

Autonomous combinatorial colour barcoding for multiplexing single molecule RNA visualization

Yong-Sheng Cheng^{1*}, Yue Zhuo¹, Katharina Hartmann¹,
Peng Zou¹, Gözde Bekki¹, Heike Alter¹ & Hai-Kun Liu^{1*}

¹Division of Molecular Neurogenetics, German Cancer
Research Center (DKFZ), DKFZ-ZMBH Alliance, Im
Neuenheimer Feld 280, Heidelberg, 69120, Germany.

*Correspondence: yongsheng.cheng@dkfz.de and
l.haikun@dkfz.de

Supplementary Figures

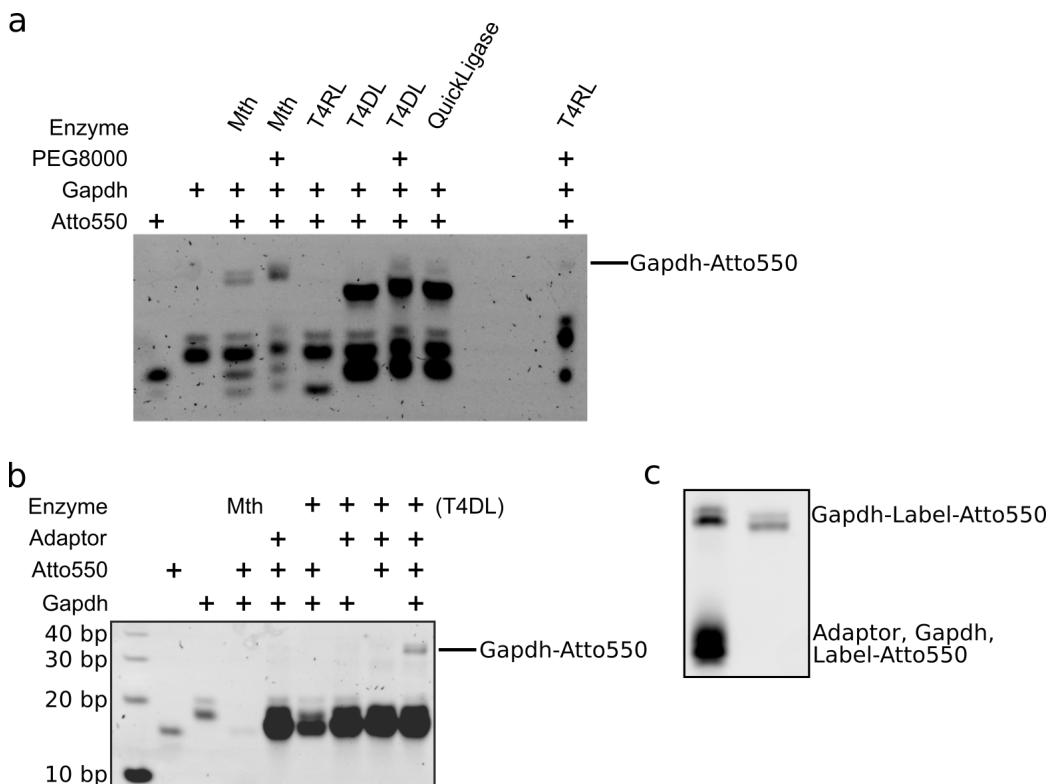


Figure 1: T4DL based HuluFISH 1.0 labelling and purification. (a) Various enzymes used for conjugating Gapdh oligo pool. Mth for Thermostable 5' AppDNA/RNA Ligase (NEB, M0319S). T4RL for T4 RNA ligase 1 (NEB, M0204S). QuickLigase (NEB, M2200S). The adaptor for T4DL was not 3' end amine blocked, therefore there is a thick band below Gapdh-Atto550 band. (b) Quality control reaction for HuluFISH labelling. Single oligo component or T4DL ligation reaction single component knockout condition. TrackIt™ 10 bp DNA Ladder (ThermoFisher, 10488019) was used as marker. Gel were stained with 3×GelRed in water. (c) Urea-PAGE gel purification of HuluFISH probe. The upper band is ligated HuluFISH probe, it runs slightly faster after purification (2nd lane).

