# End-to-End Solution for PGT-A by LP-WGS

## Background

**Preimplantation genetic testing for Aneuploidy (PGT-A)** is a genetic screening procedure performed on embryonic cells obtained by in vitro fertilization (IVF) prior to uterine transfer, with the aim of identifying chromosomal aneuploidies and select- ing embryos suitable for transfer. Recent studies indicate that PGT-A can significantly improve implantation rate, clinical pregnancy rate, ongoing pregnancy rate and live birth rate in women of advanced maternal age, while reducing the risks of miscarriage and congenital anomalies and decreasing the vertical transmission risk of hereditary disorders.

PGT-A technologies have evolved from early fluorescence in situ hybridization (FISH) to microarray platforms (aCGH/SNP array) and, more recently, to widely adopted next-generation sequencing (NGS) methods. With advances in blastocyst culture and molecular diagnostics, NGS owing to its higher resolution and accuracy, has gradually become the preferred choice for PGT-A in reproductive medicine. In clinical practice, PGT-A commonly employs low-pass whole-genome sequencing (LP-WGS) to gener- ate read-depth information across the whole genome that can be analyzed to detect whole-chromosome aneuploidies, segmental aneuploidies, and, to a certain extent, mosaicism.

Responding to these technological advances and clinical demands, LexigenBio developed the **End-to-End Solution for PGT-A by LP-WGS**. By integrating optimized whole-genome amplification (WGA), NGS library preparation, and bioinformatics analysis pipelines, this solution provides an integrated workflow from single-cell or ultra-low input gDNA samples through to automated reporting, enabling laboratories to increase throughput and turnaround while maintaining sensitivity and accuracy, thereby delivering reliable technical support for reproductive decision-making.

