

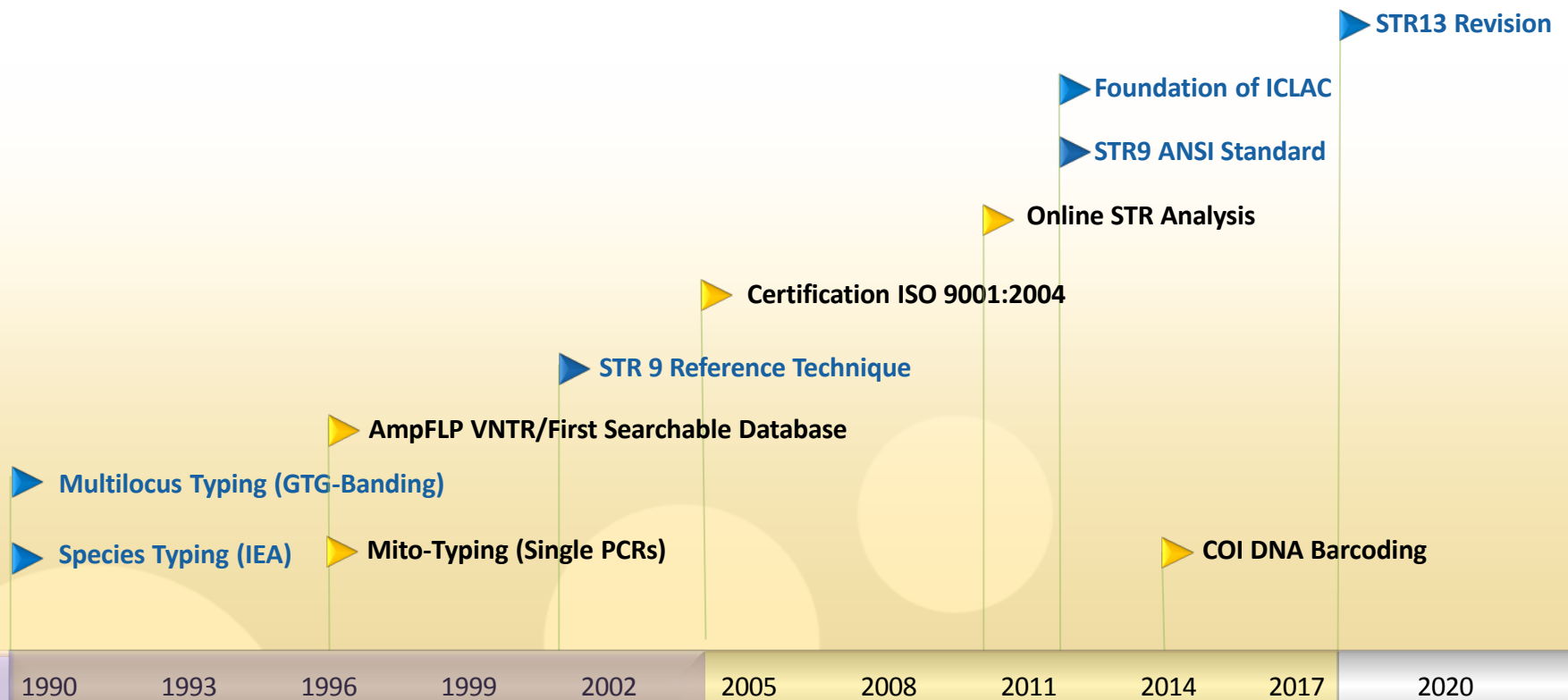
Reliability of Scientific Data Using Human Cell Lines as Tumor Model Systems - Perspectives of NGCC -

Authentication Procedures - Risk Assessment - Online Tools

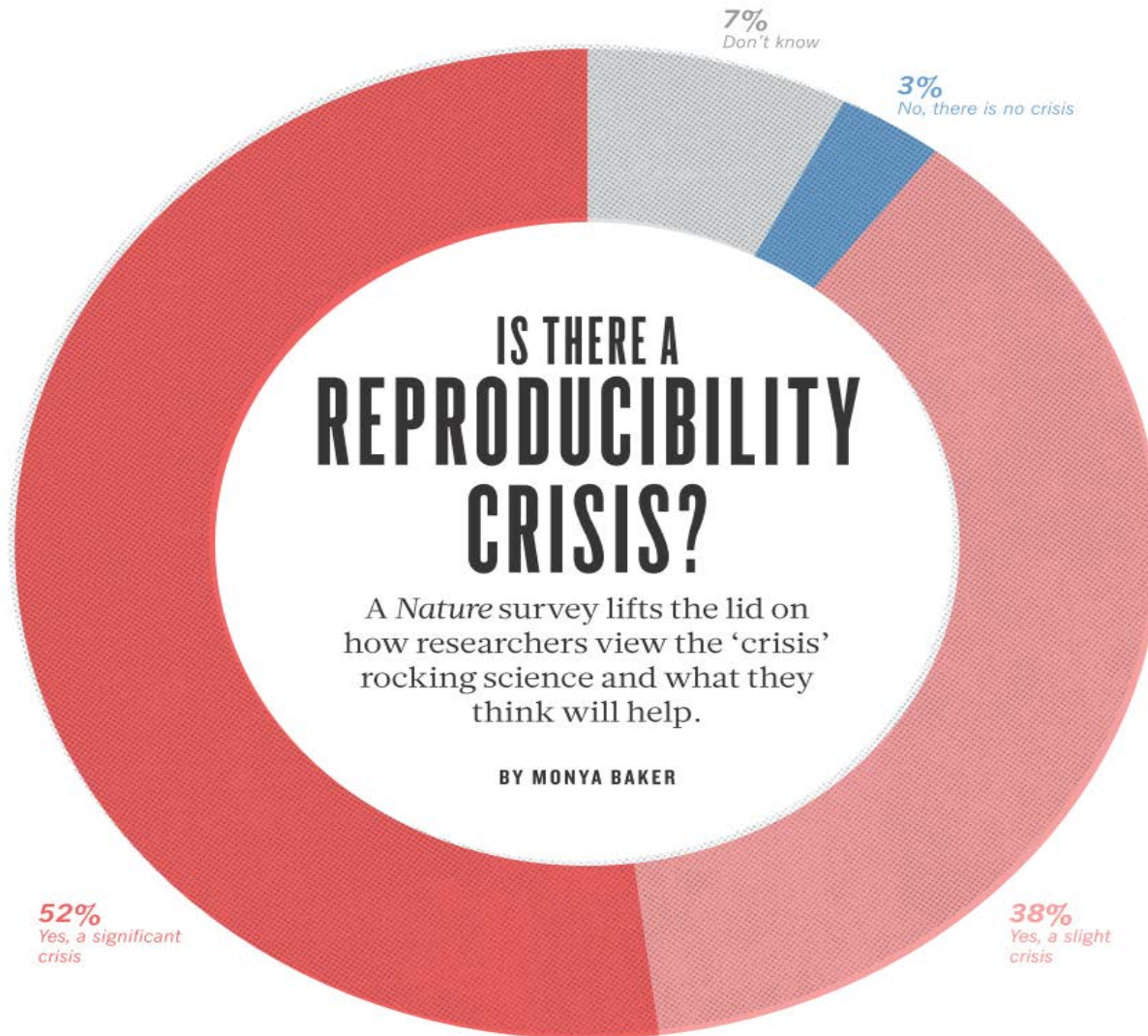
Wilhelm G. Dirks, PhD
Leibniz - Institute DSMZ - German Biological Resource Centre



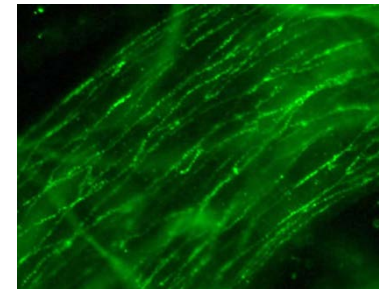
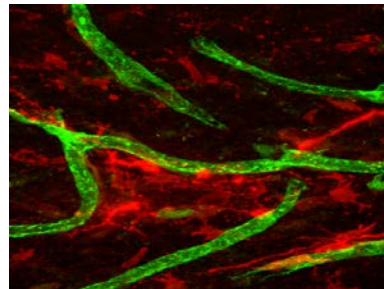
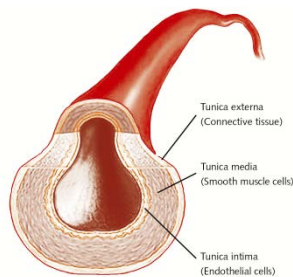
Milestones in Cell Line Identity Validation



Reliability of Scientific and Pre-clinical Data



Case Report: Model System ECV-304



Dirks et al.: ECV-304 (endothelial) is really T-24 (bladder carcinoma): cell line cross-contamination at source. In Vitro Cell Dev Biol Anim, 1999, 35 (10):558-9

ECV-304 is not of HUVEC origin and an inappropriate cell line to study endothelial or vascular cell biology!

