[Protocol 4.01] Testing for remaining Sendai virus_CytoTune2.0

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No tags associated

Created: 08.12.2021 11:01
Last modified: 12.12.2023 09:48

No custom dates added

Protocol 4.01Testing for remaining Sendai virus_CytoTune2.0
Version: 1.0 (12.12.2023)

Media and Reagents:
RNeasy Plus Mini Kit (Qiagen, 74134)
SuperScript III First-Strand Synthesis System
(Thermo Fisher,18080051)
Taq polymerase (Thermo Fisher, 10342020)
100 mMdNTP Set (Thermo Fisher,10297-018)
Agarose (Thermo Fisher,15510-027)
GelRed (Biotium, 41003)
10x BlueJuice Gel Loading Buffer (Thermo Fisher,10816015)
100 bp Ladder (Thermo Fisher,15628-019)

Materials and Equipment:
0,2 ml PCR tubes
1,5 ml tubes
PCR Cycler
Electrophoresis chamber
Nanodrop photometer

1. Introduction and Purpose

This protocol describes a RT-PCR assay to test for remaining Sendai virus genome and transgenes in iPSC cultures after reprogramming with CytoTune-iPS 2.0 Reprogramming Kit (Thermo Fisher, A16517).

2. RNA isolation and reverse transcriptase
1. Isolate RNA using the RNeasy Plus Mini Kit following manufactures instructions. Isolate RNA from two separate wells of a six well plate using separate columns for each wells.
2. Determine the amount and quality of RNA using a Nanodrop photometer.
3. Use 1 µg RNA with SuperScript III First-Strand Synthesis System for reverse transcription following the manufacturer's protocol (The half amount of the recommended protocol is sufficient).
4. Dilute cDNA after reverse transcription 1:10 (20 µl + 180 µl) with water.

3. Procedure Sendai Virus PCR
1. The primer pairs for CytoTune Sendai virus detection and for a control housekeeping gene (Hu18SRNA) are listed in the table below.

Target	Internal primer name	Primer sequence	T_m ° C	Product size [bp]
SeV	SeV_F	GGA TCA CTA GGT GAT ATC GAG C*	55	181
	SeV_R	ACC AGA CAA GAG TTT AAG AGA TAT GTA TC*	55	
Klf4	SeV_Klf4_F	TTC CTG CAT GCC AGA GGA GCC C	55	410
	SeV_Klf4_R	AAT GTA TCG AAG GTG CTC AA*	55	
cMyc	SeV_cMyc_F	TAA CTG ACT AGC AGG CTT GTC G*	55	532
	SeV_cMyc_F	TCC ACA TAC AGT CCT GGA TGA TGA TG	55	
KOS	SeV_KOS_F	ATG CAC CGC TAC GAG TGA GCG C	55	528
	SeV_KOS_R	ACC TTG ACA ATC CTG ATG TGG	55	
Hu18SRNA	Hu18SRNA_F	GTA ACC CGT TGA ACC CCA TT	50	151
	Hu18SRNA_R	CCA TCC AAT CGG TAG TAG CG	50	

*Primer contains SeV genome sequences. Pairing of these primers with transgene-specific primers allows specific detection of transgenes carried by the CytoTune Sendai reprogramming vectors.

2. Use 2 µL of diluted cDNA for Sendai Virus PCR.
3. PCR is set up as follows:

PCR ingredient	µl per reaction
10x PCR buffer	2
dNTP-Mix (10 mM each)	0.8
50mM MgCl2	0.6
Forward primer (100 pmol /µl)	0.5
Reverse primer(100 pmol /µl)	0.5
Water	13.5
Taq polymerase (5 U/µl)	0.1
diluted cDNA	2
total	**20 µl**

Note:
Negative control 2 µl of diluted cDNA from a control PSC line not derived using Sendai virus
Positive control 2 µl of diluted cDNA from generated iPS (passage 2 or 3) after reprogramming

4. Close and spin all PCR tubes briefly, load the PCR cycler and start the program.

Cycler program:

1 cycle	94 °C	3 min
35 cycles	95 °C	30 sec
	55 °C	30 sec
	72 °C	30 sec
1 cycle	72 °C	10 min
	4 °C	∞

5. Cast a 2 % standard agarose gel with DNA stain GelRed 5µl/100 ml.
6. Pipette 2 µl of 10x BlueJuice to each PCR reaction. Use 10 µl of PCR reaction for gel.
7. Use 1 µl of 100 bp Ladder mixed with 1 µl of 10x BlueJuice and 8 µl of water as size and running control.
8. Run agarose gel at 80 -100 V for 20 - 40 minutes.

Relevant applicable documents:
Manual CytoTune-iPS 2.0 Reprogramming Kit (Thermo Fisher, A16517)
Manual SuperScript III First-Strand Synthesis System (Thermo Fisher,18080051)
Manual RNeasy Plus Mini Kit (Qiagen, 74134)