

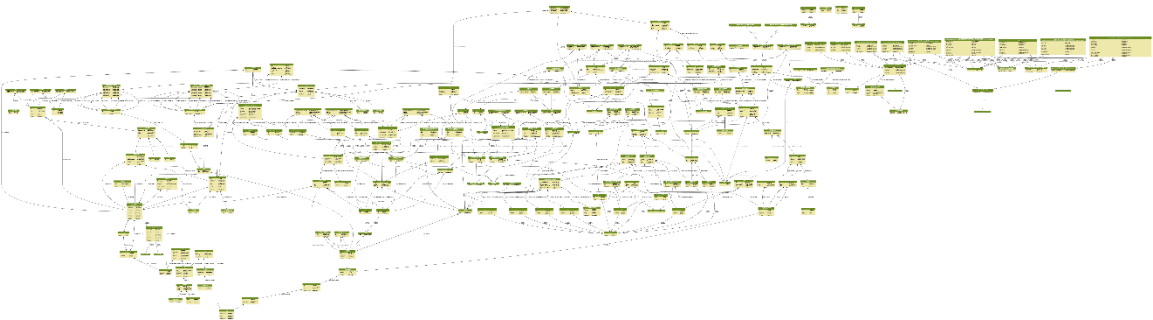
Supplemental Information:

Summary of STRs target genotyping protocols

Tissue	DNA	Target Enrichment	Calling Method	Majority STR Type	Targets	Purpose	Refs
human blood	Bulk	multiplex PCR	Capillary Electrophoresis	hexa-	~20	Forensic	CODIS(Bruce Budowle)
Human	Bulk	Array capture	Next Generation Sequencing	all types	7851	Mutation Discovery	Guilmatre <i>et al</i> (Guilmatre et al. 2013)
human blood	Bulk	RNA Probes	Next Generation Sequencing	tri- and longer	10764	Mutation Discovery	Jorge Duitama <i>et al</i> (Duitama et al. 2014)
A.thaliana	Bulk	MIPs	Next Generation Sequencing	tri- and hexa-	102	Evolution phylogeny	Carlson <i>et al</i> (Carlson et al. 2015)
human leukemia	scWGA	multiplex PCR	Capillary Electrophoresis	di-	128	Lineage Discovery	Shlush <i>et al</i> (Shlush et al. 2012)
human cancer	scWGA	Access Array	Next Generation Sequencing	di-	~2000	Lineage Discovery	Biezuner <i>et al</i> (Biezuner et al. 2016)
human cancer or normal	scWGA	duplex MIPs	Next Generation Sequencing	di- and mono-	12473 ~50,000	Lineage Discovery	(current study)

Supplemental Table1. STR capture methods summary

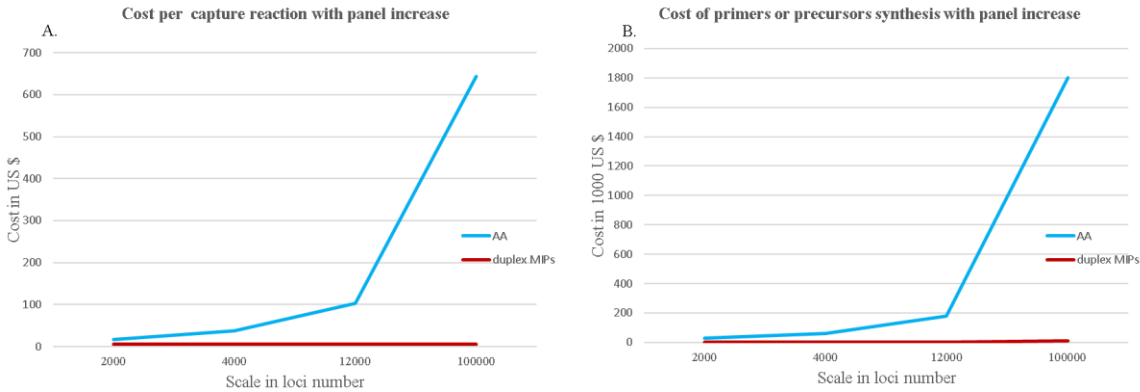
Entity relation diagram (ERD) of the cell lineage database structure, a full super high resolution figure is in supplemental file4.



Supplemental Figure1.Entity relation diagram (ERD) of the cell lineage database structure.

The scalability of duplex MIPs

The cost trend of duplex MIPs pipeline and Access Array pipeline while scaling up.



Supplemental Figure2. Cost and Scalability between Access Array and duplex MIPs

General cost of duplex MIPs capture pipeline was listed below.

The synthesis cost of duplex MIPs pipeline is ~2,200\$ for 12,000 loci panel compared with over 30,000\$ for 2000 AA primer pairs. Duplex MIPs capture reaction per cell is ~2.33\$, while the AA reaction cost per cell is ~19.69\$.

Reagents	Cat.No	Cost(\$)	Total Volume(ul or reactions)	Volume per Reaction (ul)	Cost per Reaction (\$)
duplex MIPs	Home made	2200	9400000	1	0.000234043
Betaine solution 5M	B0306 1VL Sigma	49	1500	4	0.13
Phusion High-Fidelity DNA Polymerase	NEB-M0530L	424	250	0.4	0.68
Ampligase 10X Reaction Buffer	A1905B EPICENTRE	66	5000	2	0.03
Ampligase DNA Ligase W/O Buffer	A3210K EPICENTRE	693	2000	1	0.35
Exonuclease I (E.coli)	NEB-M0293L	268	750	0.175	0.06
Exonuclease III (E.coli)	NEB-M0206L	236	250	0.18	0.17
RecJf	NEB-M0264L	272	167	0.1	0.16
Exonuclease T - 1,250 units, T7	NEB-M0265L	280	250	0.08	0.09
Exonuclease Lambda	NEB-M0263L	248	500	0.4	0.20
Exonuclease	M0262L	268	1000	0.02	0.01
NEBNext Ultra II Q5 MasterMix	NEB-M0544L	395	12500	10	0.32
MinElute PCR Purification Kit	QIAGEN 28006	594	250reactions	2reaction/Run	0.02
Qubit® dsDNA HS Assay Kit,	Q32854	269	500reactions	2reaction/Run	0.01
Agencourt Ampure XP Beads	BeckmanCoulter A63881	1485	600000	16	0.04
2% Agarose, dye-free, BluePippin, 100 - 600,	BDF2010	475	50reactions	1reaction/Run	0.05
TapeStation Screen Tap	5067-5582	211	112 reactions	2reaction/Run	0.02
TapeStation Reagents	5067-5583	90.33	112 reactions	2reaction/Run	0.01
Cost per Cell					2.33

Supplemental Table 2. The cost of duplex MIPs capture pipeline. The cost was calculated by 200 cells/run, WGA cost and sequencing run cost were not included.