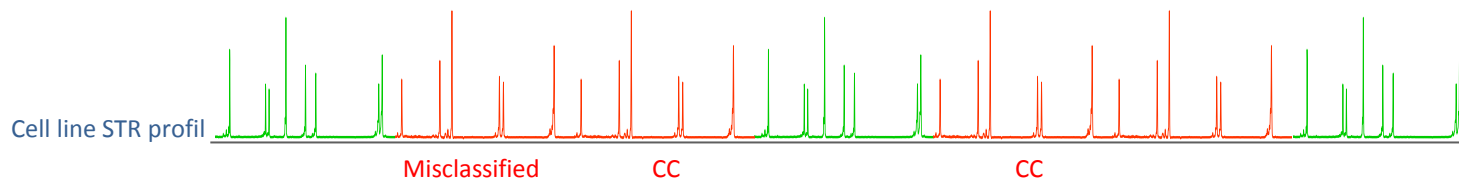
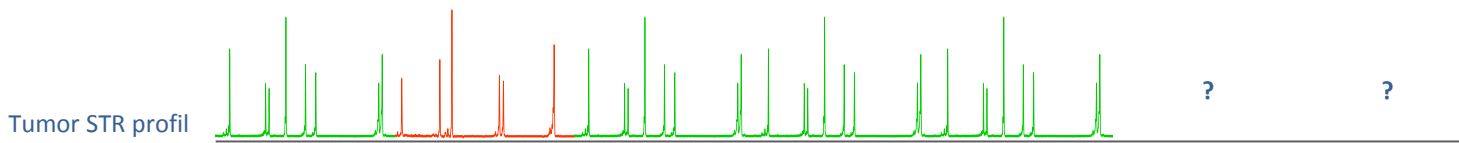
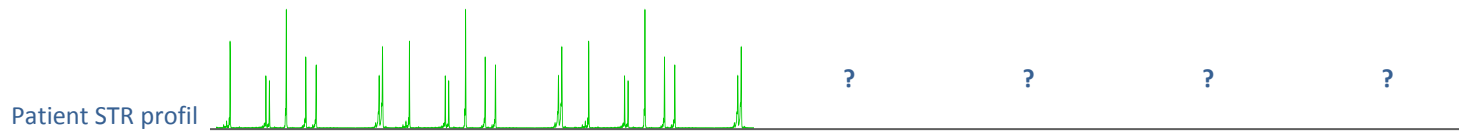
DSMZ alerts Cell Banks ATCC, JCRB, ECACC, and RIKEN in 1999 to stop distribution of ECV-304

Scientific publications using ECV-304 as a model system (pubmed, NIH, highwire press, Stanford university
keywords ECV-304/ECV304, endothelial

1990 - 1999: **148**

1999 - 2013: **1580**

How to Generate STR Reference STR Data



full authentication

fractional authentication

fractional authentication



Nonaplex STR Typing (STR9) of Human Cells

8012-8017 | PNAS | July 3, 2001 | vol. 98 | no. 14

www.pnas.org/cgi/doi/10.1073/pnas.121616198

Short tandem repeat profiling provides an international reference standard for human cell lines

John R. Masters^{a,b}, Jim A. Thomson^c, Bernadette Daly-Burns^a, Yvonne A. Reid^d, Wilhelm G. Dirks^e, Phil Packer^f, Lorraine H. Toji^g, Tadao Ohno^h, Hideyuki Tanabeⁱ, Colin F. Arlett^j, Lloyd R. Kelland^k, Maureen Harrison^l, Arvind Virmani^m, Timothy H. Wardⁿ, Karen L. Ayres^o, and Paul G. Debenham^c

^aInstitute of Urology, University College London, 3rd Floor Research Laboratories, 67 Riding House Street, London W1W 7EY, United Kingdom; ^cLGC, Queens Road, Teddington, Middlesex TW11 0LY, United Kingdom; ^dAmerican Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209; ^eDSMZ German Collection of Cell Cultures, Mascheroder Weg 1b, 38124 Braunschweig, Germany; ^fEuropean Collection of Animal Cell Cultures, Centre for Applied Microbiology and Research, Salisbury, Wiltshire SP4 0JG, United Kingdom; ^gCoriell Institute for Medical Research, 401 Haddon Avenue, Camden, NJ 08103; ^hThe Institute of Physical and Chemical Research (Japan) (RIKEN) Cell Bank, Koyadai 3-1-1, Tsukuba Science City, 305-0074, Japan; ⁱJapanese Collection of Research Bioresources Cell Bank, National Institute of Health Sciences, 1-18-1 Kami-Yoga, Setagaya-ku, Tokyo 158-8501, Japan; ^jMRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9RR, United Kingdom; ^kCRC Center for Cancer Therapeutics, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, United Kingdom; ^lImperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, United Kingdom; ^mHamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, 6000 Harry Hines Boulevard, Dallas, TX 75235-8593; ⁿDepartment of Drug Development, Paterson Institute for Cancer Research, Wilmslow Road, Manchester M20 4BX, United Kingdom; and ^oDepartment of Applied Statistics, University of Reading, P.O. Box 240, Earley Gate, Reading RG6 6FN, United Kingdom

Edited by Stanley M. Gartler, University of Washington, Seattle, WA, and approved April 16, 2001 (received for review December 22, 2000)



Workgroup ASN-002 (ANSI)/ICLAC

MODELS OF CANCER SERIES — SCIENCE AND SOCIETY

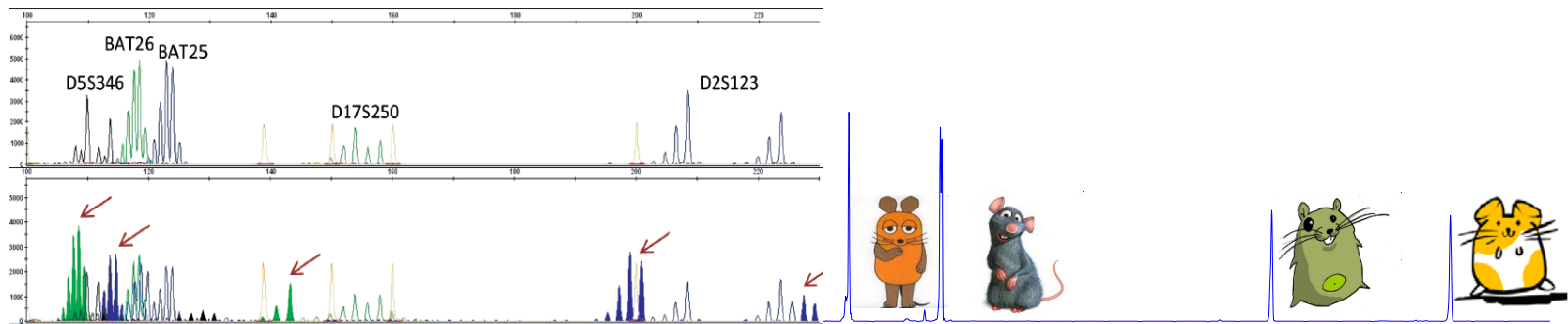
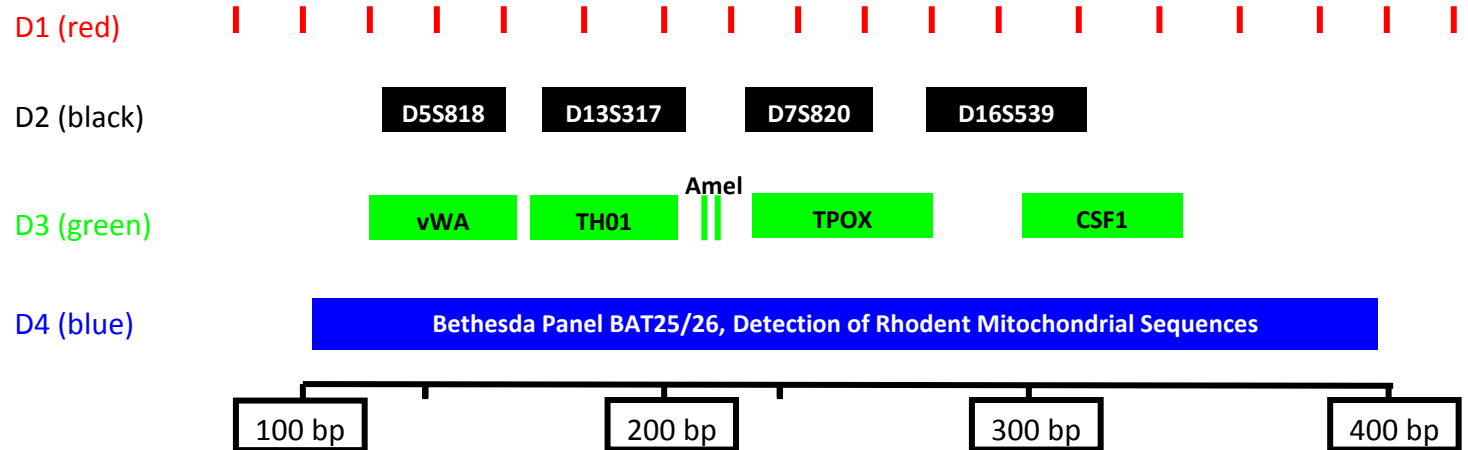
Cell line misidentification: the beginning of the end