Supplementary Tables

Table 1: Oligo sequences for HuluFISH

Name	Sequences	Notes
Label Atto550 (Hulu 1.0)	CTAAATCCAT _z A	z, Amino-C6-dT with Atto550
Adaptor for Label Atto550	ATGGATTTAGNNNN	
Adaptor for L1+2+3 (Hulu 2.0)	ACCGTGTCTGGGCGATAC-CGATTAGGGTGAGTT-TAGTCGAGC	
L1	AAAC _z CACCCTAATC	z, Amino-C6-dT with Atto/Alexa dyes
L2	GGTA _z CGCCC	z, Amino-C6-dT with Atto/Alexa dyes
L3	AGGACACGGT	3' end modified with Atto/Alexa dyes

Table 2: HuluFISH probe sequences

Oligo_Name	Sequence
Gapdh_old	ATGAACCTAAGCTGGGA
Gapdh_old	AGGAAACACTCTCCTGA
Gapdh_old	TCACCATTTTGTCTACG
Gapdh_old	CCAAATCCGTTCACACC
Gapdh_old	TCTCCACTTTGCCACTG
Gapdh_old	AAGGGGTTCGTTGATGGCAA
Gapdh_old	GACCATGTAGTTGAGGT
Gapdh_old	TGGAGTCATACTGGAAC
Gapdh_old	GTGCCGTTGAATTTGCC
Gapdh_old	CTTCCCATTCTCGGCCTTGA
Gapdh_old	AAGATGGTGATGGGCTT
Gapdh_old	ATGTTAGTGGGGTCTCG
Gapdh_old	ACGACATACTCAGCACC

Gapdh_old	CCATGGTGGTGAAGACA
Gapdh_old	AGATGATGACCCTTTTG
Gapdh_old	ACAAACATGGGGGCATC
Gapdh_old	GACAATCTTGAGTGAGT
Gapdh_old	AGTTGGTGGTGCAGGAT
Gapdh_old	CCAAAGTTGTCATGGAT
Gapdh_old	GGTCATGAGCCCTTCCACAA
Gapdh_old	TCTTCTGGGTGGCAGTGAT
Gapdh_old	ATGATGTTCTGGGCAGC
Gapdh_old	AGTGAGCTTCCCGTTCA
Gapdh_old	TAGGAACACGGAAGGCCAT
Gapdh_old	ACTTGGCAGGTTTCTCC
Gapdh_old	ACCACCTTCTTGATGTC
Gapdh_old	AAGATGCCCTTCAGTGG
Gapdh_old	GTTGAAGTCGCAGGAGA
Gapdh_old	AGGTGGAAGAGTGGGAGTT
Gapdh_old	TTGAGAGCAATGCCAGC
Gapdh_old	GGAAATGAGCTTGACAA
Gapdh_old	AGCCGTATTCATTGTCA
Nr2e1_old	AATGCGGCTTGTTGATC
Nr2e1_old	TAGACCCCGTAGTGCTT
Nr2e1_old	TTGAAGAATCCGGAGCA
Nr2e1_old	GGTCCTATTCCTTCGAA
Nr2e1_old	GGTTTCCAGACTTGCAG
Nr2e1_old	TGTCTTGTCTACGGGGCAT
Nr2e1_old	ACGCCCTGCATTGGTTT
Nr2e1_old	TCCAAACACTTCTTCAG
Nr2e1_old	GGCATCTTTGTTTCATGT
Nr2e1_old	TGTTTGCGGATGGTGGA
Nr2e1_old	TGTGAAGAAAGCAGGGG
Nr2e1_old	TTCAGGAGTGGCAGACA
Nr2e1_old	TTCATGGGGATACTTGG
Nr2e1_old	AGATACATTGGGGTCCC
Nr2e1_old	TTCACACACGGACTCAG
Nr2e1_old	TAAAGAGAAGCCTGGCA
Nr2e1_old	CTCTTTGCCCACTTGAT
Nr2e1_old	CAAAGTGGAAAAGGCTG
Nr2e1_old	TGGCCCATTGTGCTATT