Cost comparison between duplex MIPs pipeline and Access Array pipeline

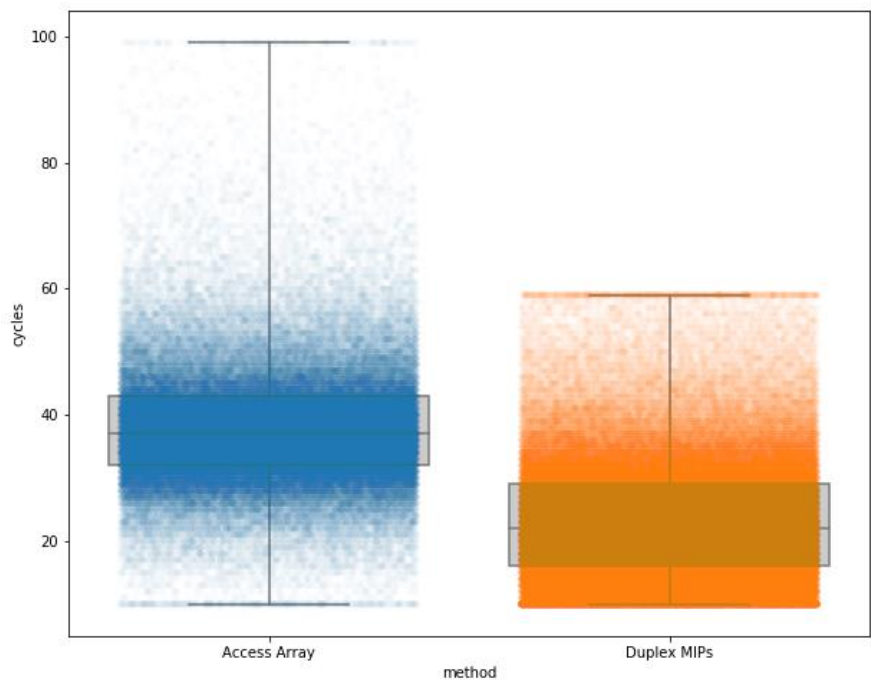
The sequencing cost per cell per 1000 loci is 0.012\$ in duplex MIPs pipeline vs 0.016\$ in AA pipeline. In total, the cost per cell is ~35.6\$ for 12,000 loci with duplex MIPs platform compared to 39.4\$ per cell for 2000 loci in AA pipeline.

Name	duplex MIPs for 12,000 loci(\$)	Access Array for 2,000 loci(\$)
WGA Cost per cell	4.26	4.26
Target Enrichment cost per cell	2.330032758	19.69
Sequencing cost per cell per 1000 loci	0.0120833	0.0160625
Total Cost per cell	35.59003276	39.37
Initial Cost*	8523.33	32762.95

Supplemental Table 3. Cost comparison between duplex MIPs pipeline and Access Array pipeline.

Initial Cost* including primers or duplex MIPs precursors synthesis, targeting enrichment reagents

The amplification cycles comparison between Access Array pipeline and duplex MIPs platform



Supplemental Figure3. The amplification cycles comparison between Access Array pipeline and duplex MIPs platform

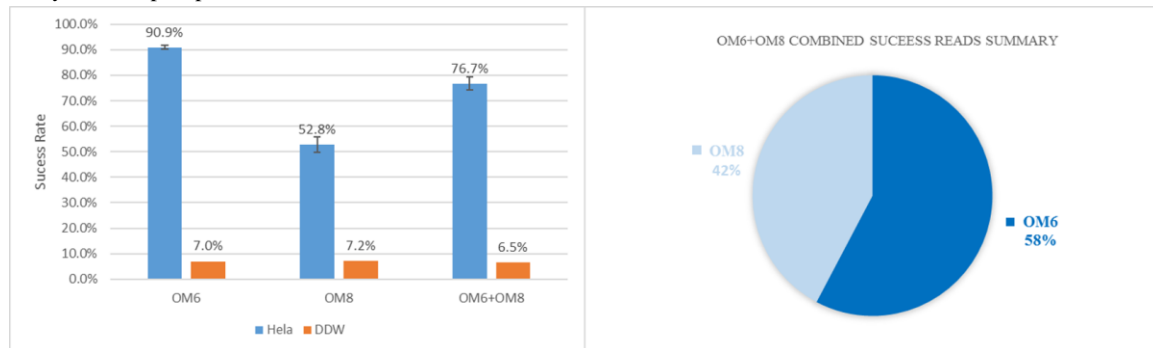
Scale-up test at 25K and 50K panel

Barcodecontent ID	Total Reads	Success Read	Success Rate	# Loci (>=1X)	Panel Name	Template	Panel Size
11094	83946	64784	77.2%	15425	OM9	Hela	50K
11098	86039	66589	77.4%	15531	OM9	Hela	50K
11102	46	9	19.6%	9	OM9	DDW	50K
11730	205862	163105	79.2%	13784	OM6+OM8	Hela	25K
11731	189982	140744	74.1%	13312	OM6+OM8	Hela	25K
11732	184	12	6.5%	12	OM6+OM8	DDW	25K

Supplemental Table4. 25K and 50K panel test

The combination of two independent panel OM6 and OM8

To test the feasibility of combine two independent panel of duplex MIPs, an independent panel of duplex MIPs OM8 (Supplemental File3), no shared targets between OM8 and OM6, was prepared and tested with OM6. 8 nM OM6 and 8 nM OM8 were pooled by 1:1 and used to capture in a single reaction with a modified protocol where 59.7 °C was used as Hyb and Gap steps instead of 56 °C.

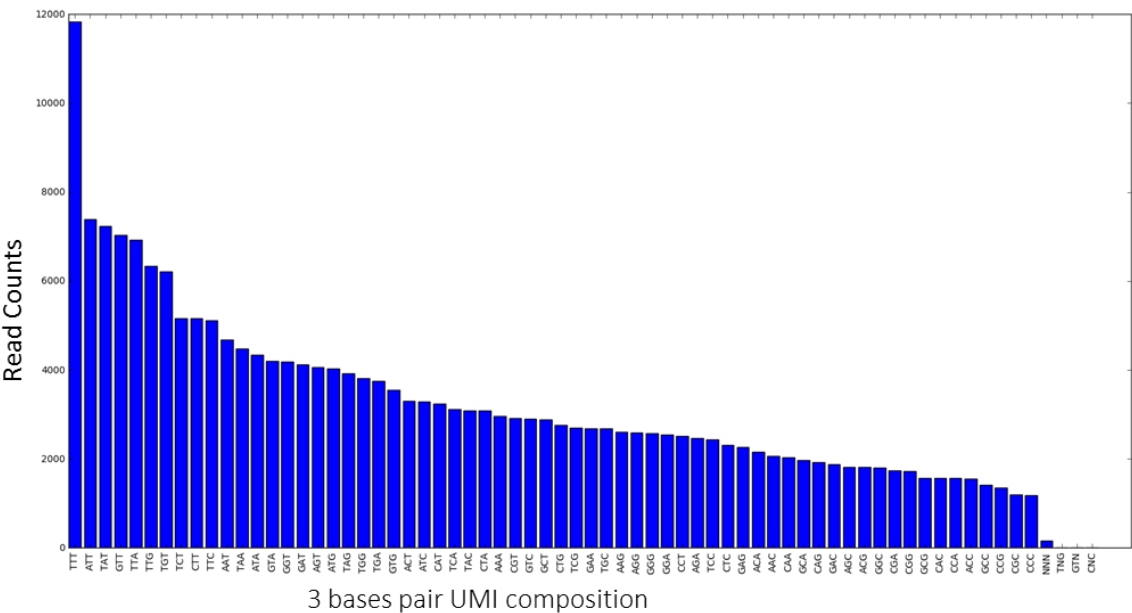


Supplemental Figure4. The combination of two independent panel OM6 and OM8 | Hela bar for OM6 is average value of two replicates. Hela bar for OM8 is average value of three replicates. OM6+OM8 bar is average value of two replicates. DDW is negative control, no replicates. Pie chart is average value of two OM6+OM8 replicates value

An example of Unique Molecular Identifier (UMI) read counts in the MiseqR33

Samples from MiseqR33 was analyzed. All 64 different UMIs were detected in all the samples. The sample with barcodes number 743 from MiseqR33 was shown as an example. The reads mapping to their reference targets were collected and UMIs were counted by reads contained this UMI. Counts ranking from high to low by UMI compositions was shown in Sup. Fig2.