- Christine Alston-Roberts, Standards Specialist, ATCC, 10801 University Boulevard, Manassas, VA 20110, USA
- Rita Barallon, Ph.D., Service Business Manager, Life and Food Sciences Life Sciences, LGC, Queens Road, Teddington, Middlesex, TW11 0LY, UK
- Steven R. Bauer*, Ph.D., FDA/Center for Biologics Evaluation and Research, Chief, Cell and Tissue Therapy Branch, Division of Cellular and Gene Therapies, Office of Cellular, Tissue and Gene Therapies, NIH Building 29B 2NN10 HFM-740, 8800 Rockville Pike, Bethesda, MD 20892, USA
- John Butler, Ph.D., Biochemical Science Division (831), Advanced Chemical Science Laboratory (227), Room B226, NIST, 100 Bureau Drive, Stop 8312, Gaithersburg, MD 20899-8312, USA
- Amanda Capes-Davis, Ph.D., CellBank Australia, Children's Medical Research Institute, Westmead, New South Wales, Australia
- Wilhelm G. Dirks, Ph.D., Molecular Biology, DSMZ — German Collection of Microorganisms and Cell Cultures, Inhoffenstr. 7b, 38124 Braunschweig, Germany
- Roderick A.F. MacLeod, Ph.D., Cytogenetics Laboratory, DSMZ — German Collection of Microorganisms and Cell Cultures, Inhoffenstr. 7b, 38124 Braunschweig, Germany
- John R. W. Masters, Ph.D., FCRPath, Professor of Experimental Pathology, University College London, 67 Riding House Street, London, W1W 7EJ, UK
- Mark Nardone, Director, Bio-Trac Program, The Foundation for the Advanced Education in the Sciences at the National Institutes of Health, Bethesda, MD 20892, USA
- Roland M. Nardone, Ph.D., Professor Emeritus, Catholic University of America, Cell and Molecular Biology, 620 Michigan Avenue NE, Washington, DC 20064, USA
- Raymond W. Nims, Ph.D., Consultant, RMC Pharmaceutical Solutions Inc., 2150 Miller Drive, Suite A, Longmont, CO 80501, USA
- Paul J. Price, Ph.D., CSO, Research and Development, Room B-33, D-Finitive Cell Technology, 1023 Wappoo Rd, Charleston, SC 29407, USA
- Yvonne A. Reid, Ph.D., Collection Scientist, Cell Biology Collection, ATCC, 10801 University Boulevard, Manassas, VA 20110, USA

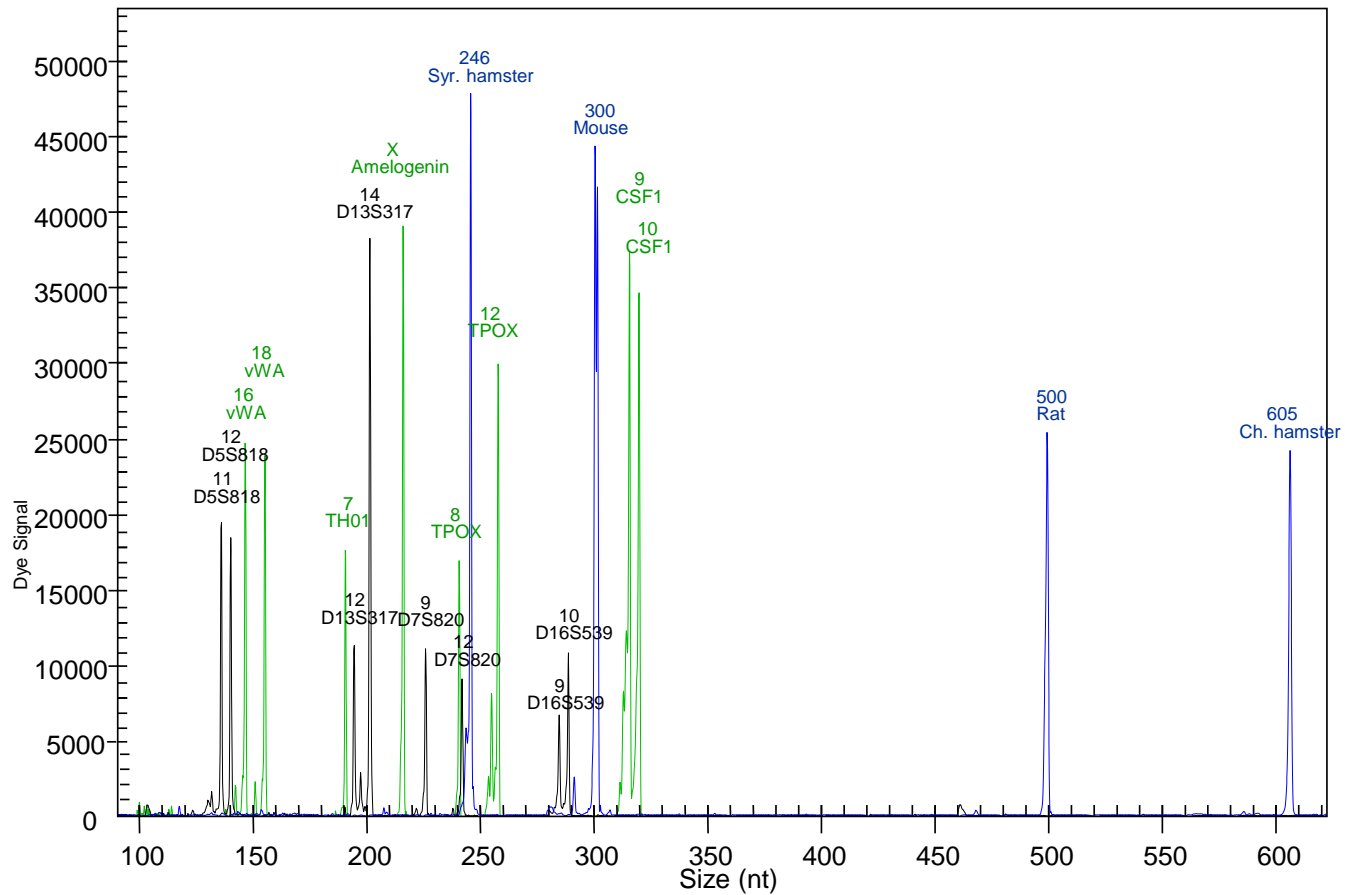
ICLAC is an independent committee with support from the scientific community. Thank you to ICLAC Members and Partner Organizations for your support.



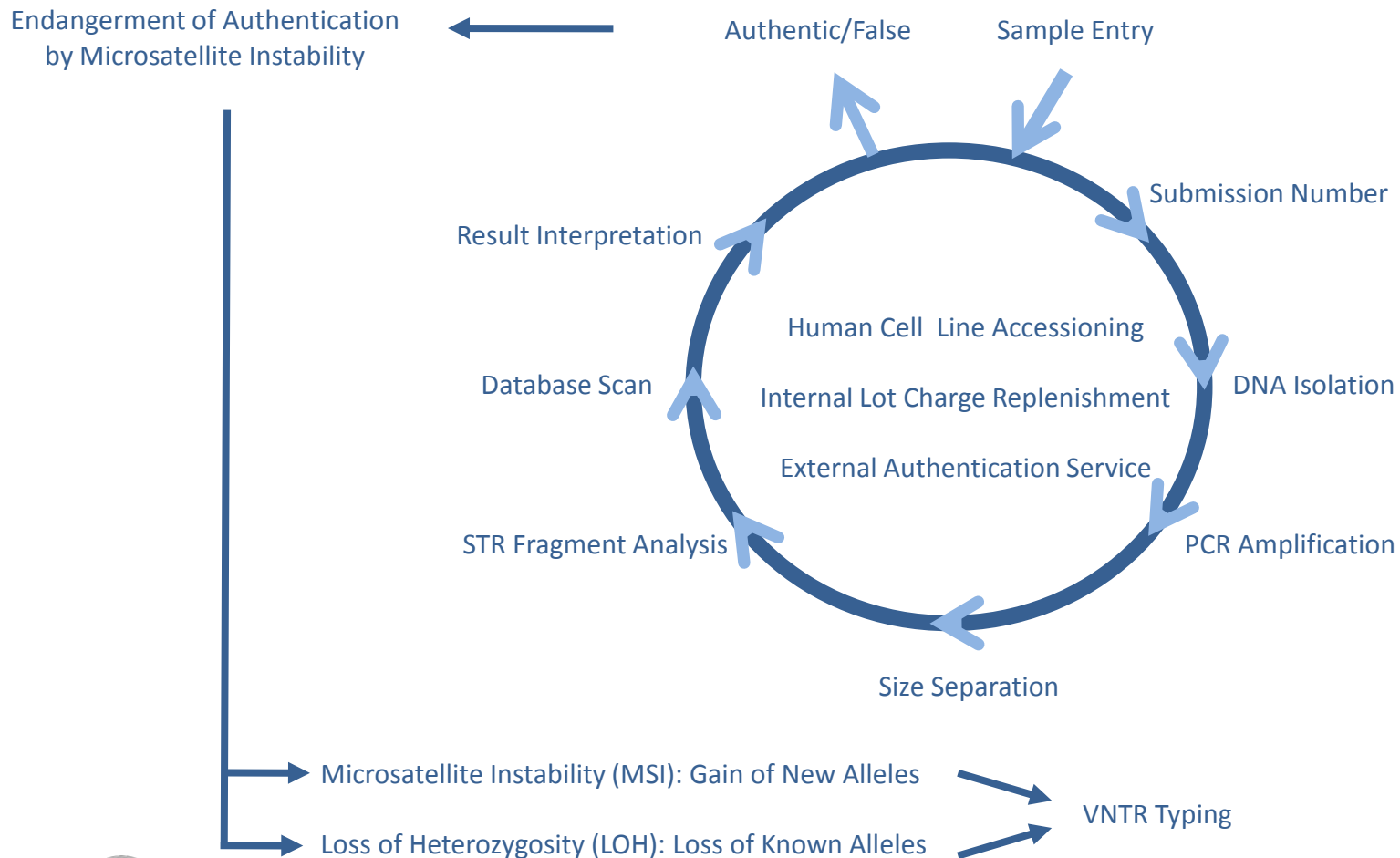
STR9, Bethesda Panel, Rhodentia Detection



