# Final QC report:
# Generation of a Dual Reporter iPSC line

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# GLOSSARY

| Abbreviation / acronym | Description |
|---|---|
| TNNI3 | Troponin I (Cardiac muscle) |
| TNNI1 | Troponin I, type 1 (Cardiac slow muscle) |
| iPSC | Induced pluripotent stem cells |
| CRISPR | Clustered regularly interspaced short palindromic repeats |
| WCB | Working Cell Bank |

## AIM

The project aims at generating a heterozygous insertion of IRES-H2B-mCherry at the C-terminal end of the TNNI3 gene in the BIONi010-C wt Induced pluripotent stem cell (iPSC) line. When the insertion and line has been evaluated, a heterozygous insertion of IRES-d2EGFP at the C-terminal end of the TNNI1 gene will be carried out resulting in a dual reporter iPSC line.

## EXECUTIVE SUMMARY

This report details the generation of a reporter line in the BIONi010-C wt iPSC line containing an IRES-H2B-mCherry in the C-terminal part of the TNNI3 gene. Furthermore, the TNNI3 reporter line was used to establish the a dual TNNI3/TNNI1 reporter iPSC line containing an additional IRES-d2EGFP in the C-terminal part of the TNNI1 gene. The project was carried out upon request of Pfizer Inc. Primary Pharmacology Group, Groton, CT, USA

Following gene editing and single cell cloning, first a TNNI3 and subsequently a dual TNNI3/TNNI1 iPSC reporter line were successfully isolated. The lines were expanded to cell banks of 10 vials and characterized for the following analyses: Sterility, Viability/morphology, Mycoplasma testing, Identity by STR analysis, Genotyping, Karyology (G-banding), Single Nucleotide Polymorphism (SNP) analysis. 10 vials of each line containing 2-3 million cells each were shipped to Pfizer, US.

# BRIEF PROJECT DESCRIPTION - TNNI3-IRES-MCHERRY REPORTER

The gene TNNI3 encodes the Troponin I (Cardiac muscle) protein which is expressed in mature cardiomyocytes.

In brief, a homologous donor construct encoding an IRES-H2B-mCherry flanked by homologous arms matching the C-terminal part of the TNNI3 gene was designed and ordered from GeneArt. The endofree plasmid was co-nucleofected together with the Cas9 protein and a sgRNA designed to target the last exon (Exon 7) of the TNNI3 gene after the STOP codon (Figure 1 and Appendix).

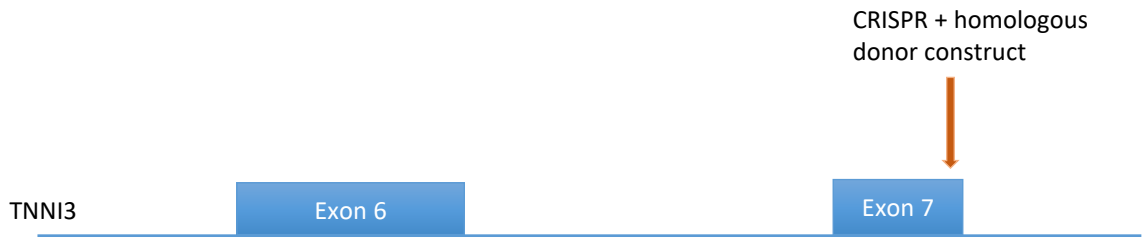



***Figure 1:*** *A single sgRNA and a CRISPR-Cas9 protein was used to insert the reporter in the last Exon after the STOP codon of the TNNI3 gene.*

# MAIN SECTION

### *Gene editing*

The wt iPSC line BIONi010-C was nucleofected with CRISPR-Cas9 and edited clones were analyzed using TNNI3 screening and absence primers (see appendix). Several potential clones were identified and isolated for further analysis by PCR and sequencing. A TNNI3 reporter clone, named TNNI3-IRES-mCherry reporter cl. 84-4, was sequence verified and subjected to further single cell cloning to ensure the purity before expansion and banking in 15 vials.

### *QC of TNNI3-IRES-mCherry reporter cl. 84-4*

Following banking of 15 vials, a single vial of each line was thawed and the following QC analyses were performed:

1) Sterility, Viability Test and Morphology
2) Identity by STR Analysis
3) Mycoplasma Test
4) Sequencing Analysis
5) Karyology by G-Banding

## Sterility Test

1 ml of the supernatant from the BIONi010-C TNNI3 reporter line was transferred to 10 mL of LB medium without antibiotics. After 48 hours incubation in a 37ºC shaker, the LB medium was still clean, indicating that no bacterial contamination was present in the bank.

## Viability Test and Morphology

Survival of the BIONi010-C TNNI3 reporter line and typical iPSC morphology was confirmed by microscope analysis 1 day after thawing in 2 wells of a 6-well dish (Figure 2).

TNNI3-IRES-mCherry reporter cl. 84-4

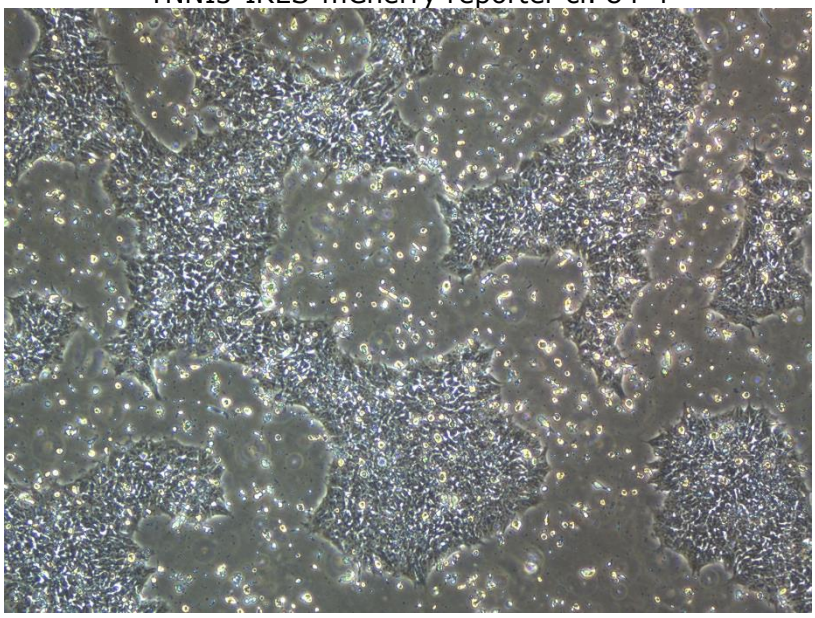


***Figure 2:*** *Microscope analyses was carried out 1 day after thawing in 2 wells of a 6-well dish.*

## Mycoplasma Test

A mycoplasma test by PCR revealed that no mycoplasma contamination was present in the culture of the TNNI3 reporter line (Figure 3).