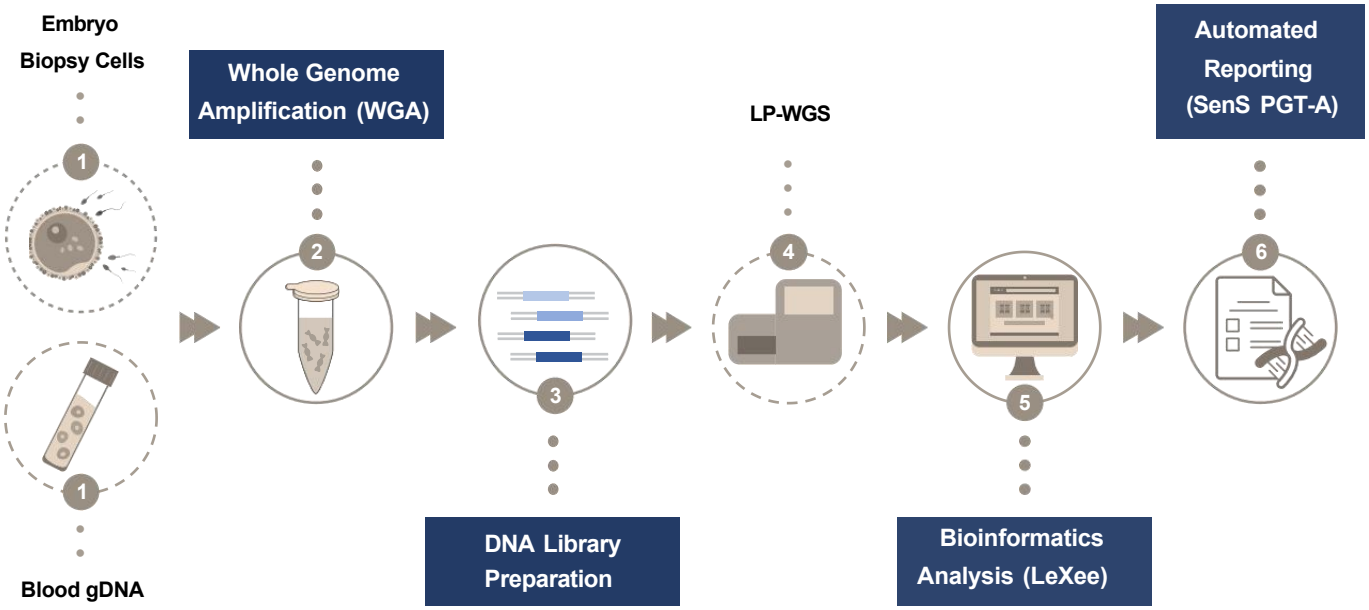
## Introduction

**End-to-End Solution for PGT-A by LP-WGS** (hereafter referred to as **Comprehensive PGT-A Solution**) provides a stream- lined, scalable workflow for NGS-based PGT-A using LP-WGS. The solution integrates the **LeXPrep Single Cell WGA Kit**, **LeXPrep DNA Library Preparation Kit** series with **LeXee Bioinformatics Visualization System** and prebuilt analysis pipeline (**SenS PGT-A**) to enable automated reporting and interpretation.

**Comprehensive PGT-A Solution** has been optimized for the reliable detection of CNVs, including whole-chromosome, mosaic and segmental aneuploidies, from DNA derived from single cells or ultra-low amounts of purified gDNA. The complete workflow begins with cell lysis and WGA, followed by NGS library preparation and incorporation of unique dual indexes (UDIs). Final libraries can be pooled for multiplexed sequencing of up to 768 samples per run on mainstream sequencing platforms.

Due to the prebuilt pipeline in LeXee, **Comprehensive PGT-A Solution** delivers accurate calls of aneuploidies, reliable detection of low-level mosaicism (~30%) in defined mixtures, and the capability to call chromosomal aneuploidies at 5 Mb-resolution with as few as 5 M read pairs. By delivering high-quality, reproducible data, **Comprehensive PGT-A Solution** is expected to become a powerful technological tool to gain deeper insights into chromosomal variation in PGT-A applications.

## Workflow




## Feature

### Complete Solution for PGT-A by LP-WGS

- **Rapid:** sample-to-insight TAT commonly in less than 12 hr
- **High-throughput:** supports multiplexing of up to 768 samples in a single run
- **Flexible:** broadly compatible with mainstream sequencing platforms
- **Automated:** streamlined, user-friendly analysis pipeline with reporting and interpretation

### Accurately Detection and Calling of Aneuploidies

- Reliably calls whole-chromosome and segmental aneuploidies down to a size of 10 Mb, as well as low (30%) mosaicisms for defined mixtures of samples with known aneuploidies.
- Capable of calling chromosomal deletion/duplication at 5 Mb-resolution with as few as 5 M read pairs.

## Performance

### Accurate Detection of Whole-chromosome Aneuploidies in gDNA Standards

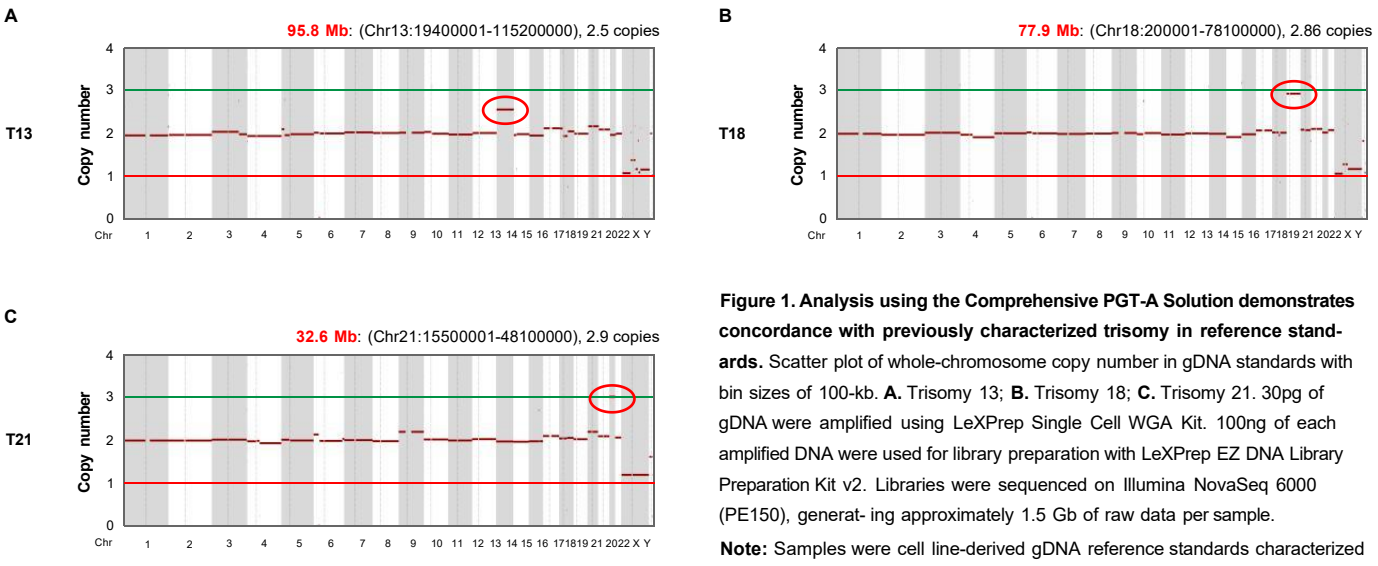
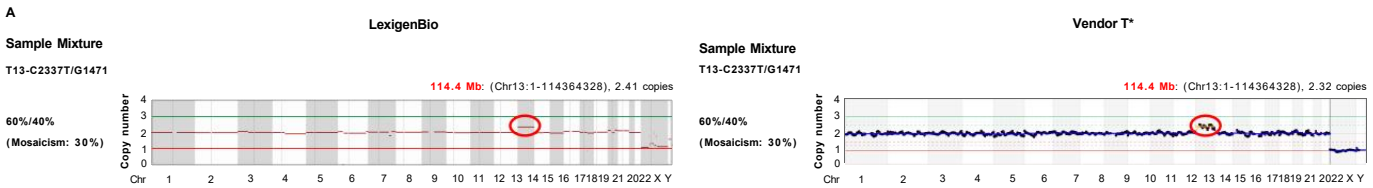



