



GenoPlexs Express

Library Construction Protocol

Molbreeding Biotechnology LLC

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1 Overview

1.1 Introduction

The GenoPlexs Express is a streamlined amplicon library construction solution that utilizes a two-step Multiplex PCR system, enabling rapid and efficient screening of low-to medium-density target regions ($\leq 6,000$ loci). GenoPlexs Express delivers high-quality data with standardized data with full automation compatibility and low per-sample cost, offering a fast, cost-effective solution for large-scale genotyping.

1.2 Kit components

Component	MPE000104 96 rxns	MPE000125 960 rxns	MPE000126 9600 rxns	Storage
GenoPlexs Express Library Prep Kit				
GenoPlexs 3 × T Master Mix	384 μ L	3.84 mL	38.4 mL	-15~-25°C
GenoPrep DNA Clean Beads	192 μ L	1.92 μ L	19.2 μ L	2~8°C
GenoPlexs PCR Enhancer	96 μ L	960 μ L	9.6 mL	-15~-25°C

1.3 Genomic DNA input requirements

- The minimum input DNA amount is 20 ng, we recommend to use 50 - 150 ng per sample for library preparation.
- For genomic DNA extracted using the GenoPrep Plant Genomic DNA Quick Extraction Kit (Cat.no. DN004524), it is recommended to add 1 μ L of GenoPlexs PCR Enhancer into the Input DNA.

1.4 Required reagents

The following kits and materials are essential for successful execution of this protocol. All items listed with catalog numbers are available through MolBreeding.

Item	Catalog Number	Description
GenoPlexs Express Panel	Predesigned or Custom Design	Predesigned Panel Primers Custom Panel Primers
GenoPlexs Express Library	MPE000104 (96 rxns)	For library prep
Prep Kit	MPE000125 (960 rxns)	
	MPE000126 (9600 rxns)	
GenoPlexs CDI Barcode Mix for Illumina	GP001325 P001-P018	Indexing plate A total of 1728 combinations
GenoPrep dsDNA Quantitation Kit	DN000109	For library quantification
Nuclease-free Water	Various supplier	For volume adjustment
10 mM Tris-Cl (pH 8.0)	Various supplier	For library elution
Fresh 80% ethanol	Various supplier	Washes during library prep

1.5 Required equipment & consumables

■ Equipment and accessories

- Pipettes & tips: Single- or multi-channel (for 384-well workflows).
- Thermal cycler (heated lid, compatible with 0.2 mL tubes).
- Centrifuge (adapters for 384-well plates and 2-5 mL tubes).
- Magnetic rack (e.g., DynaMag™-96 Side Magnet, Thermo Fisher #12331D).
- Fluorometer (e.g., Qubit®, QIAxpert®, for DNA/library quantification).
- Vortex mixer (for reagent mixing).
- Automated pipetting workstation (for Step 2 PCR products transfer and Step 4 libraries purification).

■ Essential consumables

- 384 Pin tool.
- 384-well plate reagent reservoir.
- 2-5 mL Low-binding microcentrifuge tubes.
- 384-well plate with a working volume of 25 µL (with compatible sealing caps).

2. Workflow

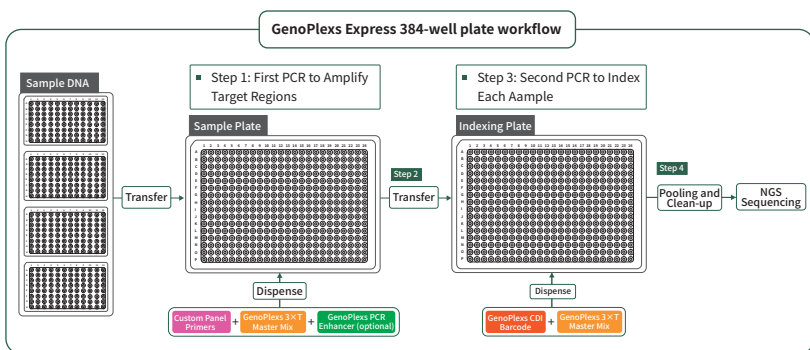
1 First PCR to amplify target regions

2 Transfer the first PCR products into the prepared second PCR reactions

3 Second PCR to index each sample

4 Libraries pooling and purification

5 QC of the library and perform sequencing



Step 1: First PCR to amplify target regions

- Prepare First PCR Reaction Mix (Total Volume: 6 μ L):

3 μ L Input DNA

1 μ L Custom Panel Primers

2 μ L GenoPlexs 3 $\times$ T Master Mix

- Run the following thermal program:

Step	Temperature	Incubation time	Number of cycles
1	95°C	3 min	1
2	95°C	30 sec	16
	60°C	4 min	
3	72°C	4 min	1
4	4°C	∞	Hold

- II Safe Stop:** When the program reaches 4 °C, store the PCR product at -20 °C overnight or proceed to Step 2.

