Author:

**GNxxxxxx-xxx**

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

ddPCR

# Objective

Absolute quantification of vector genome titers for gene therapy project samples via Droplet Digital PCR (ddPCR).

# Background (Method status)

Projects:

TAK-686 Huntington

* Qualified for intended use for BDS and FDP according to Method Readiness Report RPT-005106

Samples Nr. \_\_\_\_\_ - \_\_\_\_\_\_

* Fit for purpose for Intermediates according to Method History File RPT-005102

Samples Nr. \_\_\_\_\_ - \_\_\_\_\_\_

* For information only

Samples Nr. \_\_\_\_\_ - \_\_\_\_\_\_

ID TAG (DP0073) Hunter

* Qualified for intended use for BDS, FDP and Intermediates according to Method Readiness Report RPT-007513

Samples Nr. \_\_\_\_\_ - \_\_\_\_\_\_

* For information only

Samples Nr. \_\_\_\_\_ - \_\_\_\_\_\_

TAK-748 FIX

* Fit for purpose according to MHF RPT-004766

FVIII / ITR

* For information only

IDT / ITR

* Fit for purpose according to MHF RPT-007369

FXN-BT / ITR

* For information only

# Results

**Table 1** presents the mean value between two independent tests from GNxxxxxx-xxx and GNxxxxxx-xxx. These mean values are regarded as final reportable results.

**Table 1: Two-fold Test Analysis**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **GN** | **target** | **rep#** | **Sample name** | **mean A [vg/mL]** | **mean ITR [vg/mL]** | **ratio A/ITR** |
|  |  | **1** |  |  |  | #DIV/0! |
|  | **2** |  |  | #DIV/0! |
|  |  |  | **final result (mean)** | **#DIV/0!** | **#DIV/0!** | #DIV/0! |
|  |  |  | **CV%** | **#DIV/0!** | **#DIV/0!** |  |

Attention!!! Please adjust controls specifically to project(s)!

**Table 2: Results of the Experiment**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| nr. | target | sample name | result HT2 or IDT or FIX or FVIII or FXN-BT [vg/ml] | result ITR [vg/ml] | comment |
| 1 |  |  | **Complies:**  **yes**  / **no** | **Complies:**  **yes**  / **no** |  |
| 2 |  |  |  |  |  |
| 3 |  |  |  |  |  |
| 4 |  |  |  |  |  |
| 5 |  |  |  |  |  |
| 6 |  |  |  |  |  |
| 7 |  |  |  |  |  |
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| 25 |  |  |  |  |  |
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| 33 |  |  |  |  |  |
| 34 |  |  |  |  |  |
| 35 |  |  |  |  |  |
| 36 |  |  |  |  |  |
| 37 |  |  |  |  |  |
| 38 |  |  |  |  |  |
| 39 |  |  |  |  |  |
| 40 |  |  |  |  |  |
| 41 |  |  |  |  |  |
| 42 |  |  |  |  |  |
| 43 |  |  |  |  |  |
| 44 |  |  |  |  |  |
| 45 |  |  |  |  |  |
| 46 |  |  |  |  |  |
| 47 |  |  |  |  |  |
| 48 |  |  |  |  |  |

# Experiment Set-Up

See experiment file

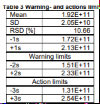
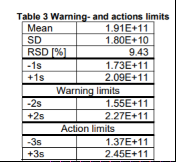
# Assessment Criteria

Control Limits

**HT2**

**Plasmid control** pMY017 Lot # 190122/AL/02 **Reference control** RPP686\_1903\_FDP

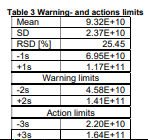
(GN000168-010) (GN000168-015)

**FIX**

**plasmid control** Cyan22 Lot#N2006/1 043 **Reference control** CRM22 ELT

(GN000168-011) (GN000168-012)

 Computergenerierter Alternativtext:
3 
"mits 
1.72E+11 
2.57É+10 
RSD 
14.01 
1.46E+11 
1.98É+11 
Warn i 
limits 
121E+11 
224É+11 
Action limits 
gyE+10 
2.49É+11 

**IDT/ITR**

**plasmid control** pXL29\_SacI; Lot#190820/01/AL **Reference control** PP073\_1933\_FDP

**IDT** (GN000168-014) **IDT** (GN000168-021)

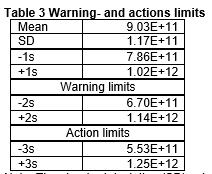
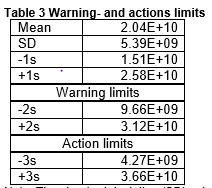
Computergenerierter Alternativtext:
Table 3 Warni 
Mean 
SD 
RSO % 
• and actions I 
6.20E+10 
6.81E+09 
1098 
S.52E+10 
6.88E+10 
-2s 
Warnin lim•ts 
4,84E+10 
7.56E+10 
Action limits 
4,16E+10 
8.24E+10  Computergenerierter Alternativtext:
Table 3 Wamin 
Mean 
SD 
RSD % 
-1s 
Warnin 
4-2 S 
+3s 
- and actions limits 
I .32E+l I 
1.97E+10 
15.01 
1.12E+ll 
1.51E+ll 
limits 
9.20E+10 
1.71E+ll 
Action limits 
7.23E+10 
1.91E+ll 

