Establishment of cell lines from embryos of *Nothobranchius* annual killifish

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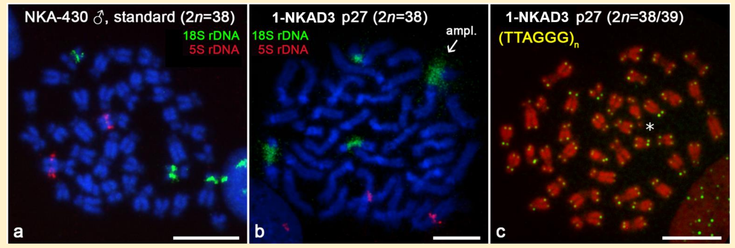
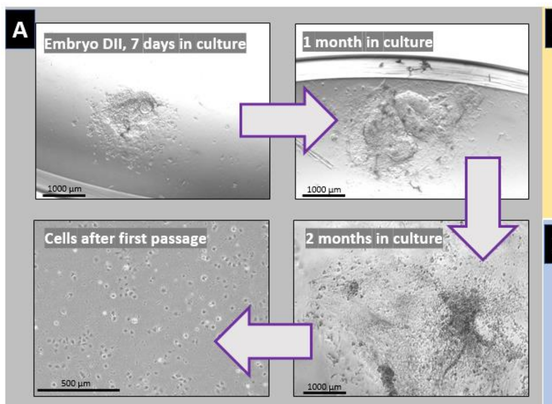
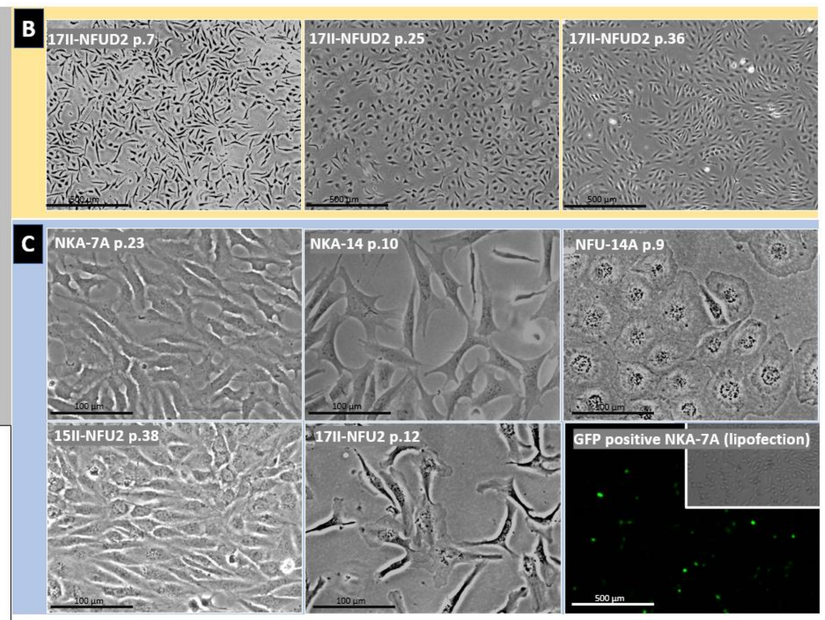
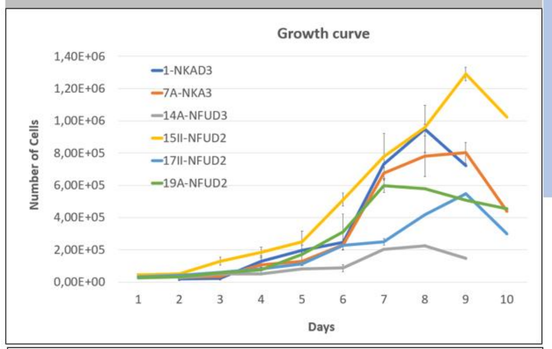
- In this study, we have established and characterized 10 cell lines derived from single DII or DIII stage embryos of annual killifish *Nothobranchius furzeri* (MZM-0410) and *N. kadleci* (NKA-430).
- Cell lines exhibited either fibroblast-like or epithelial-like cell morphology and remained homogeneous and uniform even after subcultivation for more than 60 passages (over 40 passages for lines with lower proliferation rate).
- Fluorescence in situ hybridization (FISH) pointed to some karyotype changes compared to patterns observed on standard metaphase spreads prepared from fin tissue of individuals belonging to the same *N. furzeri* and *N. kadleci* strain, respectively.
- The established cell lines could become an useful *in vitro* model for conducting research in various fields.



