Autonomous combinatorial colour barcoding for multiplexing single molecule RNA visualization

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Supplementary Figures

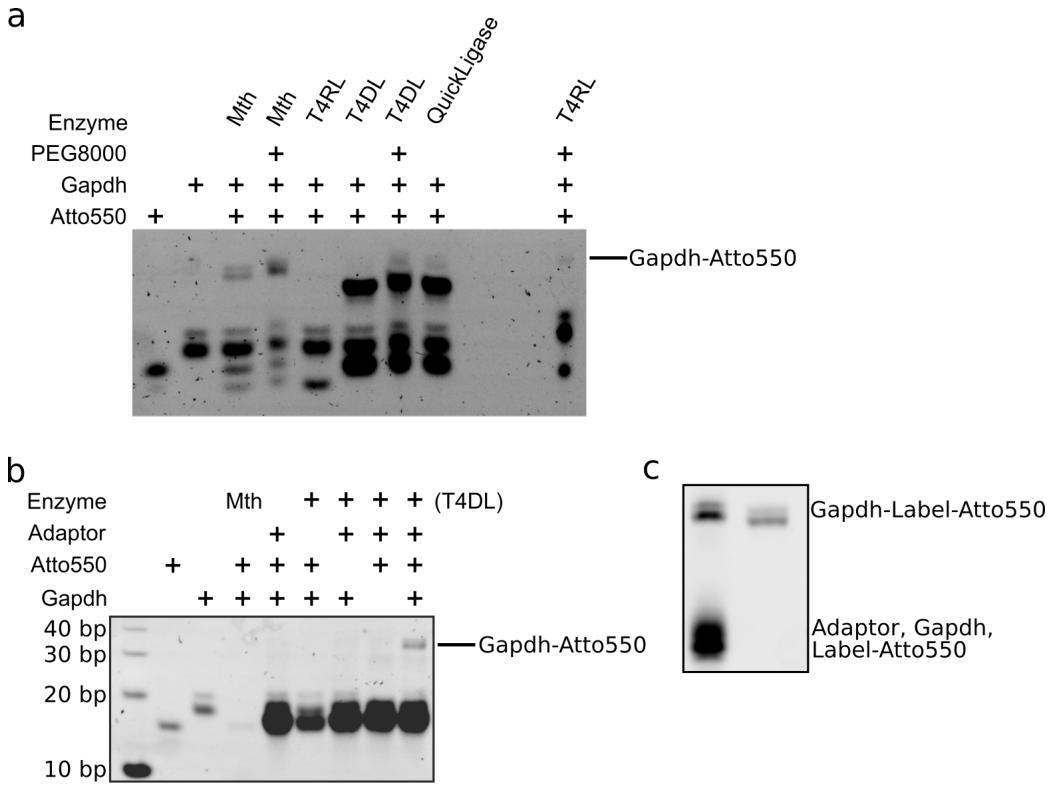

Figure 1: T4DL based HuluFISH 1.0 labelling and purification. (a) Various enzymes used for conjugating Gapdh oligo pool. Mth for Thermostable 5' AppDNA/RNA Ligase (NEB, M0319S). T4RL for T4 RNA ligase 1 (NEB, M0204S). QuickLigase (NEB, M2200S). The adaptor for T4DL was not 3' end amine blocked, therefore 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$^{\text{TM}}$ 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 (2$^{\text{nd}}$ lane).

