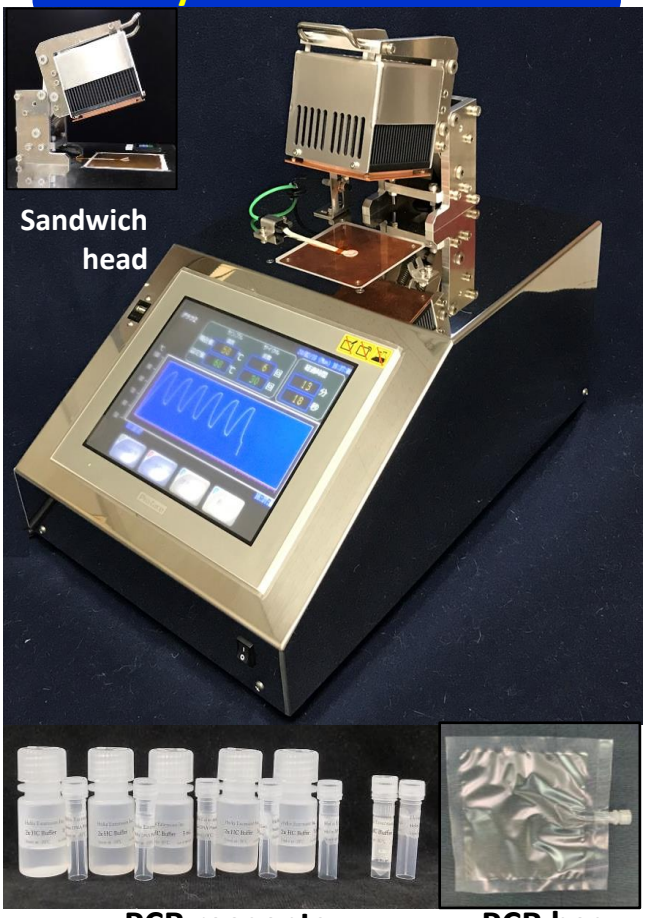
Large scale PCR system

～ Large scale DNA production by PCR for mRNA template and gene therapy ～

A PCR device for large scale DNA production

Helix Extension Co., Ltd., started a business from August 4th, 2023, will commercialize a group of large-scale PCR technologies together with Yanagiya Machinery, Yamaguchi University, ABLE and other partner companies.

The First Sandwich Thermal Cycler in the World!



PCR reagents　　PCR bag

Large-scale PCR device

Helix Sandwich Thermal Cycler（Helix cycler）

- Throughput:　1～10 mL
- Weight:　18.5 kg
- Size:　W350-D500-H350（open　H460）
- Electric power:　AC 100V , 50/60Hz , 500W
- Programming: 2- or 3-step temperature program
- Heating and cooling method Peltier type
- Pre-heating　15°C – 105°C
- Control temperature range　15°C – 105°C