Mitotic metaphases of *N. kadleci*-derived cell line 1-NKAD3 and non-cultured metaphase spread prepared from fin tissue of individuals belonging to the same *N. kadleci* strain (NKA-430).
(a-b) FISH with 18S and 5S rDNA probes. Rather standard rDNA patterns were detected in *N. kadleci*-derived cell lines. **(b)** Note amplification of 18S rDNA repeats in 1-NKAD3 (the most prominent one indicated by an arrow). **(c)** Standard pattern of FISH with telomeric repeats; metaphase contains additional small unpaired chromosomal fragment (indicated by asterisk). Chromosomes were counterstained with DAPI (blue; pseudocolored to red in telomeric FISH). Diploid chromosome number (2*n*) is indicated for each metaphase. Scale bar = 10 µm.



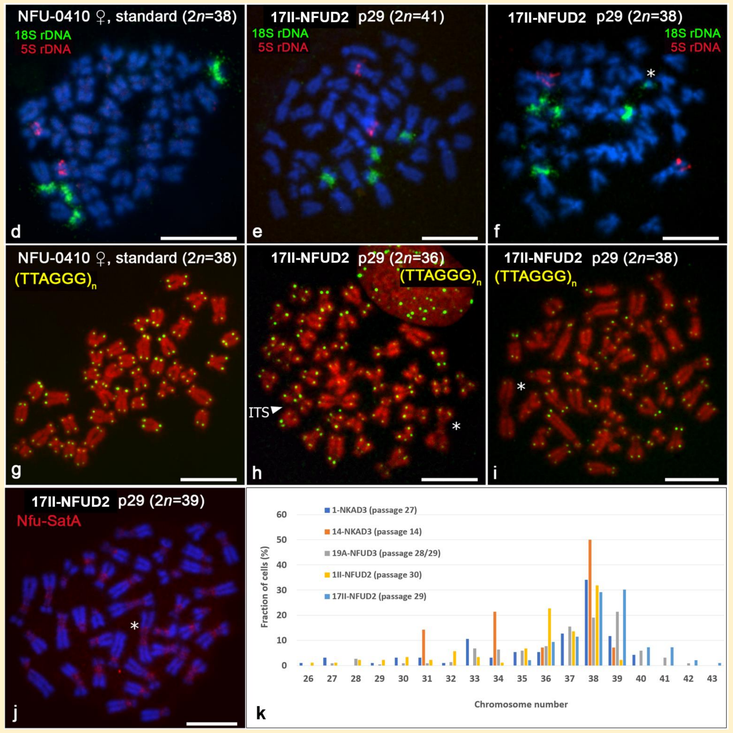




Cultivation conditions
L-15 Medium, 20% fetal bovine serum, glutamax and non-essential amino acids, Penicillin+Streptomycin
Optimum temperature 29°C, no need for CO_2 supply
Detaching: Trypsin-EDTA for 8 min at 29°C
Plastique: Plates and T25 flasks with base for matrix-dependent tissue cultures
Long-term storage of cells
Freezing medium: Bambanker (Lymphotec); liquid nitrogen/-80°C