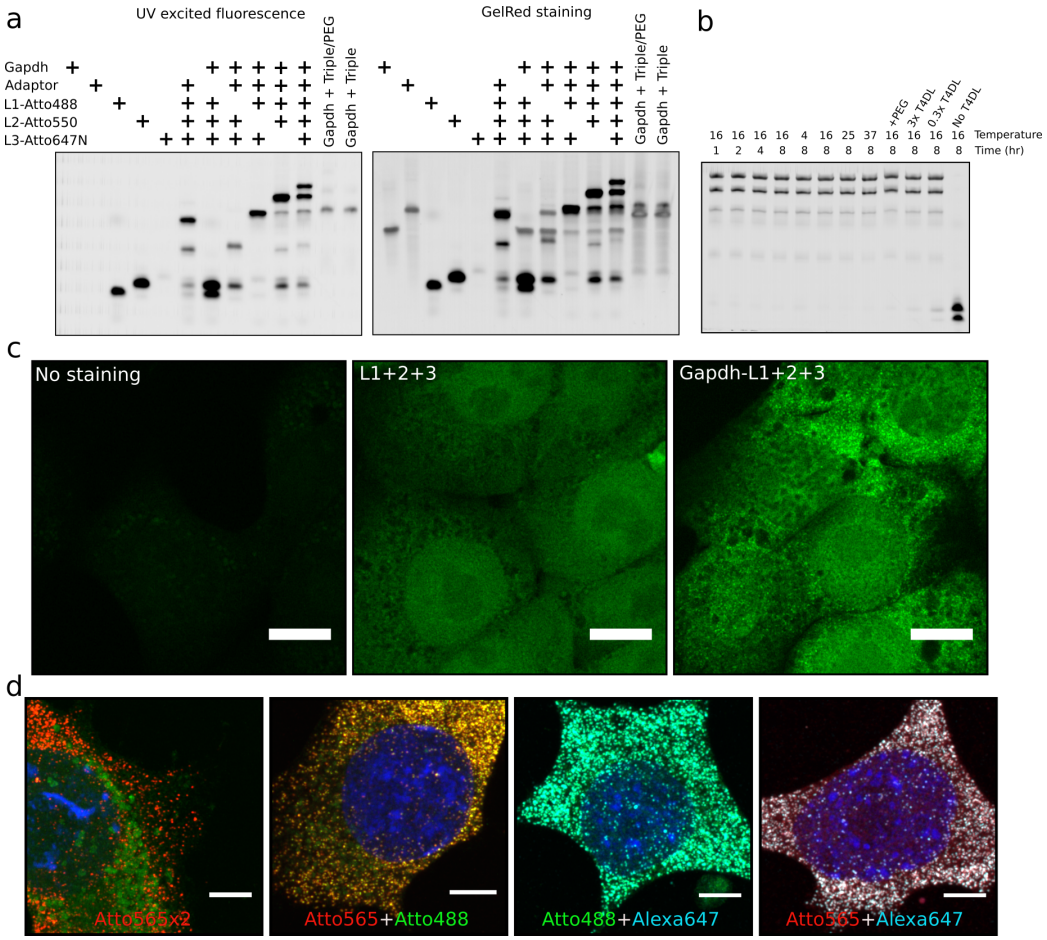

Figure 2: Multiple-way ligation for HuluFISH probe. (a) Quality control reaction for multiple piece ligation. Gapdh oligo pool, adaptor, and 3 singly labelled common label oligos were ligated together with various component knockout conditions. Gel was imaged with GelDoc (Bio-Rad) before and after GelRed staining. Only Atto488/550 can be properly excited in the unstained gel, Atto647N is weakly excited. (b) Condition scouting for multiple way ligation. Various times, temperatures and T4DL concentrations, or addition of PEG were tested for ligation efficiency. The top band is the triple labeled Gapdh probe. Scale bar 10 μm. (c) Control staining for multiple-way ligation probes. Triple fluorophore probe alone (L1+2+3) and Gapdh-conjugated L1+2+3 were pre-annealed with adaptor and compared with non-stained control in Hepa 1-6 cells. (d) 4 different colour combinations for Gapdh individual staining. Scale bar 5 μm.

Supplementary Tables

Table 1: Oligo sequences for HuluFISH

Name	Sequences	Notes
Label Atto550 (Hulu 1.0)	CTAAATCCATzA	z, Amino-C6-dT with Atto550
Adaptor for Label Atto550	ATGGATTTAGNNNN	
Adaptor for L1+2+3 (Hulu 2.0)	ACCGTGTCCTGGGCGATAC-CGATTAGGGTGAGTT-TAGTCGAGC	
L1	AAACzCACCCTAATC	z, Amino-C6-dT with Atto/Alexa dyes
L2	GGTAzCGCCC	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	AAGGGGTCGTTGATGGCAA
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	GGCATCTTTGTTCATGT
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	ATCTTGGAGAGCGGCAA
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
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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
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Tlx_new	TTGGTTGTGCGCTGCCCTGCT
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Sox2_tag	CGTTTCGCTGCGGAGATTTTTacgctcgact
Sox2_tag	GGCATCACGGTTTTTGCGTTAacgctcgact
Sox2_tag	CCCCAAAAAGAAGTCCCAAGAacgctcgact
Sox2_tag	CCCTCAGGTTTTCTCTGTACAacgctcgact
Sox2_tag	TACATGGTCCGATTCCCCCGCacgctcgact
Sox2_tag	GCCTAACGTACCACTAGAACTacgctcgact
Hes5_tag	GGAGCTCTGGAGGCGATTAGacgctcgact
Hes5_tag	CACGGTACTTGGGGCCATacgctcgact
Hes5_tag	TGGGACTGAGCATCTCCACCGacgctcgact

Hes5_tag	TCTTCTCCACCACCGGCTTCCacgctcgact
Hes5_tag	TGCTGTTGATGCGGTCCCacgctcgact
Hes5_tag	AGCTTGGAGTTGGGCTGGTGCacgctcgact
Hes5_tag	CTCCAGGATGTCGGCCTTCTCacgctcgact
Hes5_tag	TGTGTTTCAGGTAGCTGACGGacgctcgact
Hes5_tag	TCGCTGTAGTCCTGGTGCAGacgctcgact
Hes5_tag	AACTGTACCGCCTCCTGCacgctcgact
Hes5_tag	CAGCTTCATCTGCGTGTCGacgctcgact
Hes5_tag	ATCCTCTGGTCCGCTCCAacgctcgact
Hes5_tag	CCACATCCAGAGGAACGAGCTCacgctcgact
Hes5_tag	ACTGCGGCTGGGGAATGTCTTCacgctcgact
Hes5_tag	AGAAGGTAGCGGCCAACCTGacgctcgact
Hes5_tag	AATGACCCTCCTGCTGGCCAacgctcgact
Hes5_tag	CAACTCTGCACACACATTCTCTacgctcgact
Hes5_tag	GGCCCTGATTATCCCCAAATGAacgctcgact
Hes5_tag	GCAAACACAAAACAACCCCACGacgctcgact
Hes5_tag	GTGACCAGGAACTTCGCAGAacgctcgact
Hes5_tag	AAATATCATAGAACCCCCGGTGacgctcgact
Hes5_tag	GTGAGCCAACCCCCGACTCTAacgctcgact
Hes5_tag	CCCTGAAGAAAGTCCTCTACGGacgctcgact
Hes5_tag	GCAGTTCCGCCTTCACAAAAGacgctcgact
Hes5_tag	AGCGCGCATCAGACAGCCAAGacgctcgact
Hes5_tag	AGGATCATCGTGGAGACCCCacgctcgact
Hes5_tag	CCACCCATACAAAGGAATCCTacgctcgact
Hes5_tag	TACAAAATCGTGCCCACATGCacgctcgact
Hes5_tag	CCCACATGACCAAGAGTTCAAAacgctcgact
Hes5_tag	GCCTTCAGAACAGCCTGTGacgctcgact