SUPPLEMENTARY MATERIALS
to the article A.V. Smirnov, A.N. Korablev, I.A. Serova, A.M. Yunusova, A.A. Muravyova, E.S. Valeev, N.R. Battulin "Studying concatenation of the Cas9-cleaved transgenes using barcodes"

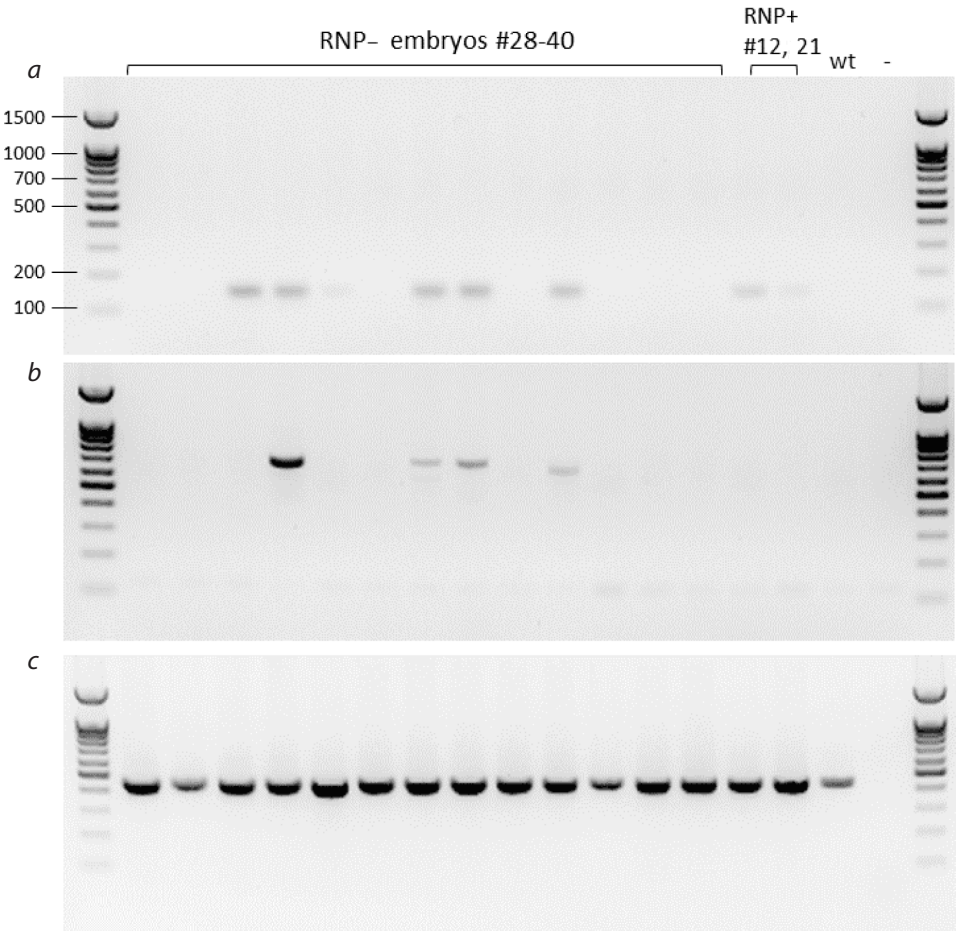

Fig. S1. PCR genotyping of the embryos.

a – PCR with primers for the Cherry gene (expected product size – 145 bp); *b* – PCR with primers for the transgene-transgene junctions (expected product size – 699 bp); *c* – PCR with primers for the mouse Smc2 gene (DNA quality control) (expected product size – 410 bp). Legend: (embryos) – 13 embryos from the RNP– experiment; (+) – positive embryos from the Cas9 experiment (2/40); (w) – wild-type DNA; (–) – negative control.

