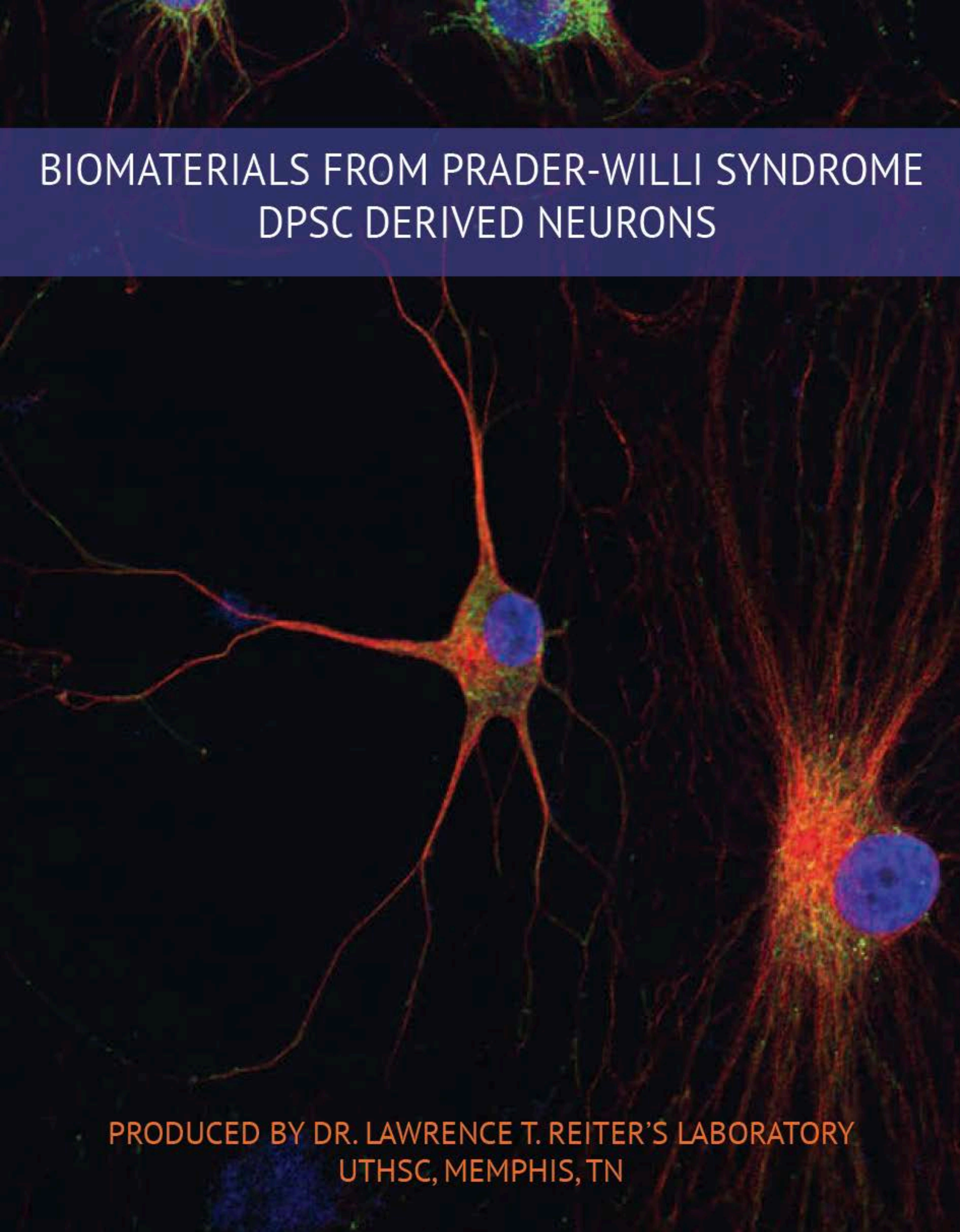
BIOMATERIALS FROM PRADER-WILLI SYNDROME DPSC DERIVED NEURONS



PRODUCED BY DR. LAWRENCE T. REITER'S LABORATORY
UTHSC, MEMPHIS, TN

Contact Information:

Lawrence T. Reiter, PhD
Professor
The University of Tennessee Health Science Center
Department of Neurology
855 Monroe Avenue, Link 415
Memphis, TN 38163

lreiter@uthsc.edu

Office: 901.448.2635

INTRODUCTION

The Reiter Laboratory at the University of Tennessee Health Science Center, with funding from the Foundation of Prader-Willi Research (FPWR), has created a unique collection of biomaterials (RNA and protein) from Prader-Willi syndrome (PWS) dental pulp stem cell (DPSC) derived neurons. This collection includes samples from male and female subjects across PWS genetic subtypes (deletion, uniparental disomy, and imprinting center defects) as well as neurotypical control subjects. In addition to the genetic information collected for each line, we have performed remote autism assessments for each subject. The Social Communication Questionnaires (SCQ) is a validated parent questionnaire, determining the likelihood of autism spectrum disorder (ASD) in an individual. The DPSC cell lines were established from "baby teeth" using the protocol described in Goorha et al. 2017 (1). After the DPSC culture became confluent we differentiated the cells into neurons using a previously published protocol (2).

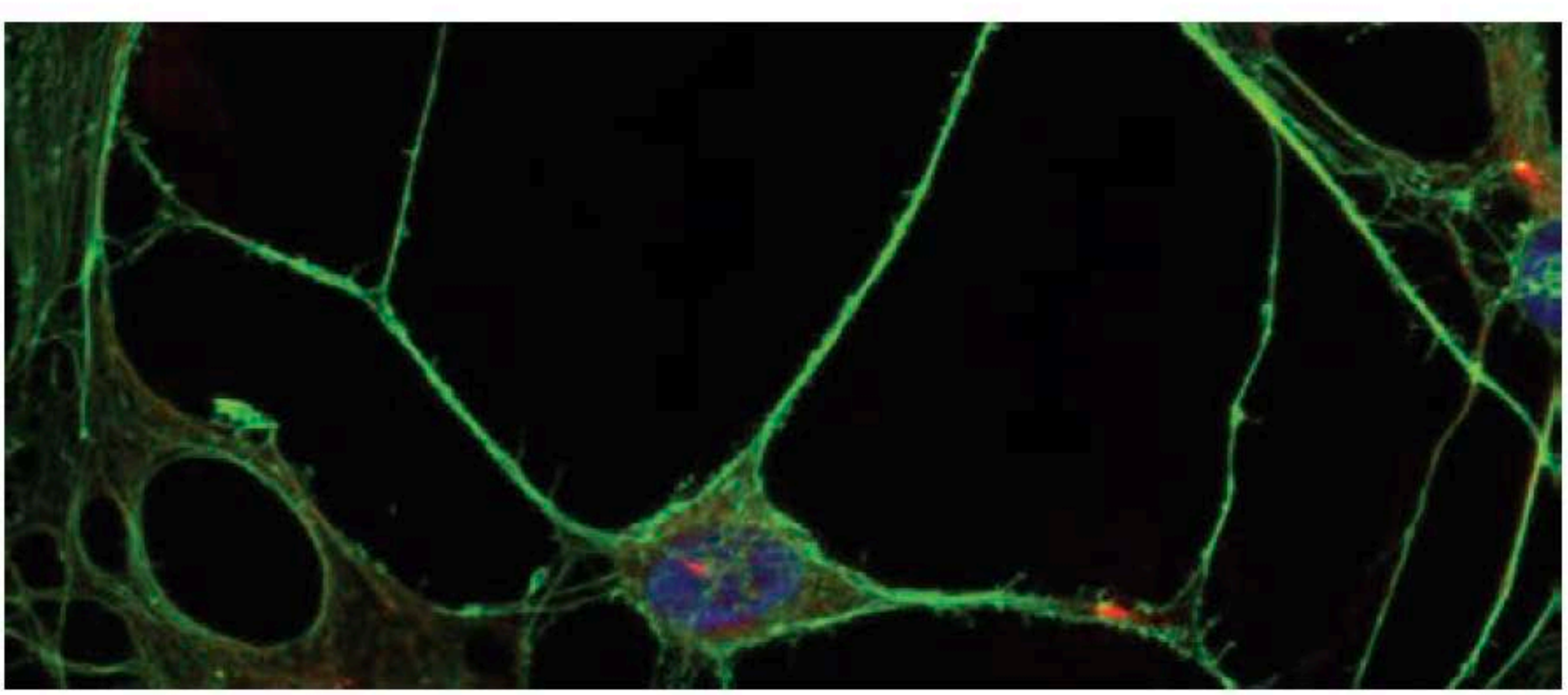
Briefly, the cells are subjected to epigenetic reprogramming using 5-azacytidine treatment. Following the epigenetic reprogramming, neurotrophic factors and PKC and cAMP activators were used to promote the neuronal differentiation. Finally, the cultures were maintained in a neuronal maturation media for 4 weeks. After maturation, the neuronal cultures were pelleted for RNA or protein extractions. Total RNA was isolated using the Zymo Direct-zol RNA Kit (Zymo Research) and protein was extracted using N-PER Neuronal Protein Extraction Reagent (ThermoFisher). RNA and protein were aliquoted and stored at -80 degrees.