**ITR (**GN000168-003) **ITR (**GN000168-007)

Computergenerierter Alternativtext:
Table 3 Warnin 
Mean 
SD 
RSD % 
-1s 
Warnin 
+2s 
- and actions limits 
5.94E+10 
6.77E+09 
11.40 
5.26E+10 
6.62E+10 
limits 
4.59E+10 
7.30E+10 
Action limits 
3.91E+10 
7.97E+10 Computergenerierter Alternativtext:
Table 3 Warnin 
Mean 
SD 
RSD % 
Warnin 
- and actions limits 
1.87E+11 
2.14E+ 10 
11.46 
1.65E+11 
2.08E+11 
limits 
1.44E+11 
2.30E+11 
Action limits 
1.23E+11 
2.51E+11 

**FVIII/ITR**

**Reference control** PP754\_1931TMAE\_E\_FILT **Plasmid control** Orth04 Amplikon

FVIII (DFM13390) FVIII (DFM13368) 

ITR (GN000168-022)

Computergenerierter Alternativtext:
Table 3 Warnin 
Mean 
SD 
RSD % 
- and actions limits 
5.17E+11 
4.33E+10 
8.38 
4.73E+11 
5.60E+11 
Warnin limits 
4.30E+11 
6.03E+11 
Action limits 
3.87E+11 
6.47E+11 

**FXN/ITR**

**PP754\_1931TMAE\_E\_FILT AAVCAM948C.5/scTR-EF1a-hFXN-huGHpA (BDS)**

ITR (GN000168-022) (GN00168-060)

|  |  |  |
| --- | --- | --- |
| **Parameter** | **FXN** | **ITR** |
| Mean | 3.76E+10 | 4.27E+10 |
| SD | 6.79E+09 | 7.62E+09 |
| RSD [%] | 18.04 | 17.85 |
| -1s | 3.08E+10 | 3.51E+10 |
| +1s | 4.44E+10 | 5.03E+10 |
| Warning limits | | |
| -2s | 2.40E+10 | 2.74E+10 |
| +2s | 5.12E+10 | 5.79E+10 |
| Action limits | | |
| -3s | 1.73E+10 | 1.98E+10 |
| +3s | 5.80E+10 | 6.55E+10 |

Computergenerierter Alternativtext:
Table 3 Warnin 
Mean 
SD 
RSD % 
- and actions limits 
5.17E+11 
4.33E+10 
8.38 
4.73E+11 
5.60E+11 
Warnin limits 
4.30E+11 
6.03E+11 
Action limits 
3.87E+11 
6.47E+11 

\*The control charts for applied controls are under development. Corresponding limits will be calculated, documented and defined as assessment criteria for control samples as soon as sufficient control results are provided.

Assay validation criteria:

* Threshold was defined at:
* 2000 for HT2, IDT, FVIII, FIX
* 1000 for FXN-BT
* 3000 for ITR
* Each target was assessed independently.
* Negative controls (NTC and negative control) remain negative or lie at least 1 logarithimic scale below the lower limit of the given range (in vg/µL).
* This applies for all samples reflecting either a “fit for purpose” or “qualified for intended use” status.
* This criterion can be neglected for samples with “for information only” results.

Sample validation criteria:

* The number of generated droplets requires to be >10 000 (for values implied in the analysis).
* The coefficient of variation is required to be ≤20% for tested samples. There is no limit or no acceptance criteria regarding the coefficient of variation for negative controls or for samples with a result below the detection limit.

**Method Range for “Fit for purpose” Samples:**

Measured concentrations in vg/µL need to lie within the following defined ranges:

* Huntington: **10 vg/µL to 4690 vg/µL** (<10vg/µl=<LOQ= < 5,00E+09vg/ml)
* ID Tag: **15 vg/µL to 3360 vg/µL** (<15vg/µl=<LOQ= < 7,50E+09vg/ml)
* FIX: **17 vg/µL to 2675 vg/µL** (<17vg/µl=<LOQ= < 8,50E+09vg/ml)
* FVIII/ITR: **15 vg/µL to 3360 vg/µL** (<15vg/µl=<LOQ= < 7,50E+09vg/ml)

Those limits can be neglected for samples with a “for information only” result.

For further target specific criteria, see SOP-051000.

# Result Calculation

Final results are defined by the concentration of the vector genome per milliliter (vg/mL) present in the sample.

Measured values are displayed by the software in vg/µL.

Calculation of the final result occurs by following the formula below.

All dilution factors implied during sample preparation are considered in the formula which converts the final result into vg/mL.

|  |
| --- |
| **X[vg/ml] = [(A \* Y) \* (1000/B)] \* D** |

**X = Concentration of the vector genome per milliliter [vg/mL]**

**A =** ddPCR Volume = 20 µL

**Y =** ddPCR Readout = vg/µL (Software Quantasoft)

**1000 =** Converting factor from µl to mL

**B =** Sample volume in the ddPCR reaction = 2 µL

**D =** final dilution factor of the sample

1:2 (10 µL sample + 10 µL DNase)

1:5 (20 µL sample + 6 µL EDTA + 74 µL ddPCR Pluronic dilution buffer)

1:ZZ (100 µL sample + specific dilution factor, see experiment file)

# Comments

N.Z.

# List of Attachments

|  |
| --- |
| GNxxxxxx-xxx – attachments.pdf |
| GNxxxxxx-xxx – experiment.pdf |
| GNxxxxxx-xxx – datafile.docx |

Run file is archived in OpenLAB ECM.