Step 2: Transfer the first PCR products into the prepared second PCR reactions

- Prepare Second PCR Reaction Mix (Total Volume: 6 μ L):

2 μ L GenoPlexs 3 $\times$ T Master Mix

2 μ L GenoPlexs CDI Barcode

2 μ L Nuclease-free Water

- Use the 384 Pin tool to dip into Step 1 first PCR products, then transfer and dip into the second reactions.

Step 3: Second PCR to index each sample

- Run the following thermal program:

Step	Temperature	Incubation Time	Number of cycles
1	95°C	3 min	1
2	95°C	15 sec	9
	58°C	15 sec	
	72°C	30 sec	
3	72°C	4 min	1
4	4°C	∞	Hold

Step 4: Libraries pooling and purification

- Combine 3 μL from each well into a 2 mL tube. Alternatively, combine all products into a 5 mL tube and vortex to mix thoroughly.

Optional step: Invert the 384-well plate into a reagent reservoir and centrifuge briefly to combine all products.

- Transfer 60 μL from the tube to a new 0.2 mL tube (or 96-well plate).
- Add 48 μL (0.8 $\times$) GenoPrep DNA Clean Beads to each well, mix thoroughly.
- Incubate 5 min (RT), spin briefly.
- Separate beads on magnet ≥ 3 min until clear, discard supernatant.
- Wash beads with 100 μL 80% ethanol (30 sec), then fully remove ethanol.
- Air-dry beads ≥ 5 min (until cracked surface).
- Elute the beads using 35 μL of 10 mM Tris-HCl (pH 8.0).

Step 5: QC of the library and perform sequencing

- Assess both the concentration and size distribution of the purified libraries.
- Perform sequencing according to the instructions for NGS sequencer.

3 Protocol steps

⚠ Notes before starting

- Equilibrate GenoPrep DNA Clean Beads at room temperature (20 - 25°C) for 30 min prior to use. Other reagents should be thawed at 4°C.
- Prepare 100 µL of 80% ethanol solution and 35 µL of 10 mM Tris-HCl (pH 8.0) per pooled sample.

Step 1: First PCR to amplify target regions

1. **Preparation of first PCR reaction mix:** Refer to Table 1 for guidance.

Table 1. First PCR reaction mix

Component	Volume per well	Volume per plate (384 samples)
Input DNA	3 µL	1170 µL
Custom Panel Primers	1 µL	390 µL
GenoPlexs 3 × T Master Mix	2 µL	780 µL
Total volume	6 µL	2340 µL

2. **Sealing and mixing:** Seal the plate with a adhesive film, vortex thoroughly, and then briefly centrifuge to collect the contents at the bottom of the wells.
3. **Thermal cycling:** Place the 384-well plate in the thermal cycler, and run the following program with the heated lid set to 105°C.

Table 2. First PCR program

Step	Temperature	Incubation time	Number of cycles
1	95°C	3 min	1
2	95°C	30 sec	16
	60°C	4 min	
3	72°C	4 min	1
4	4°C	∞	Hold
 Note: The correspondence between amplification cycles and custom panel primers is detailed in Appendix B.			

- ⏸ **Safe Stop:** When the program reaches 4 °C, store the PCR product at -20 °C overnight or proceed to Step 2.

Step 2: Transfer the first PCR products into the prepared second PCR reactions

1. Vortex thoroughly, and briefly centrifuge the 384-well plate to collect the contents at the bottom of the wells.
2. **Preparation of second PCR reaction Mix:** Refer to Table 3 for guidance.

Table 3. Second PCR reaction mix

Component	Volume per well	Volume per plate (384 samples)
GenoPlexs 3×T Master Mix	2 µL	780 µL
GenoPlexs CDI Barcode	2 µL	780 µL
Nuclease-free water	2 µL	780 µL
Total volume	6 µL	2340 µL

3. **Transfer of the amplification product:** Use the 384 Pin tool to dip into the Step 1 amplification product, then transfer and dip into the second reactions.
4. **Sealing and mixing:** Seal the plate with a clear adhesive film, vortex thoroughly, and then briefly centrifuge to collect the contents at the bottom of the wells.