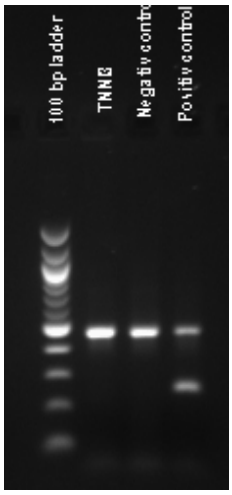



***Figure 3:*** *Mycoplasma test for the TNNI3 reporter cl. 84-4*

## Identity by STR Analysis

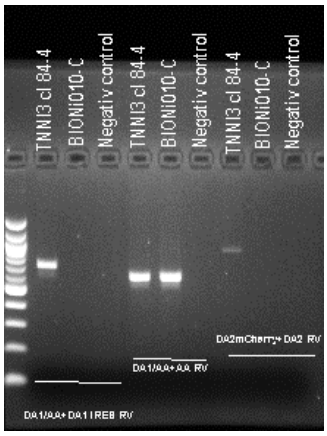
To confirm the correct identity of the gene-edited clone, an STR analysis was carried out on the TNNI3 reporter line after banking, which was identical to the original BIONi010-C wt iPSC line (Table 1)

***Table 1:*** *STR analysis of TNNI3 reporter clone*

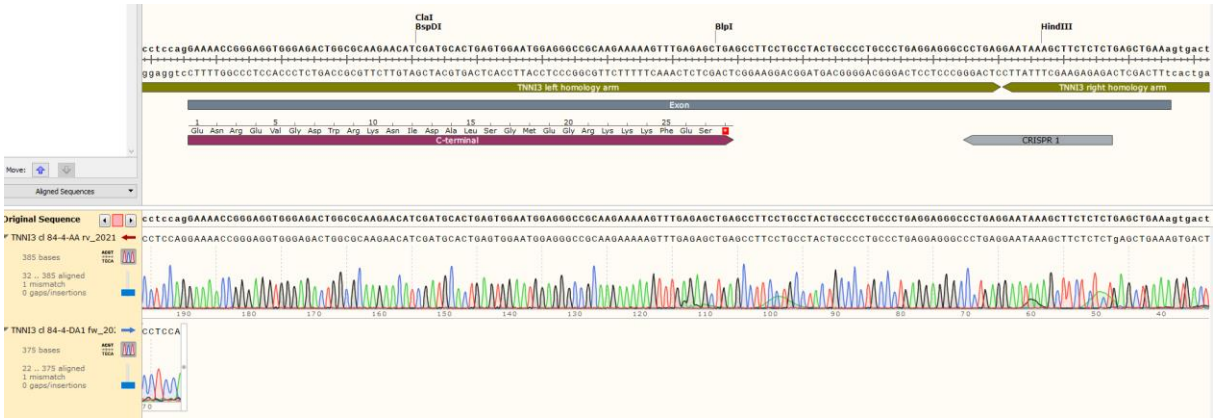
| | **BIONi010-C** | | **TNNI3-IRES-mCherry reporter cl. 84-4** | |
|---|---|---|---|---|
| **Marker** | Allele 1 | Allele 2 | Allele 1 | Allele 2 |
| AMEL | X | Y | X | Y |
| CSF1PO | 10 | 12 | 10 | 12 |
| D13S317 | 11 | 12 | 11 | 12 |
| D16S539 | 10 | 12 | 10 | 12 |
| D18S51 | 13 | 16 | 13 | 16 |
| D19S433 | 13 | 14.2 | 13 | 14.2 |
| D21S11 | 28 | 32.2 | 28 | 32.2 |
| D2S1338 | 22 | 23 | 22 | 23 |
| D3S1358 | 15 | 16 | 15 | 16 |
| D5S818 | 11 | 13 | 11 | 13 |
| D7S820 | 9 | 11 | 9 | 11 |
| D8S1179 | 13 | 14 | 13 | 14 |
| FGA | 21 | 24 | 21 | 24 |
| TH01 | 7 | 9.3 | 7 | 9,3 |
| TPOX | 8 | 11 | 8 | 11 |
| vWA | 17 | | 17 | |

## PCR and Sequencing

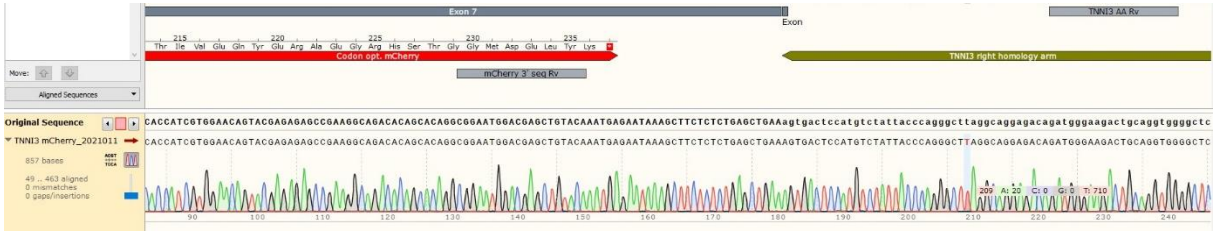
PCR and Sequencing analysis confirmed that the TNNI3-IRES-mCherry reporter cl. 84-4 contained both the IRES and the mCherry in one allele, as well as an intact wt allele (Figure 4).




TNNI3 cl. 84-4 sequencing validation of IRES sequence




TNNI3 cl. 84-4 sequencing validation of mCherry sequence




TNNI3 cl. 84-4 wild-type allele sequencing

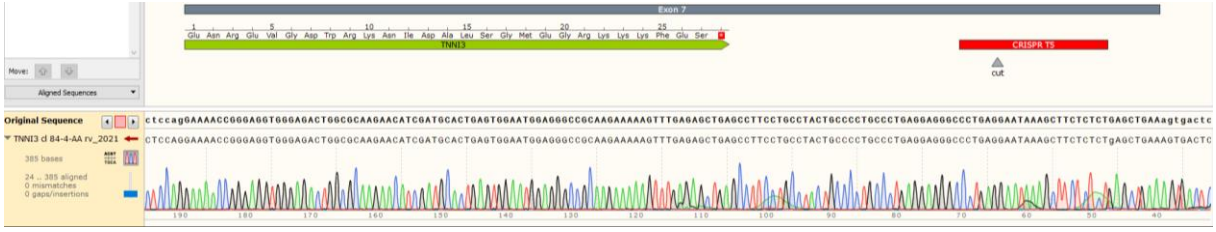



***Figure 4:*** *PCR and sequencing analysis of the TNNI3 reporter. Sequencing showed a 100% match with the expected sequence.*

## Karyotype

The Karyotype of the TNNI3 reporter line was investigated by a conventional G-banding analysis. The analysis revealed a normal male karyotype (46, XY) (Figure 5 and appendix).

