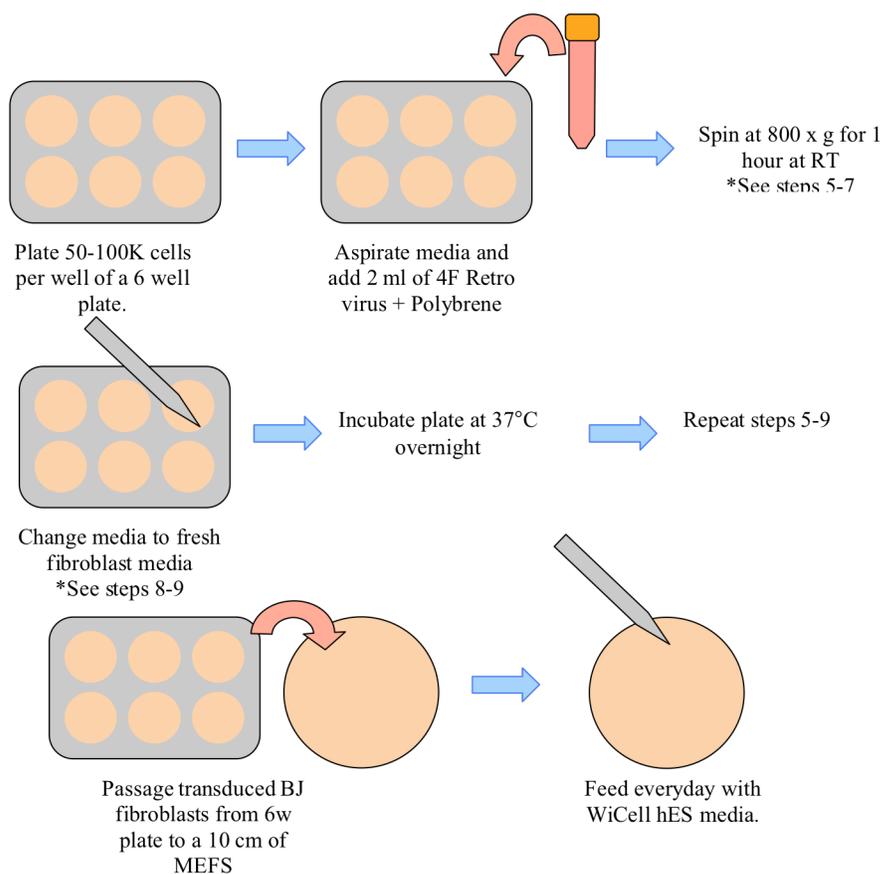


General Spinflection Protocol	Prepared by: Berggren, Travis tberggren@salk.edu
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Adapted from	Salk STEM Cell Core in-house protocols
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Introduction:

This protocol was developed in the Salk STEM Cell Core to enable researchers to consistently and reproducibly produce reprogrammed cells. It assumes that the four factor retrovirus being used has a high titer and that the BJ Fibroblasts being used are growing robustly. After transduction, colonies should start to appear within 3 to 5 weeks.

Flow Chart:



Protocol Steps:

Prepare Plate For Spinflection:

1. Prepare four factor (Oct4, Sox2, Klf4, c-myc) retrovirus to transduce fibroblasts. Note: This can be made months ahead of time and stored in the -80C freezer.