1. Goorha, S. and L.T. Reiter, Culturing and Neuronal Differentiation of Human Dental Pulp Stem Cells. Curr Protoc Hum Genet, 2017. 92(21): p. 21 6 1-21 6 10.
2. Kiraly, M., et al., Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons. Neurochemistry International, 2009. 55: p. 323-332.

ACKNOWLEDGEMENT

We ask that the following acknowledgement be included in any publications using this resource:

"DPSC neuronal extracts were provided by the Reiter laboratory through a grant from the Foundation for Prader-Willi Research."



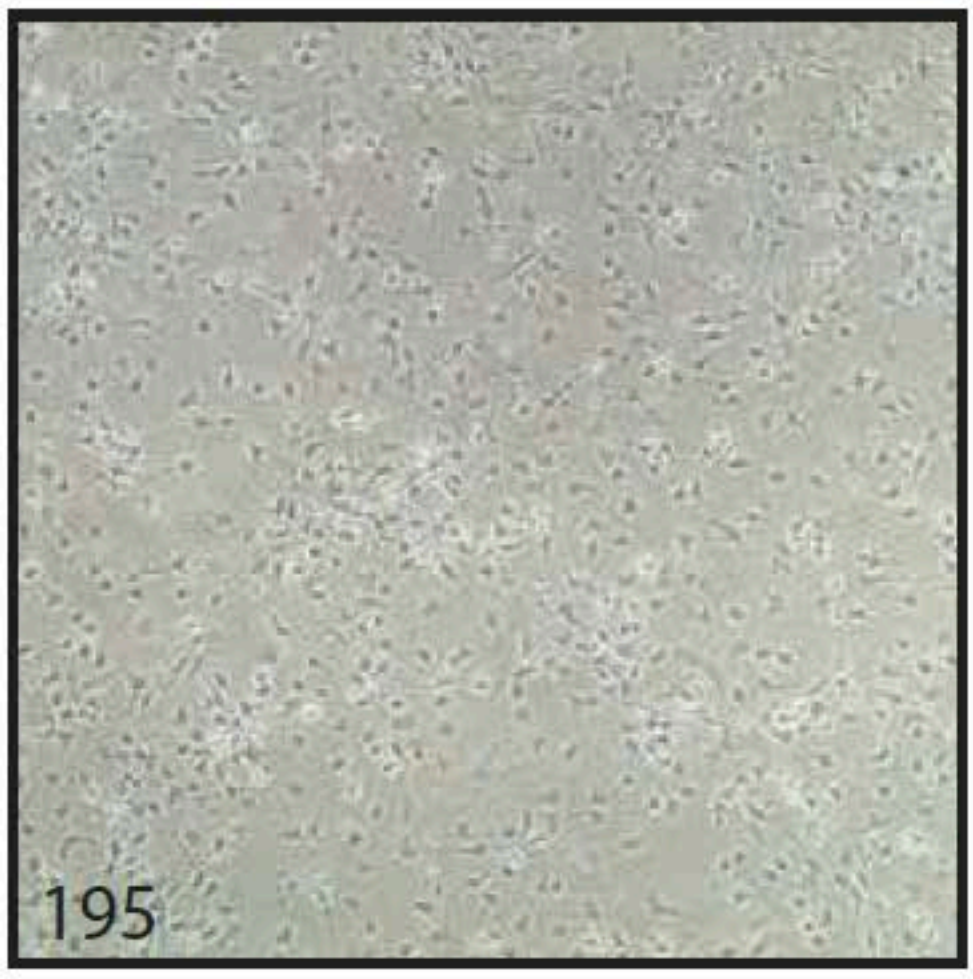
AVAILABLE CELL LINES				
Cell Line	**Diagnosis**	**Sex**	**Age**	**SCQ Score**
195	Neurotypical Control	M	4.4	8
238	Neurotypical Control	M	8.3	0
312	Neurotypical Control	M	8.0	2
182	Neurotypical Control	F	12.8	6
301	Neurotypical Control	F	11.4	2
297	Deletion	M	5.6	9
298	Deletion	M	5.8	10
192	Deletion	F	13.3	11
258	Deletion	F	5.5	7
106	IC Defect*	M	9.8	23
319	IC Defect*	F	5.3	15
191	UPD	M	7.1	6
228	UPD	M	10.2	18
94	UPD	F	5.1	30
162	UPD	F	4.6	7
249	UPD	F	8.1	4
268	UPD	F	6.6	25

