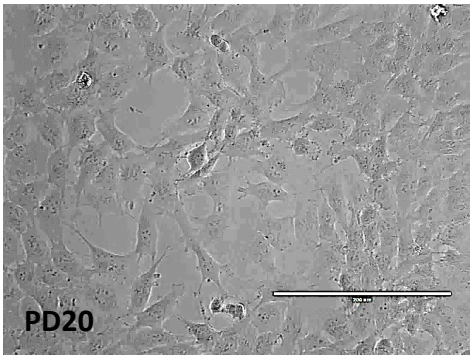
FANCD2 Immortalized primary FA human fibroblasts

PD20 (*FANCD2*$^{-/-}$)
PD20 RV:D2 (*FANCD2*$^{-/-}$ plus RetroViral FANCD2 transgene)

Description

Organism: *Homo sapiens*, human
Tissue: Skin sample donated by FA patient to Oregon Health and Science University.
Synonyms: PD20, FANCD2, FA group D cell line PD20, SGC34603



References

Immortalization and D2 cloning achieved using these procedures:

Immortalization of Four New Fanconi Anemia Fibroblast Cell Lines by an Improved Procedure. P.M. Jakobs, et al., Somatic Cell and Molecular Genetics. 1996

Positional Cloning of a Novel Fanconi Anemia Gene, FANCD2. Cynthia Timmers et al., Molecular Cell. 2001

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Growth media

Alpha MEM (HyClone, SH30265.01), 15% fetal bovine serum (FBS, Hyclone Laboratories, SH30071.03), and 1% penicillin-streptomycin (Gibco #15140122).

Antibiotic Resistance

The PD20 RV:D2 line carries a pMMP-puro expression vector and can be maintained, or intermittently selected, in 1µg/mL puromycin to ensure a pure transgenic culture.

The PD20 carries a G418 (neo) resistance marker residual from complementation assays and not essential to the *FANCD2*$^{-/-}$ phenotype. If you would like to maintain the line in G418, the recommended dose is 500µg/mL.

Cell Line	Puromycin	G418 (neo)
PD20		+
PD20 RV:D2	+	

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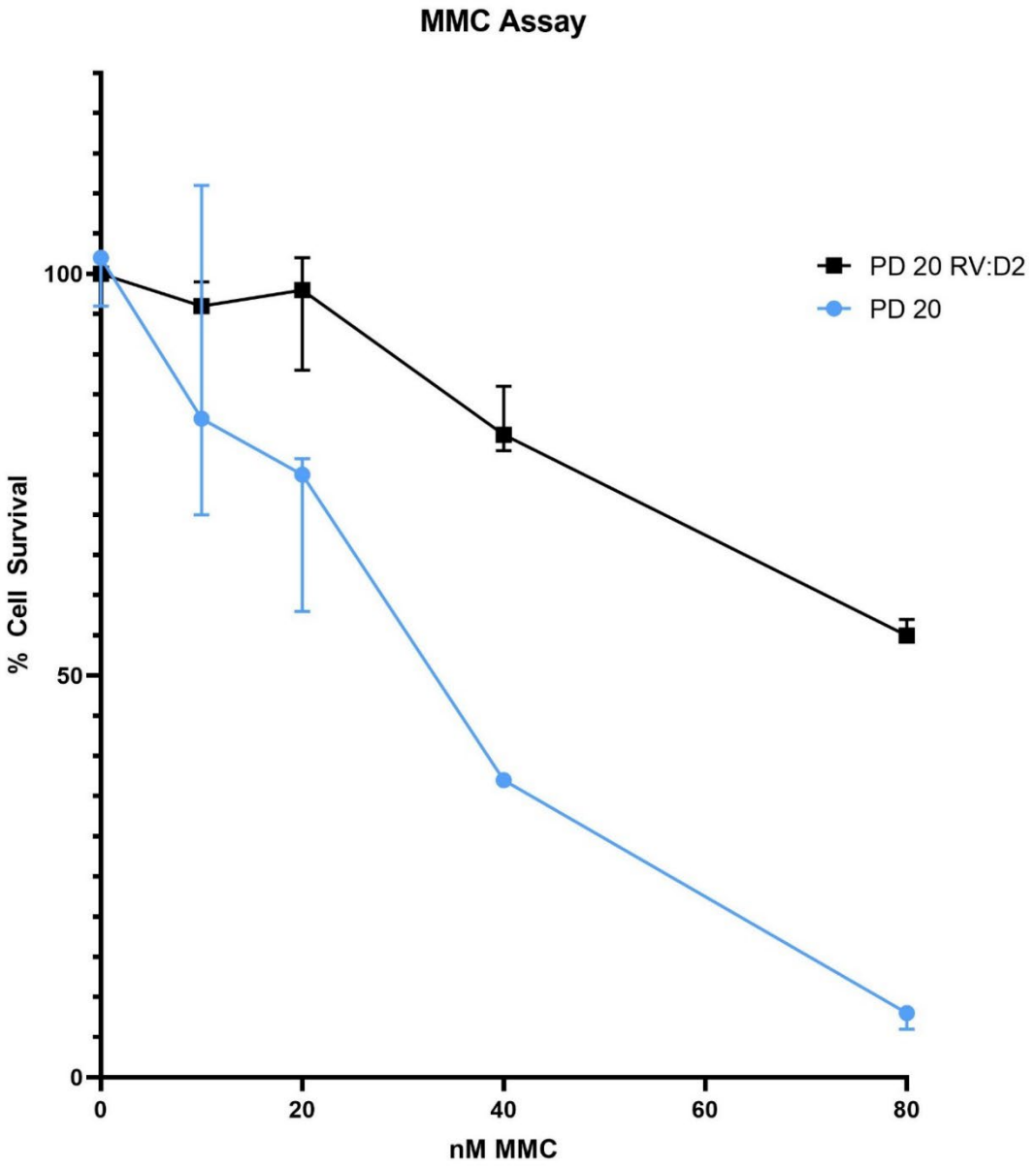
MMC Assay

