Simple strategies for reducing the risks of cell line contamination

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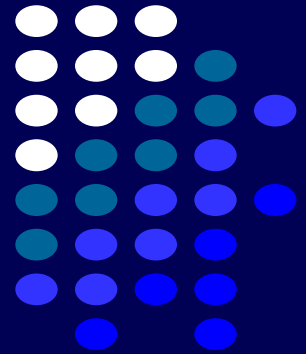

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Cell Lines

Cells from a single source that grow indefinitely

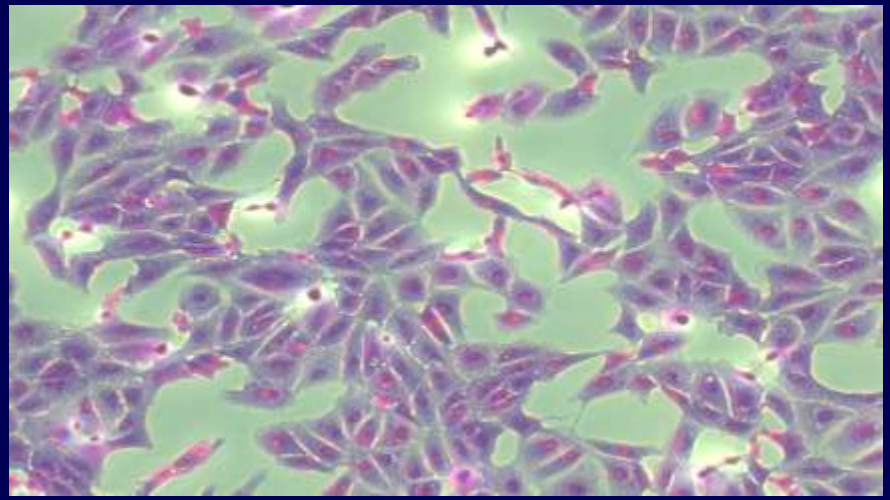

Photo: CellBank Australia

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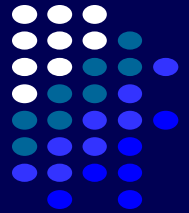
Cell line contamination



An additional cell population present within a cell line that makes it “impure”

Contaminating cells may be microbial or from another cell line

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Cell line contamination

Table 5. Effects of mycoplasma contamination on cell cultures

- General effects on eukaryotic cells:
  - Altered levels of protein, RNA and DNA synthesis
  - Alteration of cellular metabolism
  - Induction of chromosomal aberrations (numerical and structural alterations)
  - Change in cell membrane composition (surface antigen and receptor expression)
  - Alteration of cellular morphology
  - Induction (or inhibition) of lymphocyte activation
  - Induction (or suppression) of cytokine expression
  - Increase (or decrease) of virus propagation
  - Interference with various biochemical and biological assays
  - Influence on signal transduction
  - Promotion of cellular transformation
  - Alteration of proliferation characteristics (growth, viability)
  - Total culture degeneration and loss
- Specific effects on hybridomas:
  - Inhibition of cell fusion
  - Influence on selection of fusion products
  - Interference in screening of monoclonal antibody reactivity
  - Monoclonal antibody against mycoplasma instead of target antigen
  - Reduced yield of monoclonal antibody
  - Conservation of hybridoma

Drexler HG & Uphoff CC, *Cytotechnology* **39**:75-90 (2002)

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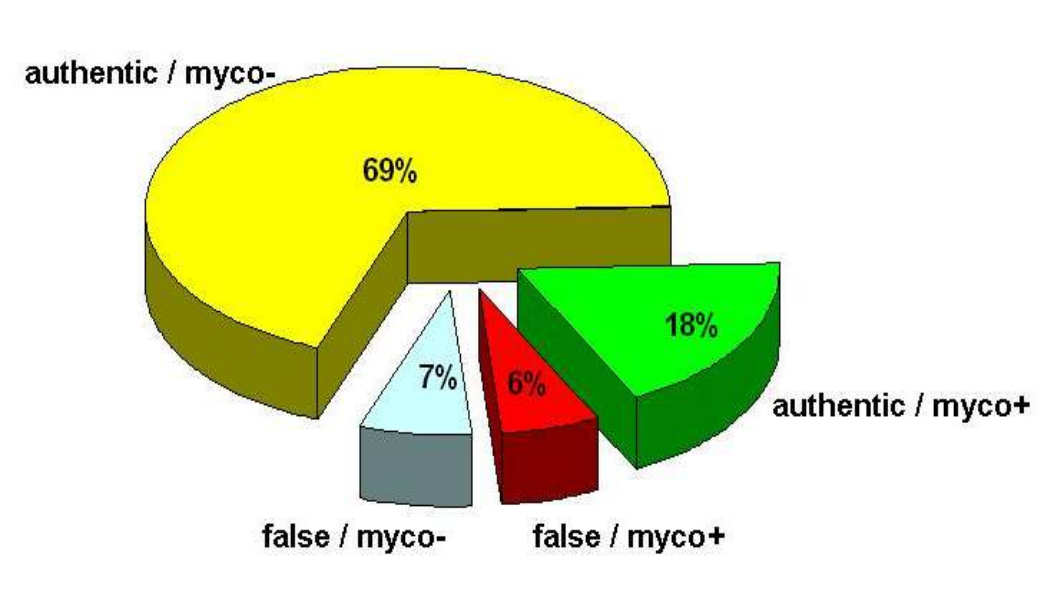
Incidence of contamination