Step 3: Second PCR to index each sample

1. **Thermal cycling:** Place the 384-well plate in the thermal cycler, and run the following program with the heated lid set to 105°C.

Table 4. Second PCR program

Step	Temperature	Incubation Time	Number of cycles
1	95°C	3 min	1
2	95°C	15 sec	9
	58°C	15 sec	
	72°C	30 sec	
3	72°C	4 min	1
4	4°C	∞	Hold

Step 4: Libraries pooling and purification

1. Vortex thoroughly, and briefly centrifuge the 384-well plate to collect the contents at the bottom of the wells.
2. Combine 3 μL from each well into a 2 mL tube. Alternatively, combine all products into a 5 mL tube and vortex to mix thoroughly.

Optional step: Invert the 384-well plate into a reagent reservoir and centrifuge briefly to combine all products.

3. Transfer **60 μL from the tube to a new 0.2 mL tube (or 96-well plate)**.
4. Add 48 μL ($0.8\times$) GenoPrep DNA Clean Beads to each well of the 96-well plate from Step 3, Vortex to mix thoroughly, and then incubate at room temperature for 5 minutes. Briefly centrifuge to collect the contents.
5. Place the 96-well plate on the magnetic rack and allow it to sit until the liquid turns completely clear, which should take at least 3 minutes.
6. While keeping the 96-well plate on the magnetic rack, carefully discard the supernatant, ensuring that the beads remain undisturbed.
7. Keeping the plate still on the magnetic rack, add 100 μL of 80% ethanol to each well and incubate for 30 seconds at room temperature. Discard the supernatant, ensuring no residual ethanol remains.
8. Keep the plate on the magnetic rack and air-dry the beads at room temperature for at least 5 minutes, until the bead surface appears slightly cracked.



Note: Ensure no residual ethanol is present, and the beads have just cracked on the surface. The residual ethanol, and over-drying beads may result in low library yield.

9. Remove the 96-well plate from the magnetic rack, add 35 μL 10 mM Tris-HCl to each well. Vortex to mix thoroughly and incubate at room temperature for 5 minutes to elute DNA from the beads. Briefly spin down to collect the contents.

10. Place the 96-well plate on the magnetic rack and wait until the liquid becomes completely clear, which typically takes at least 3 minutes.
11. Transfer the elute DNA into a new 96-well plate, DO NOT disturb the beads.
12. For DNA library quantification, we recommend using the GenoPrep® dsDNA Quantitation Kit. Mix the purified libraries in equal amounts to form the final sequencing library, which can be directly used for sequencing or stored at -20°C.



Note: The libraries stored at -20°C for no more than one week.

Step 5: QC of the library and perform sequencing

1. **Quantify:** Use Qubit reagents or equivalent dsDNA fluorescence kit (e.g. GenoPrep dsDNA Quantitation Kit, Catlog DN000109) for concentration determination. The optimal concentration of the library should be 2 - 20 ng/μL. We do not recommend to proceed with sequencing if the concentration is below 2 ng/μL.
2. **Size check:** Use Gel electrophoresis or Agilent 2100 Bioanalyzer to validate the size range of each library. The optimal size of the library should be between 250bp - 500 bp.
3. **Perform sequencing according to the instructions for NGS sequencer.**

The maximum pool size is calculated as: $\text{Sample Capacity} = \frac{\text{Total Chip Input (M reads)}}{\text{Per-Sample Requirement (M reads)}}$. For example, a 100M-read chip can accommodate 138 samples ($100\text{M} \div 0.72\text{M}$), ensuring both data quality and throughput efficiency.

4 Appendix

Appendix A: Instructions for GenoPlexs CDI Barcodes

- The 8-base GenoPlexs CDI Barcodes are arranged in 96-well plates, with **5 reactions per well** and 96 barcodes per plate, for a total of 1,728 combinations across 18 plates.
- GenoPlexs CDI Barcodes are available through a custom service, allowing up to 7,680 unique combinations to be customized.
- For the base sequence information of GenoPlexs CDI Barcodes, please directly contact the technical support.

Appendix B: Relationship between amplification cycles and panel primers

Panel primers	Recommended number of amplification cycles
30-50	20
51-96	18
97-192	17
193-384	16
385-768	15
769-1536	14
1537-3072	13
3073-6144	12

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