Table S1. Comparison of DNA array-based spatial transcriptomic assays

ST method	DNA features	Barcode sequencing for each array	Feature diameter(s) (μm)	Feature center distance(s) (μm)	Percentage of array area covered by features[a]	Feature density (features per mm^2)	Array substrate[b]	Tissue used for ST assays[c]	cDNA synthesis & amplification methods[d]	Mean UMIs[e]	Data
Visium	Spotted DNAs	No	55	100	27%	~110	Glass	Mouse OB, 10-μm thickness, tissue fixation & permealization	TSO & PCR	15,377/55 μm	*GSE153859 (10.1101/2020.08.24.252296)*
DBiT-seq	Microfluidic wells	No	1) 10 2) 20 3) 50	1) 20 2) 40 3) 100	25%	1) 2500 2) 625 3) 100	Microfluidic channel-divided tissue	Mouse embryo, 7· μm thickness, tissue fixation & permealization	TSO & PCR	~5,000/10 μm	*GSE137986 (10.1016/j.cell.2020.10.026)*
Slide-seqV2	Assembled beads	Yes	10	10	78%	1×10^4	Glass	Mouse OB, 10-μm thickness, fresh frozen	RPE & PCR	494/10 μm	*(10.1038/s41587-020-0739-1)*
HDST	Assembled beads	Yes	2	3	34%	1.07×10^5	Silicon	Mouse OB, 10-μm thickness, tissue fixation & permealization	T7 aRNA	12/10 μm or 2/2 μm	*GSE130682 (10.1038/s41592-019-0548-y)*
Seq-Scope	Illumina DNA clusters	Yes	≥ 0.5	≤ 0.8	≤ 70%	$\leq 1.5 \times 10^6$	Linear PAA coating	Mouse liver, 10-μm thickness, tissue fixation & permealization	RPE & PCR	~1000/10 μm	*GSE169706 (10.1016/j.cell.2021.05.010)*
Stereo-seq	DNA nanoballs	Yes	0.22	1) 0.5 2) 0.715	1) 15% 2) 7%	1) 4×10^6 2) 1.96×10^6	Silicon	Mouse OB, 10-μm thickness, tissue fixation & permealization	RPE & PCR	~1450/10 μm or 59/2 μm	*GSE153164 (10.1016/j.cell.2022.04.003)*
Pixel-seq	Polonies	No	~1	~1	> 90%	$\leq 1 \times 10^6$	Crosslinked PAA gel	Mouse OB, 10-μm thickness, fresh frozen	TSO & PCR	977/10 μm or 47/2 μm	*GSE186097*

Notes:

[a]: The percentages for Visium, DBiT-Seq, Slide-seqV2, HDST, and Stereo-seq were calculated based on reported feature sizes, densities, and spatial patterns. The percentage for Seq-scope was measured by analyzing a reported cluster image of the flowcell hybridized with the highest template concentration (100 pM).

[b]: PAA, polyacrylamide.

[c]: Different RNA capturing conditions were used in ST methods and some captured RNAs from multiple cell layers from a whole tissue section.

[d]: TSO, template-switching oligo; RPE, random priming and extension; T7 aRNA, T7 RNA polymerase-based amplification. Different amplification methods have different yields. RPE can increase the capture efficiency.

[e]: UMI counts were obtained from the original publications.

Table S2. Consumable lists for the stamping and sequencing-based polony gel fabrication

