MPXV Synthetic DNA Plasmid Guidance

NIST Research Grade Test Material 10223

Sample Description

Each unit of RGTM 10223 contains approximately 110,000 copy/µL linearized plasmid DNA suspended in 250 µL of 1× TE buffer + approximately 5 ng/µL yeast tRNA to act as a carrier. The plasmid contains PCR targets for MPXV assays. The concentration was estimated using digital PCR assays (dPCR).

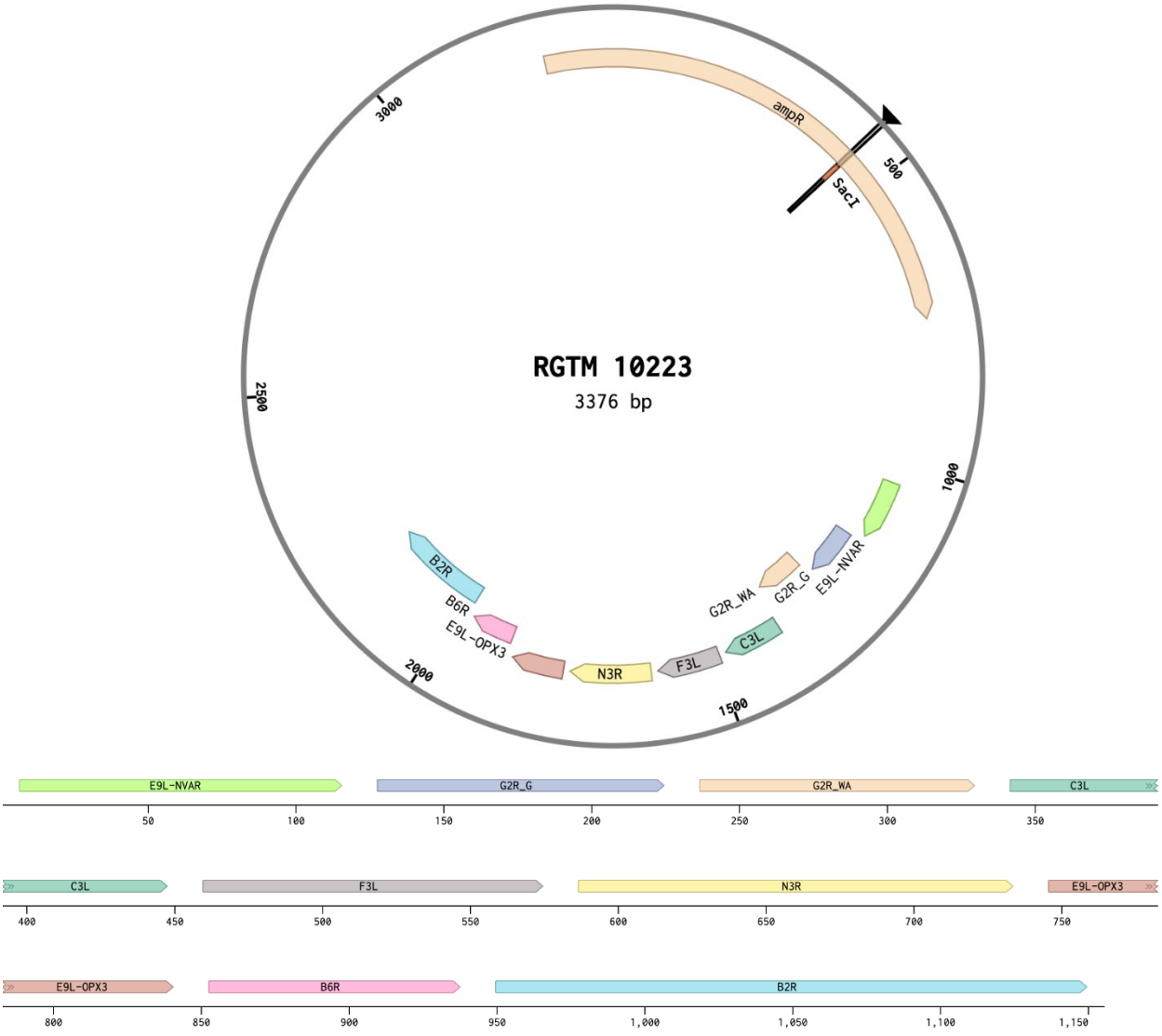

Figure 1. Vector (top) and insert (bottom) schematics. RGTM 10223 is provided as a linearized DNA that was cut at the ScaI site.

One unit of material contains two tubes – one with plasmid and one with dilution buffer. NIST is currently evaluating the stability of the diluted plasmid using this buffer, and will update with new data when available.

Purpose

- Act as a positive control for MPXV PCR assays
- Serve to generate standard/calibration curve for MPXV PCR assays

Storage

Store material at 4 °C. **DO NOT FREEZE.**

Notices and Warnings

This plasmid does not contain any complete coding regions from the monkeypox genome, it should be handled according to your institution's practices regarding synthetic nucleic acid or it should be handled using BSL-1 practices.

