# *PlasmidExpress*

## AAV-ITRs Plasmid Sequencing

Adeno-associated virus (**AAV**) vectors are the leading platform for gene delivery for Gene Therapy. AAV-mediated gene replacement, gene silencing, and gene editing have helped AAV gain popularity as the ideal therapeutic vector. **AAV** is a single-stranded DNA virus. At each end of the viral genome is a GC-rich element known as the inverted terminal repeat (**ITR**). The ITRs play a key role in directing intermolecular and intramolecular homologous recombination of **AAV** genomes. The use of hybrid **ITR AAV** vector genomes provides new strategies to manipulate viral genome conversion products and to direct intermolecular recombination events required for efficient dual-**AAV** vector reconstitution of the transgene.

Sequencing the **ITR** regions by Sanger DNA sequencing technology is a challenging task due to the high GC-content and large palindromic sequences (~160 bp) that fold back into a T-shaped hairpin structure and interferes with polymerases commonly used for Sanger cycle sequencing.

The **Poochon-Quintarabio DNA Sequencing Center** developed a rapid and cost-effective **AAV-ITR** whole plasmid sequencing Service, *PlasmidExpress,* which accurately sequences the entire plasmid including the **ITR** regions. To better support your viral vector testing and drug discovery pipeline, we offer 24 hour data-delivery rapid sequencing services for any ITR-AAV Plasmids, Vectors, and Packaged Products.

### Sample Preparation and Submission

- **Sample Type:** Plasmid DNA including any AAV-ITR vector/plasmids up to 30 kb
- **Submission of Samples**: Please consider to use 96-well plates for ≥ 36 samples; tubes or 8-well strips for < 36 samples. Plasmid DNA up to 30 kb, or purified PCR product ≥ 2 kb, at 100-200 ng/µl with **an OD260/OD280** reading ≥ 1.80 in 5-10 µl is preferred. Send to Poochon at ambient temperature using our drop-box service or any overnight shipping service. To submit sample forms online, please visit https://www.quintarabio.com/corder/plsexpress
- **Turnaround Time:** Sequencing data is available for download in 24 hours
- **Unit Price:** $15 per sample

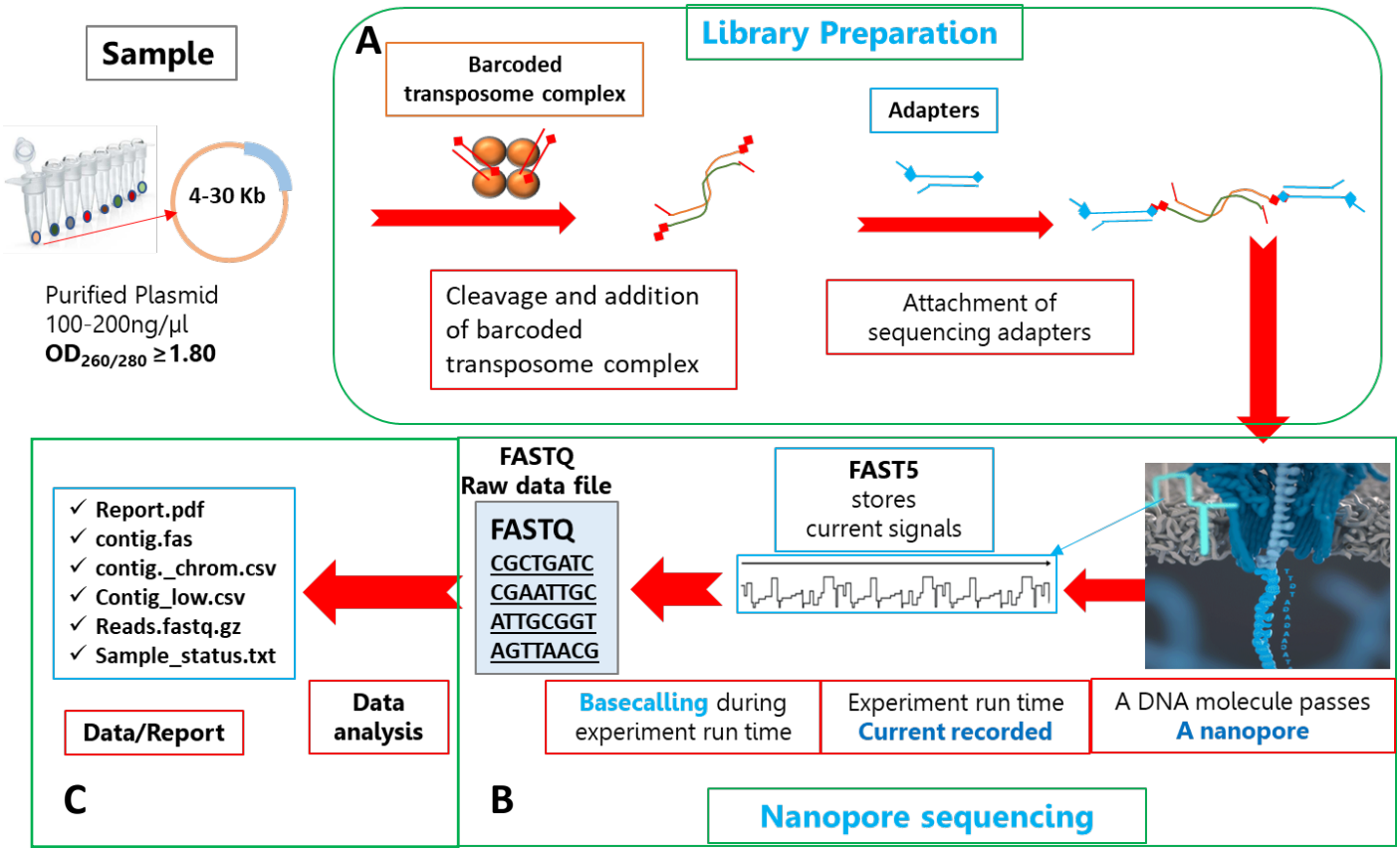
5350 Partners Court, Suite C, Frederick, MD 21703
Email: support@poochonscientific.com
Tel: 301-761-4835
www.poochonscientific.com