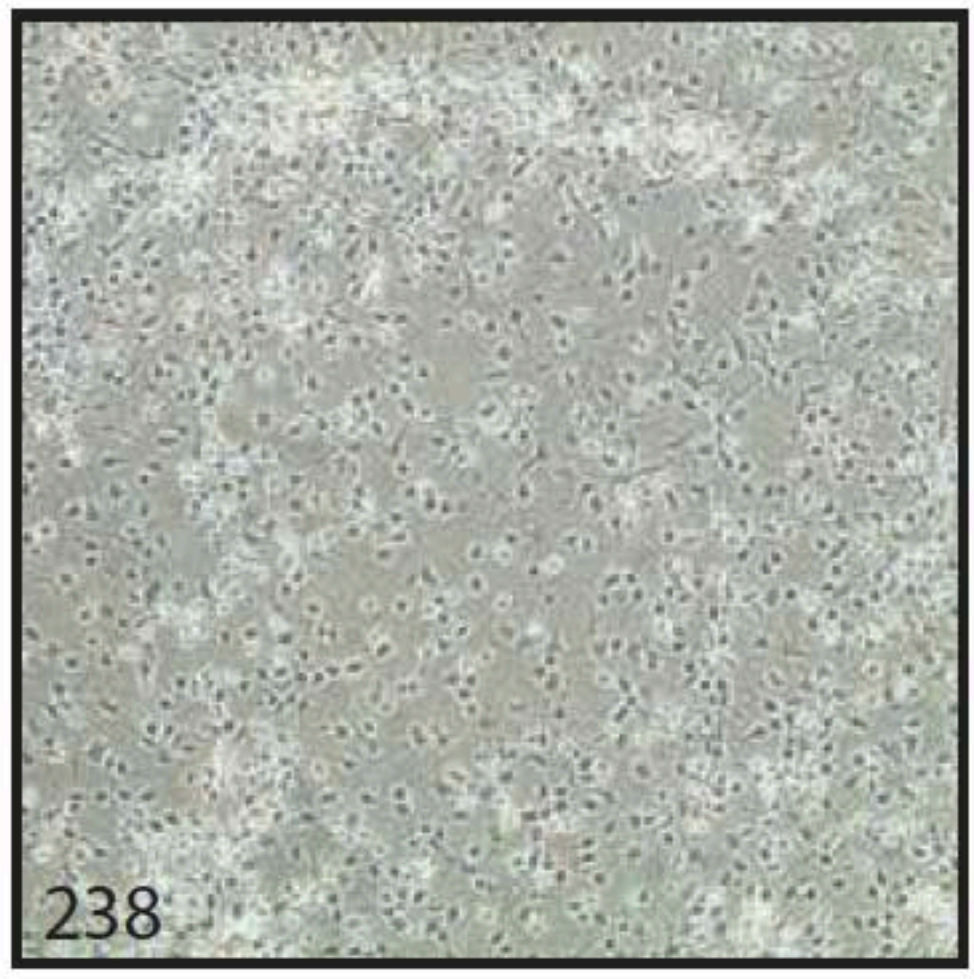
All cell lines are negative for mycoplasm.

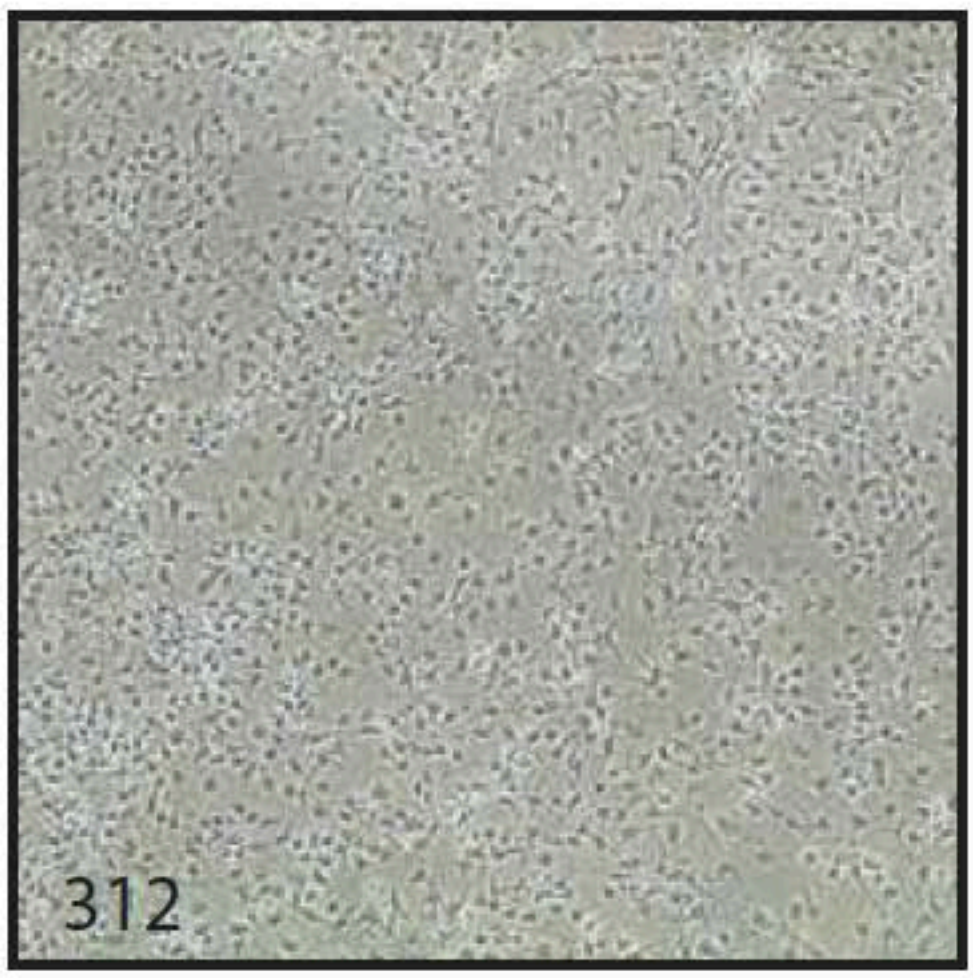
SCQ (Social Communication Questionnaire) score $\geq$15 = possible ASD

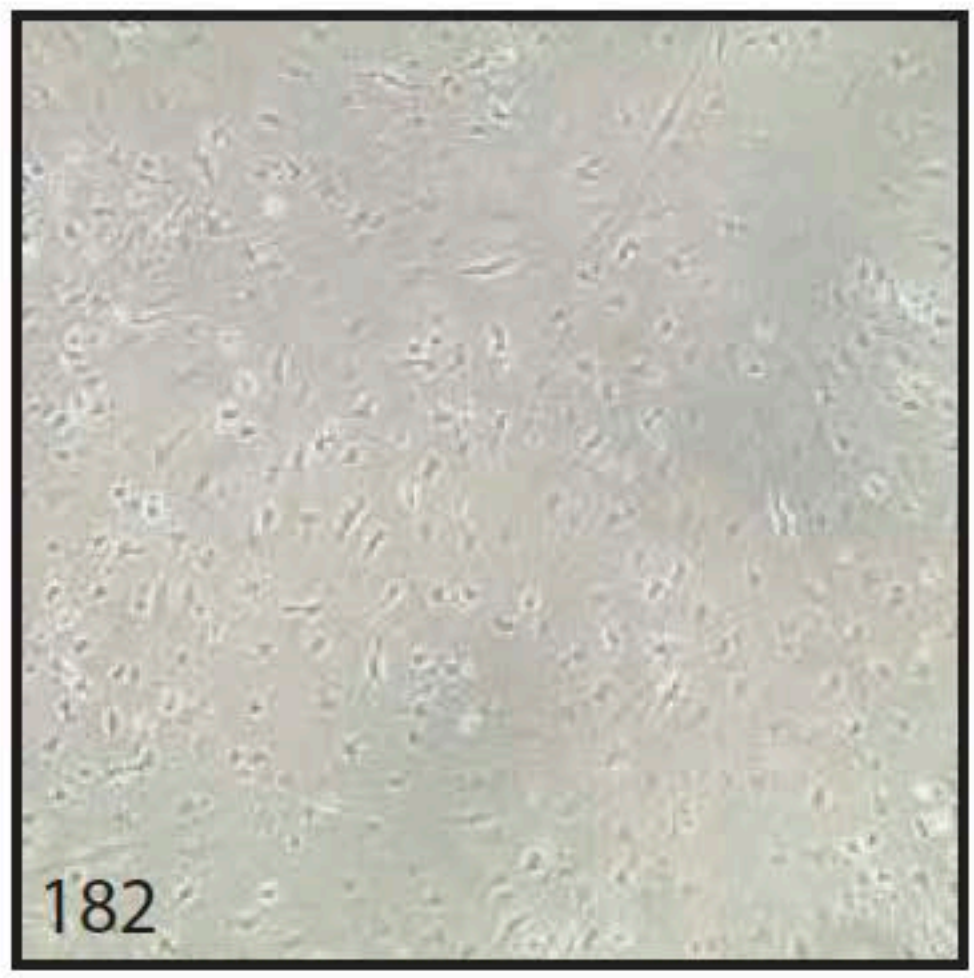
*Deletion and UPD ruled out by FISH and methylation testing