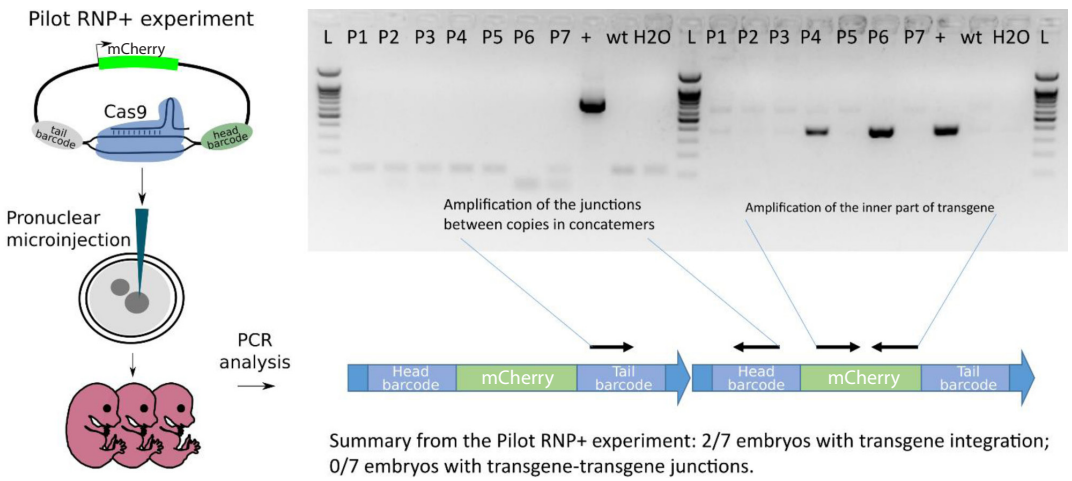

Fig. S2. Results of the Pilot RNP+ experiment.

Left: scheme of experiment. Pilot RNP+ experiment differs from RNP+ experiment from the main text (Fig. 1) with the plasmid library used. Right: Results of PCR analysis. Seven embryos (P1–P7) were analyzed. Two embryos (P4, P6) had integration of the transgene into genome. No embryos with transgene-transgene junctions were identified.

Table S1. Primers used for PCR

Primer name	Sequence 5′–3′	PCR experiment/length (bp)
AAVS1 CAG Cherry F	TCGGTACCGAGAACCGGGCAGGTCACGC	AAVS1 amplification for cloning 493
AAVS1 CAG Cherry R	GTGGTACCCTAGCTCTTCCAGCCCCCTG	
CAG bc far F	GGTTATTGTCTCATGAGCGGA	Junction, inverse PCR, genotyping (Table S2)
CAG bc 1F	AATAAACAAATAGGGGTTCCGCGC	
CAG bc 2F	TAGGGGTTCCGCGCACATTTC	
CAG bc far R	GCGGAACTCCATATATGGGCTAT	
CAG bc 1R	TATGAACTAATGACCCCGTAATTG	
CAG bc 2R	TGGGCTATGAACTAATGACCC	
CAG bc 3R	CCCCGTAATTGATTACTATTAATAAC	
CAG bc 4R	TCAATAATCAATGTCGCTCCTACG	
CONCAT AAVS1 JUNC F	GCTCCAGGAAATGGGGGTGTG	Internal junctions PCR in combination with primers from Table S2
CONCAT AAVS1 JUNC R	AGGGATCCTGTGTCCCCGAG	
ddPCR mCher F	GAACGGCCACGAGTTCGAGA	PCR genotyping (Cherry) 145
ddPCR mCher R	CCTCAGTTCATGTACGGCTCCAAG	

Table S2. Primers used to generate PCR products for the NGS analysis

Embryo/PCR fragment	Primer pairs	Length (bp)
#31 left bc junction	CAG bc 1F + AAVS1 JUNC R	459
#31 right bc junction	AAVS1 JUNC F + CAG bc 1R	471
#31 inverse	CAG bc 1F + CAG bc 1R	206
#34 left bc junction	CAG bc 2F + AAVS1 JUNC R	449
#34 right bc junction	AAVS1 JUNC F + CAG bc 2R	476
#34 inverse	CAG bc 2F + CAG bc 2R	201
#35 left bc junction	CAG bc far F + AAVS1 JUNC R	504
#35 right bc junction	AAVS1 JUNC F + CAG bc far R	491
#35 inverse	CAG bc far F + CAG bc far R	271
#37 left bc junction	nd	nd
#37 right bc junction	AAVS1 JUNC F + CAG bc 3R	331
#37 inverse	CAG bc 2F + CAG bc 3R	183

Table S3. Number of NGS reads

Library	Reads with barcodes
Original barcoded plasmids library	17096822
E31_junctions	30342
E31_inverse_PCR	33597
E34_junctions	30847
E34_inverse_PCR	28330
E35_junctions	18744
E35_inverse_PCR	18369
E37_junctions	45449
E37_inverse_PCR	1296
E12_inverse_PCR	17248
E21_inverse_PCR	69581

Table S4. Primers used for droplet digital PCR

Primer name	Sequence 5′–3′
mCherry-F	GCGCCTACAACGTCAACATC
mCherry-R	CTTGTACAGCTCGTCCATGC
mCherry probe	HEX-TGGAACAGTACGAACGCGCCGAGGG-BHQ2
Emid1-F	GCCAGGACTGGGTAGCAC
Emid1-R	TAGGGGTTCCGCGCACATTTC
Emid1 probe	FAM-GCGGAACTCCATATATGGGCTAT-BHQ1