PCR reagents

Product Summary（10 mL PCR x5, 1 mL PCR x1）

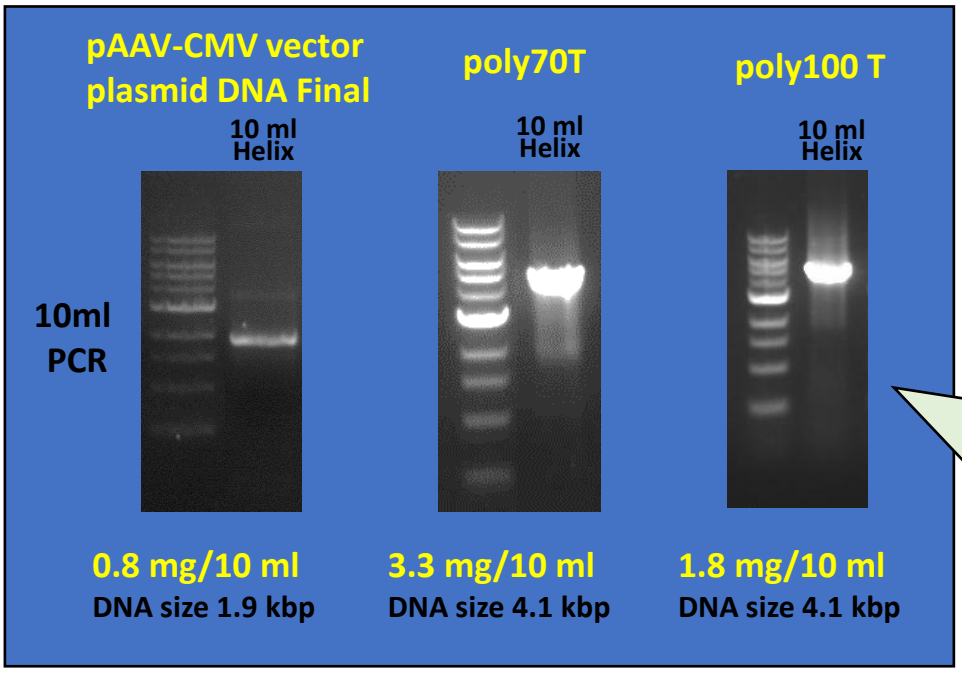
- Helix DNA polymerase*1
  Quantity：200 µL×5, 20 µL×1 for test PCR
- 2×HC buffer (dNTP included)*1
  Quantity：5 mL×5, 100 µL×5 for test PCR

*1: Optimized for large scale PCR reactions

PCR bag

Product Summary

- Bag for large-scale PCR
- Size　10cm×10cm



Performance

PCR reaction size: **～ 10 mL**

Yield: **100 µg – A few mg/reaction**

Amplification size: **~10 kbp**

PCR product accuracy: **~99.9%**

Addition of poly-A tails sequence of mRNA
ITR region of AAV, etc.

Amplification possible ！

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Applications

・Nucleic acid amplification/PCR ・DNA gene therapy development
・Preparation of template DNA for mRNA synthesis
・Preparation of virus vectors for gene therapy
・Preparation of cloning vectors for gene engineering
・Development of nucleic acid raw materials and drug substances for gene therapy including AAV （for mRNA drugs）
・Gene expression in cultured animal cells using PCR amplified DNA

Services provided

・Large-scale PCR device
・Helix DNA polymerase and the buffer
・TF Enhancer Reagent
・Plastic reaction bag
・Primer design for large-scale PCR
・Contract manufacturing of PCR DNA

Comparison of DNA production by *E. coli* and PCR

Comparison	E. coli plasmid DNA	PCR DNA
DNA shape	Annular	**Straight chain**
DNA yield	Unstable when containing repetitive sequences or GC	**Fixed yield without the influence of E. coli host**
Equipment	E. coli culture, bacterial collection/lysis, depth filter equipment	**Large-scale PCR device only**
Production timeframe	Days to Weeks or Months	**Hours to Days**
Cellular purification process	Required	**Not Required**
Cartagena law	Required	**Not Required**
Fidelity	Approx. 10^{-6}	**200 mL PCR (3 kb): Approx. 10^{-5}**

Features of large-scale PCR

Fast temperature control is difficult by a large-scale PCR due to the large reaction volume.
The sandwich Peltier devise with thin plastic reaction bag allows efficient thermal control.
The Helix DNA polymerase is a highly thermostable polymerase and shows high fidelity.

Comparison of mutation error measured by a next-generation sequencing	DNA amplified under optimized condition
A commercial DNA polymerase (World highest fidelity)	0.14%
Helix DNA polymerase	0.14%

Thirty cycle of 200 mL-PCR for 3.2 kbp amplification showed 137 mg DNA yield. Larger scale PCR-DNA manufacturing will be performed for your desired sequences.

References
・Akada et al., Large-scale DNA production for gene therapy by PCR instead of E. coli. mRNA health conference, poster, 2021
・Akada et al., mL-scale DNA production by a large-scale PCR machine. Bio Industry, 36, 58-67, 2019 (in Japanese)
・Conforti et al., A linear DNA endoding the SARS-CoV-2 receptor binding domain elicits potent immune resonse and neutralizing antibodies in domestic cats. Molecular Therapy, 28, 238-248, 2023

Manufacturers
Helix cycler: Yanagiya Machinery
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