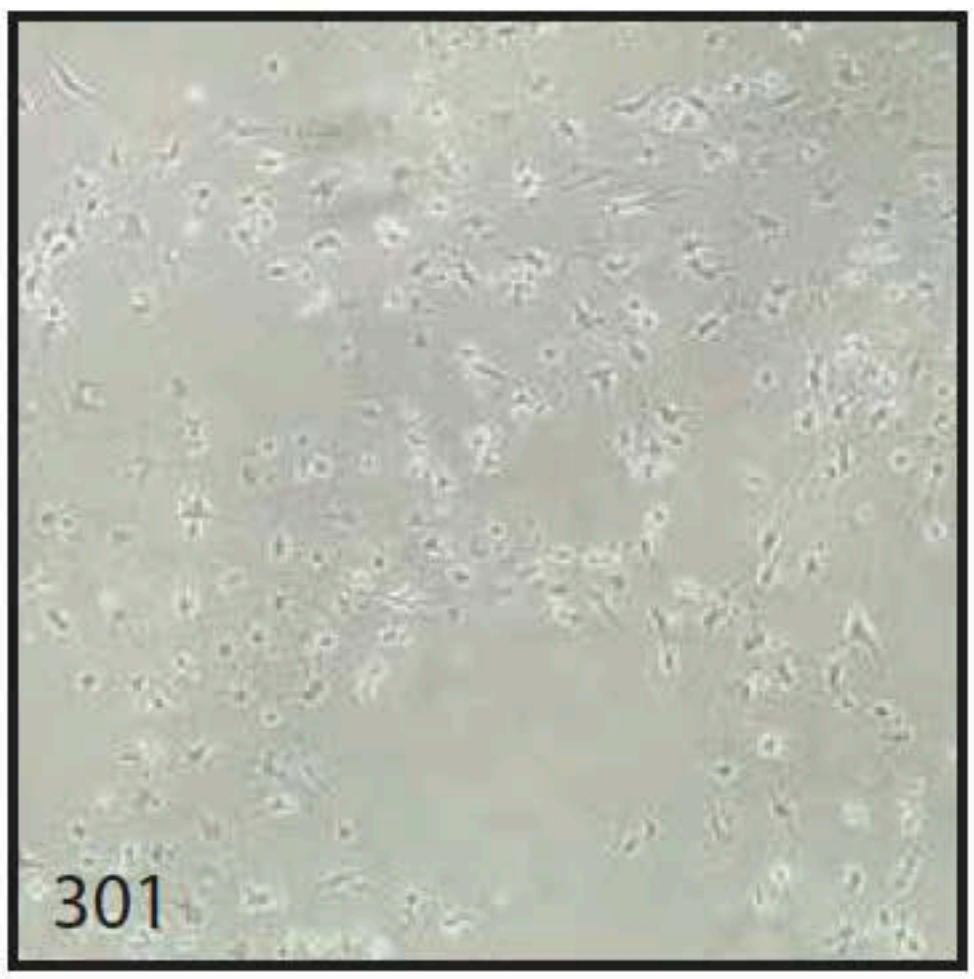
Representative Images of Neuronal Cultures