**Figure 1. Analysis using the Comprehensive PGT-A Solution demonstrates concordance with previously characterized trisomy in reference standards.** Scatter plot of whole-chromosome copy number in gDNA standards with bin sizes of 100-kb. **A.** Trisomy 13; **B.** Trisomy 18; **C.** Trisomy 21. 30pg of gDNA were amplified using LeXPrep Single Cell WGA Kit. 100ng of each amplified DNA were used for library preparation with LeXPrep EZ DNA Library Preparation Kit v2. Libraries were sequenced on Illumina NovaSeq 6000 (PE150), generat- ing approximately 1.5 Gb of raw data per sample.

**Note:** Samples were cell line-derived gDNA reference standards characterized by pronounced trisomy 13/18/21 (LDT Bioscience, C2337T/C2338T/C2339T).

## High Concordance in Calling SegmentalAneuploidies as Well as Low (30%) Mosaicisms

A




B

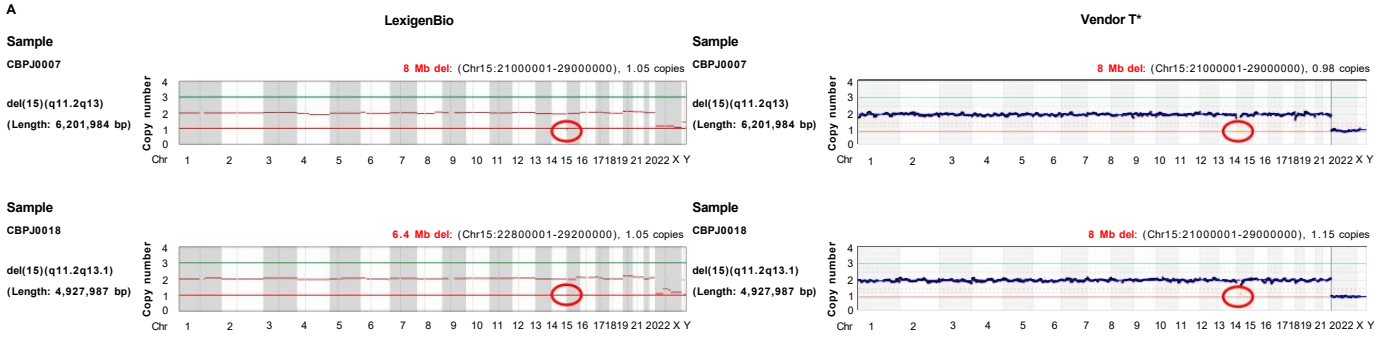
| Sample Mixture | | CNV Event Called | | Interpreted Karyotype | | Observed Mosaicism (%) | |
|---|---|---|---|---|---|---|---|
| | | LexigenBio | VendorT* | LexigenBio | VendorT* | LexigenBio | VendorT* |
| T13-C2337T/G1471 60%/40% | Rep-1 | Mosaic Low | Mosaic Low | 46,XY,+13M | 46,XY,+13M | 41 | 32 |
| | Rep-2 | Mosaic Low | Mosaic Low | 46,XY,+13M | 46,XY,+13M | 35 | 35 |