POOCHON SCIENTIFIC

# PlasmidExpress

## AAV-ITRs Plasmid Sequencing

## Whole Plasmid Sequencing Workflow




**(A)** **Creation of the library:** High purity plasmid samples (100-200 ng/µl with **OD260/OD280** ≥ 1.80) were processed for linearization and barcoding followed by the attachment of adaptors to the barcoded linear DNA molecules.

**(B)** **Nanopore Sequencing and Basecalling:** The library preparation is loaded onto a nanopore sequencer (flow-cell). Changes in current (signal) caused by the strand of DNA or RNA as it passes through the pore were recorded as FAST5 files. The signal stored within FAST5 files was then processed by Basecalling Algorithms to decode the sequence of bases into FASTQ files.

**(C)** **Data Analysis (Bioinformatics):** The FASTQ files (sequence data) were processed by bioinformatics tools to filter the data for quality based on length and scores. As a result, a report in PDF file format and five other data files per sample were generated.


5350 Partners Court, Suite C, Frederick, MD 21703
Email: support@poochonscientific.com
Tel: 301-761-4835
www.poochonscientific.com


POOCHON SCIENTIFIC

# PlasmidExpress

## AAV-ITRs Plasmid Sequencing

## AAV-ITR Plasmid Analysis Example: pAAV Plasmid

**Sample:** Purified plasmid DNA, 100 ng/µl, OD260/OD280 = 1.82.

→ 5 µl was used for the analysis

→ A Report in PDF file format and five other data files were generated for this sample

1. **Report.pdf – Next page**
2. **contig.fas –** ***Open this file using SnapGene viewer (free download: https://www.snapgene.com/snapgene-viewer), or other software to visualize the annotation and analyze the sequence (example of MGIN circle map opened using SnapGene Viewer on a following page).***
3. **contig._chrom.csv – The list of whole sequence determined; open this file using Microsoft Excel**
4. **Contig_low.csv – The list of nt sites with low score/confidence; open this file using Microsoft Excel**
5. **Reads.fastq.gz – Raw data file**
6. **Sample_status.txt – Data statistics and barcode number**

5350 Partners Court, Suite C, Frederick, MD 21703
Email: support@poochonscientific.com
Tel: 301-761-4835
www.poochonscientific.com

