pregnancy and lower costs by allowing the prioritization of embryos for implantation, thereby reducing the total number of IVF cycles.

Samples for PGT are typically obtained by taking a biopsy of a few cells, referred to as a trophectoderm biopsy. Although a variety of PGT methods have been used in the past (such as fluorescence *in situ* hybridization [FISH] and array comparative genomic hybridization [aCGH] assays), next-generation sequencing (NGS) is currently preferred as the most advanced and comprehensive PGT method. NGS-based PGT involves extracting DNA from the trophectoderm biopsy, amplifying the DNA to appropriate input levels for NGS library prep, sequencing, and analyzing data to examine chromosome structure and number. This allows the determination of whether an embryo is euploid, aneuploid, mosaic, and/or carrying single-gene mutations. This information enables the physician to select an embryo for transfer that has the greatest chance of success.

The majority of PGT workflows have incorporated Takara Bio's PicoPLEX whole genome amplification (WGA) technology into their testing protocols. PicoPLEX technology is designed and optimized for unbiased amplification of single-copy genomic DNA from inputs as low as a single cell or cell-free DNA from liquid biopsies. The simple protocol reduces handling errors, dramatically improves time to results, and reduces background. It is trusted as the gold-standard DNA amplification technology for PGT due to its accuracy and reproducibility.

PicoPLEX technology: how does it work?

The WGA reaction (Figure 1) starts with cell lysis, and then DNA is used as a template for preamplification using a quasi-linear amplification approach. This ensures the DNA template is reprimed and amplified in the same manner to create a library of hairpin molecules that cannot be further amplified. These hairpin molecules are a key intermediate ensuring that all amplified molecules are copies of the original DNA template rather than copies of copies. This approach ensures that low-level errors that may be introduced during PCR are not amplified, reducing false signals in the final libraries. These hairpins, in turn, are directly amplified into bulk quantities of DNA used for downstream PGT applications. A highly optimized change in the amplification conditions releases the hairpins to be further amplified in bulk with universal primers, leading to a high-complexity mixture of DNA molecules with minimal amplification bias.

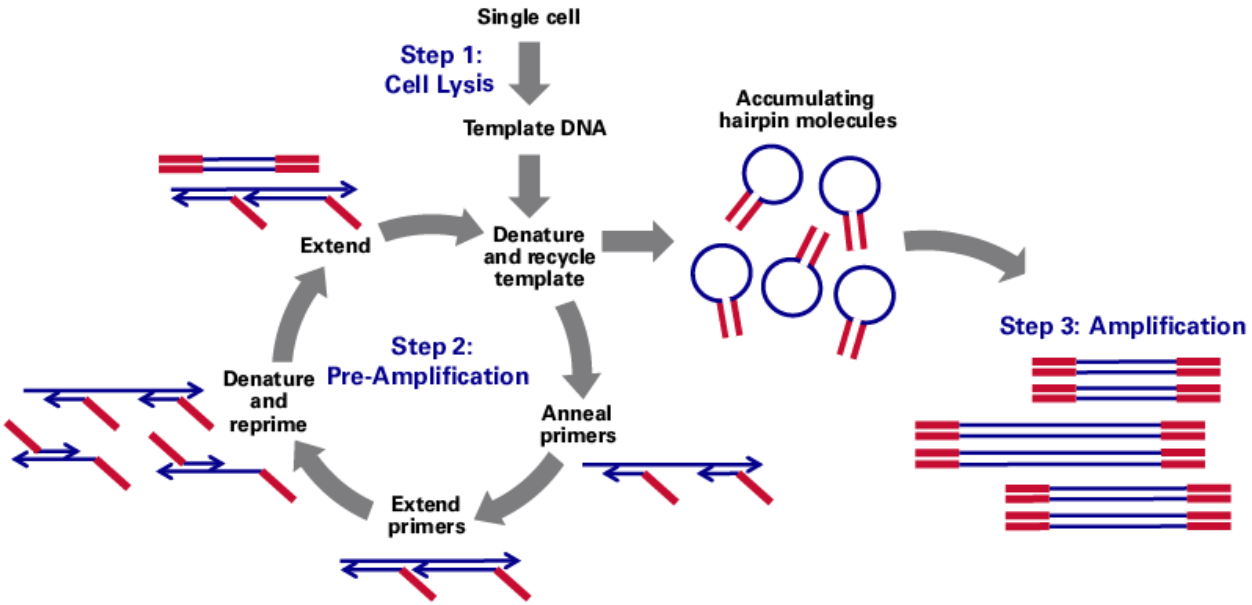


Figure 1. PicoPLEX technology provides high levels of reproducibility and accuracy. Widely used for whole genome amplification prior to downstream PGT applications, this hairpin sequestering approach ensures that amplicons are copies of the original template, minimizing amplification bias and maximizing fidelity. Takara Bio offers a research-use-only version of this technology: the PicoPLEX WGA Kit (Cat. # R30050).