**Figure 2. Evaluation of the minimum detectable level of mosaicisms in mosaic samples demonstrates concordance between results from Comprehensive PGT-A Solution and Vendor T*. A.** Scatter plot of whole-chromosome copy number; **B.** Calling and interpretation of chromosomal mosaicisms. All the CNVs analysis and interpretation was performed with SenS PGT-A in 100-kb bin sizes.

**Note:** Artificial mosaic samples were generated by mixing two gDNA standards [C2337T and G1471 (Promega)] at the indicated ratio, containing mosaic aneuploidies at 30% across chromosome 13.

## Reliably Calls Chromosomal Deletion at 5 Mb-resolution with as Few as 5 M Read Pairs

A




B

| Sample | | CNV Event Called | | Interpreted Karyotype | | Size Range | |
|---|---|---|---|---|---|---|---|
| | | LexigenBio | Vendor T* | LexigenBio | Vendor T* | LexigenBio | Vendor T* |
| CBPJ007 | Rep-1 | Segmental Aneuploid | SegmentalAneuploid | 46,XY,-15S | 46,XY,-15S | 7 Mb | 8 Mb |
| | Rep-2 | Segmental Aneuploid | SegmentalAneuploid | 46,XY,-15S | 46,XY,-15S | 8 Mb | 8 Mb |
| CBPJ008 | Rep-1 | Segmental Aneuploid | SegmentalAneuploid | 46,XY,-15S | 46,XY,-15S | 6.4 Mb | 8 Mb |
| | Rep-2 | Segmental Aneuploid | SegmentalAneuploid | 446,XY,-15S | 46,XY,-15S | 5.5 Mb | 8 Mb |

**Figure 3. Comprehensive PGT-A Solution demonstrates higher resolution in detecting segmental aneuploidies compared to Vendor T* in standards with known chromosomal abnormalities. A.** Scatter plot of whole-chromosome copy number; **B.** Calling and interpretation of chromosomal segmental aneuploidies.

**Note:** Samples were Prader-Willi syndrome (46,XY,del(15)(q11.2q13)) Reference Standard (COBIOER, CBPJ0007) and Prader-Willi syndrome (46,XY,del(15)(q11.2q13.1)) Reference Standard (COBIOER, CBPJ0018).

## Ordering Information

| Type | Product | Detail | Catalog# |
|---|---|---|---|
| Single Cell WGA Kit | LeXPrep Single Cell WGA Kit, 24 rxn | 24 rxn | LX30101 |
| | LeXPrep Single Cell WGA Kit, 96 rxn | 96 rxn | LX30102 |
| DNA Lib Prep Module | LeXPrep DNA Library Preparation Module v2, 24 rxn | 24 rxn | LX02421 |
| | LeXPrep DNA Library Preparation Module v2, 96 rxn | 96 rxn | LX02422 |
| | LeXPrep EZ DNA Library Preparation Module v2, 24 rxn | 24 rxn | LX02601 |
| | LeXPrep EZ DNA Library Preparation Module v2, 96 rxn | 96 rxn | LX02602 |
| Adapter Module | LeXPrep Universal Stubby Adapter (UDI) Module series, 24/96/1152 rxn | #1-768 | LX03240 etc. |
| | LeXPrep Universal Adapter (MDI) Module (for MGI) series, 24/96/1152 rxn | #1-384 | LX03711 etc. |
| Visual Online Analysis Platform | LeXee Bioinformatics Analysis Visual System | Unit | LX09101 |
| Automated Reporting Software | Prebuilt Pipelines (SenS PGT-A) | Unit | LX09102 |

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