Cell line	Split ratio	Passage details	Short characteristics and morphology	Derived from single embryo
1-NKAD3	1:3	1-2×/week	Fast growth, 2 cell types, triangle and spindle shape	NK 430 stage DIII
7A-NKAD3	1:2/1:3	1-2×/week	Fast growth, triangle shape, very homogenous	NK 430 stage DIII
14-NKAD3	1:2	1×/10-20 days	Very slow growth, form net, fibroblast/neuronal-like	NK 430 stage DIII
12A-NFUD3	1:2	1×/7-10 days	Slow growth, rounded/triangle shape	NF MZM 0410 stage DIII
14A-NFUD3	1:2	1×/7-10 days	Slow growth, rounded/triangle shape	NF MZM 0410 stage DIII
19A-NFUD3	1:3	2-3×/week	Fast growth, triangle and spindle shape, very homogenous	NF MZM 0410 stage DIII
1II-NFUD2	1:3	2-3×/week	Fast growth, spindle shape	NF MZM 0410 stage DII
15II-NFUD2	1:3	2-3×/week	Fast growth, 2 cell types, rounded and spindle shape	NF MZM 0410 stage DII
17II-NFUD2	1:3	2-3×/week	Fast growth, triangle/spindle shape, very homogenous	NF MZM 0410 stage DII
20II-NFUD2	1:3	2-3×/week	Fast growth, spindle shape, signs of senescence after passage 45	NF MZM 0410 stage DII

(A) Development of cell lines from DII embryos. Eggs were collected after natural mating and stored at 19°C for up to one year to develop naturally via diapause. Prior dechorionation, eggs were sterilized by bleaching. Embryos were disintegrated mechanically (by pipetting up and down in complete L-15 medium) and transferred to 48-well plates. Cultivation medium was refreshed twice a week. After 2-2.5 months of cultivation, primary cultures consisting of heterogeneous cell mass were trypsinized and cell suspensions were seeded to a 24-well plates. To obtain cell lines with a single cell type, we continued with splitting twice a week. Cultures became homo-geneous after 4-6 passages.
To establish cell lines from DIII embryos, we disintegrated embryos by cutting them into smaller pieces and pipetting. Tissue fragments were cultured in L-15 medium for 2-3 weeks. After that we continued with regular splitting of cultures to obtain homogenous cell lines.
(B) Cell cultures did not change morphological features during continuous subcultivation or after extended storage in liquid nitrogen (18 months).
(C) Cell morphology of representative lines. *Nothobrachius* cells are transfectable using Lipofectamine 3000 Reagent (Invitrogen), illustrated by transfection of a CMV:eGFP construct (19319; Addgene) that results in expression of green fluorescent protein (GFP).



Mitotic metaphases of *N. furzeri*-derived cell line 17II-NKAD2 and standard non-cultured metaphase spreads prepared from fin tissue of individuals belonging to the same *N. furzeri* strain (MZM-0410).

(d-f) FISH with 18S and 5S rDNA probes. Rather standard rDNA patterns were detected in *N. furzeri*-derived cell lines. **(f)** Note remarkably small unpaired chromosome (indicated by asterisk) bearing additional 18S rDNA cluster in cell line 17II-NKAD2. **(g-i)** FISH with telomeric repeats. Standard pattern found in metaphase spreads from fin tissue **(g)**. Unpaired large metacentric chromosome in cell line 17II-NKAD2 is indicated by asterisk **(h,i)**. Rare finding of strong interstitial telomeric signal (ITS) in another metacentric chromosome is indicated by arrowhead. **(j)** Example of FISH with centromere-specific 77-nt long minisatellite sequence Nfu-SatA. Chromosomes were counterstained with DAPI (blue; pseudocolored to red in telomeric FISH). Diploid chromosome number (2*n*) is indicated for each metaphases. Scale bar = 10 µm.
Distribution of chromosome number in representative cell lines (we analysed 88- 319 metaphases for each cell line) **(k)**.

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