Protocol: 2,500 cells/well were plated in 48 well plates in 250 ul of complete growth medium in triplicate followed by the addition of a two-fold serial dilution of mitomycin-C (Research Products International CAS# 50-07-7) starting from a final concentration of 80 nM over 4 successive wells. Cells were grown for 4d in the presence of drug without refeeding prior to quantifying cell survival using Cell Counting Kit-8 (Bimake CAT #B34304). Data were normalized versus control wells that had received no MMC.



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Western Blot:

Contributing Researcher: Swarna Beesetti Swarna.Beesetti@STJUDE.ORG
Date: June 2021

Experimental Set-Up:

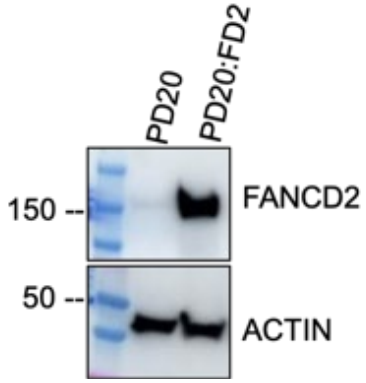
Cell Line: **PD20 RV:D2**
Control(s): PD20
Treatment: PD20 RV: D2 cells maintained in Puromycin; No Specific treatment.
Protein: FANCD2

Western Blot Conditions:

Samples Loaded: 10ug
SDS Page: 4-20% gradient gel
Transfer Condition: Semi dry standard
Detection: Chemiluminescence

	Primary Antibody: **Anti-FANCD2 Antibody (FI17): sc-20022**	Secondary Antibody: **NAME**
Source	Santa Cruz Biotechnology	Mouse (Jackson)
Concentration	200ug/ml	200ug/0.5ml
Dilution	1:2000	1:5000
Final Concentration	0.1ug	10ug
Incubation Temp/Time	4c, overnight	Room temperature, 1hr

Results:



Interpretation:

Confirmed the FANCD2 expression in PD20 RV:D2 cells.

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Quality Control Testing

- Terminal expansion samples were sent July 2021 to IDEXX BioAnalytics (Columbia, MO, USA) and authenticated using the Cell Check 16 Plus service as well as tested for *Mycoplasma* and interspecies contamination from mouse, rat, African green monkey and Chinese hamster.

Cell Line	Mycoplasma sp.	mouse	rat	Human	Chinese Hamster	African Green Monkey
PD20	-	-	-	+	-	-
PD20 RV:D2	-	-	-	+	-	-

- A Human 16 species-specific **STR marker profile** has been established for the cell lines and used for comparative analysis with available published profiles to confirm their unique identity. These genetic profiles can be used for future comparisons of these cell lines.

	IDEXX Case #	**22285-21-01**	**22286-21-01**
	Cell Line ID	**PD20 FANCD2**	**PD20 RV:D2 FANCD2 (Tg)**
X, Y	AMEL	X, Y	X, Y
Chr 5	CSF1PO *	10, 12	10, 11, 12, 13
Chr 13	D13S317	11	11
Chr 16	D16S539	11, 12	11, 12
Chr 18	D18S51 *	12, 20	12, 19, 20
Chr 21	D21S11	29, 30	29, 30
Chr 3	D3S1358	14, 15	14, 15
Chr 5	D5S818	9, 12	9, 12
Chr 7	D7S820	9	9
Chr 8	D8S1179	13, 14	13, 14
Chr 4	FGA	22, 22.2	22, 22.2
Chr 21	Penta_D	12, 13	12, 13
Chr 15	Penta_E *	12, 15	12
Chr 11	TH01	9.3	9.3
Chr 2	TPOX	11	11
Chr 12	vWA	16, 17	16, 17

*Differences in CSF1PO, D18S51, and Penta_E are from somatic mutation, trisomy or duplication events in selected (Tg) clone.

To submit a sample for STR profiling go to https://www.idexxbioanalytics.com/authenticate to get a guide on Cell Line Authentication and https://www.idexxbioanalytics.com/cellcheck to order. Request your samples are compared to "PD20 FANCD2 (IBA# 22285-21-01)" and/or "PD20 RV:D2 FANCD2 (Tg) (IBA# 22286-21-01)".

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