Flow Cytometry and qPCR under GMP

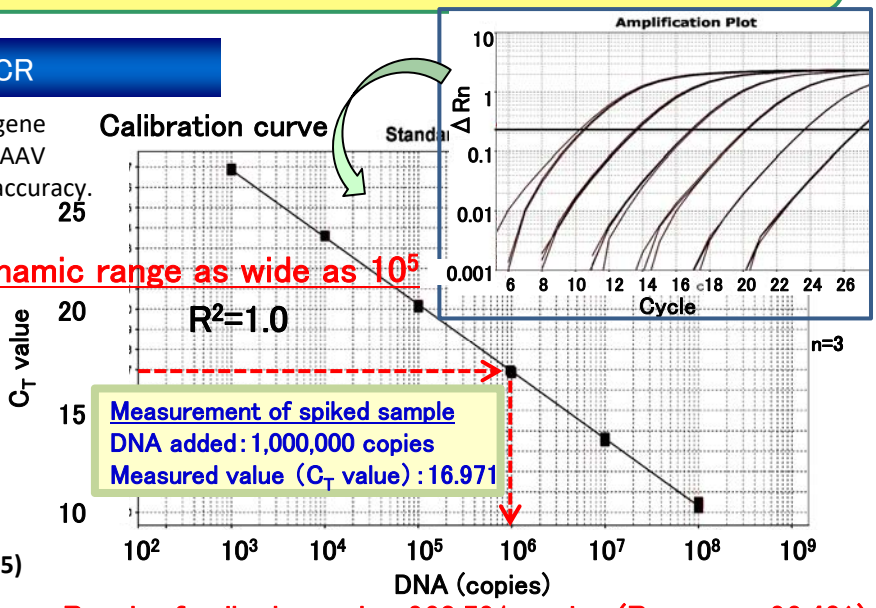
We have introduced flow cytometer and qPCR in order to analyze cell therapy and gene therapy products. In preparation for regulatory submission, we are operating these instruments under Good Manufacturing Practice (GMP). Here we show some examples of these analyses.

Analysis of AAV derived DNA by qPCR

Adeno-associated virus(AAV) is widely used in gene therapy. We analyzed plasmid DNA containing AAV derived sequence and evaluated linearity and accuracy.

qPCR (Thermo Fisher Scientific, Quant Studio 5)

SOP number：NME16540 "Operation and maintenance procedure for qPCR"

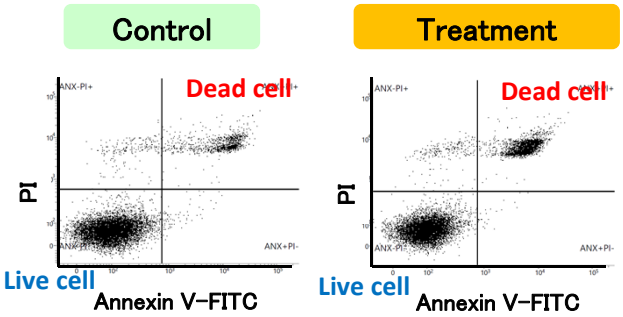


Result of spiked sample： 963,591 copies (Recovery：96.4%)

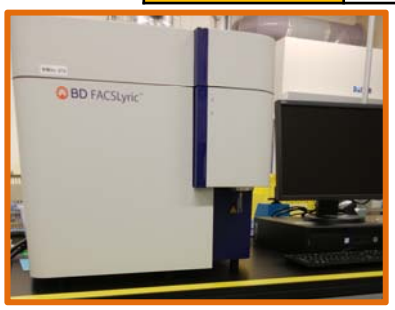
We obtained satisfactory R^2 value for calibration curve and recovery of spiked sample.

Analysis of cell death by flow cytometer

We evaluated cell death and apoptosis by Annexin V-FITC and PI staining.



	Live	Dead
Control	87.2%	10.1%
Treatment	74.9%	22.7%



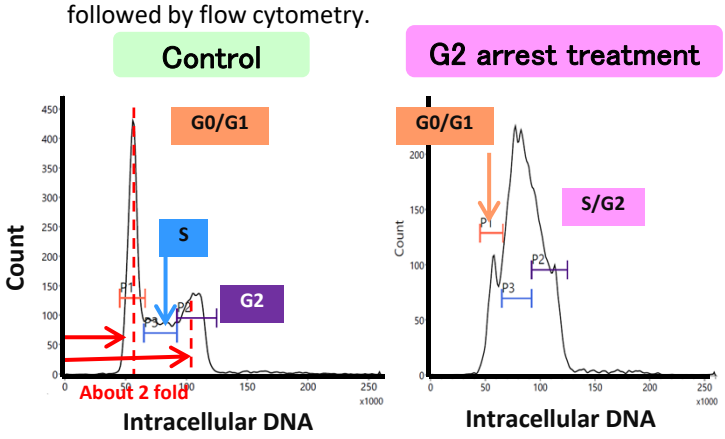
Percentage of dead cell (Annexin V positive and PI positive) were increased by treatment.

Flow Cytometer (Becton Dickinson, FACSLyric)

SOP number：NME14110
"Operation and maintenance procedure for flow cytometer"

Analysis of cell cycle by flow cytometer

We calculated cell cycle phase by quantifying DNA amount per cell. DNA quantification was performed by PI staining followed by flow cytometry.



	G0/G1	S	G2
Control	41.8%	24.2%	30.2%
G2 arrest treatment	13.3%	50.3%	33.3%

Percentage of S phase and G2 phase were increased by G2 arrest control.

PI: Propidium Iodide. It binds to double strand nucleic acid and is not membrane-permeable.
FITC: Fluorescein Isothiocyanate