POOCHON SCIENTIFIC

# PlasmidExpress

## AAV-ITRs Plasmid Sequencing

**PlasmidExpress Report:** *pAAV*

***QuintaraBio* 2023-06-01**
PlasmidExpress

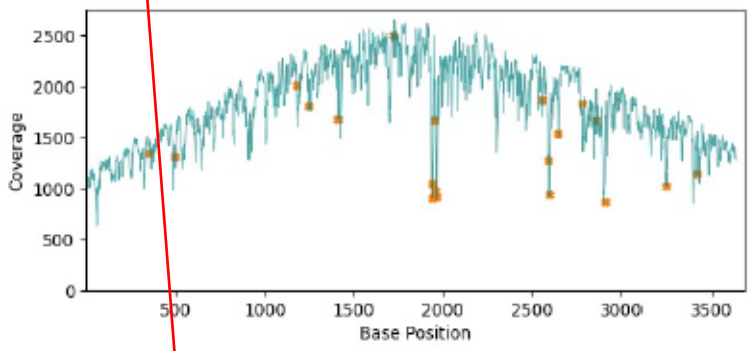
| Name | Reads | Bases |
|---|---|---|
| Total | 3299 | 10477389 |
| Host Genomic DNA | 0 | 0 |

**Assembly Status: form 1 contig**

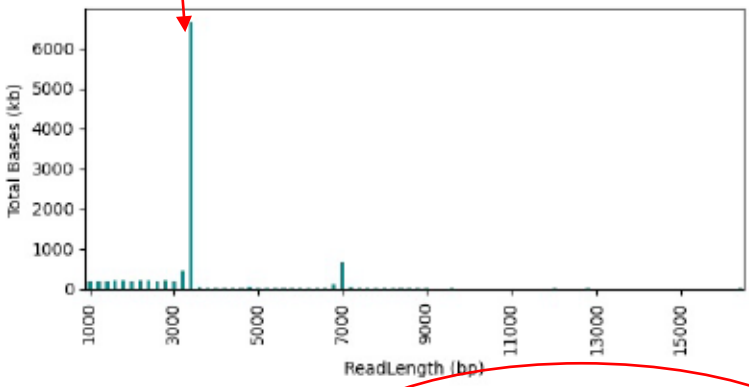
| Contig | Length (bp) | Read Count | Bases Mapped |
|---|---|---|---|
| pSCAAV_contig | 3635 | 3169 | 9950994 (94.98%) |

**JON_contig Coverage Map**

low confidence base positions are markered with x




**Read Length Distribution**




**Files Included**

- read fastq file
- contig fasta
- base information for each contig position
- low confidence bases for each contig

**Good Data Indicators**

- number of reads >= 500
- read length peaks at expected plasmid size
- all reads form 1 contig only
- more than 90% bases mapped back to contig sequence