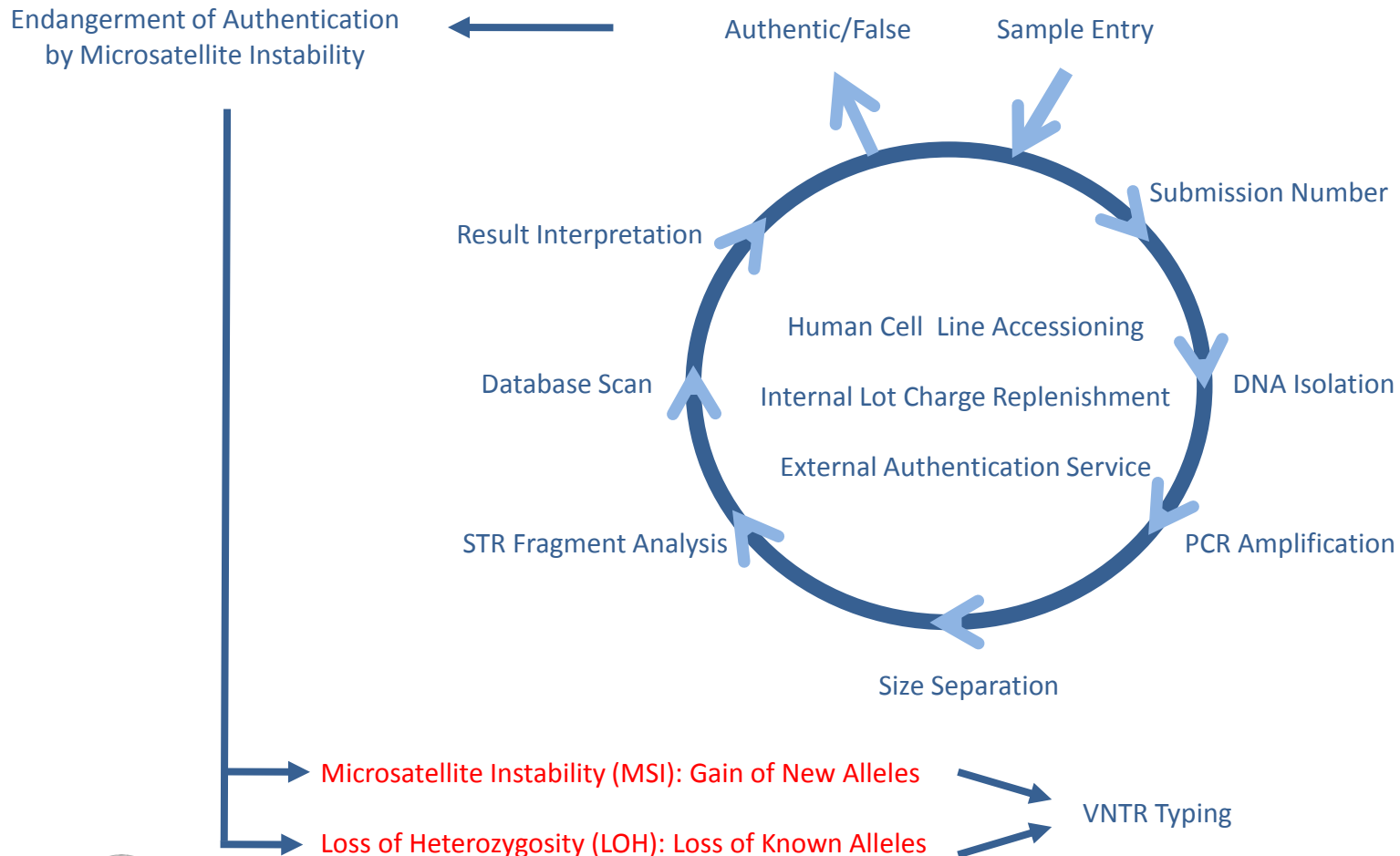
Analysis for Intra- and Inter-species CC



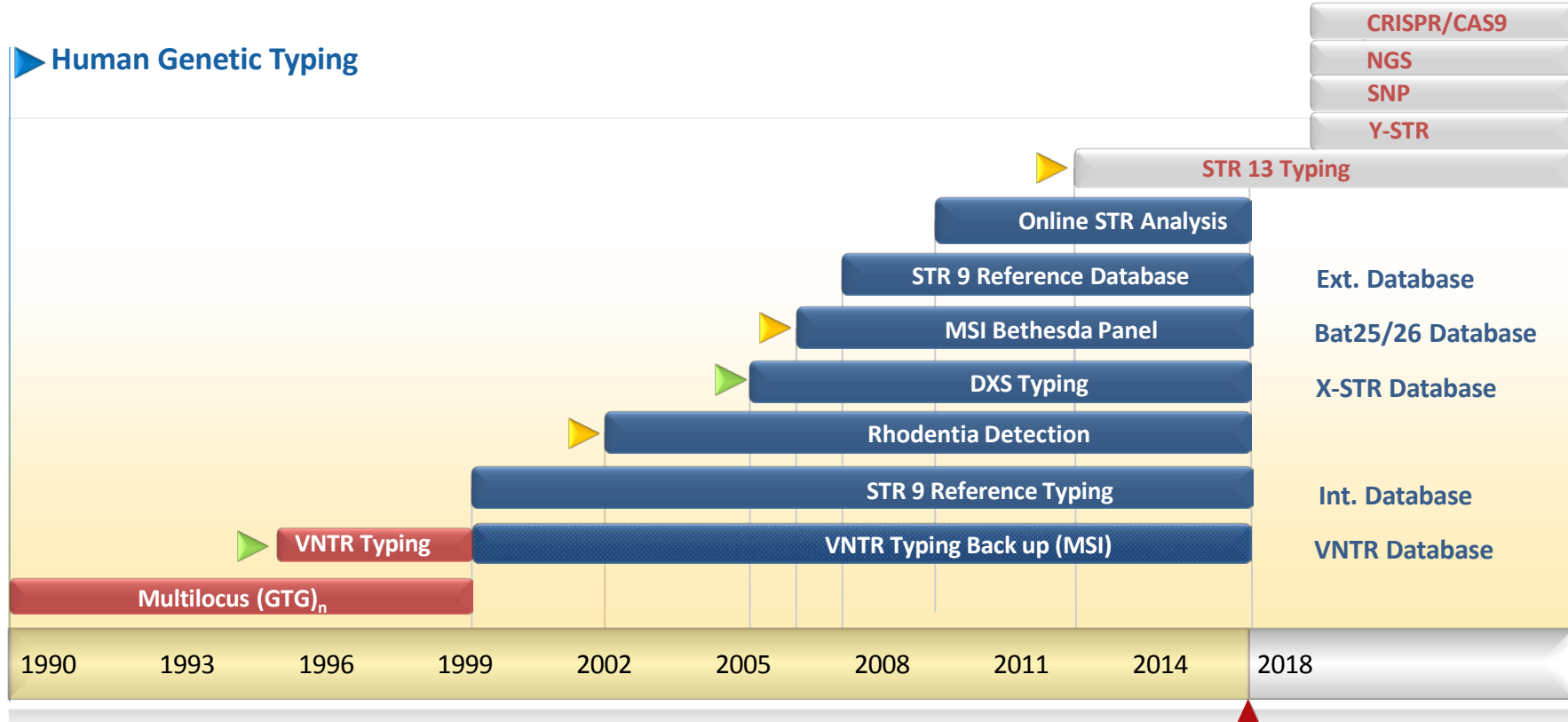
Limitations of STR Typing Technology



Limitations of STR Typing Technology



Applied Techniques for Authentication



Search Engine for STR Profiles



STR matching analysis by your data

	Go STR analysis with EV over 0.90 after entering your data below.								
Locus name:	D5S818	D13S317	D7S820	D16S539	VWA	TH01	AM	TPOX	CSF1PO
Enter your STR data:->	9,11	10,14	8,11	10,12	16,20	7,9	X,X	8,11	12,12
Sample data (HeLa):	11,12	12,13,3	8,12	9,10	16,18	7	X	8,12	9,10