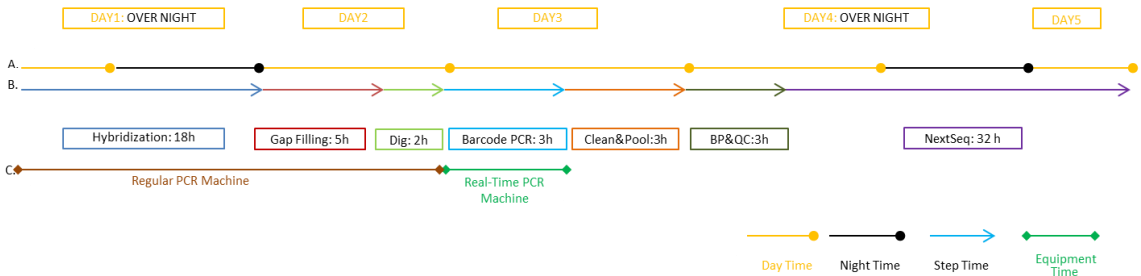
The counts of UMI bases of this sample: 'T': 17658, 'A': 11363, 'G': 10063, 'C': 8060, 'N': 28, biased towards 'T'.



Supplemental Figure5. An example of UMI read counts in the MiseqR33.

Duplex MIPs workflow timeline

The whole workflow of duplex MIPs pipeline took 5days from hybridization to data analysis, with roughly 3-hour hands on time.



Supplemental Figure6. Duplex MIPs workflow timeline (A). Day counts (B). Reaction step time count (C). Machine time count

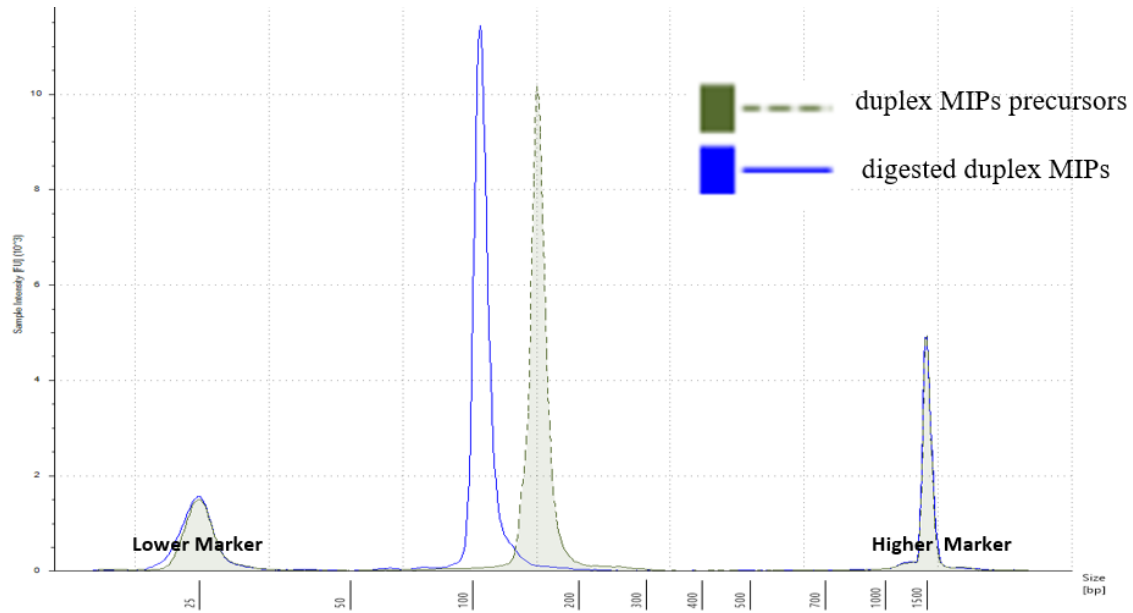
List of major reagents using in duplex MIPs pipeline

Reagents	Catalog #
KOD Hot Start DNA Polymerase	Merck 71086
dNTP 25mM each	Bioline BIO-39053
Betaine solution 5M	Sigma B0306 1VL
Phusion High-Fidelity DNA Polymerase	NEB M0530L
Ampligase 10X Reaction Buffer 5ml	EPICENTRE A1905B
Ampligase DNA Ligase W/O Buffer	EPICENTRE A3210K
Exonuclease I (E.coli) - 15,000 units	NEB M0293L
Exonuclease III (E.coli) - 25000 units	NEB M0206L 1
RecJf - 1,000 units	NEB M0264S
Exonuclease T - 1,250 units	NEB M0265L
T7 Exonuclease - 5,000 units	NEB M0263L
Lambda Exonuclease	EPICENTRE LEO32K
NEBNext Ultra II Q5 Master Mix	NEB M0544S
MinElute PCR Purification Kit (250)	QIAGEN 28006
Qubit® dsDNA HS Assay Kit	Life Technologies Q32854
Agencourt Ampure XP Beads	Beckman Coulter A63881
2% Agarose, dye-free, w/ internal standards	Sage Science ES-BDF2003
SYBR Green I	Lonza 50513

Supplemental Table5. List of major reagents using in duplex MIPs pipeline

Quality control step used in duplex MIPs preparation.

The size of duplex MIPs precursor is ~150bp. Duplex MIPs precursors were first amplified by 30 cycle PCR and further digested by MlyI (NEB) in order to create a ready-to-run duplex MIPs. Expected size for precursor amplification product is ~150bp and following digestion, the size of ready-to-run duplex MIPs is ~105bp.



Supplemental Figure7. The size of duplex MIPs precursor and the digested duplex MIPs | The dashed green peak in the middle is duplex MIPs precursors; the solid blue peak in the middle is duplex MIPs.

The calibration of duplex MIPs pipeline.

Three major steps: hybridization, gap-filling, digestion in the MIPs capture pipeline were calibrated in 18 different conditions. Hybridization were tested in 2, 4, 18 hours; Gap filling were tested in 1, 2, 4 hours; while the Digestion in 1, 2 hours (Data from MiseqR31, MiseqR32, and MiseqR33).