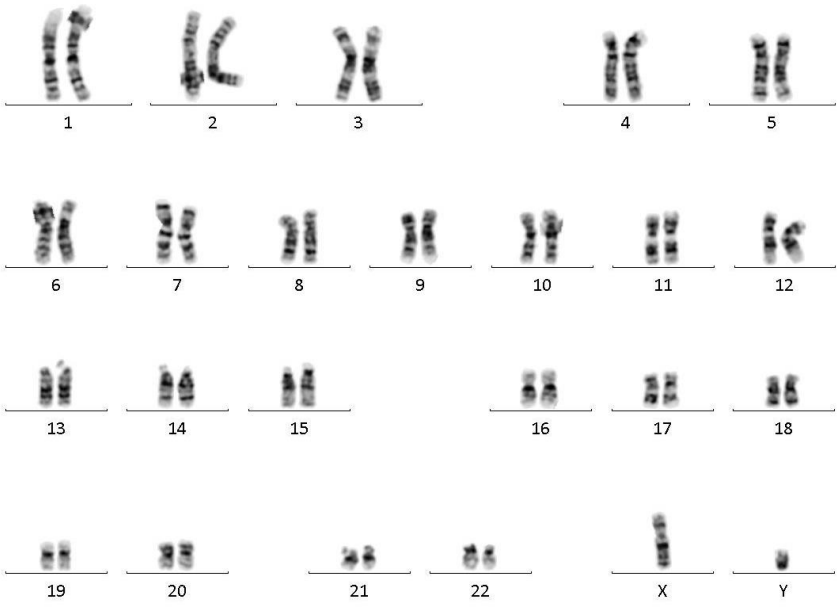



***Figure 5:*** *G-banding karyotype analysis revealed a normal male karyotype (46, XY).*

# BRIEF PROJECT DESCRIPTION - BIONI010-C-55

The gene TNNI1 encodes the Troponin I, type 1 protein (Cardiac slow muscle) expressed in mature cardiomyocytes.

In brief, a homologous donor construct encoding an IRES-d2EGFP flanked by homologous arms matching the C-terminal part of the TNNI1 gene was designed and ordered from GeneArt. The endofree double stranded plasmid was nucleofected together with the Cas9 protein and an sgRNA designed to target the 3'UTR of TNNI1 (Figure 6 and Appendix).

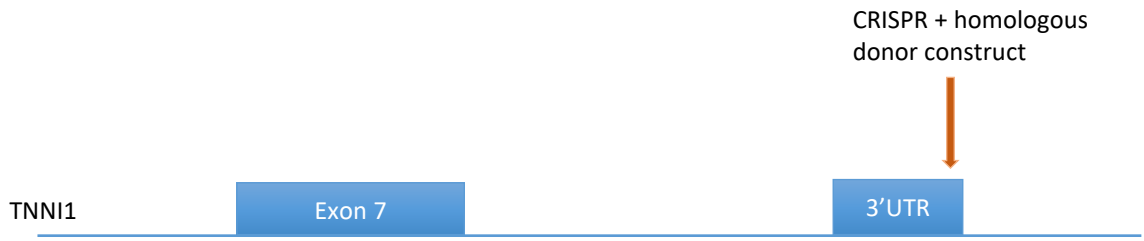



***Figure 6:*** *A single sgRNA and a CRISPR-Cas9 protein was used to insert the reporter in the 3' UTR of the TNNI1 gene.*

# MAIN SECTION

### *Gene editing*

The iPSC line TNNI3-IRES-mCherry reporter cl. 84-4 described above was used for establishment of the dual TNNI3/1 reporter. The line was thawed and nucleofected using CRISPR-Cas9 and edited clones were analyzed using TNNI screening and absence primers (see appendix). Several potential clones were identified and isolated for further analysis by PCR and sequencing. A single TNNI1 reporter clone, named BIONi010-C-55 (dual TNNI3/TNNI1 reporter cl. 74) was sequence verified before expansion and banking in 50 vials.

### *QC of BIONi010-C-55 dual reporter*

Following banking, a single vial was thawed and the following QC analyses were performed:

6) Sterility, Viability Test and Morphology
7) Identity by STR Analysis
8) Mycoplasma Test
9) Sequencing Analysis
10) Karyology by G-Banding
11) High Resolution Karyotype (SNP) using ddPCR (StemGenomics)

## Sterility Test

1 ml of the supernatant from the BIONi010-C-55 dual reporter line was transferred to 10 mL of LB medium without antibiotics. After 48 hours incubation in a 37ºC shaker, the LB medium was still clean, indicating that no bacterial contamination was present in the bank.

## Viability Test and Morphology

Survival of the BIONi010-C-55 dual reporter line and typical iPSC morphology was confirmed by microscope analysis 1 day after thawing in 2 wells of a 6-well dish (Figure 7).

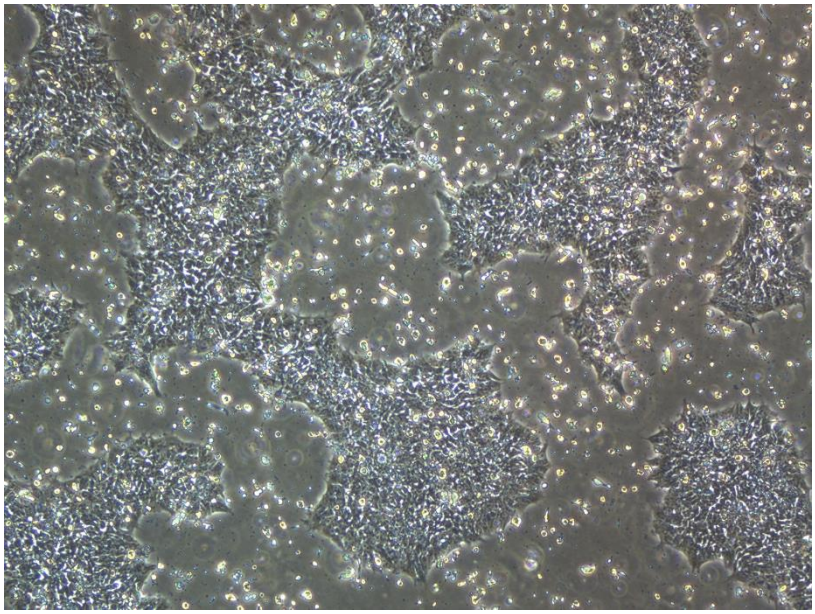


***Figure 7:*** *Microscope analyses was carried out 1 day after thawing in 2 wells of a 6-well dish.*

## Mycoplasma Test

A mycoplasma test by PCR revealed that no mycoplasma contamination was present in the culture of the BIONi010-C-55 dual reporter line (Figure 8).

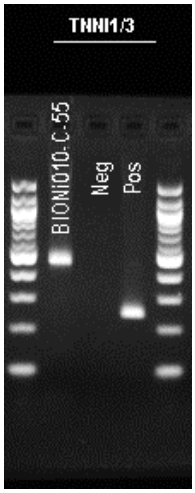



***Figure 8:*** *Mycoplasma test for BIONi010-C-55 dual reporter*

## Identity by STR Analysis

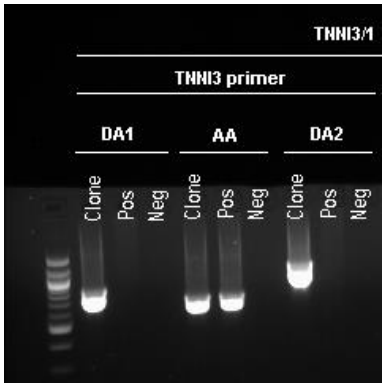
To confirm the correct identity of the gene-edited clone, an STR analysis was carried out on the BIONi010-C-55 dual reporter line after banking, which was identical to the original BIONi010-C wt iPSC line (Table 1)