• EV = evaluation value

The human STR profile database includes about 570 cell lines from DSMZ.
[A method for the EV calculation.](#)



Online Identification Tool

STR matching analysis by entering your data.
- DSMZ Profile Database -

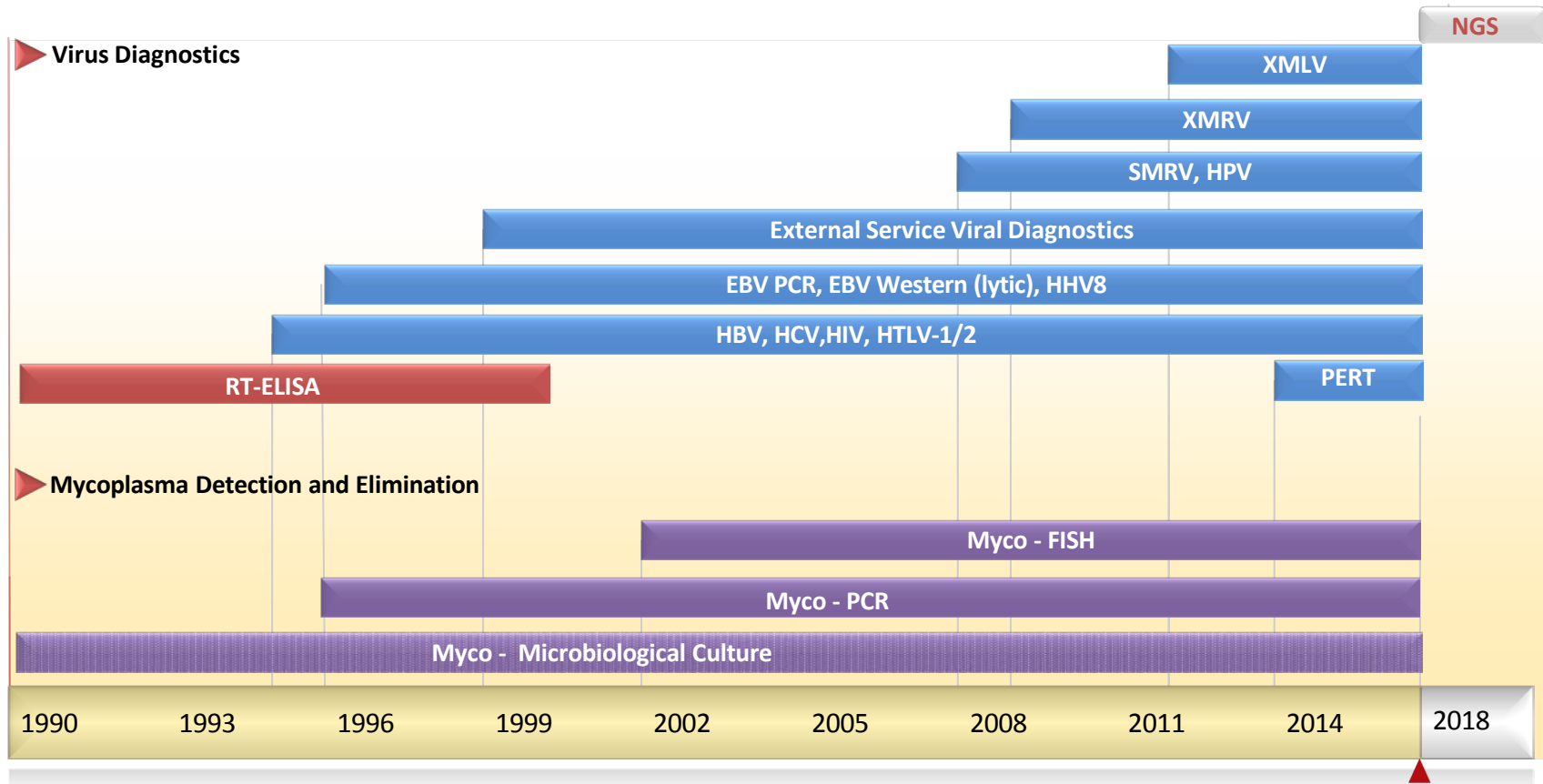
LOOK! graphical analysis shown at the bottom of this page.

EV	Cell No.	Cell name	Locus names									Figures
			D5S818	D13S317	D7S820	D16S539	VWA	TH01	AM	TPOX	CSF1PO	
		<i>Query (Your Cell)</i>	9,11	10,14	8, 11	10,12	16,20	7, 9	x	8,11	12	
1.06(36/34)	7	CML-T1	9,11	10,14	8,11	10,12	16,20	7,9	X,X	8,11	12,12	-

Analyze again after changing the EV

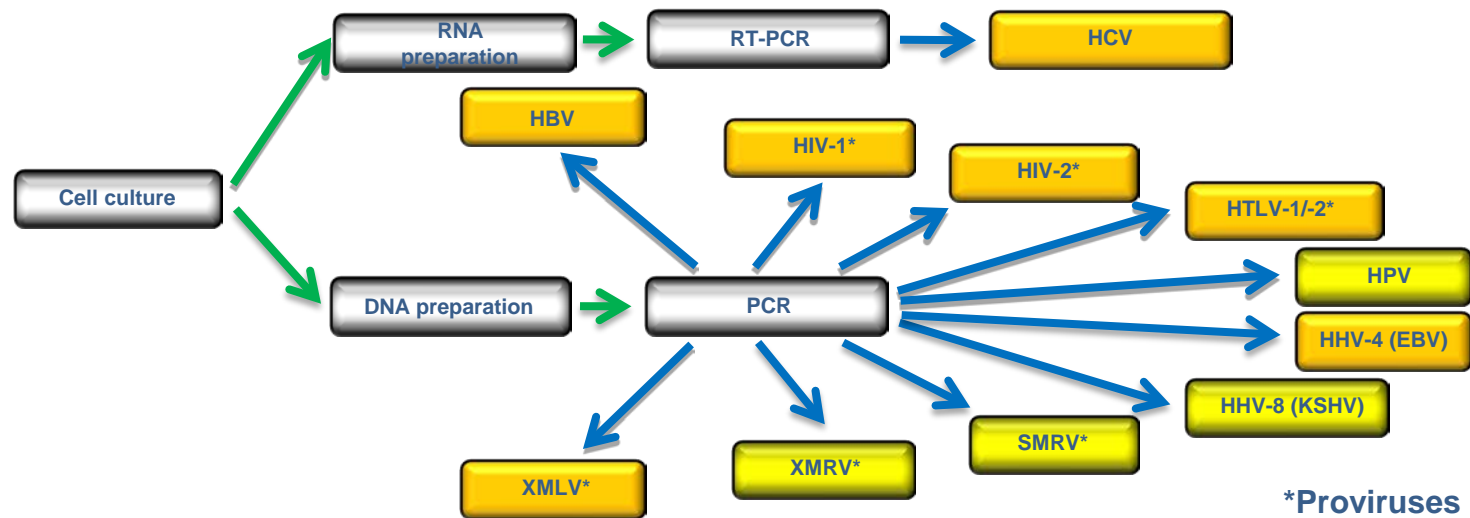
EV	Cell No. Scored	Graph
0.95~1.00	1	
0.90~0.95	0	
0.85~0.90	0	
0.80~0.85	0	
0.75~0.80	3	
0.70~0.75	10	
0.65~0.70	20	
0.60~0.65	18	
0.55~0.60	88	
0.50~0.55	101	
0.45~0.50	105	
0.40~0.45	100	
0.35~0.40	64	
0.30~0.35	20	
0.25~0.30	30	
0.20~0.25	10	