Probe Type	DNA	Hyb(hr)	Gap(hr)	Dig(hr)	Total Reads	Total Success	Success Rate	Loci>0	Loci>4	Loci>9
OM6	Hela	2	1	1	91805	18568	20%	6568	854	121
OM6	Hela	2	1	1	115167	23632	21%	7293	1322	243
OM6	Hela	2	1	2	121728	71250	59%	8892	4125	1770
OM6	Hela	2	1	2	114540	71036	62%	9229	4508	1960
OM6	Hela	2	2	1	199923	39214	20%	8365	2536	694
OM6	Hela	2	2	1	195185	79740	41%	9451	4911	2267
OM6	Hela	2	2	2	100563	56274	56%	8787	3641	1337
OM6	Hela	2	2	2	88212	51594	58%	8605	3247	1098
OM6	Hela	2	4	1	151143	48412	32%	8854	3198	997
OM6	Hela	2	4	1	141481	45520	32%	8390	3000	902
OM6	Hela	2	4	2	157111	84307	54%	9506	5147	2480
OM6	Hela	2	4	2	129168	88406	68%	9498	5333	2611
OM6	Hela	4	1	1	212479	111956	53%	10162	6138	3348
OM6	Hela	4	1	1	234372	133546	57%	10269	6808	4101
OM6	Hela	4	1	2	129933	52523	40%	8995	3295	1127
OM6	Hela	4	1	2	141878	62774	44%	9369	4097	1566
OM6	Hela	4	2	1	291192	151906	52%	10468	7360	4635
OM6	Hela	4	2	1	261932	154769	59%	10503	7442	4729
OM6	Hela	4	2	2	2279390	960410	42%	8474	8086	7674
OM6	Hela	4	2	2	158861	119662	75%	10064	6275	3624
OM6	Hela	4	4	1	258732	93063	36%	10062	5689	2785
OM6	Hela	4	4	1	175854	107480	61%	10156	6287	3512
OM6	Hela	4	4	2	207550	156801	76%	10395	7339	4781
OM6	Hela	4	4	2	146975	112963	77%	10028	6267	3519
OM6	Hela	18	1	1	108935	75979	70%	9946	5124	2297
OM6	Hela	18	1	1	281556	218901	78%	10831	8540	6092
OM6	Hela	18	1	2	229945	82983	36%	9935	5247	2571
OM6	Hela	18	1	2	161878	80571	50%	9948	5148	2376
OM6	Hela	18	2	1	112089	80908	72%	10092	5458	2587
OM6	Hela	18	2	1	191178	154354	81%	10649	7833	5016
OM6	Hela	18	2	2	97018	39422	41%	8628	2692	893
OM6	Hela	18	2	2	111756	57099	51%	9508	4006	1576
OM6	Hela	18	4	1	105243	87278	83%	10100	5780	2814
OM6	Hela	18	4	1	240644	200976	84%	10795	8679	6224
OM6	Hela	18	4	2	223929	95769	43%	10204	6009	3099
OM6	Hela	18	4	2	183300	145216	79%	10607	7781	4816

Supplemental Table6. Calibration of duplex MIPs process: Hyb -Gap-Dig | Hyb means hybridization, the first step in duplex MIPs capture protocol. Gap means gap filing, the second step. Dig is the third step, linear DNA digestion. Green

highlighted the protocol we chosen as standard. The success rate calculated as mapped reads/total reads. The loci captured defined as loci that has at least one mapped read.

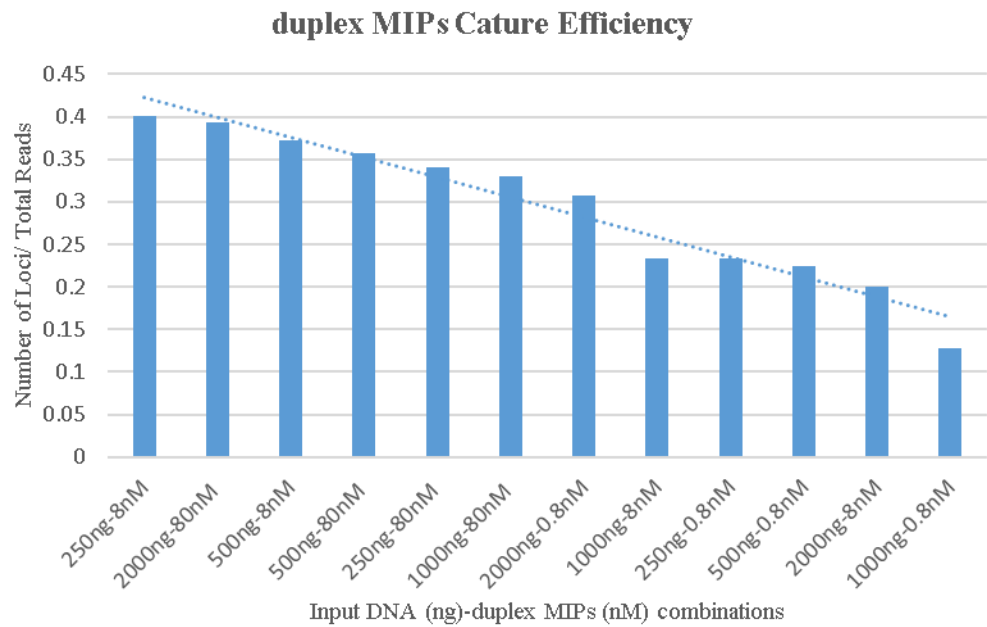
Calibration of Sequencing Library Size Selection

BluePippin Size (bp)	Name	Total Reads	Total Success	Success Rate	Loci >0
300	W151020 p2-C9	61816	57226	92.6%	7944
240-340	W151020 p2-C9	144518	130517	90.3%	9783
270-310	W151020 p2-C9	87924	82158	93.4%	8791
300	H1- 090215-B3	87665	83768	95.6%	3075
240-340	H1- 090215-B3	164359	155680	94.7%	4046
270-310	H1- 090215-B3	122252	117106	95.8%	3574
300	H1- 090215-B3	85631	81251	94.9%	2891
240-340	H1- 090215-B3	178585	168311	94.2%	3985
270-310	H1- 090215-B3	123557	117945	95.5%	3411
300	H1- 090215-B6	129546	123914	95.7%	5568
240-340	H1- 090215-B6	387493	368533	95.1%	6850
270-310	H1- 090215-B6	213020	203982	95.8%	6209
300	H1- 090215-E9	114190	109002	95.5%	5194
240-340	H1- 090215-E9	460648	436100	94.7%	6728
270-310	H1- 090215-E9	137124	131168	95.7%	5569
300	H1- 090215-A1	77196	73307	95.0%	5230
240-340	H1- 090215-A1	154987	146026	94.2%	6327
270-310	H1- 090215-A1	120505	114812	95.3%	5882
300	H1- 090215-F5	14620	13535	92.6%	3706
240-340	H1- 090215-F5	184932	170304	92.1%	7744
270-310	H1- 090215-F5	22392	20930	93.5%	4533
300	PC2	12488	11149	89.3%	5004
240-340	PC2	95192	81596	85.7%	10347
270-310	PC2	9078	8208	90.4%	4454

Supplemental Table7. Calibration of Sequencing Library Size Selection | PC2 was bulk DNA; all the other samples were single cell WGA DNA

Efficiency comparison between different duplex MIPs: template ratio

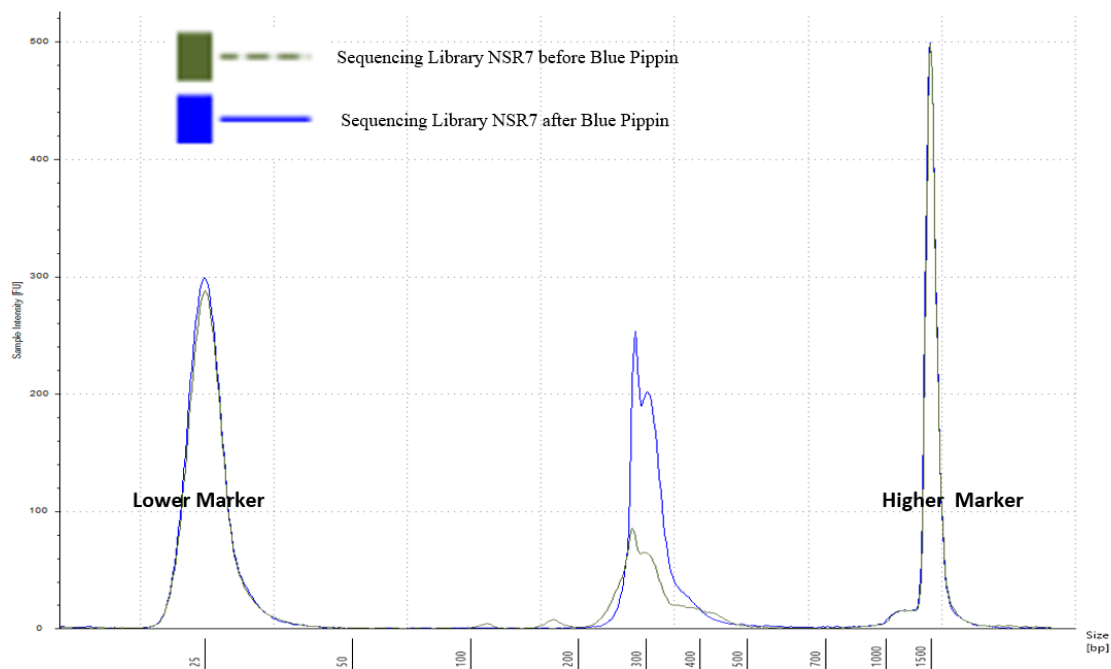
Considered the yield of single cell WGA reaction, we decided to use 8nM MIPs and 250ng to 500 ng single cell WGA DNA as the template DNA for our standard protocol. This combination is corresponding to a ratio ~70:1 duplex MIPs to DNA template.