PicoPLEX inside: NGS-based PGT

The following publications demonstrate NGS-based PGT-A utilizing PicoPLEX technology to improve embryo screening outcomes, understand mosaicism (which can significantly complicate diagnoses), and discover changes in gene expression during embryo development.

Vendrell, X. *et al.* New protocol based on massive parallel sequencing for aneuploidy screening of preimplantation human embryos. *Syst. Biol. Reprod. Med.* **63,** 162–178 (2017).

The authors developed a highly reliable assay for detecting aneuploidy in single blastomeres and samples of 5–10 trophectoderm cells. Their approach—a low-coverage whole genome sequencing method from WGA libraries plus an original algorithm and copy number viewer—depended on PicoPLEX technology to amplify nanogram amounts of gDNA from picogram-level inputs for their NGS protocol. They combined PicoPLEX's limited displacement preamplification with barcodes, followed by PCR amplification, and achieved both a high level of useful reads as well as full correlation of chromosome dose with samples processed using a CGH-BAC microarray approach. They found no differences in sequencing and mapping parameters between unamplified control DNA samples and WGA products from single blastomeres, indicating an unbiased coverage using PicoPLEX technology. Their assay enabled the accurate detection of segmental aneuploidy, with rearrangements of segments down to 1 Mb in length.

Vera-Rodríguez, M. *et al.* Distribution patterns of segmental aneuploidies in human blastocysts identified by next-generation sequencing. *Fertil. Steril.* **105,** 1047–1055.e2 (2016).

The authors evaluated a commonly used NGS-based PGT-A technique for the detection of segmental and whole-chromosome aneuploidies from trophectoderm biopsies and compared these results to the typical aCGH approach. The overall concordance rate between NGS and aCGH was 99.8%, and 92.9% of segments detected by both NGS and aCGH were confirmed by FISH. PicoPLEX technology was used for whole genome amplification, followed by library preparation. The NGS approach detected segmental aneuploidies down to 10 Mb with the same efficiency as aCGH; furthermore, NGS detected several mosaic segmental aneuploidies that were not detected by aCGH. Additionally, the authors determined that most of the segmental aneuploidies resulted from mitotic errors leading to mosaicism, and this mosaic pattern was reliably detected by NGS.

Macaulay, I. C. *et al.* G&T-seq: Parallel sequencing of single-cell genomes and transcriptomes. *Nat. Methods* **12,** 519–522 (2015).

The authors introduced a new technique, genome and transcriptome sequencing (G&T-seq), which enables a more in-depth characterization of single cells through simultaneous full-length RNA sequencing, whole-genome or targeted sequencing, and genome-wide copy number variation (CNV) detection. Their method involves bead-based isolation of polyA+ mRNA from gDNA, and then separate amplification, library preparation, and sequencing. PicoPLEX technology was chosen to amplify DNA for downstream CNV analysis. The authors produced several interesting and novel findings using their G&T-seq method: 1) chromosomal copy number in a single cell is corroborated by the expected changes in gene expression in that cell; 2) an aneuploidy event that occurs during a single cell division tracks with gene expression dosage; 3) within a single cell, there is high concordance of single nucleotide variation detected in gDNA and mRNA; and 4) a previously unknown fusion and the causative chromosomal rearrangement underlying the fusion were discovered.

Fuchs Weizman, N. *et al.* Towards improving embryo prioritization: parallel next generation sequencing of DNA and RNA from a single trophectoderm biopsy. *Sci. Rep.* **9,** 2853 (2019).

The authors created a clinically applicable method, called preimplantation genetic testing for aneuploidy and transcriptome (PGT-AT), that prepares a biopsy of 4–6 trophectoderm cells for simultaneous DNA- and RNA-seq to determine the ploidy status and transcriptome profile. In this study, two gold-standard Takara Bio technologies were employed: SMART-Seq chemistry for cDNA library preparation from full-length mRNA and PicoPLEX chemistry for cell lysis, whole genome amplification, and library preparation. In addition to achieving 100% concordance of CNV detection between the standard PGT-A method and the PGT-AT method performed on the same blastocysts, they discovered that pathways regulating gene expression and energy metabolism were downregulated in the euploid samples. The authors expressed confidence that the transcriptome profiling aspect could be safely introduced into the current clinical PGT-A workflow, and doing so could greatly improve our embryo prioritization abilities.

Tortoriello, D. V., Dayal, M., Beyhan, Z., Yakut, T. & Keskintepe, L. Reanalysis of human blastocysts with different molecular genetic screening platforms reveals significant discordance in ploidy status. *J. Assist. Reprod. Genet.* **33,** 1467–1471 (2016).

The authors reanalyzed blastocysts that returned abnormal PGT-A results to determine the reliability of different assays run by different labs. They examined the levels of concordance between the first and second rounds of trophectoderm biopsies analyzed by two different PGS labs using aCGH or SNP array (first biopsy/lab) and SNP array or NGS (second biopsy/lab). For NGS, WGA was performed with PicoPLEX technology. Chaotic results were obtained, including normal karyotype or gender-discrepant results upon second biopsy, and it could not be determined whether the lack of concordance was due to mosaicism, differences between platforms, or human error. The authors recommended regular, independent oversight verifying test performance and accuracy.