2. Add Polybrene to the four factor retrovirus mixture. Note: Final concentration of Polybrene is 4-8 ug/ml.
3. Thaw retrovirus and mix all four factors together in equal amounts. Note: Use non-concentrated virus. Mix 0.5ml of each retrovirus factor together for a total of 2mls.
4. Plate 1 or more wells of a 6-well plate with an actively growing population of BJ fibroblasts. Note: The seeding density of the well to be transduced should be between 50,000 to a 100,000 cells per well (of a 6-well). *

Perform Spinfection:

5. Aspirate the fibroblast media from the 6-well plate of BJ fibroblasts.
6. Add 2 mls of warmed four factor retrovirus + Polybrene to each well to be reprogrammed.
7. Perform the spinfection at 800 xg (or 1980 rpm) for 1 hour at room temperature.
8. After the spin completes, aspirate the retrovirus media and add 2mls fresh fibroblast media.
9. Allow the cells to recover for at least 6 hours (or overnight) in a 37C incubator.
10. Repeat steps 5 through 7.
11. After the final spinfection, allow the cells to recover for 8-24 hours.
12. Passage 1 well of transduced BJ fibroblasts onto 1x10cm plate of mitotically inactivated-MEFs. Notes: Passage the BJ fibroblasts using the enzyme Accutase and plating into fibroblast media. The MEFs are plated at a density of 1.2 million cells per 10cm plate.
13. Alternatively, for a feeder free method, passage 1 well of transduced BJ fibroblasts onto 3 matrigel coated wells. Note: Split the fibroblasts with accutase and plate in fibroblast media. **
14. The next day, switch to feeding with WiCell hES media.
15. Feed plates every day.
16. Colonies will begin to appear within 3 to 5 weeks.

Materials:

Product	Amount Needed	Supplier	Catalog #
Allegra X-15R Centrifuge or equivalent with plate adapter	1	Beckman Coulter	BK392932
4 factor retrovirus	≥ 4 mls	Made in-house	
6 well TC treated plate	1	USA Scientific	CC7682-7506
10 cm TC treated dish	1	USA Scientific	CC7682-3394
Serological Pipets	Numerous	Corning	

Polybrene	2-4 ul of 8 mg/ml stock	Millipore	TR-1003-G
BJ Fibroblasts	1 well of a 6 well	ATCC	CRL-2522
Mitotically-inactivated MEFs	1x10cm dish plated with 1.2 million MEFs	Global Stem	GSC-6001G
Fibroblast Media	See below		
WiCell hES Media	See below		
mTeSR1 (optional)		Stemcell Technologies	05850

Fibroblast Media

Ingredient	Amount (mls)	Supplier	Catalog #
DMEM	90	Life Technologies	11965-118
FBS	10	Gemini or Various Sources	100-106
NEAA	1	Life Technologies	11140050

WiCell hES Media

Ingredient	Amount (mls)	Supplier	Catalog #
DMEM/F12	80	Life Technologies	11330057
Knock-Out Serum Replacement	20	Life Technologies	10828-028
Glutamax	1	Life Technologies	35050061
NEAA	1	Life Technologies	11140050
Beta-Mercaptoethanol 1000x	.001	Life Technologies	21985-023
FGF2 (50ug/ml conc.)	.02	Various sources	

Notes:

*Retroviral integration is dependent on actively dividing cells. Reprogramming efficiencies also have been shown to increase with increased proliferation of the starting cell population. For these reasons it is important to start reprogramming experiments with robust and actively dividing/growing cells. For human fibroblast we typically start with 50,000-100,000 cells per well (of 6 well). We typically transduce the fibroblast a day or two after passaging when the cells are sub-confluent, but are touching or nearly touching each other, and that ample open areas between the cells are still present. An extra well can be plated and counted at the time of infection if desired.

**We have had success using WiCell hES Media or mTeSR1. WiCell hES media (made in-house) is significantly less expensive than commercially purchased mTeSR1. We have had success plating transduced fibroblast onto matrigel coated wells and feeding with WiCell media (no MEFs, no conditioned media). Then, switching to mTeSR1 once colonies start to emerge and picking colonies directly onto matrigel/mTeSR1 conditions for expansion and characterization.

Alternatively (traditional approach) the transduced cells are passaged onto mitotically inactivated MEF feeder cells, and fed every day with WiCell (or similar) hES cell media.

Make fresh media, store at 4 deg C. and use within 2 weeks. Make fresh media as needed.