Nr2e1_old	AGAGTGTTAGCATCAAC
Nr2e1_old	ATTCATGCCAGATACAG
Nr2e1_old	TCTGGGAGTCTGTGTTG
Nr2e1_old	GCAAAGCCTGTATTTCA
Nr2e1_old	CTAATCGGAGCTGTCTG
Nr2e1_old	AGACAGGCAAATTCAGT
Nr2e1_old	GGAACAGCTTTGAAAGT
Nr2e1_old	CTCAGTTCAGAACCACT
Nr2e1_old	ATCTTGAGAGCGGCAA
Nr2e1_old	TGTTGAGAGTTAGCTGA
Nr2e1_old	GGGTATCTGGTATGAAT
Nr2e1_old	CTAATTGACCGTAAAGC
Nr2e1_old	GTCACTGGATTTGTACA
Gapdh_new	AACCTAAGCTGGGACCCC
Gapdh_new	GGACGAGGAAACACTCTCCTG
Gapdh_new	CGACCTTCACCATTTTGTCT
Gapdh_new	TGACCAGGCGCCCAATAC
Gapdh_new	CAATCTCCACTTTGCCACTG
Gapdh_new	ATGAAGGGGTCGTTGATGGCA
Gapdh_new	CCGTGAGTGGAGTCATACTGG
Gapdh_new	CCTTGACTGTGCCGTTGAATT
Gapdh_new	ATGACAAGCTTCCCATTCTCG
Gapdh_new	CTGGAAGATGGTGATGGGCTT
Gapdh_new	TGATGTTAGTGGGGTCTCGCT
Gapdh_new	CACGACATACTCAGCACCGG
Gapdh_new	GGCTCCACCCTTCAAGTG
Gapdh_new	GGGCGGAGATGATGACCCTTT
Gapdh_new	TCACAAACATGGGGGCATCGG
Gapdh_new	GGCTAAGCAGTTGGTGGTGC
Gapdh_new	CCTTCCACAATGCCAAAGTTG
Gapdh_new	GTGATGGCATGGACTGTGGT
Gapdh_new	GGCCATCCACAGTCTTCT
Gapdh_new	TCACGCCACAGCTTTCCAGAG
Gapdh_new	GATGATGTTCTGGGCAGCC
Gapdh_new	GGATGACCTTGCCCACAG
Gapdh_new	GTGAGCTTCCCGTTCAGC
Gapdh_new	AGGAACACGGAAGGCCATGC
Gapdh_new	CATACTTGGCAGGTTTCTCC

Gapdh_new	CTTCACCACCTTCTTGATGTC
Gapdh_new	TCAGTGGGCCCTCAGATGCCT
Gapdh_new	TCAGTGTAGCCCAAGATGCC
Gapdh_new	TGCTGTTGAAGTCGCAGGAGA
Gapdh_new	TTGAGAGCAATGCCAGCCCCG
Gapdh_new	GCTGTAGCCGTATTCATTGTC
Gapdh_new	AGTGTCCCTTGCTGGGGTGGGT
Tlx_new	TTGGTTGTGCGCTGCCCTGCT
Tlx_new	TGATCCGGCGGGCTTGCTCAT
Tlx_new	CCACACACTTTGCAAGGGATA
Tlx_new	TAGTGCTTCCCGGAGCTG
Tlx_new	TTGAAGAATCCGGAGCAGCC
Tlx_new	TCCAGACTTGCAGACATAGGT
Tlx_new	TTGTCTACGGGGCATCCTCC
Tlx_new	CCTGCATTGGTTTCTGTGTGT
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Hes5_tag	GTGACCAGGAACTTCGCAGAcgctcgact
Hes5_tag	AAATATCATAGAACCCCCGGTGacgctcgact
Hes5_tag	GTGAGCCAACCCCCGACTCTAacgctcgact
Hes5_tag	CCCTGAAGAAAGTCCTCTACGGacgctcgact
Hes5_tag	GCAGTTCGCGCTTCACAAAAGacgctcgact
Hes5_tag	AGCGCGCATCAGACAGCCAAGacgctcgact
Hes5_tag	AGGATCATCGTGGAGACCCacgctcgact
Hes5_tag	CCACCCATACAAAGGAATCCTacgctcgact
Hes5_tag	TACAAAATCGTGCCACATGCacgctcgact
Hes5_tag	CCCACATGACCAAGAGTTCAAacgctcgact
Hes5_tag	GCCTTCAGAACAGCCTGTGacgctcgact