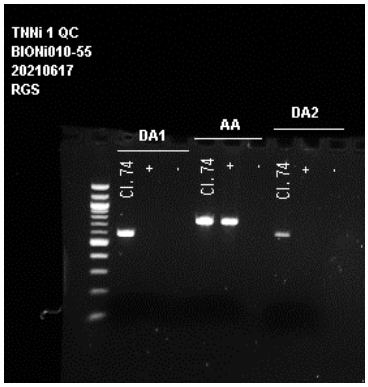
***Table 2:*** *STR analysis of BIONi010-C-55 dual reporter*

| | **BIONi010-C** | | **BIONi010-C-55 (dual TNNI3/TNNI1 reporter cl. 74)** | |
|---|---|---|---|---|
| **Marker** | Allele 1 | Allele 2 | Allele 1 | Allele 2 |
| AMEL | X | Y | X | Y |
| CSF1PO | 10 | 12 | 10 | 12 |
| D13S317 | 11 | 12 | 11 | 12 |
| D16S539 | 10 | 12 | 10 | 12 |
| D18S51 | 13 | 16 | 13 | 16 |
| D19S433 | 13 | 14.2 | 13 | 14.2 |
| D21S11 | 28 | 32.2 | 28 | 32.2 |
| D2S1338 | 22 | 23 | 22 | 23 |
| D3S1358 | 15 | 16 | 15 | 16 |
| D5S818 | 11 | 13 | 11 | 13 |
| D7S820 | 9 | 11 | 9 | 11 |
| D8S1179 | 13 | 14 | 13 | 14 |
| FGA | 21 | 24 | 21 | 24 |
| TH01 | 7 | 9.3 | 7 | 9.3 |
| TPOX | 8 | 11 | 8 | 11 |
| vWA | 17 | | 17 | |

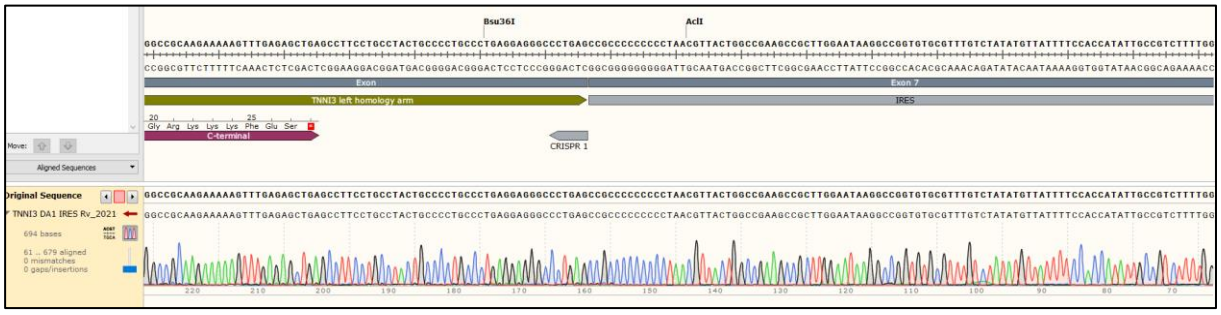
## PCR and Sequencing

PCR and Sequencing analysis confirmed that the BIONi010-C-55 dual reporter contained both the IRES-mCherry as well as an intact wt allele in TNNI3 and the IRES-d2EGFP as well as an intact wt allele in TNNI1 (Figure 9).