Leukemia-lymphoma cell lines submitted for deposit at DSMZ

Capes-Davis A et al., *Int. J. Cancer* **127**:1-8 (2010)

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Strategy 1

CHECK FOR KNOWN CONTAMINATION OR MISIDENTIFICATION

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ECV304

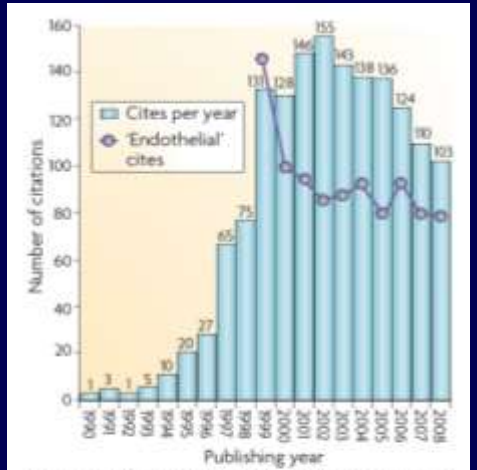

Figure 2 | **Citations of T24 bladder cancer cells referred to as normal endothelial cells.** The demonstration that ECV304 cells are not endothelial cells had little effect on its use as a model for endothelial cells in publications, as shown by the graph. Data generated courtesy of R.A.F. MacLeod, National Institute of Standards and Technology.

ATCC SDO Workgroup ASN-0002, *Nat. Rev. Cancer* **10**: 441-448 (2010)

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List of contaminated cell lines

Database of Cross-Contaminated or Misidentified Cell Lines
Amanda Capes-Davis and R. Ian Freshney

Version 6.7 Table 1
Publication Date 27/06/2011

Attributions in the last two columns indicate where these misidentifications were reported and in no way imply responsibility for the cause by the authors or institutions.
This table is meant as a preliminary guide to avoiding suspect cell lines, but all recently acquired cell lines should be tested (e.g. by STR profiling for human cell lines) and compared to reference stock before use.
The "Contaminating Cell Line", in most cases, will have overgrown the claimed original, or will have replaced it by a technical error, and the original cells will no longer exist. Genuine examples of some cell lines, such as RT-4 human bladder carcinoma, may still exist (see Table 2); their identity would be confirmed by profiling.
We would be grateful to anyone who can confirm the existence of authentic stocks of any of the lines listed; please contact Ian Freshney [i.freshney@ntlworld.com] and copy to Amanda Capes-Davis [acapdav@gmail.com].
We would also be grateful for evidence of other misidentified lines not listed here or any other relevant information.
Confusion may also arise from two different cell lines having the same name; information on these would also be welcome.
Observations made in these lists are based on published reports and details obtained from cell banks, their websites, and Wikipedia. "Reference PubMed ID" refers to the unique ID number assigned by the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/).
The authors take no credit nor responsibility for any of the primary observations and have merely attempted to collate data previously available on other sites.