Supplemental Figure8. Efficiency comparison between different probes: template ratio | Efficiency was calculated by captured loci/ total reads. Average value of two replicates for each test was used in the figure.

Sequencing library quality control

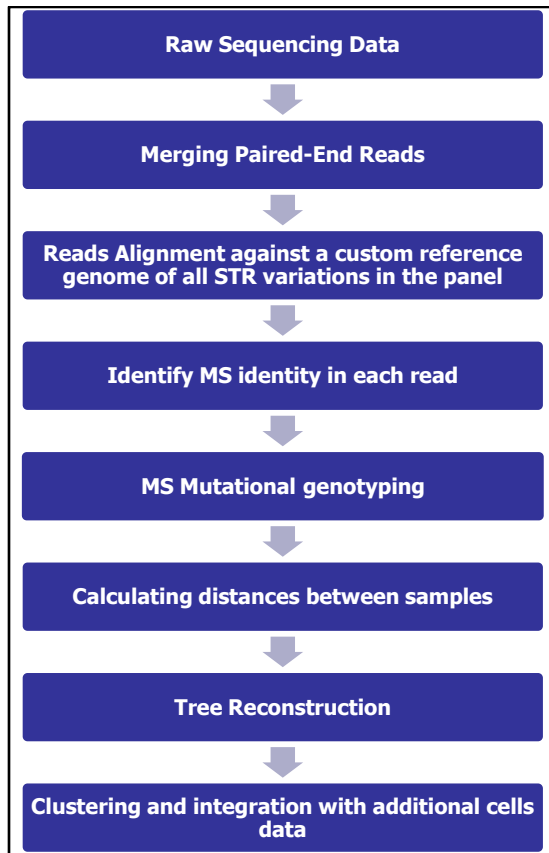
As a sequencing library quality control step, TapeStation was applied to the libraries before and after Blue Pippin. 240bp~340bp range size selection setting was used on 2% V1 cassette. Two side-product peaks were removed by BluePippin as shown below.



Supplemental Figure9. Library Quality Control: Tape Station before Blue Pippin and after Blue Pippin

A schematic diagram of the computational pipeline

A new mapping strategy was replaced the one in our previous work(Biezuner et al. 2016). Reads were aligned against a custom reference genome of all possible STR variations in the panel. This improved the computing efficiency. All the source code was available in <https://github.com/ofirr/clineage>



Supplemental Figure10.

A schematic diagram of the computational pipeline

Sampling documentation user interface

Individuals

Sampling events

Samples

Separation events

Cells/Bulks

Plates

Each individual (e.g. patient, mouse...) is documented with some basic background.

Add Individual

▼ Filters

Name

HH:MM

HH:MM

Name	Taxa	Sex	Born	Comment	Background	Location	Sacrificed
795-09	Homo sapiens	F	[no data]	Primary breast tumor	Cancer patient	Germany	[no data]
MM16-412	Homo sapiens	M	[no data]	lymph node resection after diagnosed malignant melanoma	Melanoma patient	Germany	[no data]
cell line P53	Homo sapiens	M	[no data]		[no data]	[no data]	[no data]
Healthy donor - F	Homo sapiens	F	[no data]		Healthy	Rambam Medical Center, Haifa	[no data]
IMR-90_072017_weizmann	Homo sapiens	F	[no data]		Caucassican	Weizmann Ins	[no data]
MM16-DSS-Check	Homo sapiens	M	[no data]	lymph node resection after diagnosed malignant melanoma	Melanoma patient	Germany	[no data]
U937 052017_Weizmann	Homo sapiens	M	[no data]		Leukemic patient	Weizmann Ins	[no data]
Healthy donor	Homo sapiens	M	[no data]		Healthy	Rambam Medical Center, Haifa	[no data]
U937 042017_Rambam	Homo sapiens	M	[no data]	Cell Line	Leukemic patient	Rambam Medical Center, Haifa	[no data]
MM16-423	Homo sapiens	M	[no data]	lymph node resection after diagnosed malignant melanoma Chair of experimental medicine and therapy research	Melanoma patient	Germany	[no data]
TP53 bulk DNA	Homo sapiens	F	[no data]	CLL Patient Gender unknown	Leukemic patient	Rambam Medical Center, Haifa	[no data]
IDH1 / DNMT3a bulk DNA	Homo sapiens	F	1939-05-02 00:00:00	IDH1 / DNMT3a	Leukemic patient	Rambam Medical Center, Haifa	[no data]
IDH2 bulk DNA	Homo sapiens	F	1951-09-23 00:00:00	NPM1 /IDH2	Leukemic patient	Rambam Medical Center, Haifa	[no data]
FLT3-ITD/NPM1			1943-04-04			Rambam Medical	

Supplemental Figure11. Individual

Individuals

Sampling events

Samples

Separation events

Cells/Bulks

Plates

Each individual (e.g. patient, mouse...) is documented with some basic background.

Add Individual

▼ Filters

Name

Name

Taxa

H1-ex vivo seeding started 12/1/2015	Homo sa
LCL15	Homo sa
AA control-mouse	Mus mus
MD4954	Mus mus
MD4953	Mus mus

Individual editing form

Taxa: Homo sapiens

Name: YUCLAT

Sex: Male

Born:

Received from Ruth Halaban

Comment:

Background:

Location: Yale

Sacrificed:

Partner: RuthHalaban

OK

Cancel

round	Location	Sacrificed
	Rivka's fridge No1. Meyer building	2015-01-12 00:00:00
D-, NPM1-	Rambam Medical Center, Haifa	[no data]
	Rivka's fridge No1. Meyer building	[no data]
5/Lgr5-ki	Hubrecht Institute, Utrecht, the Netherlands	2013-12-04 00:00:00
5/Lgr5-ki	Hubrecht Institute, Utrecht, the Netherlands	2013-04-12 00:00:00

YUCLAT	Homo sapiens	M	[no data]	Received from Ruth Halaban	[no data]	Yale	[no data]
TCGA-06-0128	Homo sapiens	M	1944-01-01 00:00:00		[no data]	[no data]	[no data]
TCGA-06-0152	Homo sapiens	M	1946-01-01 00:00:00	68 y/o	[no data]	[no data]	[no data]
donor ITEM-001	Homo sapiens	M	[no data]	Cristoph Klein	[no data]	[no data]	[no data]
CW14	Homo sapiens	M	1945-12-01 00:00:00	Diagnosed for CLL on 9.7.2001	Caucassican	Boston (DFCI)	[no data]
10448VPM	Homo sapiens	M	[no data]		[no data]	[no data]	[no data]
AA control-Human	Homo sapiens	M	[no data]		[no data]	[no data]	[no data]
Healthy patient - Liran	Homo sapiens	M	[no data]	Healthy patient control for - Liran Shlush experiment	Wild type	Totonto-Liran	[no data]
8056R - I Iran	Homo sapiens	M	[no data]	I Iran Shlush patient	I leukemic patient	Totonto-I Iran	[no data]