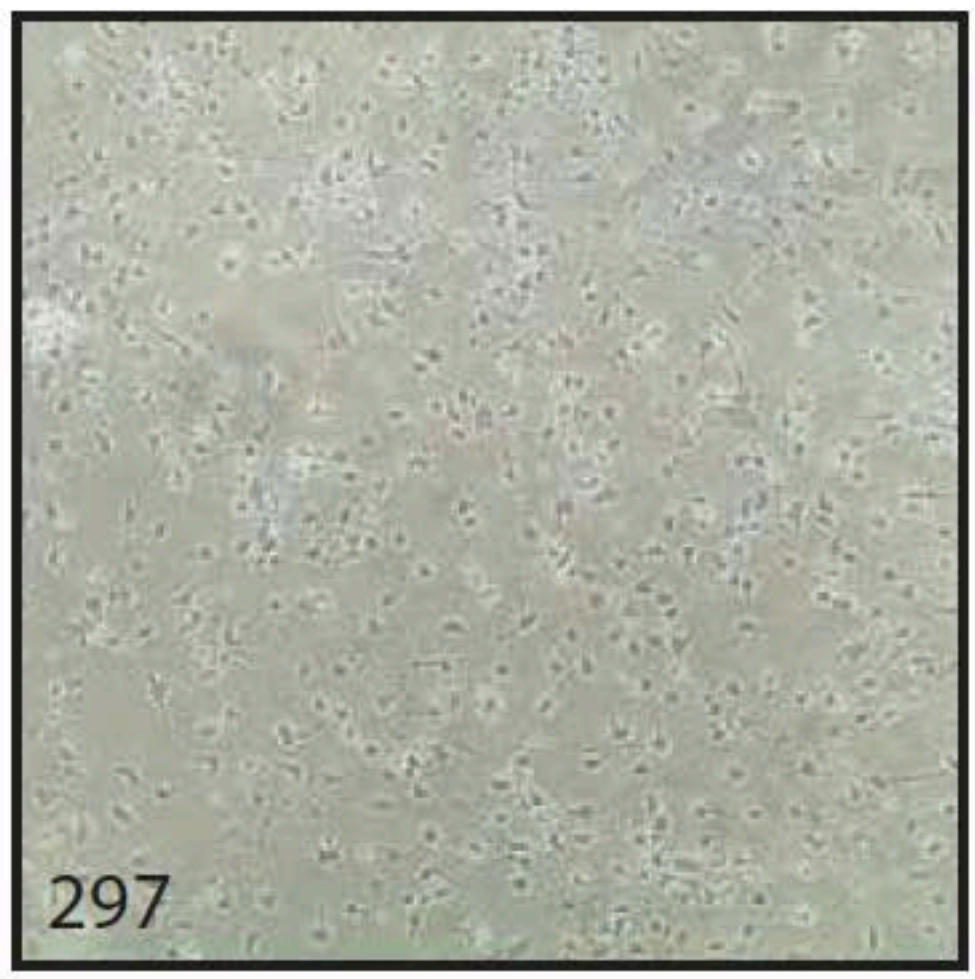


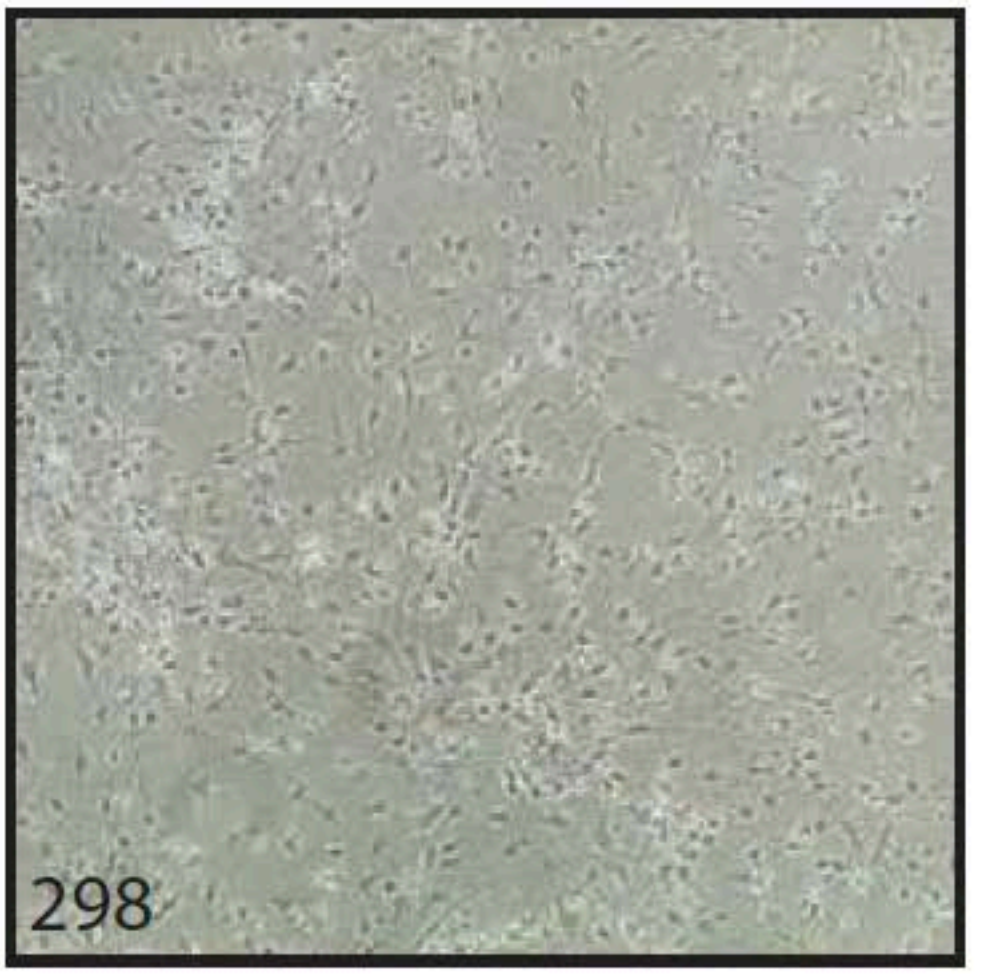








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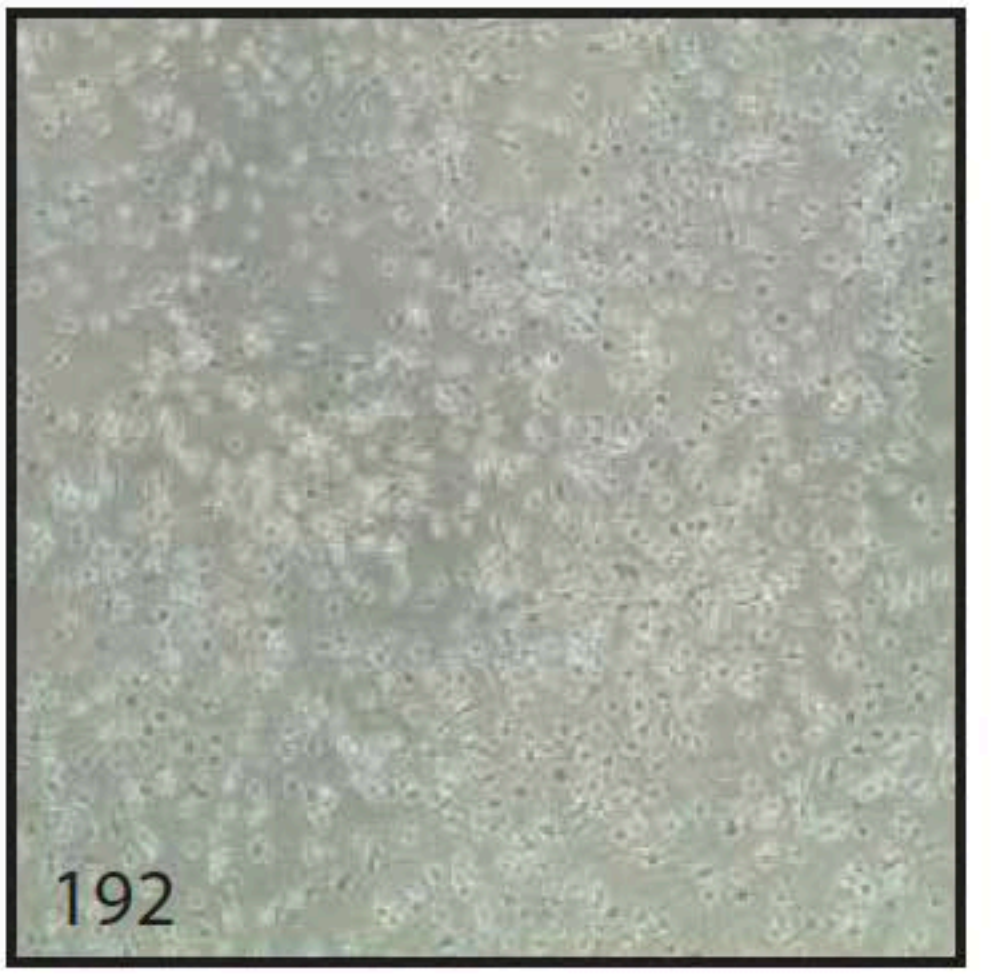
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