Development of Technologies: Virus Diagnostics



Detection of Viruses in Human Cell Lines

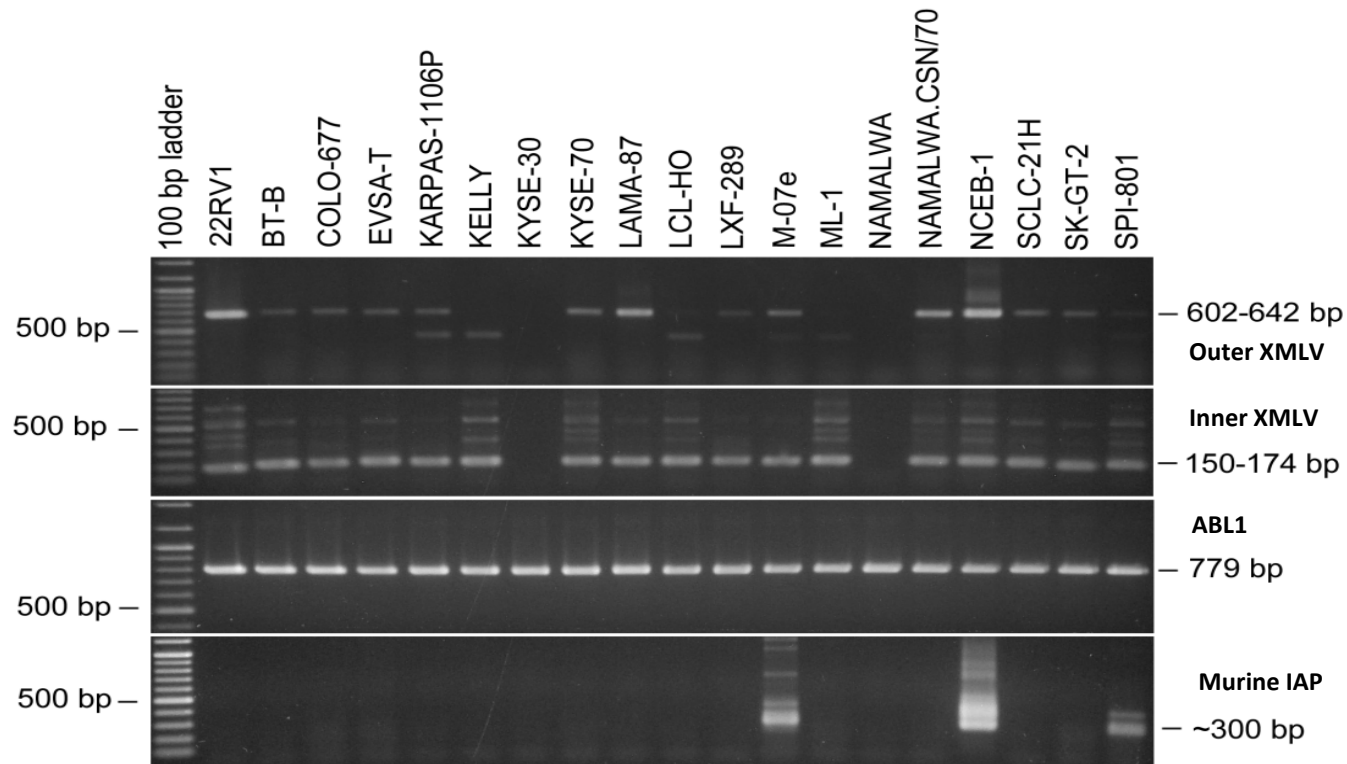
Cellular Viral Sequences



Sequences and Lytic Phase Proteins of Active Viruses



XMLV-Detection in Human Cell Lines




Risk Assessment for Bio Safety Classification

to product search »

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Catalogue Microorganisms
 Catalogue Human and Animal Cell Lines
 Catalogue Plant Cell Lines
 Catalogue Plant Viruses and Antisera
 DZIF-Sammlung der DSMZ

Member of 

Cell line: CRO-AP2

DSMZ no.: ACC 48

Species: human (*Homo sapiens*)

Cell type: B cell lymphoma

Origin: established in 1996 from the diagnostic pre-treatment pleural effusion sampled shortly before death of a 49-year-old HIV+ homosexual man with primary effusion lymphoma (PEL) and previous history of Kaposi's sarcoma; autopsy revealed involvement of serous membranes without formation of solid tumor mass; cells were described to be EBV+, HHV-8+ and to carry a BCL6 point mutation

Reference(s): [15032](#), [14417](#), [14483](#)

Biosafety level: 2

Risk assessment: The cell line is positive for EBV (HHV-4) by PCR analysis. However, expression of immediate-early protein BZLF-1 and lately expressed capsid protein were negative by western blot and immunostaining, respectively, in untreated and phorbol ester / sodium butyrate stimulated cells. The infection was classified as latent infection without production of active viruses. A transmission of EBV during handling of the cells is unlikely. Additionally, the cell line carries HHV-8 sequences which were detected by PCR and Southern blot. We did not determine the infection mode of this herpes virus and cannot exclude the secretion of the viruses. The cell line is thus categorized biosafety level 2.

Permissions and restrictions: A, B

DSMZ Cell Culture Data:

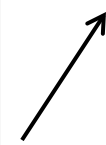
Morphology: lymphoblast-like cells growing in clumps or as single cells in suspension

Medium: 80% RPMI 1640 + 20% h.i. FBS

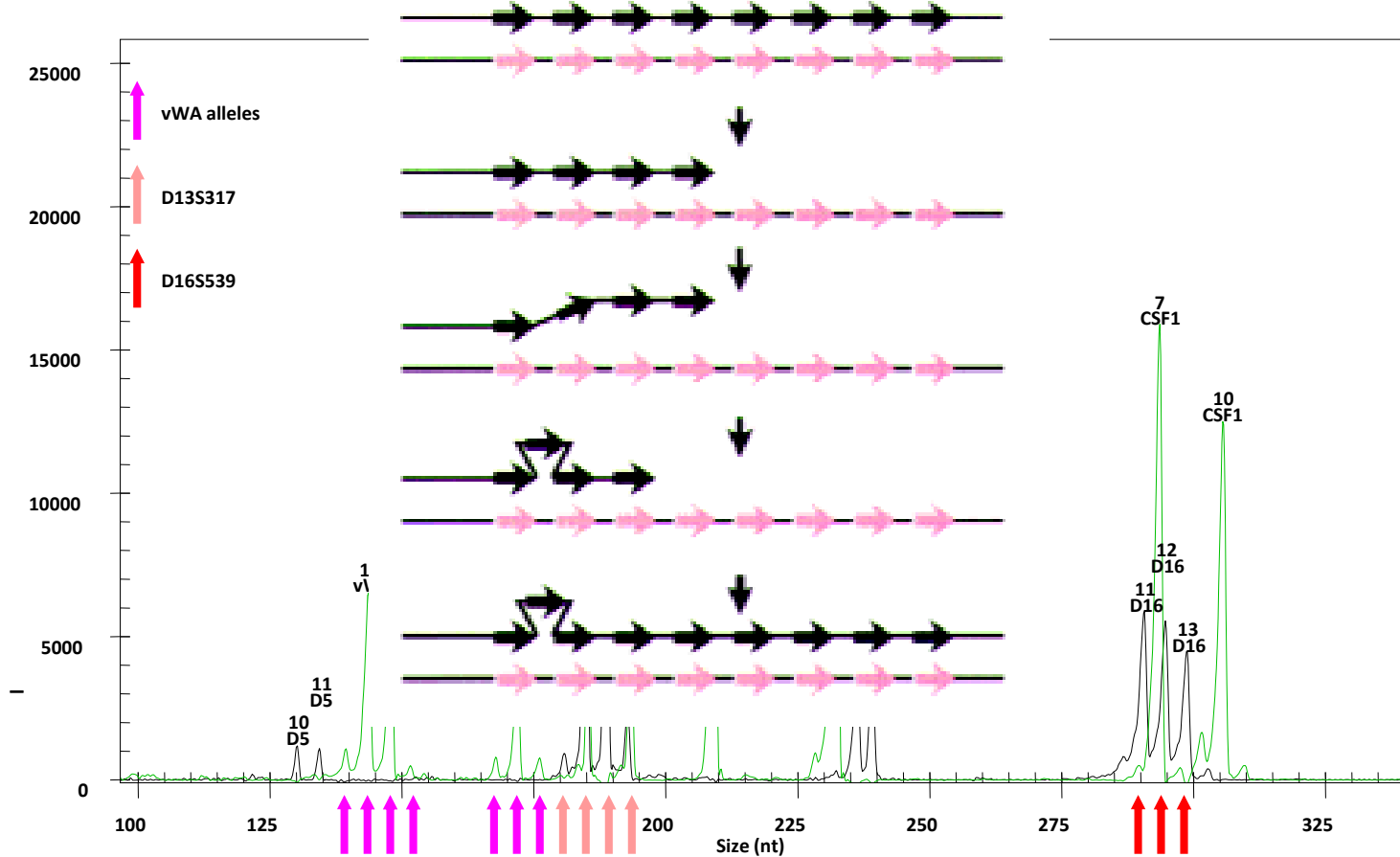
Subculture: solit saturated culture about 1:2 to 1:3 everv 2-4 days: maintain at

TRBA 468 „Liste der Zelllinien und Tätigkeiten mit Zellkulturen“

Zelllinie	Ursprungsorganismus	Zusätzlicher biologischer Arbeitsstoff	Schutzstufe
COLO-678	Mensch		1
COLO-679	Mensch		1
COLO-680N	Mensch		1
COLO-699	Mensch		1
COLO-704	Mensch		1
COLO-720L	Mensch	Humanes Herpesvirus 4 (HHV-4) keine Virusabgabe	1
COLO-783	Mensch		1
COLO-800	Mensch		1
COLO-818	Mensch		1
COLO-824	Mensch		1
COLO-829	Mensch		1
COLO-849	Mensch		1
COS	Affe (Grüne Meerkatze)		1
COS-1	Affe (Grüne Meerkatze)	Simian-Virus 40 (SV40) großes T-Antigen	1
COS-7	Affe (Grüne Meerkatze)	Simian-Virus 40 (SV40) großes T-Antigen	1
CPC-N	Mensch		1
CR	Erite		1
CRE	Maus		1
CRFK	Katze		1
CRIP	Maus		1
CRO-AP2	Mensch	Humanes Herpesvirus 8 (HHV-8); Humanes Herpesvirus 4 (HHV-4) keine Virusabgabe	2
CRO-AP3	Mensch	Humanes Herpesvirus 8 (HHV-8)	2
CRO-AP5	Mensch	Humanes Herpesvirus 8 (HHV-8); Humanes Herpesvirus 4 (HHV-4) keine Virusabgabe	2
CRO-AP6	Mensch	Humanes Herpesvirus 8 (HHV-8); Humanes Herpesvirus 4 (HHV-4) keine Virusabgabe	2
CSMalpha1H	Maus		1
CSMalpha6C	Maus		1
CT26.CL25	Maus	Simian-Virus 40 (SV40)-Sequenzen	1
CT26.WT	Maus		1
CTV-1	Mensch		1
CV-1	Affe (Grüne Meerkatze)		1
GW13.20-383 (clone of BCL)	Maus		1
CX-1	Mensch		1
D1.1	Mensch		1
D10.G4.1	Maus		1
D-11	Fisch (Regenbogenforelle)		1
D17	Hund		1
D1B	Maus		1
D22	Hund		1
D283 Med	Mensch		1
D2N	Maus		1
D3	Maus		1
D341 Med	Mensch		1