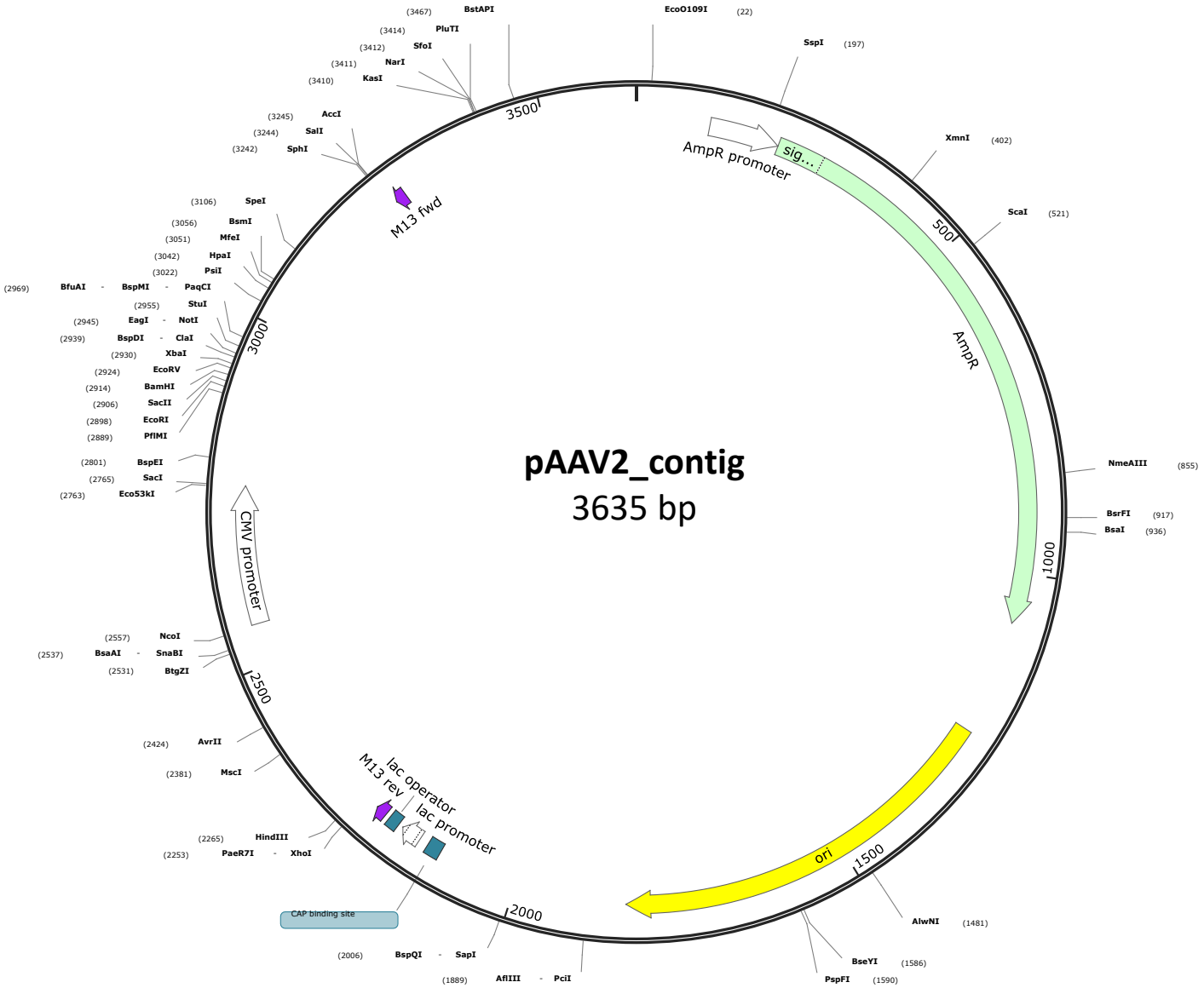

5350 Partners Court, Suite C, Frederick, MD 21703
Email: support@poochonscientific.com
Tel: 301-761-4835
www.poochonscientific.com


POOCHON SCIENTIFIC

# PlasmidExpress

## AAV-ITRs Plasmid Sequencing

## **pAAV_contig.fas** (SnapGene Viewer)





5350 Partners Court, Suite C, Frederick, MD 21703
Email: support@poochonscientific.com
Tel: 301-761-4835
www.poochonscientific.com


POOCHON SCIENTIFIC

# PlasmidExpress

## AAV-ITRs Plasmid Sequencing

## **pAAV_contig.fas - 3565 bp** (exported from SnapGene Viewer)

ACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACT
TTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGC
TTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGC
TCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATC
CTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGC
AAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGT
AAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCT
TTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGA
TGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGC
GGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGT
ATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGAC
AGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTT
TTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCC
GTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTT
GTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTA
GTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCG
TGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGC
GAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAG
CGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTG
AGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCC
TTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGA
ACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCA
GCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACA
CTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCTCG
AGATCAGAAAGCTTCCCGGGGGGATCTG**GGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCC**
**GGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTGGAGGGGTGGAGTCGTGA**CCTA
GGCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGG
ACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTG
GATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGG
ACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAG
CTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCGGACTCTAAGGTAAATATAAAATTTTTAAGTGTATAATGTGTTA
AACTACTGATTCTAATTGTTTCTCTCTTTTAGATTCCAACCTTTGGAACTGAATTCCGCGGGCCCGGGATCCGAGATATCCCTC
TAGACCATCGATGCGGCCGCTAGGCCTCACCTGCGATCTCGATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTA
ACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTT
TTTTAAACTAGT**CCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTG**
**CCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAC**AGATCCGGGCCCGCATGCGTCGACAATTCACTGGCCGTCGTTTTACA
ACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGC
ACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACC
GCATATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGC
TTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTCATC

Notes: **Left ITR; Right ITR**

5350 Partners Court, Suite C, Frederick, MD 21703
Email: support@poochonscientific.com
Tel: 301-761-4835
www.poochonscientific.com

POOCHON SCIENTIFIC