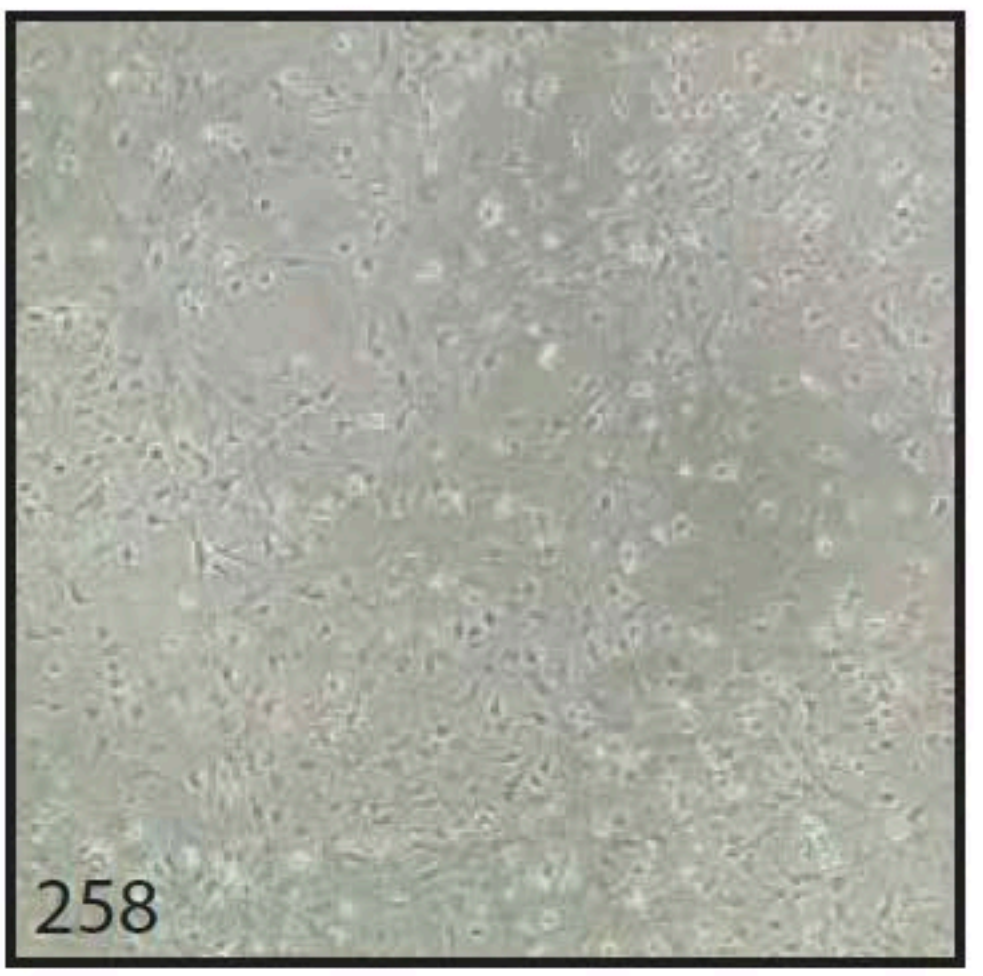
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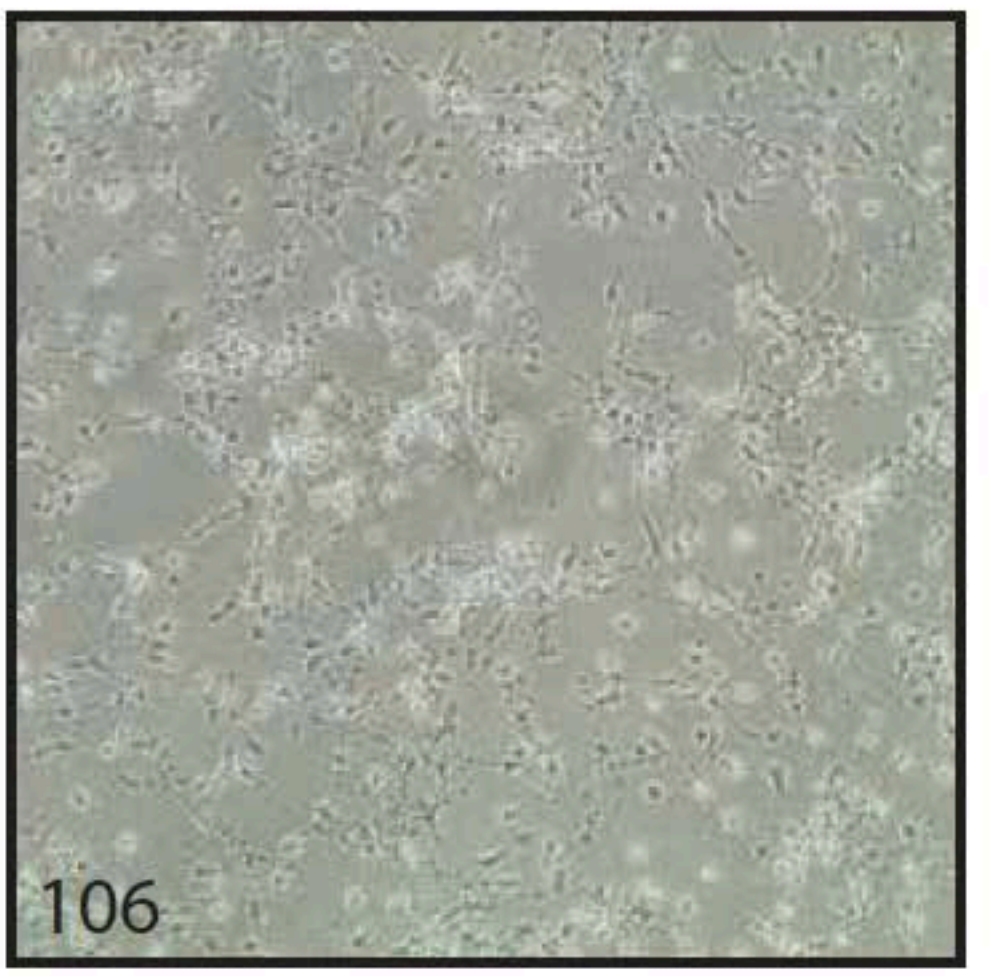
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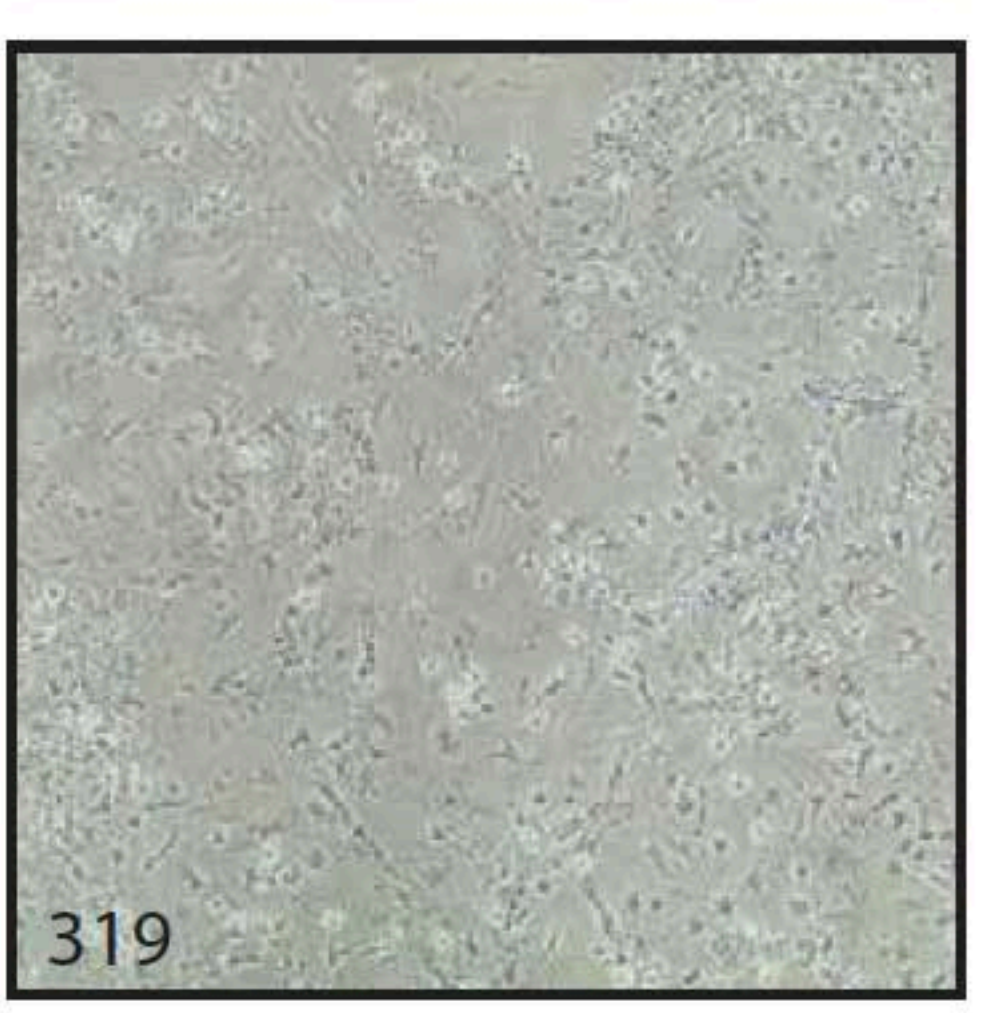