Please review the Safety Data Sheet provided with the material. The Safety Data Sheet can also be downloaded from the website https://www.nist.gov/programs-projects/mpxv-monkeypox-synthetic-dna-pcr-standards.

Instructions for Use

Vortex briefly (approximately 5-10 s) and spin to recollect the mixed contents. Recap the vial immediately after use to avoid altering the concentration or introducing contaminants.

The included tRNA dilution buffer should be used for diluting this material (e.g. positive controls, dilution curves). This buffer will maintain the stability of the DNA in solution such that there is no observable change in the copy number for years. To date (7/22/22) a 1:100 dilution of this material has been stable. You may also use the dilution buffer as a no-template control (NTC).

Follow the procedure specific to your assay.

Estimated Values

The copy number concentration was estimated using droplet digital PCR. "Assay" is a NIST-designation and may not match other naming conventions.

Target	Assay	Mean concentration (copy number / µL)	Coefficient of Variation
E9L	E9L-NIST	1.10E+05	<10 %
B2R	B2R-NIST	1.10E+05	<10 %
G2R_G	G2R_G-NIST	1.11E+05	<10 %

For the 3 targets were tested, no significant difference in concentration was observed. This indicates that the linearized plasmid contains a single copy of each target. The full sequence of the insert can be found on our website in the "Access Data" section.

Target Selection

We selected targets were based upon published assays of relevant targets for MPXV. The plasmid contains only the sequence fragment for the PCR assay, typically ~100-200 bp. Each fragment is separated by a short sequence to help identify the target as synthetic.

Target	Assay	Primer/Probe Sequences*	Source
E9L	E9L_NVAR	F- TCAACTGAAAAGGCCATCTATGA P- *TET*-CCATGCAATATACGTACAAGATAGTAGCCAAC R- GAGTATAGAGCACTATTTCTAAATCCCA	doi:10.1016/j.jcv.2006.03.012
OPX	E9L_OPX	F- TCAAATATTGATCGTCCAACGA P- *FAM*-TAACATCCGTCTGGAGATATCCCGTTAGA-*BHQ1* R- TGGATGAATTTCTCAATATTAGTTGG	doi:10.4269/ajtmh.2010.09-0716
G2R_G	G2R_G	F- GGAAAATGTAAAGACAACGAATACAG P- *FAM*-AAGCCGTAATCTATGTTGTCTATCGTGTCC-*BHQ1* R- GCTATCACATAATCTGGAAGCGTA	doi:10.1016/j.jviromet.2010.07.012
G2R_WA	G2R_WA	F- CACACCGTCTCTTCCACAGA P- *FAM*-AACCCGTCGTAACCAGCAATACATTT-*BHQ1* R- GATACAGGTTAATTTCCACATCG	doi:10.1016/j.jviromet.2010.07.012
C3L	C3L	F- TGTCTACCTGGATACAGAAAGCAA P- *FAM*-CCCATATATGCTAAATGTACCGGTACCGGA-*BHQ1* R- GGCATCTCCGTTTAATACATTGAT	doi:10.1016/j.jviromet.2010.07.012
F3L	F3L	F- CTCATTGATTTTTCGCGGGATA P- *6FAM*-CATCAGAATCTGTAGGCCGT-*MGBNFQ* R- GACGATACTCCTCCTCGTTGG	doi:10.1038/labinvest.3700143
N3R	N3R	F- AACAACCGTCCTACAATTAAACAACA P- *6FAM*-TATAACGGCGAAGAATATACT-*MGBNFQ* R- CGCTATCGAACCATTTTTGTAGTCT	doi:10.1038/labinvest.3700143
B6R	B6R	F- ATTGGTCATTATTTTTGTCACAGGAACA P- *MGB/DarkQuencher*-AGAGATTAGAAATA-*FAM* R- AATGGCGTTGACAATTATGGGTG	doi:10.1016/j.jcv.2006.03.012
B2R	B2R	multiple	doi:10.1038/s41586-019-0928-6

* These are the original assays as published; NIST has not tested or verified their utility.

The plasmid length is 3376 nt and consists of a cloning vector with ampicillin resistance and the insert. The insert size is 1155 nt. The sequence of the insert was confirmed using Sanger sequencing.

Feedback

Since NIST produced the material for no-charge, we ask you to provide feedback on the material. Your feedback regarding use of this material will help us further test, develop, and improve these materials for future development. Please provide your feedback by August 31, 2023. After that date, please contact us directly using MPXV-RGTM@nist.gov.

Feedback form

NIST Additional Information

NIST generated assays for dPCR on each target to ensure they could all be measured using the same reaction conditions. These may not match the published assays. Those assays will be available at a later date.

NIST Contact Information

General Inquiries (MPXV-RGTM@nist.gov)