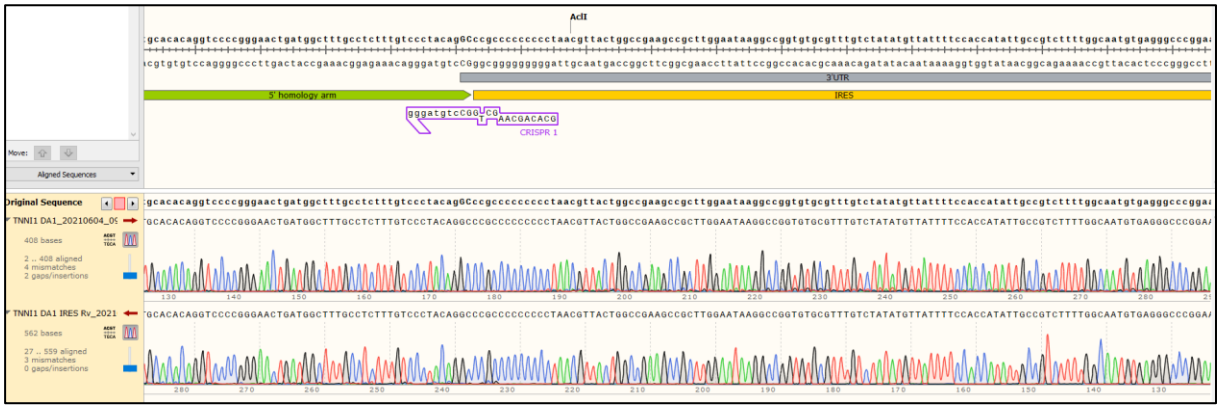

BIONi010-C-55 dual reporter sequencing of TNNI3-IRES-mCherry at the 5’ end



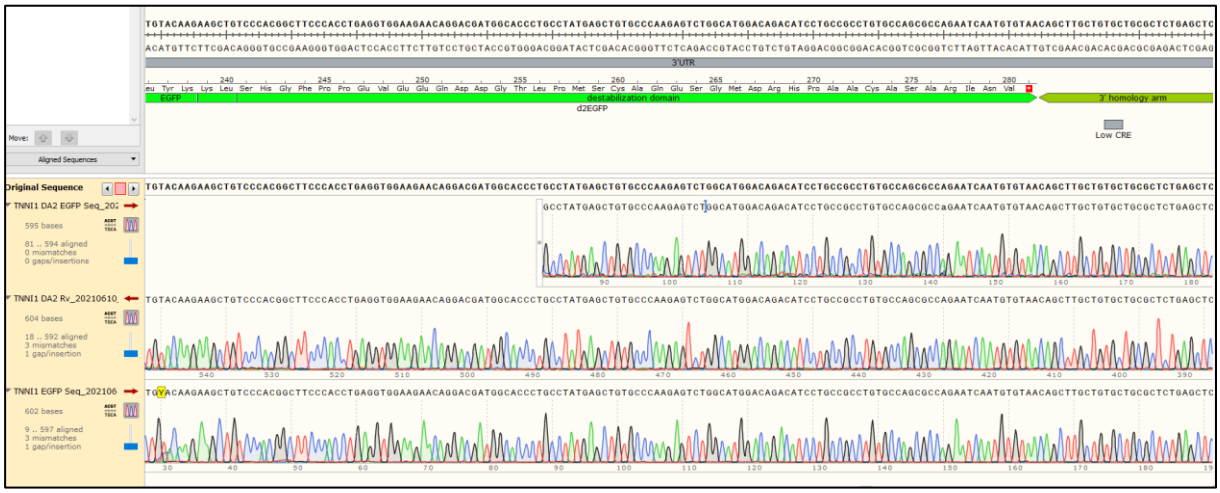

BIONi010-C-55 dual reporter sequencing of TNNI3-IRES-mCherry at the 3’ end


BIONi010-C-55 dual reporter wild-type TNNI3 sequencing

BIONi010-C-55 dual reporter sequencing of TNNI1-IRES-d2EGFP at the 5’ end




BIONi010-C-55 dual reporter sequencing of TNNI1-IRES-d2EGFP at the 3’ end




BIONi010-C-55 dual reporter wild-type TNNI1 sequencing

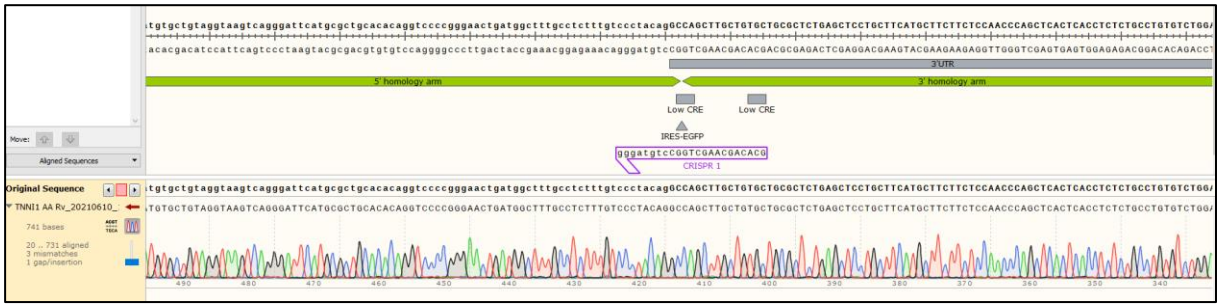



***Figure 9:*** *PCR and sequencing analysis of the BIONi010-C-55 dual reporter showed a 100% match with the expected sequences.*

## Karyotype

The Karyotype of the BIONi010-C-55 dual reporter line was investigated by a conventional G-banding analysis. The analysis revealed a normal male karyotype (46, XY) (Figure 10 and appendix).

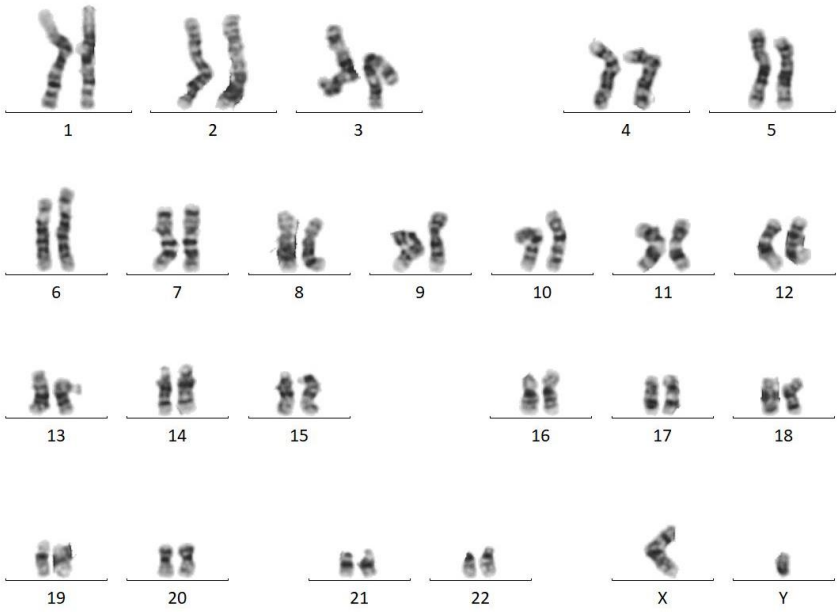
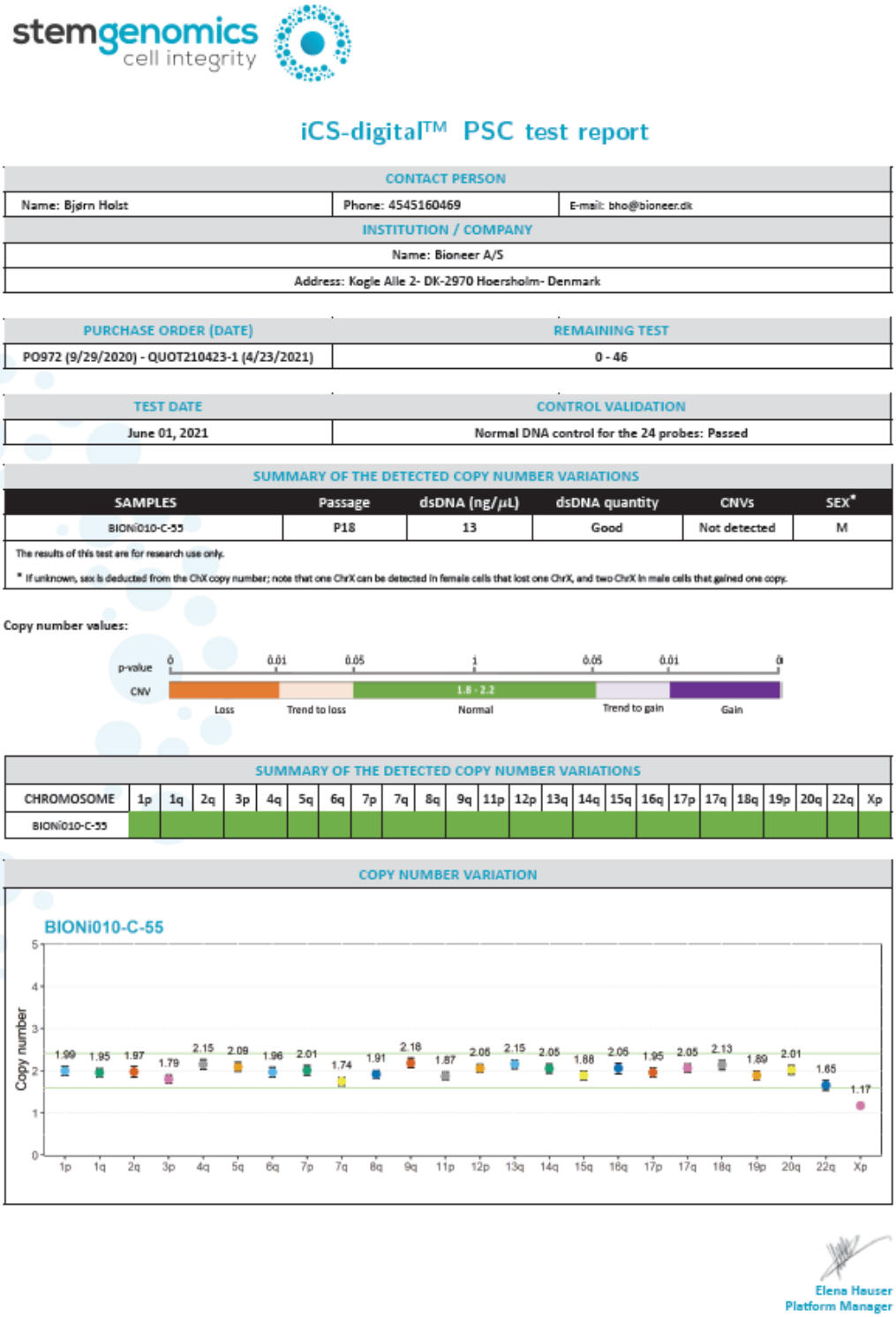



***Figure 10:*** *G-banding karyotype analysis revealed a normal male karyotype (46, XY).*

## High Resolution Karyotype Analyses

Single nucleotide polymorphisms (SNP) was analyzed using digital droplet PCR detection of the 24 most common SNP's in iPSC's (Stemgenomics). The dual reporter BIONi010-C-55 did not show any abnormalities (Figure 11).



stemgenomics cell integrity

iCS-digital™ PSC test report

| CONTACT PERSON | | |
|---|---|---|
| Name: Bjørn Holst | Phone: 4545160469 | E-mail: bho@bioneer.dk |
| **INSTITUTION / COMPANY** | | |
| Name: Bioneer A/S | | |
| Address: Kogle Alle 2- DK-2970 Hoersholm- Denmark | | |

| PURCHASE ORDER (DATE) | REMAINING TEST |
|---|---|
| PO972 (9/29/2020) - QUOT210423-1 (4/23/2021) | 0 - 46 |

| TEST DATE | CONTROL VALIDATION |
|---|---|
| June 01, 2021 | Normal DNA control for the 24 probes: Passed |

SUMMARY OF THE DETECTED COPY NUMBER VARIATIONS

| SAMPLES | Passage | dsDNA (ng/µL) | dsDNA quantity | CNVs | SEX* |
|---|---|---|---|---|---|
| BIONi010-C-55 | P18 | 13 | Good | Not detected | M |

The results of this test are for research use only.