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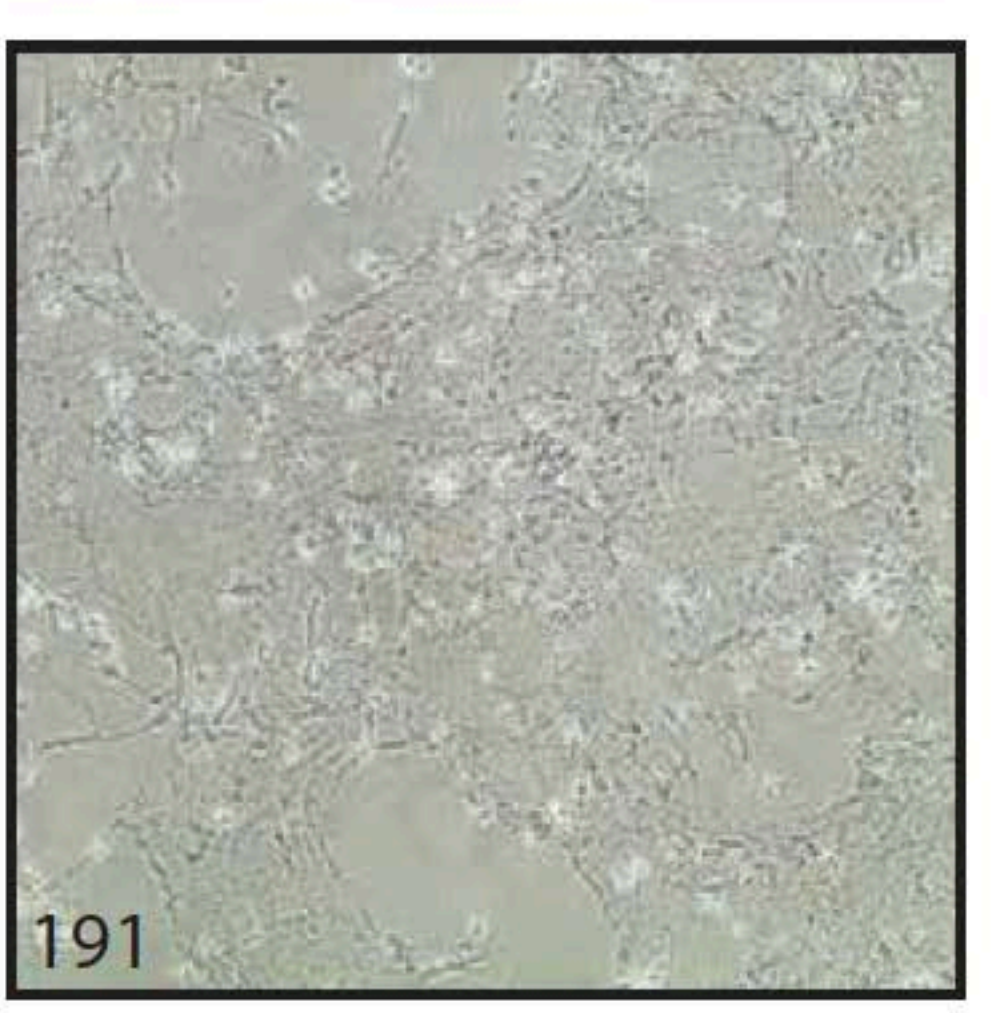
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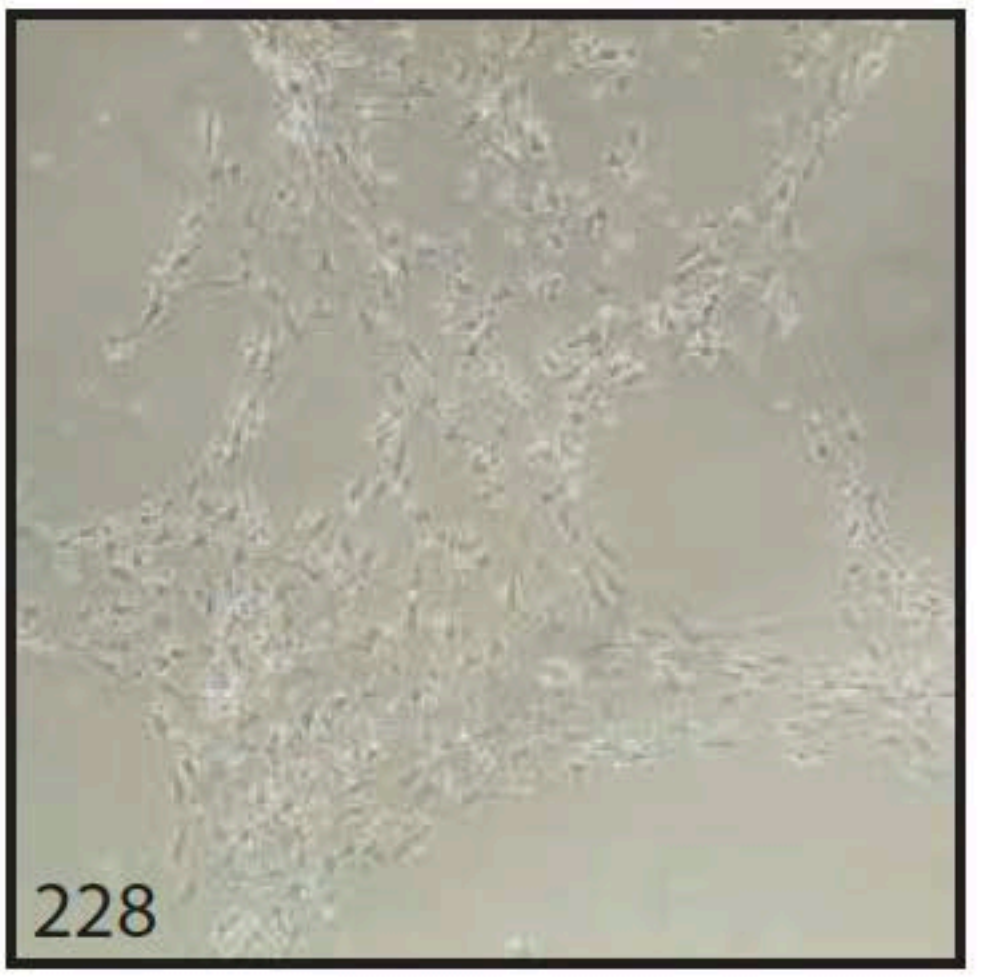
