Simultaneous Detection of Drug-Induced Liver Injury Protein and microRNA biomarkers using dynamic chemical labelling on a Luminex MAGPIX system

Antonio Marín-Romero [a, b, c], Mavys Tabraue-Chávez [a], Bárbara López-Longarela [a], Mario A. Fara [a], Rosario M. Sánchez-Martín [b, c], James W. Dear [d], Hugh Ilyine [e], Juan J. Díaz-Mochón [b, c, *] and Salvatore Pernagallo [a, *]

[a.] DESTINA Genomica S.L. Parque Tecnológico Ciencias de la Salud (PTS), Avenida de la Innovación 1, Edificio BIC, Armilla, Granada 18100, Spain.

[b.] GENYO - Centre for Genomics and Oncological Research: Pfizer / University of Granada / Andalusian Regional Government, PTS Granada - Avenida de la Ilustración, 114 - 18016 GRANADA, Spain

[c.] Universidad de Granada. Facultad de Farmacia. Departamento de Quimica Farmacéutica y Orgánica, Campus Cartuja s/n, 18071 Granada, Spain

[d.] Pharmacology, Therapeutics and Toxicology, Centre for Cardiovascular Science, University of Edinburgh, The Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK.

[e] DESTINA Genomics Ltd, 7-11 Melville St, Edinburgh EH3 7PE, UK

[†] Corresponding author: salvatore@destinagenomics.com, juan@destinagenomics.com

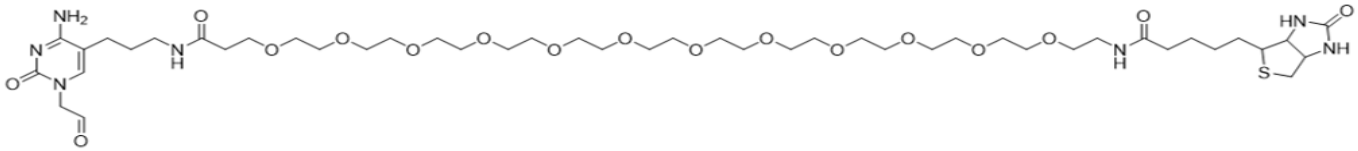
Electronic Supplementary Information (ESI)

Table S1 – Sequences

Sequence ID	**Name**	**Peptide with abasic position (N'-C')**
1	DGL 122	xx-CACCATT*GT*_AC*ACT*CCA
		miRNA sequence (5'-3')
2	Target miR-122	UGGAGUGU**G**ACAAUGGUGUUUG

xx = amino-PEG-linker; "*" = propanoic acid side chain at the gamma position; "_" = abasic unit containing a propanoic acid side chain at the gamma position. The underlined sequence miR-122 is the region that hybridises with the DGL 122. The "G" in position 9 from 5' (in bold) is opposite to the abasic unit monomer and allows the specific incorporation of the aldehyde-modified biotinylated cytosine nucleobase (Figure S1).

Figure S1 – Chemical structure of **aldehyde-modified biotinylated cytosine nucleobase**



Section S1 – Reagents for reactions

Assay buffer, Anti-ARG1 Beads and detection antibody were purchased from Merck (Cat # HLINJMAG-75K). The proprietary Stabiltech buffer was purchased from DESTINA Genomica S.L. Wash buffer contains 0.1% Tween 20 in PBS 1X.

Section S2 – Luminex MagPlex beads coupling with DGL 122

Five million microspheres were suspended in MES Buffer (50 µL, 0.1 M, pH 5) in an eppendorf tube and combined with (i) a solution with 200 pmoles of abasic probe (2 µL, 100 µM), (ii) EDC (2.5 µL, 10 mg/mL in de-ionised water). The solution was then vortexed and incubated for 30 min at room temperature with shaking taking place in darkness. Following this, 2.5 µL of a second EDC solution (10 mg/mL freshly prepared) was added, and the solution incubated for further 30 min at room temperature in darkness. Beads were washed once with 1 mL of 0.02% Tween 20 and once with 1 mL of 0.1% SDS. Following the washings, the beads were then resuspended and incubated with 200 µL of Ethanolamine (50 mM) in 0.1% Tween 20 for 1h, under vortexing at room temperature in darkness. The beads were then washed one more time in 1 mL of 0.02% Tween 20 solution, and 1 mL of 0.1% SDS solution. Finally, the beads were resuspended in 100 µL of PBS solution with 0.1% Tween20 and 10% PEG10K.

Table S2. ARG1 calibration curve data. n = 3

Standard	Expected concentration (pg/mL)	MFI Average	SD	CV
Standard 7	50000.00	19518.50	799.74	4.10%
Standard 6	16666.67	12795.50	222.03	1.74%
Standard 5	5555.56	5945.50	30.41	0.51%
Standard 4	1851.85	1674.50	21.92	1.31%
Standard 3	617.28	416.00	120.21	28.90%
Standard 2	205.76	218.50	17.68	8.09%
Standard 1	68.59	101.75	8.13	7.99%
Standard 0	0.00	67.50	12.02	17.81%

Table S3. miR-122 calibration curve data. n = 3

Standard	Expected concentration (pM)	MFI Average	SD	CV
Standard 7	1050.00	3723.17	173.35	4.66%
Standard 6	350.00	2009.00	63.15	3.14%
Standard 5	116.67	876.67	39.26	4.48%
Standard 4	38.89	367.67	30.75	8.36%
Standard 3	12.96	189.00	7.55	3.99%
Standard 2	4.32	110.33	6.43	5.83%
Standard 1	1.44	77.17	2.36	3.06%
Standard 0	0.00	----	----	----

Table S4. MFI measurement (in triplicate) of ARG1 and miR-122 in singleplex analysis and seqCOMBO. Respective concentrations (pg/mL for ARG1 and pM for miR-122) are calculated through the calibration curves reported in Figure 2. n = 3

Assay	Sample	Analyte	Average MFI	SD	CV	Predicted concentration (pg/mL or pM)
ARG1 singleplex	DILI	ARG1	3340.5	118.9	3.54%	3451.4
	No DILI		31.0	0.0	0.00%	n.d.
miR-122 singleplex	DILI	miR-122	1376.8	89.5	6.50%	207.0
	No DILI		26.0	0.0	0.00%	n.d.
seqCOMBO	DILI	ARG1	3256.0	166.9	5.13%	3372.9
		miR-122	1436.5	153.4	10.7%	219.3
	No DILI	ARG1	52.5	0.7	1.35%	n.d.
		miR-122	42.5	2.12	4.99%	n.d.

Figure S2 – Levels of ARG1 and miR-122 in DILI patient when measured via singleplex and seqCOMBO assays. Inter-CV values between singleplex and seqCOMBO are represented. n = 3.