Supplemental Figure12. Individual editing form

Individuals	Sampling events	Samples	Separation events	Cells/Bulks	Plates
Add Plate					
<div> <div>Filters</div> <div> <div>Name</div> <div>State</div> <div>Type</div> </div> </div>					
Name	Type	Barcode	Timestamp	State	Lastusedwell
AAR12	MPXs		[no data]		A01
AAR13	MPXs		[no data]		A01
AAR14	MPXs		[no data]		A01
AAR15	MPXs		[no data]		A01
AAR16	MPXs		[no data]		A01
AAR17	MPXs		[no data]		A01
AAR18	MPXs		[no data]		A01
TCGA-06_Chaya	Full Skirted 96 well PCR plate (tetrad)		2014-10-29 00:00:00		A1
MM_MPX_w/o_LB_P21	MPXs		[no data]		B4
Ruth Halaban YUTOGS Patient #1 02.11.14	Full Skirted 96 well PCR plate (tetrad)		2014-11-02 00:00:00		A1
Ruth Halaban YUCLAT Patient #2 plate 1 02.11.14	Full Skirted 96 well PCR plate (tetrad)		2014-11-02 00:00:00		A1
Ruth Halaban YUCLAT Patient #2 plate 2 02.11.14	Full Skirted 96 well PCR plate (tetrad)		2014-11-02 00:00:00		A1
MM_MPX_w/o_LB_P22	MPXs		[no data]		G1
AAR19	MPXs		[no data]		A01
AAR20	MPXs		[no data]		A01
MM_MPX_w/o_LB_P25	MPXs		[no data]		H3
AAR21	MPXs		[no data]		F6
United_hg19_Tail	Primer pairs		[no data]		A1

Supplemental Figure13. Plates

Individuals

Sampling events

Samples

Separation events

Cells/Bulks

Plates

Add Plate

▼ Filters

Name

State

Type

Name	Type	Barcode	Timestamp	State	Lastusedwell
AAR12	MPXs		[no data]		A01
AAR13	MPXs		[no data]		A01
AAR14	MPXs				A01
AAR15	MPXs				A01
AAR16	MPXs				A01
AAR17	MPXs				A01
AAR18	MPXs				A01
TCGA-06_Chaya	Full Skirted 96 well PCR plate (tetrad)				A1
MM_MPX_w/o_LB_P21	MPXs				B4
Ruth Halaban YUTOGS Patient #1 02.11.14	Full Skirted 96 well PCR plate (tetrad)		2014-11-02 00:00:00		A1
Ruth Halaban YUCLAT Patient #2 plate 1 02.11.14	Full Skirted 96 well PCR plate (tetrad)		2014-11-02 00:00:00		A1
Ruth Halaban YUCLAT Patient #2 plate 2 02.11.14	Full Skirted 96 well PCR plate (tetrad)		2014-11-02 00:00:00		A1
MM_MPX_w/o_LB_P22	MPXs		[no data]		G1
AAR19	MPXs		[no data]		A01
AAR20	MPXs		[no data]		A01
MM_MPX_w/o_LB_P25	MPXs		[no data]		H3
AAR21	MPXs		[no data]		F6
United_hg19_Tails_plt24	Primer pairs		[no data]		A1

Plate editing form

Type: Full Skirted 96 well PCR plate (tetrad)

Name: Ruth Halaban YUCLAT Patient #2 plate 2

Barcode:

Timestamp: 2014-11-02

State:

Lastusedwell: A1

OK

Cancel

Supplemental Figure14. Plates editing form

Individuals

Sampling events

Samples

Separation events

Cells/Bulks

Plates

Extraction event (e.g. operation where tumor samples were taken, mouse was sacrificed and dissected...) this is not necessarily post-mortem.

Add extraction event

▼ Filters

Name

Extracted before date

HH:MM

Extracted after date

HH:MM

Extraction event location

individual's name

user who extracted

Comment

Individual	Name	User performed	User documented	Date	Location	Comment
MM16-423	Blood	ChristophKlein	Veronika	2017-09-18 00:00:00	Germany	leukapheresis product/ blood of malignant melanoma patient
MM16-412	blood	ChristophKlein	Veronika	2017-03-08 00:00:00	Germany	Chair of experimental medicine and therapy research blood of melanoma patient
MM16-412	metastasis	ChristophKlein	Veronika	2016-05-17 00:00:00	Germany	metastasis from melanoma patient Chair of experimental medicine and therapy research
MM16-412	lymph node	ChristophKlein	Veronika	2015-02-13 00:00:00	Germany	lymph node resection after diagnosed malignant melanoma
MM16-412	metastasis	ChristophKlein	Veronika	2017-05-17 00:00:00	Germany	metastasis from melanoma patient
MM16-412	metastasis	ChristophKlein	Veronika	2017-03-06 00:00:00	Germany	metastasis from melanoma patient
MM16-412	cell line	ChristophKlein	Veronika	2016-05-17 00:00:00	Germany	Chair of experimental medicine and therapy research
795-09	17/06/2016	ChristophKlein	Shiran	2016-06-17 00:00:00	Germany	Blood from M1 patient
795-09	2009	ChristophKlein	Shiran	2009-01-01 00:00:00	Germany	Primary breast tumor Bone marrow isolation at primary surgery
MM16-412	lymph node	ChristophKlein	Shiran	2016-05-17 00:00:00	Germany	Chair of experimental medicine and therapy research
Healthy donor - F	DNA Peripheral Blood	ShlomitYehudaiReshef	Noa	2017-07-04 00:00:00	Rambam Medical Center, Haifa	
IMR-90_072017_weizmann	IMR-90_16072017	Shiran	Shiran	2017-07-16 00:00:00	Weizmann Ins	

Supplemental Figure15.

Sampling

Individuals

Sampling events

Samples

Separation events

Cells/Bulks

Plates

Extraction event (e.g. operation where tumor samples were taken, mouse was sacrificed and dissected...) this is not necessarily post-mortem.

Add extraction event

Filters

Name

Extraction event location

Individual

Individual	Name	Location	Comment
LCL15	Diagnosis		
AA control-mouse	blank		
MD4954	Second Extraction Event		
MD4954	First Extraction Event		
MD4953	Second Extraction Event		
MD4953	First Extraction Event		
YUCLAT	YUCLAT-SAT 09-887 - Metastasis 2		
YUCLAT	YUCLAT 08-707 - Metastasis 1		
YUCLAT	YUCLAT-AX 09-1058 - Metastasis 4		
YUCLAT	YUCLAT 09-1008 - Metastasis 3		
YUCLAT	YUCLAT 09-1182 - PBL		
YUTOGS	YUTOGS 11-2073 - PBL		
YUTOGS	YUTOGS-M.2 11-2216 - Metastasis 2		
YUTOGS	YUTOGS 10-1897 - Metastasis 1		
TCGA-06-0128	1293		
TCGA-06-0152	unKnown		
CW14	Blood - ex		

Extraction event editing form

Individual:

YUCLAT

Name:

YUCLAT-AX 09-1058 - M

Comment:

p1 cells

Date:

2009-06-08 00:00:00

Location:

Yale

User performed:

root

User documented:

Noa

OK

Cancel

Supplemental Figure16.