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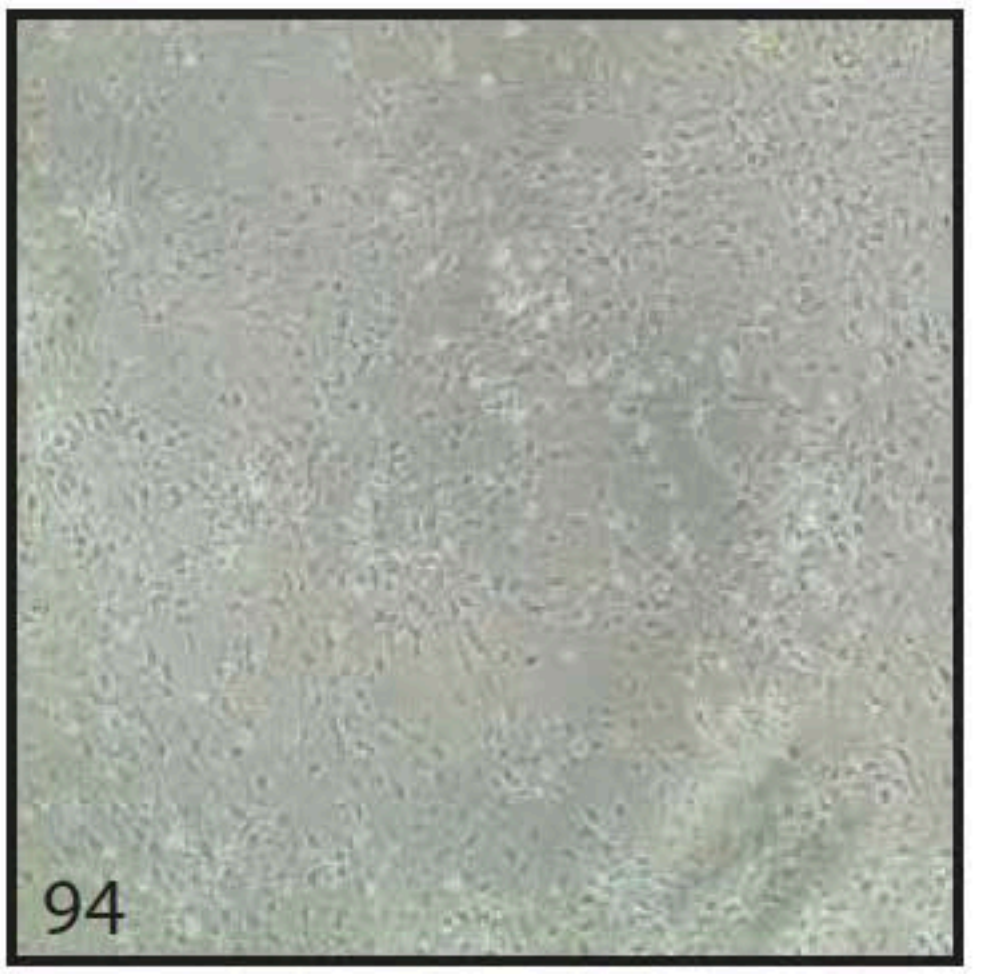
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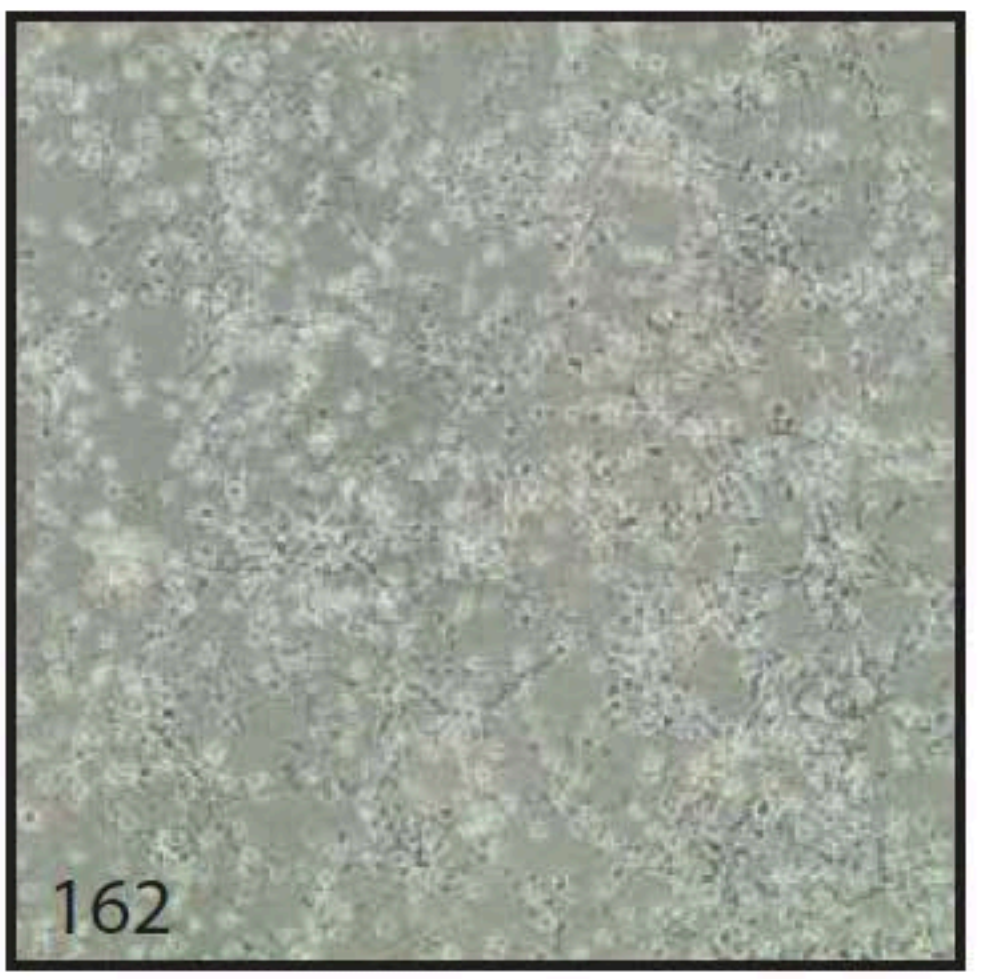
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