Onward: noninvasive PGT

Although embryo biopsy is considered a safe procedure, other less invasive techniques are of interest as alternatives that reduce the manipulation of the embryo. Recent studies have shown that blastocoel fluid and culture media can provide a source for cell-free embryonic DNA that can be used for PGT. Since cell-free DNA is present in significantly smaller amounts than cellular DNA from invasive biopsies, a WGA method with high sensitivity and minimal bias is critical to obtain enough material for downstream analysis.

The following publications aimed to answer questions about the suitability of cell-free DNA for PGT. Although their findings raise additional questions and highlight challenges, noninvasive methods have the potential to improve the safety of PGT procedures, increase confidence in genetic diagnoses and, ultimately, optimize embryo prioritization, leading to better pregnancy rates.

Ho, J. R. *et al.* Pushing the limits of detection: investigation of cell-free DNA for aneuploidy screening in embryos. *Fertil. Steril.* **110,** 467–475.e2 (2018).

The authors examined cell-free DNA from spent embryo medium (SEM) to detect ploidy and sex and to determine whether assisted hatching and morphologic grade have an impact on cell-free DNA amount and predictive value. All samples (SEM, trophectoderm biopsies, and whole embryos) were subjected to WGA with PicoPLEX technology followed by NGS-based PGT-A. A cell-free DNA concentration of 63.2 ng/µl was enough to get an accurate ploidy diagnosis, and assisted hatching did not seem to affect cell-free DNA metrics. Based on low concordance rates, the authors conclude that cell-free DNA from spent medium is not ready to replace cellular DNA in PGT-A, but it is a promising tool for noninvasive screening.

Vera-Rodriguez, M. *et al.* Origin and composition of cell-free DNA in spent medium from human embryo culture during preimplantation development. *Obstet. Gynecol. Surv.* **73,** 355–356 (2018).

The authors examined the cell-free DNA content of SEM used to culture blastocysts in order to determine cell-free DNA amount and whether a chromosomal diagnosis would be concordant between trophectoderm biopsies and their corresponding culture media samples. NGS-based aneuploidy testing and SNP analysis to detect maternal DNA contamination were performed. PicoPLEX technology was used for double WGA of cell-free DNA in SEM, while FISH was used on the whole blastocysts to detect mosaicism. The cell-free DNA in the media afforded a diagnosis but was discordant with trophectoderm biopsies at a rate of 67%, which was likely due to maternal DNA contamination (92%). Mosaicism was present in most embryos, a possible explanation for the observation of different chromosomal alterations detected between trophectoderm samples and the cell-free DNA from the matching SEM samples.

Kuznyetsov, V. *et al.* Evaluation of a novel non-invasive preimplantation genetic screening approach. *PLoS One* **13,** e0197262 (2018).

The authors were the first to assess the suitability of SEM in combination with blastocoel fluid (BF) as a noninvasive PGT tool, and they introduced a new technique for noninvasive collection of BF. PicoPLEX technology was used for WGA of SEM, BF, trophectoderm biopsy, and whole blastocyst samples, followed by NGS-based PGT-A. Relatively high levels of concordance of PGT-A results were obtained for all samples. The authors not only demonstrated high concordance between the noninvasive and invasive samples, but they also remarked that the WGA method on combined SEM + BF samples showed superior amplification rates of 100% over previously reported amplification rates from SEM or BF alone.

Tšuiko, O. *et al.* Karyotype of the blastocoel fluid demonstrates low concordance with both trophectoderm and inner cell mass. *Fertil. Steril.* **109,** 1127–1134.e1 (2018).

The authors were the first to use high-resolution NGS to compare samples from three compartments of the same blastocyst—blastocoel fluid (BF), trophectoderm (TE), and inner cell mass (ICM)—to determine whether cell-free DNA from BF represents the chromosomal status of the rest of the embryo. Using PicoPLEX technology, DNA from all samples was successfully amplified and then subjected to NGS-based PGT-A. The authors found low concordance (40%) between BF and TE or ICM samples, compared to 86% between TE and ICM. BF contained more mosaic aneuploidies and affected chromosomes than matching TE and ICM samples. They conclude that as a single source of genetic material, BF is not diagnostically acceptable with current protocols, and TE biopsy remains the most effective and safest way of determining karyotype.

Takara Bio is proud to support the reproductive health community with innovative and dependable technologies. If you are interested in talking with us about how we can support your product development, please fill out the inquiry form on the left. If you are viewing on a mobile device, please click on the hamburger icon (☰) and scroll down to the form.

Screen CNV and SNV in a single tube

Takara Bio attended PGDIS 2019 to present our novel single-tube assay that enabled the simultaneous detection of both copy number variation (CNV) across the whole genome and single-nucleotide variation (SNV) or small insertion/deletions (indels) located in the *CFTR* gene target region.

View data

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