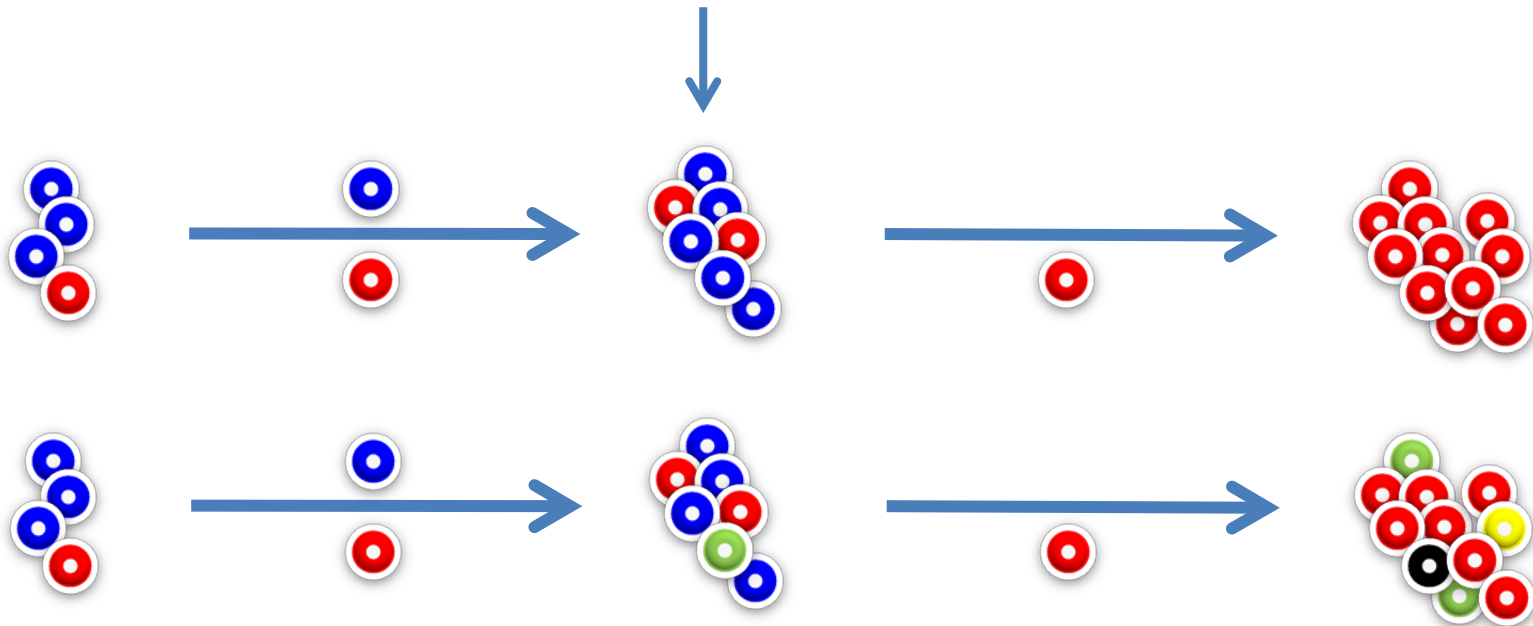
Microsatellite Instability



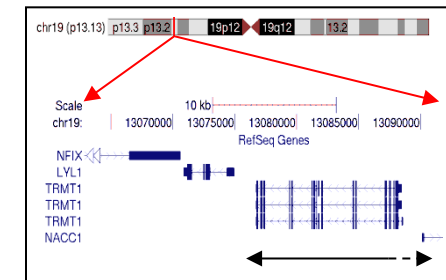
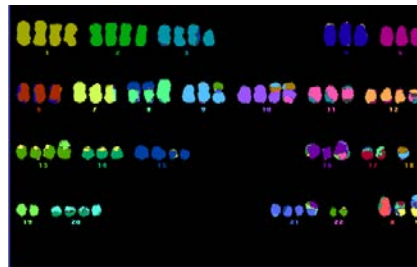
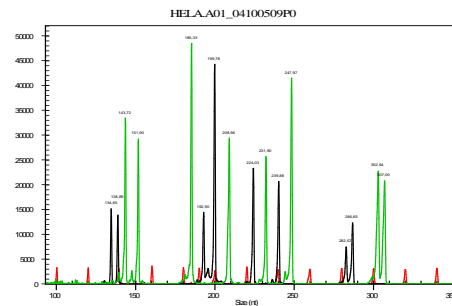
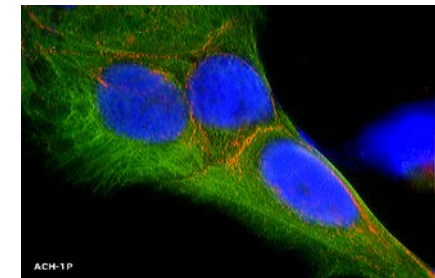
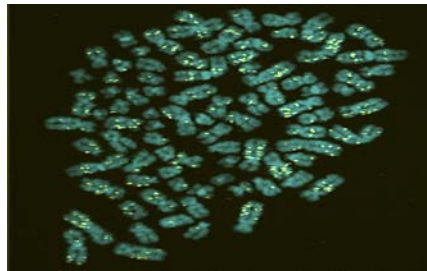
Bottlenecking Outgrowth of Subclones

ACC	FBS	Date	D5	D5'	D13	D13'	D7	D7'	D16	D16'	vWA	vWA'	TH01	TH01'	TPOX	TPOX'	CSF1	CSF1'	Amel	Amel'
128		11.01.2005	11	12	9	12	8	10	10	11	15	16	8	9	8	10	12	12	X	Y
128		08.02.2005	11	12	9	12	8	10	10	11	15	16	8	9	8	10	12	12	X	Y
128		11.01.2005	11	12	9	12	8	10	10	11	15	16	8	8	8	10	12	12	X	Y
128		08.02.2005	11	12	9	12	8	9	10	11	16	16	8	8	8	10	12	12	X	X
128		11.01.2005	11	12	9	12	8	9	10	11	15	16	8	8	8	10	12	12	X	Y
128		08.02.2005	10	12	9	12	8	9	10	11	15	16	8	8	8	10	12	12	X	Y
128		11.01.2005	11	12	9	12	8	10	10	11	15	16	8	9	8	10	12	12	X	Y
128		08.02.2005	11	11	9	12	8	10	10	11	16	16	8	8	8	10	12	12	X	Y
128	Lot 7	11.01.2005	11	12	9	12	8	10	10	11	15	16	8	9	8	10	12	12	X	Y
128	Lot 7	08.02.2005	11	12	9	12	8	10	10	11	15	16	8	9	8	10	12	12	X	Y