Sampling editing form

Individuals

Sampling events

Samples

Separation events

Cells/Bulks

Plates

Samples extracted in said extracted event, these can range from or a simple blood test (documented once) up to multiple organs and multiple tissues sampled in the same autopsy (each documented separately and all referencing the same extraction operation). We currently record organ and tissue for these samples, both within this single hierarchical level.

Add extraction

▼ Filters

Name

extraction event

▼

Organ

▼

Tissue

▼

Comment

Extraction event	Name	Organ	Tissue	Comment
MM14-23441>Separation	lymph node	Lymph node	none	Chair of experimental medicine and therapy research
MM15-127>Lymph node	LN	Lymph node	none	Chair of experimental medicine and therapy research
MM16-423>Blood	blood	Blood	Blood	Chair of experimental medicine and therapy research
MM16-412>blood	blood	Blood	Blood	Chair of experimental medicine and therapy research
MM16-412>metastasis	meta3	Blood	none	metastasis of melanoma patient
MM16-412>metastasis	meta2	Blood	none	
MM16-412>metastasis	meta1	Blood	Blood	
MM16-412>lymph node	LN	Lymph node	none	
MM16-412>cell line	cell line	Cell line	none	
795-09>17/06/2016	blood	Blood	Blood	Chair of experimental medicine and therapy research
795-09>2009	Bone marrow	Bone Marrow	Bone marrow	Chair of experimental medicine and therapy research
795-09>2009	primary tumor	Breast	none	Chair of experimental medicine and therapy research

Supplemental Figure17. Samples

Individuals

Sampling events

Samples

Separation events

Cells/Bulks

Plates

Samples extracted in said extracted event, these can range from or a simple blood test (documented once) up to multiple organs and multiple tissues sampled in the same autopsy (each documented separately and all referencing the same extraction operation). We currently record organ and tissue for these samples, both within this single hierarchical level.

Add extraction

▼ Filter

Na	
Tiss	
Extra	
MD4	
Extra	
MD4	
Extra	
MD4	
Extra	
MD4	
Extra	
YUC	
SAT	
Meta	
YUC	
08-7	
Meta	

Extraction editing form

Extraction event: YUCLAT>YUCLAT-AX 09-1058 - Metastasis 4

Name: YUCLAT - Metastasis 4

Date:

Organ: Axilla

Tissue: Axilla

Comment:

p1 cells in DMSO.
Metastasis to right axilla.

OK

Cancel

YUCLAT>YUCLAT-AX 09-1058 - Metastasis 4	YUCLAT - Metastasis 4	Axilla	Axilla	p1 cells in DMSO. Metastasis to right axilla.
YUCLAT>YUCLAT 09-1008 -Metastasis 3	YUCLAT - Metastasis 3	Scapular	Scapular	Tumor cells in DMSO. Recurrence right scapular region
YUCLAT>YUCLAT 09-1182 - PBL	YUCLAT - PBL	Blood	Blood	
TCGA-06-0128>1 293	Spheroids (GSC's) of primary tumor 1293	Brain	Other	Glioma
TCGA-06-0128>1 293	Primary tumor	Brain	Other	Glioma
TCGA-06-0152>u nKnown	Recurrent tumor of 516	Brain	Other	Glioma
TCGA-06-0152>u nKnown	Spheroids (GSC's) of primary tumor 516	Brain	Other	

Supplemental Figure18. Extraction editing form

Individuals	Sampling events	Samples	Separation events	Cells/Bulks	Plates
-------------	-----------------	---------	-------------------	-------------	--------

Separation event - documenting the separation of said sample into individual single cells. This can be done via FACS, documenting the related markers, via Laser capture, documenting the related coordinates or via more random tissue separation protocols (documenting only the protocol).

[Add sampling event](#)
[Add FACS sampling event](#)
[Add Laser Capture sampling event](#)

▼ Filters

Name	Comment
source extraction ▼	user who sampled ▼

Name	Extraction	Date	User	Comment
gp100+ DCC	MM14-23441>Separation>lymph node	[no data]	ChristophKlein	manual manipulation cryo-conserved adhesion slides
CD31+ control cell	MM14-23441>Separation>lymph node	[no data]	ChristophKlein	manual manipulation cryo-conserved adhesion slides
gp100+ DCC_manual	MM15-127>Lymph node>LN	[no data]	ChristophKlein	manual manipulation
cell from patient derived cell line	MM15-127>Lymph node>LN	[no data]	ChristophKlein	
gp100+ DCC	MM15-127>Lymph node>LN	[no data]	ChristophKlein	manual manipulation cryo-conserved adhesion slides
CD31+ control cell	MM15-127>Lymph node>LN	[no data]	ChristophKlein	cryo-conserved adhesion slides manual manipulation
NC	MM16-423>Blood>blood	2017-09-18 00:00:00	ChristophKlein	
circulating tumor cell	MM16-423>Blood>blood	2017-09-18 00:00:00	ChristophKlein	from cryo-conserved adhesion slides manual manipulation
B cells	MM16-412>blood>blood	[no data]	ChristophKlein	manual manipulation from cell suspension
T cells	MM16-412>blood>blood	[no data]	ChristophKlein	manual manipulation from cell suspension
B cell	MM16-412>blood>blood	[no data]	ChristophKlein	manual manipulation from cell suspension
disseminated cancer cell	MM16-412>metastasis>meta3	2016-05-17 00:00:00	ChristophKlein	manual manipulation from cryo-conserved adhesion slides
disseminated cancer cell	MM16-412>lymph node>lymph node	2016-05-17 00:00:00	ChristophKlein	manual manipulation from cryo-conserved adhesion slides

Supplemental Figure19. Separation events

Individuals

Sampling events

Samples

Separation events

Cells/Bulks

Plates

Separation event - documenting the separation of said sample into individual single cells. This can be done via FACS, documenting the related markers, via Laser capture, documenting the related coordinates or via more random tissue separation protocols (documenting only the protocol).

Add sampling event

Add FACS sampling event

Add Laser Capture sampling event

Sampling event editing form

Name:

YUCLAT-AX 09-1058 - M

Extraction:

YUCLAT>YUCLAT-AX 09-1058 - Metastasis 4>YUCLAT - Metastasis 4

Date:

2014-03-03

User:

Rivka

Comment:

Attachment:

Browse...

No file selected.

OK

Cancel

CellCelector	Z>YUCLAT- Metastasis 2			
YUCLAT 08-707 - Met1 CellCelector	YUCLAT>YUCLAT 08-707 - Metastasis 1>YUCLAT - Metastasis 1	2014-01-23 00:00:00	Michal	
YUCLAT-AX 09-1058 - Met4 CellCelector	YUCLAT>YUCLAT-AX 09-1058 - Metastasis 4>YUCLAT - Metastasis 4	2014-03-03 00:00:00	Rivka	
YUCLAT-2 09-1008- Met3 CellCelector	YUCLAT>YUCLAT 09-1008 -Metastasis 3>YUCLAT - Metastasis 3	2014-03-05 00:00:00	Rivka	
YUCLAT 09-1182 PBL CellCelector	YUCLAT>YUCLAT 09-1182 - PBL>YUCLAT - PBL	2014-02-27 00:00:00	Rivka	
YUTOGS 11-2073- PBL	YUTOGS>YUTGOS 11-2073 - PBL>YUTOGS - PBL	2013-02-20 00:00:00	Rivka	Done manually, isolated from whole blood
manual dissociation	TCGA-06-0128>1293>Spheroids (GSC's) of primary	2014-01-01 00:00:00	ChayaBroby	Fiction

Supplemental Figure20.

