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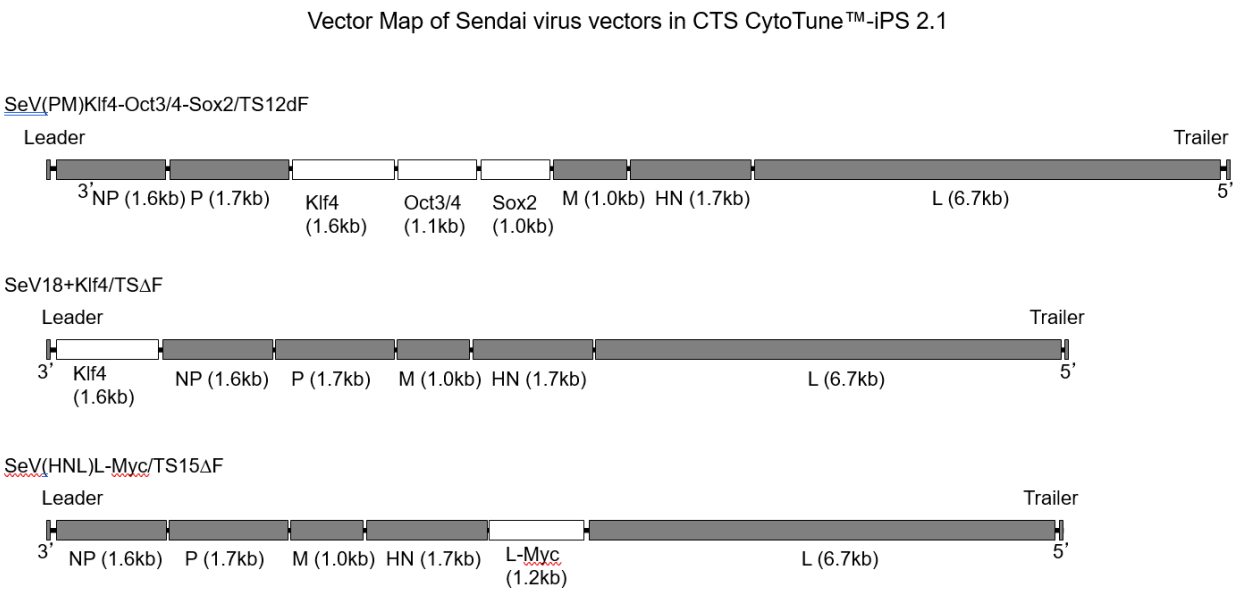
CTS CytoTune-iPS 2.1 Reprogramming Kit (A34546)

Sendai vectors are generated in African Green Monkey Kidney (*Macaca mulatta*) cells stably transfected with a plasmid containing the F (Fusion) [packaging] protein under the control of a Cre/*loxP* inducible expression system.

The country of origin for the packaging cell line is Japan and the original LLC-MK2 cells are of US origin. Cells are cultured in media containing 5% FBS. Viral vectors are purified away from the cells via chromatography, filtered, and diluted. The material does not come from a facility where work with exotic viruses affecting livestock or avian species is conducted. The material is recombinant but contains no genes and expresses no products of exotic livestock or poultry disease agents.

The parent vector is the Delta F Sendai virus vector. The F(usion) gene on the genome RNA is deleted so that infected cells produce no infectious viral particles[2].

Plasmid map – CTS CytoTune™ 2.1 Reprogramming Kit



- KOS is on the TS12 backbone (https://pubmed.ncbi.nlm.nih.gov/25479600/) which yields temperature sensitive Cytotune virus
- L-Myc is on the TS15 backbone (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3161531/) which yields temperature sensitive Cytotune virus
- Klf4 is on the TS backbone (same reference as for TS12), which does not have the mutations which respond to heat treatment.

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What are the regulatory sequences?

There are no promoters per se in the Sendai virus vectors. Transcription and translation of the viral genome is driven by viral RNA polymerases that are coded for by the virus itself (the "P" and "L" genes).

Product Vectors

Vector Name	Major elements	Origin species	Function (very short)
All CytoTune™ (Sendai viral) vectors	Nucleocapsid protein (NP)	Murine respirovirus	Forms the core nucleocapsid complex with the genome RNA.
	Phosphoprotein (P)	Murine respirovirus	Small subunit of the RNA polymerase.
	Matrix protein (M)	Murine respirovirus	Supports the envelope structure from the inside.
	Hemagglutinin-Neuraminidase (HN)	Murine respirovirus	Recognizes the cell surface receptor sialic acid.
	Large protein (L)	Murine respirovirus	Large subunit of RNA polymerase.
CytoTune™ KOS	hKlf4	Human	Transcription factors required for somatic cell reprogramming
	Oct4	Human	
	Sox2	Human	
CytoTune™ hKlf4	hKlf4	Human	
CytoTune™ hL-Myc	hL-Myc	Human	

References:

1. Baudino *et al.* (2002) *Genes & Development* **16**: 2530-2543; c-Myc is essential for vasculogenesis and angiogenesis during development and tumor progression
2. Li Ho *et al J. Virol.* (2000) **74**: 6564-9.
3. Nichols *et al. Cell* (1998) **95**:379-391; Formation of pluripotent stem cells in the mammalian embryo depends on the POU Transcription Factor 4
4. Takahashi and Yamanaka (2006) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **126:**663-673
5. Takahashi *et al.* (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell **13**:861-872
6. Zomer *et. al.* (2015) Stem Cells and Cloning: Advances and Applications **8**:125-135

We do not test Cytotune lot numbers for replication-deficiency. Therefore, it is not listed on the CoA. The main purpose of the CoA is to measure the titer.

Is homologous recombination in target cells that could reverse the ΔF defect possible?
End users want to evaluate if the production of replication-defective viral particles excludes replication-competent sendai viruses in transduced target cells.

Answer: In the production of the Cytotune (Sendai) virus particles, an SeV genome with the deleted fusion protein is used (ΔF). The deleted F-gene (*ΔF*) cannot be restored by homologous recombination in transduced target cells. The Li et al. 2000 paper is a key paper for Cytotune technology: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC112166/#B14 .

It states in section "**Construction of a packaging cell line that expresses SeV F protein" as follows:** SeV F protein is required for the formation of infectious SeV particles. Therefore, recovery of SeV from the RNA genome lacking F gene must be complemented with this gene in trans. We therefore constructed an F-expressing packaging LLC-MK2 cell line with a Cre/*loxP*-inducible expression system, see below too.