* If unknown, sex is deducted from the ChX copy number; note that one ChrX can be detected in female cells that lost one ChrX, and two ChrX in male cells that gained one copy.

Copy number values:



SUMMARY OF THE DETECTED COPY NUMBER VARIATIONS

| CHROMOSOME | 1p | 1q | 2q | 3p | 4q | 5q | 6q | 7p | 7q | 8q | 9q | 11p | 12p | 13q | 14q | 15q | 16q | 17p | 17q | 18q | 19p | 20q | 22q | Xp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BIONi010-C-55 | | | | | | | | | | | | | | | | | | | | | | | | |

COPY NUMBER VARIATION



Elena Hauser
Platform Manager

***Figure 11:*** *High resolution karyotype revealed a normal SNP profile in the BIONi010-C-55 dual reporter.*

## CONCLUSION

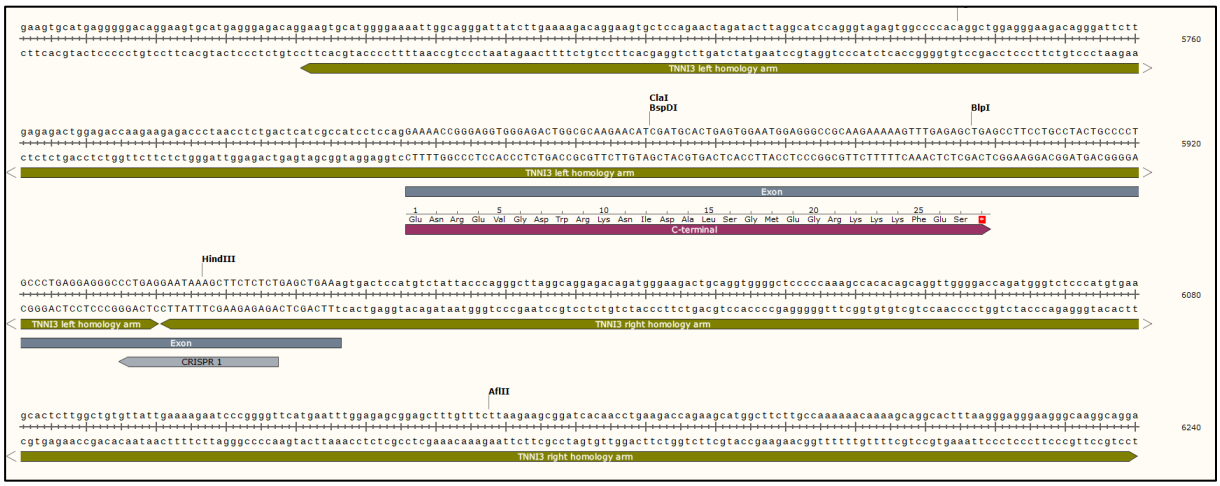
One TNNI3-mCherry reporter and one TNNI3-mCherry/TNNI1-EGFP dual reporter were successfully established in the BIONi010-C wt iPSC line using CRISPR-Cas9 technology. The cells were banked and QC'ed in detail and the QC results were all normal. 10 vials of each of the iPSC lines were shipped to Pfizer Inc. Primary Pharmacology Group, Groton, CT, USA. Furthermore, 30 vials of BIONi010-C-55 will be shipped and deposited at EBiSC2.

# APPENDIX

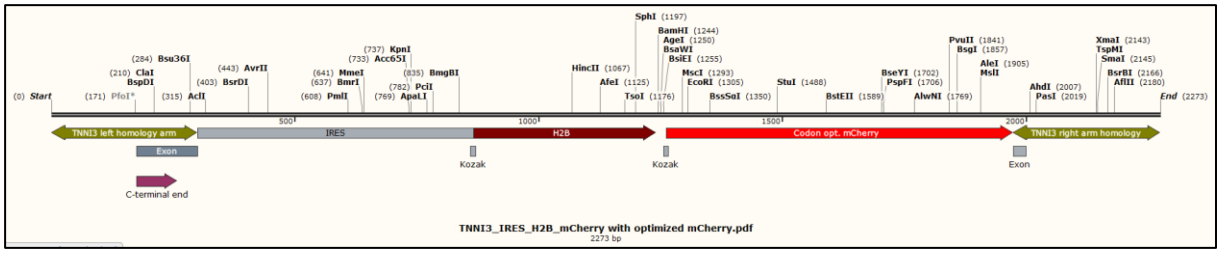
## *Gene editing strategy of TNNI3 and TNNI1*

Alignment of TNNI mRNA with corresponding chromosomal region showing how the CRISPR's are designed to target the last part of Exon 7. Screening and absence primers are also indicated.

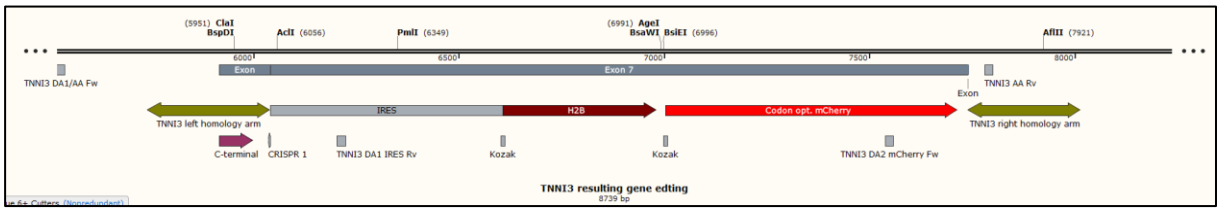
Overview of TNNI3 gene and placement of Crispr in the last Exon 7 after the STOP codon