Table 1. Cross-contaminated or Misidentified Cell Lines

Misidentified Cell Line	Claimed Species	Claimed Cell Type	Contaminating Cell Line	Actual Species	Actual Cell Type	Misidentification Reported By	Reference PubMed ID
2474680	Human	Gastric carcinoma	HT-29	Human	Colon carcinoma	MacLeod et al. 1999	10526494
2563 (MAC-21)	Human	Lung carcinoma	HeLa	Human	Cervical adenocarcinoma	Nelson-Rees et al. 1981	6451925
2957990	Human	Gastric carcinoma	HT-29	Human	Colon carcinoma	MacLeod et al. 1999	10526494
3051093	Human	Gastric carcinoma	HT-29	Human	Colon carcinoma	MacLeod et al. 1999	10526494
41M	Human	Ovarian carcinoma	OAW 28	Human	Ovarian carcinoma	Wilson et al. 1996	8795574
ACC2	Human	Salivary gland, adenoid cystic carcinoma	HeLa	Human	Cervical adenocarcinoma	Phuchareon et al. 2009	19557160
ACC3	Human	Salivary gland, adenoid cystic carcinoma	HeLa	Human	Cervical adenocarcinoma	Phuchareon et al. 2009	19557160
ACCM	Human	Salivary gland, adenoid cystic carcinoma	HeLa	Human	Cervical adenocarcinoma	Phuchareon et al. 2009	19557160
ACCNS	Human	Salivary gland, adenoid cystic carcinoma	Unknown	Mouse	Unknown	Phuchareon et al. 2009	19557160
ACCS	Human	Salivary gland, adenoid cystic carcinoma	T-24	Human	Bladder carcinoma	Phuchareon et al. 2009	19557160
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Cervical	[illegible]	[illegible]

Capes-Davis A et al., *Int. J. Cancer* **127**:1-8 (2010)
List also available at www.cellbankaustralia.com

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Strategy 2

PURCHASE FROM REPOSITORIES

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Cell line repositories

Country	Repository
Australia	CellBank Australia
Germany	German Collection of Microorganisms and Cell Cultures (DSMZ)
Japan	Japanese Collection of Research Bioresources (JCRB)
UK	European Collection of Cell Cultures (ECACC)
USA	American Type Culture Collection (ATCC)

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Strategy 3

TEST REGULARLY FOR AUTHENTICITY

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STR profiling

- Uses tetranucleotide “short tandem” repeats

- Amplify at least 8 regions, count, and compare

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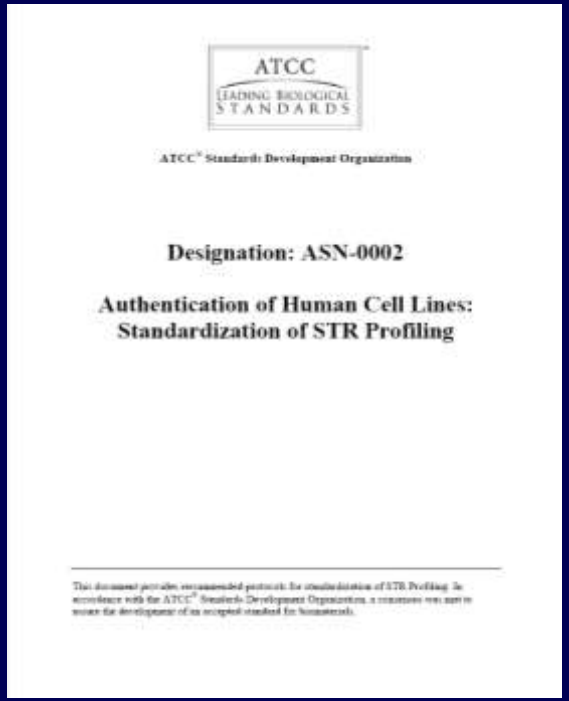
International standard



ATCC
LEADING BIOLOGICAL STANDARDS

ATCC® Standards Development Organization

Designation: ASN-0002

Authentication of Human Cell Lines: Standardization of STR Profiling

This document provides recommended protocols for standardization of STR Profiling. In accordance with the ATCC® Standards Development Organization, a consensus was met to assure the development of an accepted standard for humanity.

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Journals requiring authentication

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Strategy 4

TEST REGULARLY FOR MYCOPLASMA

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CellBank Australia approach

No. species / dose	MycoAlert®	PCR
Species	**Many**	**At least 6**
10^5-10^6 cfu mL^{-1}	Detected	Detected
10^4-10^5 cfu mL^{-1}	**Detected**	Detected
10^3-10^4 cfu mL^{-1}	Not detected	Detected
10^2-10^3 cfu mL^{-1}		**Detected**
10-100 cfu mL^{-1}		Not detected

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Conclusion

- Check for known contamination or misidentification
- Purchase from repositories
- Test regularly for authenticity
- Test regularly for Mycoplasma

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Acknowledgements

CellBank Australia

Jonathan Arthur

Elsa Moy

George Theodosopoulos

Samath Pen

Amanda Capes-Davis

Funding Partners

Cancer Institute NSW

Children's Medical Research Institute

NHMRC Enabling Grant

Cure Cancer Australia Foundation

National Breast Cancer Foundation

The Lady Mary Fairfax CellBank Australia established 2005 by a joint venture of the Children's Medical Research Institute, Cure Cancer Australia Foundation, and National Breast Cancer Foundation. Operated by CMRI from July 2007.

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