Sampling event editing form

Individuals

Sampling events

Samples

Separation events

Cells/Bulks

Plates

Single Cells or Bulks, these can be documented one by one, in group or plate by plate.

Add Cell/Bulk

Add multiple Cells

Add Cells Plate

Add Cells Plate By Names

▼ Filters

Name

Comment

sample composition

sample status

sampling event

▼

Name	Sampling	Composition	Status	Comment
negative control	MM16-423>Blood>blood>NC	Single Cell	[no data]	composition comment:negative control
MM17-224 LB wdh TZ9	MM16-423>Blood>blood>circulating tumor cell	Single Cell	[no data]	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ9
MM17-224 LB wdh TZ8	MM16-423>Blood>blood>circulating tumor cell	Single Cell	[no data]	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ8
MM17-224 LB wdh TZ7	MM16-423>Blood>blood>circulating tumor cell	Single Cell	[no data]	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ7
MM17-224 LB wdh TZ5	MM16-423>Blood>blood>circulating tumor cell	Single Cell	[no data]	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ5
MM17-224 LB wdh TZ2	MM16-423>Blood>blood>circulating tumor cell	Single Cell	[no data]	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ2
MM17-224 LB wdh TZ1	MM16-423>Blood>blood>circulating tumor cell	Single Cell	[no data]	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ1
MM17-224 LB wdh TZ12	MM16-423>Blood>blood>circulating tumor cell	Single Cell	[no data]	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ12
MM17-224 LB wdh TZ11	MM16-423>Blood>blood>circulating tumor cell	Single Cell	[no data]	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ11

Supplemental Figure21.

Cell composition documentation: single cell or bulk

Individuals

Sampling events

Samples

Separation events

Cells/Bulks

Plates

Single Cells or Bulks, these can be documented one by one, in group or plate by plate.

Add Cell/Bulk

Add multiple Cells

Add Cells Plate

Add Cells Plate By Names

Filters

Name

sampling event

Name	Comment
negative control	composition comment:negative control
MM17-224 LB wdh TZ9	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ9
MM17-224 LB wdh TZ8	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ8
MM17-224 LB wdh TZ7	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ7
MM17-224 LB wdh TZ5	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ5
MM17-224 LB wdh TZ2	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ2
MM17-224 LB wdh TZ1	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ1
MM17-224 LB wdh TZ12	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ12
MM17-224 LB wdh TZ11	Reamplification mixture at 65 and 68 °C composition comment:MM17-224 LB wdh TZ11

New Cells Plate By Names

Individual:

Sampling:

Cells name prefix:

Comment:

Existing plate:

Plate type:

Plate name:

Samples in wells:

Storage box:

Inner location:

Notes:

Timestamp:

Protocol:

OK

Cancel

Supplemental Figure22. New cells plate by names editing form

Individuals

Sampling events

Samples

Separation events

Cells/Bulks

Plates

Single Cells or Bulks, these can be documented one by one, in group or plate by plate.

Add Cell/Bulk

Add multiple Cells

Add Cells Plate

Add Cells Plate By Names

▼ Filters

Name

Comment

sample composition

sample status

09-1058 - Met4 CellCelector

Name

Sampling

Composition

Status

Comment

Sample editing form

Individual:

YUCLAT

Sampling:

YUCLAT>YUCLAT-AX 09-1058 - Metastasis 4>YUCLAT - Metastasis 4>YUCLAT-AX 09-1058 - Met4 CellCelector

Name:

tube strip 10

Composition:

Single Cell

Status:

Comment

composition comment:tube strip 10

Classification:

OK

Cancel

• YUCLAT>YUCLAT-AX 09-1058 - Metastasis 4>YUCLAT - Metastasis 4>YUCLAT-AX 09-1058 - Met4 CellCelector>tube strip 10

tube strip 9	09-1058 - Metastasis 4>YUCLAT - Metastasis 4>YUCLAT-AX 09-1058 - Met4 CellCelector	Single Cell	[no data]	composition comment:tube strip 9
tube strip 8	YUCLAT>YUCLAT-AX 09-1058 - Metastasis 4>YUCLAT - Metastasis 4>YUCLAT-AX 09-1058 - Met4 CellCelector	Single Cell	[no data]	composition comment:tube strip 8

Supplemental Figure23.

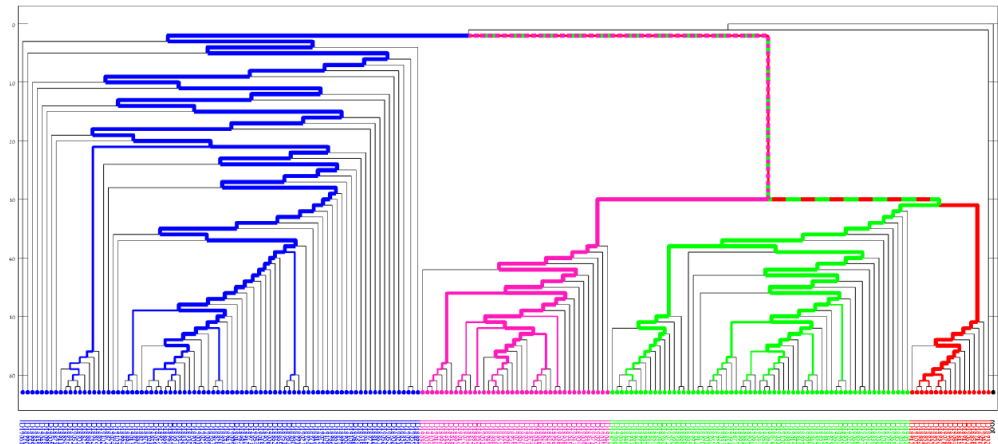
Example of YUCLAT cells documentation

Reference sequences for this loci is created for all possible STR length (3~35 repeats of AC in this case)

[illegible]

Supplemental Figure24.

Example of reference sequences for STR loci



Supplemental Figure25.

Sanity check of cell lineage discovery platform
Four colors presents four different individuals

- Biezuner T, Spiro A, Raz O, Amir S, Milo L, Adar R, Chapal-Ilani N, Berman V, Fried Y, Ainbinder E et al. 2016. A generic, cost-effective, and scalable cell lineage analysis platform. *Genome Res* **26**: 1588-1599.
- Bruce Budowle TRM, Stephen J. Niezgoda and Barry L. Brown. CODIS and PCR-Based Short Tandem Repeat Loci: Law Enforcement Tools.
- Carlson KD, Sudmant PH, Press MO, Eichler EE, Shendure J, Queitsch C. 2015. MIPSTR: a method for multiplex genotyping of germline and somatic STR variation across many individuals. *Genome Res* **25**: 750-761.
- Duitama J, Zablotzskaya A, Gemayel R, Jansen A, Belet S, Vermeesch JR, Verstrepen KJ, Froyen G. 2014. Large-scale analysis of tandem repeat variability in the human genome. *Nucleic Acids Res* **42**: 5728-5741.
- Guilmatre A, Highnam G, Borel C, Mittelman D, Sharp AJ. 2013. Rapid multiplexed genotyping of simple tandem repeats using capture and high-throughput sequencing. *Hum Mutat* **34**: 1304-1311.
- Shlush LI, Chapal-Ilani N, Adar R, Pery N, Maruvka Y, Spiro A, Shouval R, Rowe JM, Tzukerman M, Bercovich D et al. 2012. Cell lineage analysis of acute leukemia relapse uncovers the role of replication-rate heterogeneity and microsatellite instability. *Blood* **120**: 603-612.