Overview of TNNI3 homologous donor reporter construct

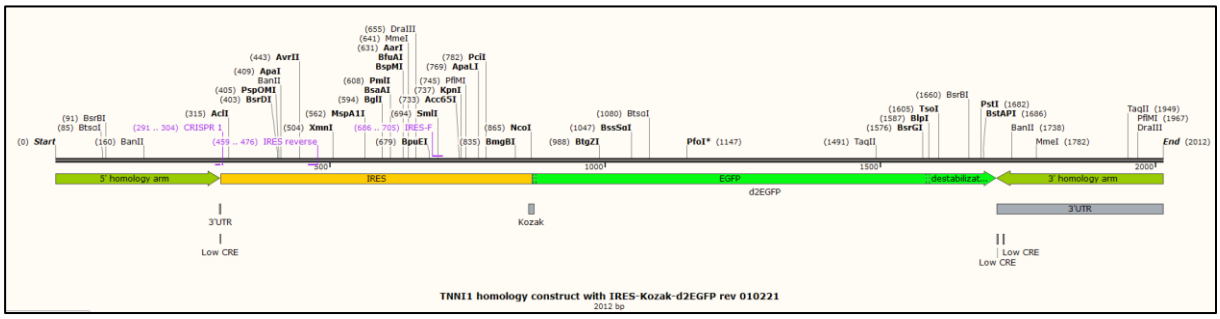



Resulting gene editing of TNNI3

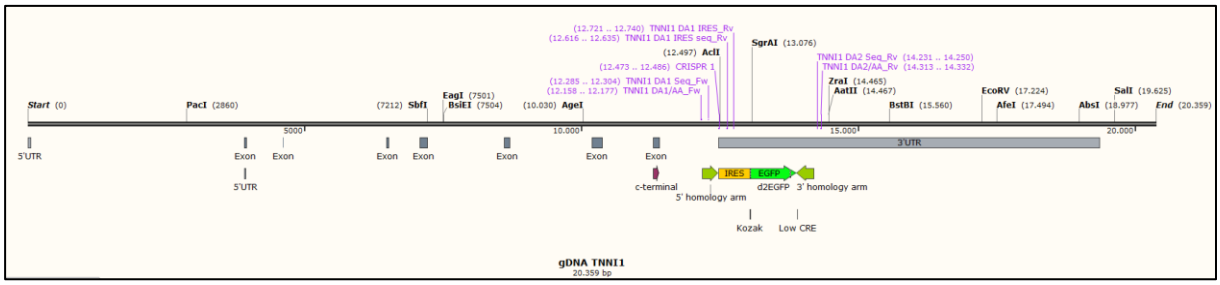


## Overview of TNNI1 gene and placement of Crispr in the 3'UTR


## Overview of TNNI1 homologous donor reporter construct




## Resulting gene editing of TNNI1



***GeneArt reporter plasmids***

# Quality Assurance Documentation

invitrogen
by Thermo Fisher Scientific

| | | | |
|---|---|---|---|
| **ProjectID:** | 2020ABHEWP | **ConstructID:** | 20ACZMJP |
| **Gene Name:** | TNNI3_IRES_H2B_mCher | **Gene Size:** | 2273 bp |
| **Designation:** | E.coli K12 DH10B™ T1R | **Vector Backbone:** | pMK-RQ (KanR) |
| **Manufacturing Date:** | 14 September 2020 | **Quantity:** | ~5 µg Plasmid DNA |

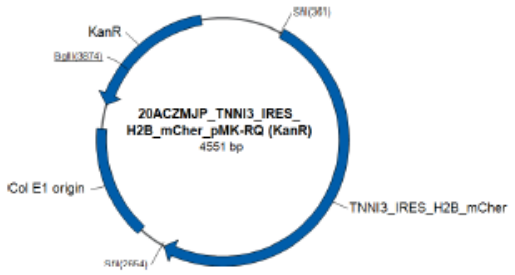
**Product Description:**

The synthetic gene TNNI3_IRES_H2B_mCher was assembled from synthetic oligonucleotides and/or PCR products. The fragment was inserted into pMK-RQ (KanR). The plasmid DNA was purified from transformed bacteria and concentration determined by UV spectroscopy. The final construct was verified by sequencing. The sequence identity within the insertion sites was 100%. 5 µg of the plasmid preparation were vacuum dried for shipping.

**Product Handling:**

The delivered DNA amounts are indicated on the individual tube labels. Centrifuge tubes prior to opening. Do not store vacuum dried DNA for a prolonged time. Add an appropriate amount of distilled water or 10 mM Tris-HCl (pH 8.5) and incubate for 1 hour at room temperature (optionally followed by an overnight incubation at 4°C). Resuspend DNA by gently pipetting up and down a couple of times. If not to be used immediately, resuspended DNA should be stored at -20°C or -80°C. Storing as aliquots helps to reduce unfavorable freeze-thaw cycles. We recommend sequence verification after each subcloning respectively transformation step.

**Plasmid Map:**




**Data Handling:**

If you have subscribed to our convenient service to deliver all Quality Assurance Documentation (QAD) for your GeneArt orders electronically through Thermo Fisher Cloud, please follow this link to access your information: https://apps.thermofisher.com/apps/geneart-qa. Alternatively, you can scan the QR code.
If you have not subscribed to this service, the QAD can be found on the CD included in your shipment. The QAD includes a .gb file containing the full vector information, a .fas file for your insert sequence only and if applicable, your ordered raw sequencing data.

# Quality Assurance Documentation

invitrogen
by Thermo Fisher Scientific

| | | | |
|---|---|---|---|
| **ProjectID:** | 2021AANAOP | **ConstructID:** | 21AAS3AP |
| **Gene Name:** | TNNI1_IRES_d2EGFP | **Gene Size:** | 2012 bp |
| **Designation:** | E.coli K12 OmniMAX™ 2 T1R | **Vector Backbone:** | pMA-T |
| **Manufacturing Date:** | 9 February 2021 | **Quantity:** | ~5 µg Plasmid DNA |

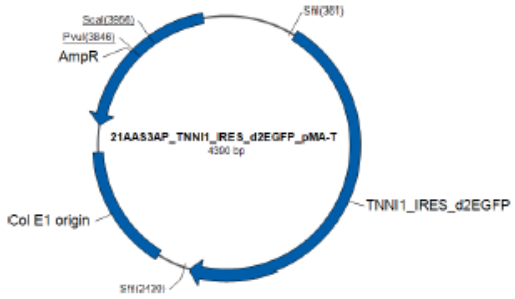
**Product Description:**

The synthetic gene TNNI1_IRES_d2EGFP was assembled from synthetic oligonucleotides and/or PCR products. The fragment was inserted into pMA-T. The plasmid DNA was purified from transformed bacteria and concentration determined by UV spectroscopy. The final construct was verified by sequencing. The sequence identity within the insertion sites was 100%. 5 µg of the plasmid preparation were vacuum dried for shipping.

**Product Handling:**

The delivered DNA amounts are indicated on the individual tube labels. Centrifuge tubes prior to opening. Do not store vacuum dried DNA for a prolonged time. Add an appropriate amount of distilled water or 10 mM Tris-HCl (pH 8.5) and incubate for 1 hour at room temperature (optionally followed by an overnight incubation at 4°C). Resuspend DNA by gently pipetting up and down a couple of times. If not to be used immediately, resuspended DNA should be stored at -20°C or -80°C. Storing as aliquots helps to reduce unfavorable freeze-thaw cycles. We recommend sequence verification after each subcloning respectively transformation step.

**Plasmid Map:**




**Data Handling:**

If you have subscribed to our convenient service to deliver all Quality Assurance Documentation (QAD) for your GeneArt orders electronically through Thermo Fisher Cloud, please follow this link to access your information: https://apps.thermofisher.com/apps/geneart-qa. Alternatively, you can scan the QR code.
If you have not subscribed to this service, the QAD can be found on the CD included in your shipment. The QAD includes a .gb file containing the full vector information, a .fas file for your insert sequence only and if applicable, your ordered raw sequencing data.