Fabrication step	Reagents & consumables	Vender	Catalog number	Size	UW price (USD)	Amount	Cost (USD)/gel
Glass cleaning	Microscope slide	Fisher Scientific	12-550D	144 pieces/pack	20.0	1 piece/gel	0.14
	Contrad 70	Decon	1003	1.33 gallon	141	25 mL/60 gels	0.01
Glass surface modification	200 proof ethanol	Decon	2701	4 × 4 gallon	58.5	360 mL/60 gels	0.01
	Blind-Silane	Sigma-Aldrich	M6514	50 mL	88.9	2 mL/60 gels	0.06
	Acetic acid	Fisher Scientific	A35-500	500 mL	33.9	4 mL/60 gels	0.01
Gel casting	Acrylamide	Sigma-Aldrich	A9099	100 g	76.1	2 g/200 gels	0.01
	N,N'-methylenebisacrylamide	Sigma-Aldrich	M7279	100 g	145	0.5 g/200 gels	0.01
	Ammonium persulfate	Sigma-Aldrich	A3678	25 g	32.2	100 mg/10 gels	0.01
	TEMED	Invitrogen	15524-010	30 mL	48.1	10 µL/10 gels	0.01
	N-(5-bromoacetamidylpentyl) acrylamide	Combi-blocks	HD-8626	1 g	3,600	70 mg/100 gels	2.52
	N, N-dimethylformamide	Sigma-Aldrich	D4551	250 mL	42.7	500 µL/200 gels	0.01
Primer grafting	Potassium phosphate dibasic trihydrate	Sigma-Aldrich	P9666	100 g	41.3	1 g/200 gels	0.01
	Potassium phosphate monobasic	Sigma-Aldrich	P9791	100 g	26.9	1 g/200 gels	0.01
	Bridge amplifiication primers	IDT	PS-BA(+)	3 µmole (3 mL × 1mM)	528	50 µL/10 gels	0.88
			PS-BA(-)	2.83 µmole (2.83 mL × 1 mM)	597	50 µL/10 gels	1.05
Polony amplification	Betaine	Sigma-Aldrich	B2629	1 kg	176	234.3 g/20 gels	2.06
	Trizma base	Sigma-Aldrich	93362	500 g	266	2.42 g/20 gels	0.06
	Ammonium sulfate	Sigma-Aldrich	A4418	500 g	79.5	1.32 g/25 gels	0.01
	Magnesium sulfate	Sigma-Aldrich	M2773	500 g	65.6	0.49 g/20 gels	0.01
	Triton X-100	Sigma-Aldrich	T8787	250 mL	90.1	1 mL/20 gels	0.02
	Dimethyl sulfoxide	Sigma-Aldrich	D8418	1 L	315	13 mL/20 gels	0.20
	Formamide (deionized)	Emdmillipore	4670-4L	4 L	447	25 mL/gel	2.79
	Bst enzyme	Lab purified	----	----	----	----	< 1
	dNTP (10 mM each)	GenScript	C01582-250	250 mL	2,500	320 µL/gel	3.20
Gel stamping	dNTP (10 mM each)	GenScript	C01582-250	250 mL	2,500	30 µL/ 20 gels	0.02
	Taq DNA polymerase	New England Biolabs	M0267X	4000 units (800 µL)	370	15 µL/20 gels	0.35
TaqI digestion	*Taq* I-v2	New England Biolabs	R0149L	20,000 units (1 mL)	210	16 µL/gel	3.35
Total cost of the stamping-based fabrication of six 7×7 mm^2 arrays on a ~55×9 mm^2 copy gel:							**$17.8**
Cost / 7×7 mm^2 array:							**$2.96**
Barcode sequencing (e.g., sequencing a gel of ~22×9 mm^2)	USER enzyme	New England Biolabs	M5505L	250 units (0.25 ml)	249	16 µL/gel	15.92
	Sequencing primer	IDT		50 nmole (5 mL × 100 µM)	137	30 µL × 100 µm/gel	0.82
	Illumina Hiseq V4 kit (no flowcell)	Illumina	FC-401-4002	50 cycles	2,805	1 kit/10 gels	280.50
Total cost of the sequencing-based fabrication of three 7×7 mm^2 arrays on a ~35×9 mm^2 gel:							**$315**
Cost / 7×7 mm^2 array:							**$105**

Table S3. Comparison of DNA cluster and DNA nanoball-based assays

	Seq-Scope[a]	Stereo-seq[b]	Pixel-seq[c]	
			Sequenced gel	Stamped gel
DNA array size(s) (mm^2)	30.4 (1 × 0.8) × 38 tiles	50 - 200 10 × 5, 10 ×10, or 20 × 10	147 (7 × 7) × 3	294 (7 × 7) × 6
Array fabrication cost (per mm^2)	~$150	~$35	~$2.20	~$0.06
Array fabrication time (hour)	~17	~9	~36	~7
Library preparation time (hour)	~19	~9	~6	
Library sequencing platform(s)	Illumina and BGI platforms	MGI DNBSEQ-Tx	NovaSeq 6000	

[a]: Array cost is based on MiSeq v3 flowcells used in the original publication. Times were calculated based on the reported protocol.

[b]: Arrays were subdivided from a large chip up to 13.2 × 13.2 cm^2. Times were calculated based on the reported protocol.

[c]: Detailed cost analysis is in Table S2. Compared with Seq-Scope and Stereo-seq, the sequencing-based gel fabrication had a much lower cost partly because polony gels were fabricated on standard coverslips or glass slides instead of using commercial sequencing flowcells, and a longer time mainly due to the use of an in-house built sequencer with a much slower imaging speed than commercial sequencers.