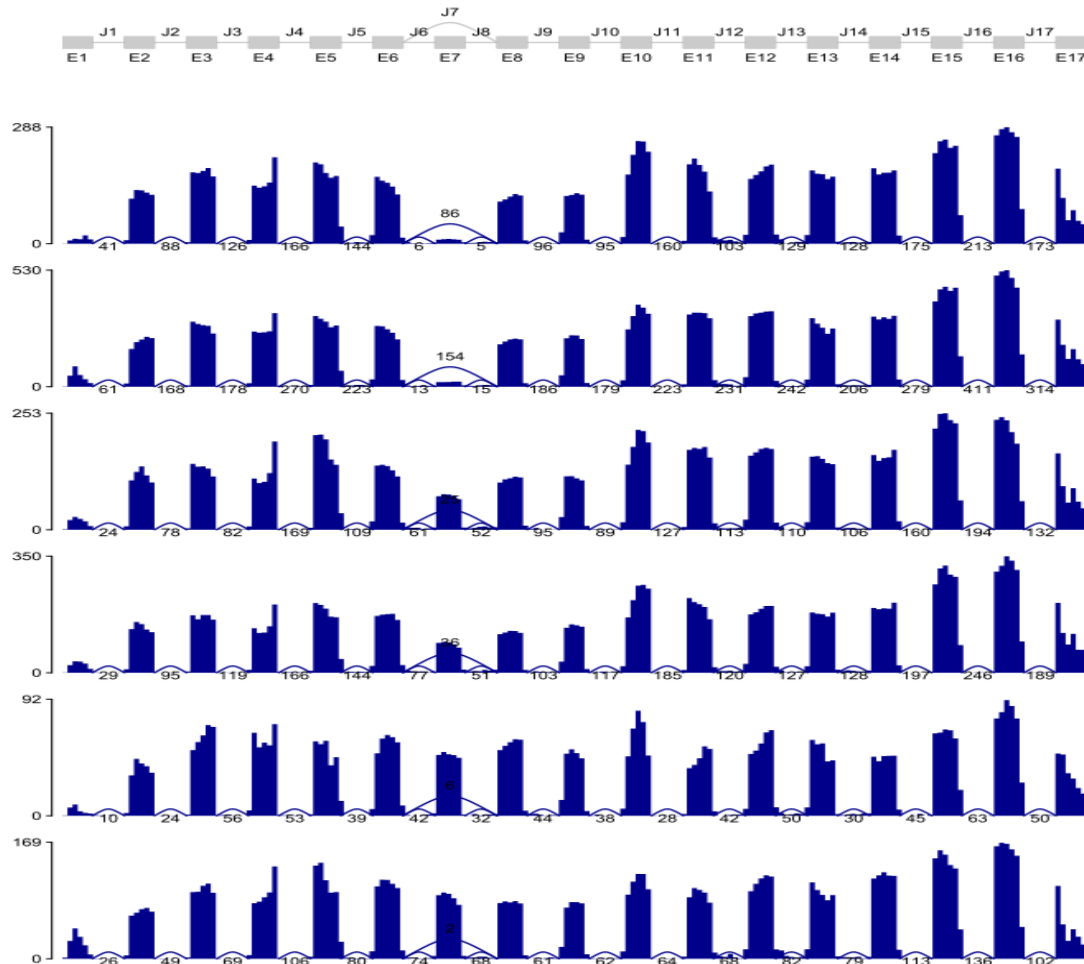
Clonal Selection or Parallel Evolution?



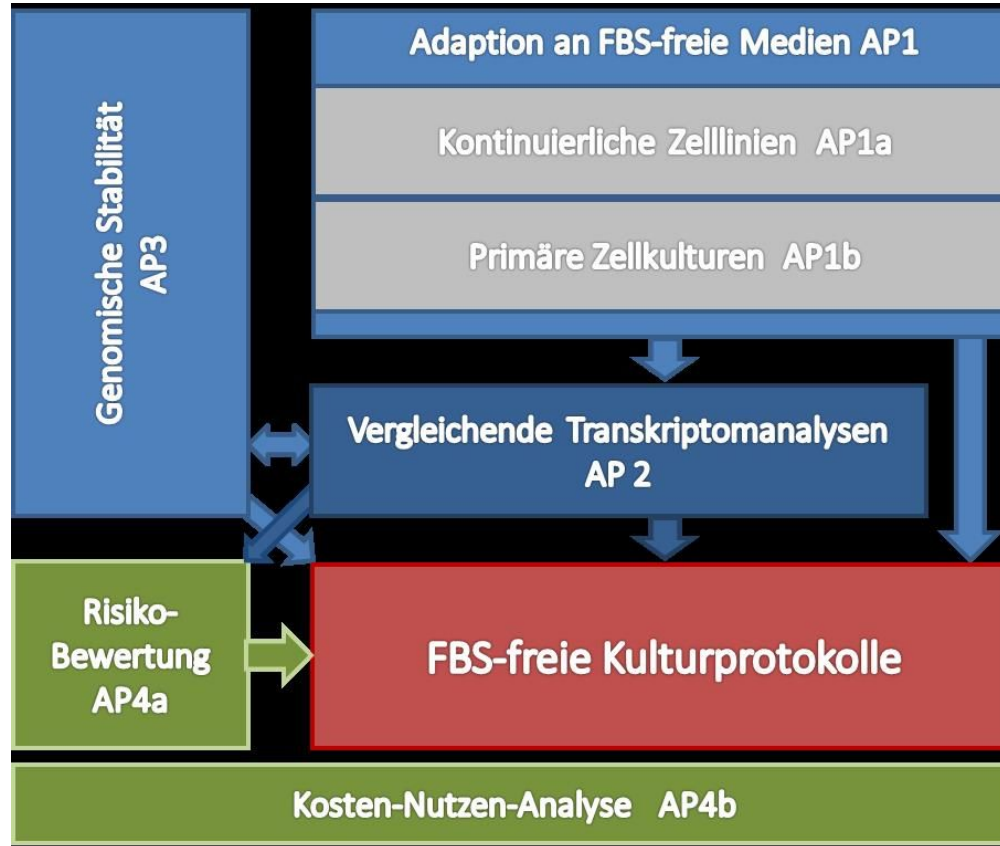
Retrospective Multidisciplinary Approach



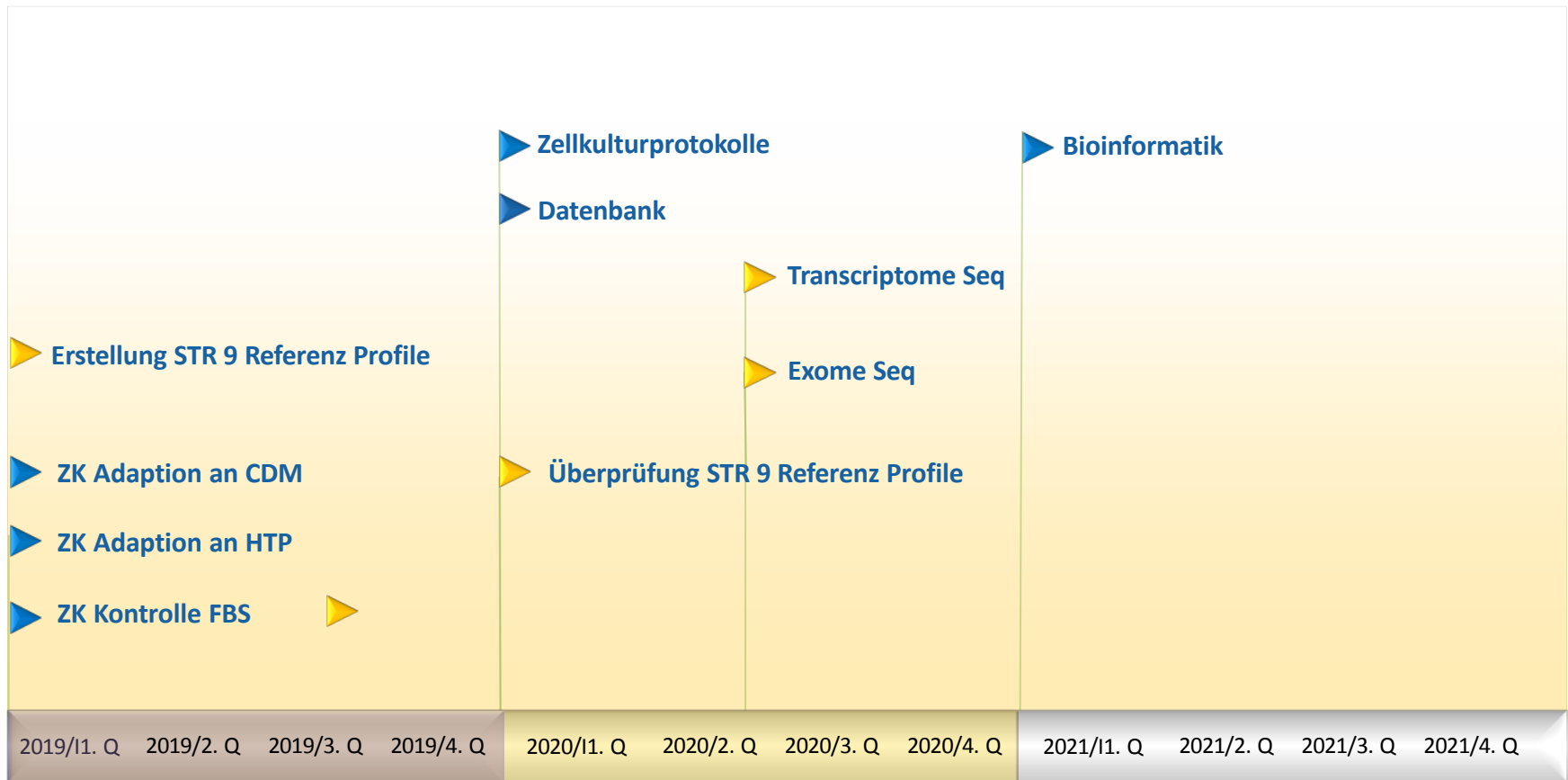
NGS is the Future!



NGS is the Future!



Meilensteine in NextGenCC



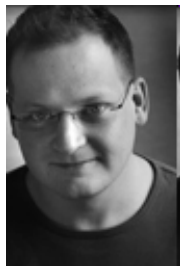
Summary

- Necessity for reliability of scientific data
- Too many false cell lines as models
- Subclones of cell lines are generated by clonal selection (via FBS)
- STR9 is a reference technique for cell line identity

Thank you for your attention!



Annika Siebert



Lennart Rösner



Franziska Rublack



Catharina Beyerle



Yvonne Merkhoffer



Silke Fähnrich



Dr. Claudia
Pomrenke



Dr. Hilmar
Quentmeier



Dr. Cord
Uphoff