***Crispr-Cas9 gene editing protocol***

Mikkel Rasmussen 10.10.2020

**Project: Generation of a dual reporter iPSC line (Pfizer #10514)**

Cells and culture conditions: BIONi010-C P27 iPSC line in mTeSR+/Matrigel without P/S

Day 0: **Cas9 Protein nucleofection:**

1. Place required amount of culture medium (mTesR) at RT and add 1:100 Revitacell.
2. Add 1 ml Matrigel (diluted 1:25 in cold DMEM/F12) pr. 2 wells of a 6-well dish pr. nucleofection. Incubate for min. 30 min at 37°C.
3. Prepare sgRNA's by diluting synthetic sgRNA's to 100µM in duplex buffer (IDT; e.g. 20µl to 2nmol).
4. Mix RNP complex by adding the following in a 1.5 ml Eppendorf tube and incubate at RT for 15 min:
    a. 2 µl sgRNA (100µM) + 2 µl Cas9 protein
5. Accutase cells for 10 min at 37°C. Transfer to 15 ml Falcon tube containing 1:3 culture medium
6. Count and spin down $1x10^6$ cells pr. nucleofection at 120g for 5 min.
7. Suck off medium and resuspend cells gently in 100 µl P3 solution (82 µl P3 buffer and 18 µl supplement) pr. nucleofection.
8. Add the following to the RNP mixes from step 4 just prior to nucleofection:
    a. 5 µl endofree donor plasmid (1 µg/µl)
9. Transfer 100µl cells in P3 solution to the Eppendorf tube with the first RNP mix, resuspend briefly up and down and transfer to tubes containing the RNP complex and further to the nucleofection cuvette without bubbles. Repeat the process for the next reaction/nucleofection.
10. Nucleofect cells using program CA167
11. Immediately add 500 µl RT culture medium (mTeSR) without P/S + 1:100 Revitacell and incubate for 5-10 min at 37°C while preparing culture dishes.
12. Suck off Matrigel from the 6-cm dishe and add 1,5 ml RT culture medium without P/S + 1:100 Revitacell to the upper wells only.
13. Transfer the nucleofected cells to the upper well of the 6-well dish, resuspend gently and transfer 1 ml to the lower wells (1:1 dilution).

Day 1: Change culture medium without Revitacell. Change medium every or every second day.

Day 5: When the cells are around 80% confluent, Accutase treat and split 250.000 cells from the upper well in a new 6-well with 1:100 Revitacell and place in the low O2 incubator for 2-3 days for single cell sorting. Spin down the remaining cells for QC lysis and PCR validation.

When confluent, EDTA split the lower well and freeze in 2 vials as a backup.

Day 7: Perform sorting of single cells into Matrigel-coated 96-well dishes in 50 culture medium + 1:100 P/S containing 1:10 CloneR using the Cytena f.sight dispenser.

Day 14: Change the medium to 200µl culture medium + 1:100 P/S without CloneR. Replace culture medium every 2nd or 3rd day.

D21: When colonies cover a good part of the wells, replica plate the 96-well dish with EDTA. Transfer half of the cells for DNA purification and the rest to a new Matrigel-coated 96-well dish with culture medium + P/S. Spin down the PCR plate and proceed with PCR screening and sequencing in the DNA lab to detect gene edited clones.

D28: When positive clones are identified by PCR and sequencing, EDTA treat and transfer individual clones to a well in a Matrigel 6-well dish for subsequent expansion and banking.

***Karyotype reports***

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Dr. rer. nat. Benjamin Schmid
Universität Kopenhagen
Bioneer A/S
Kogle Alle 2
DK-2870 Horsholm



**Report of chromosome analysis**

| | | | |
|---|---|---|---|
| **Patient:** | **ZELLSEDIMENT, Bioneer** | **date of report:** | **26.01.2021** |
| **case number** (file number): | **SEDI-210006** (20202836) | | |
| **specimen received:** | fixated iPSC | **arrival date:** | 21.01.2021 |
| **description:** | Zelllinien-Charakterisierung - TNNI3 cl84-4 P10 14-01-2021 bms, P10, m, 10551, BMS, 20.01.21 | | |

Dear Dear Dr. Schmid,

we got the above mentioned fixated cell suspension for chromosome analysis. After preparation of the fixated cells, banding and evaluation we found the following

**Karyotype (ISCN 2016):** **46,XY**
**conclusion:** **normal male karyotype**

**Humangenetic interpretation:**

**Chromosome analysis revealed a normal male karyotype.**
Up to the reached banding quality there is no hint for a numerical or structural abnormality (variable single cell abnormalities will not be reported).

| | |
|---|---|
| counted metaphases | 16 |
| structurally evaluated | 7 |
| banding quality | 350 |
| type of banding | GTG |
| structural variant | none |

**Limitations:**
Very small aberrations (e.g. microdeletions) as well as low level mosaicism might not be detected. Molecular genetic changes (DNA level) can not be diagnosed by karyotyping.

**General directions:**
Please find more information concerning cytogenetic analyses, indication, withdrawing of blood or other probes and shipment/mailing in www.uni-tuebingen.de/Klinische_Genetik/de/d-cytogenetik.html.
The use of theses results for scientific or other reasons needs authorization of our institute.

Thank you very much for sending the sample. For today we remain with kindest regards
With kind regards,

Prof. Dr. med. O. Rieß
Facharzt für Humangenetik
Institutsdirektor

Dr. med. U. Mau-Holzmann
Fachärztin für Humangenetik
Oberärztin Cytogenetik

Cytogenetische Diagnostik: Tel.: +49 7071 29-72304 oder -72305 | Fax: +49 7071 29-4433

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## Report of chromosome analysis

| | | | |
|---|---|---|---|
| **Patient:** | **ZELLSEDIMENT, Bioneer** | **date of report:** | **14.06.2021** |
| **case number** (file number): | **SEDI-210059** (20202836) | | |
| **specimen received:** | fixated iPSC | **arrival date:** | 28.05.2021 |
| **description:** | Zelllinien-Charakterisierung - BIONi010-C-55 iPSC P18 Karyo 20.05.2021 RGS, BIONi010-C-55 iPSC Karyo 20.05.2021 RGS, P18, m, 10551, RGS, 26.05.2021 | | |

Dear Dear Dr. Schmid,
we got the above mentioned fixated cell suspension for chromosome analysis. After preparation of the fixated cells, banding and evaluation we found the following

**Karyotype (ISCN 2020):** **46,XY**
**conclusion:** **normal male karyotype**

**Humangenetic interpretation:**

**Chromosome analysis revealed a normal male karyotype.**
Up to the reached banding quality there is no hint for a numerical or structural abnormality (variable single cell abnormalities will not be reported).

| | |
|---|---|
| counted metaphases | 15 |
| structurally evaluated | 6 |
| banding quality | 350 |
| type of banding | GTG |
| structural variant | none |

**Limitations:**
Very small aberrations (e.g. microdeletions) as well as low level mosaicism might not be detected. Molecular genetic changes (DNA level) can not be diagnosed by karyotyping.

**General directions:**
Please find more information concerning cytogenetic analyses, indication, withdrawing of blood or other probes and shipment/mailing in www.uni-tuebingen.de/Klinische_Genetik/de/d-cytogenetik.html.
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