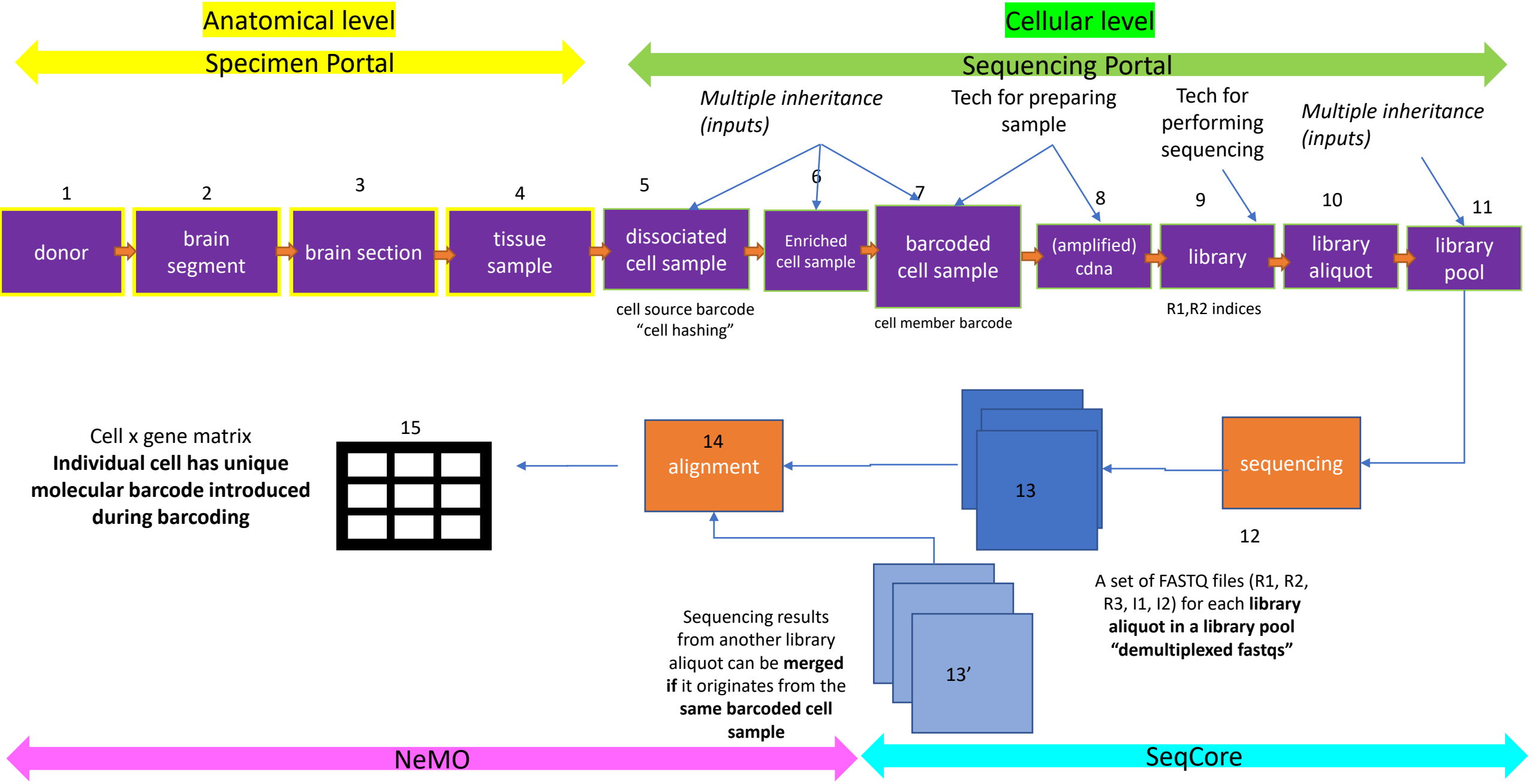
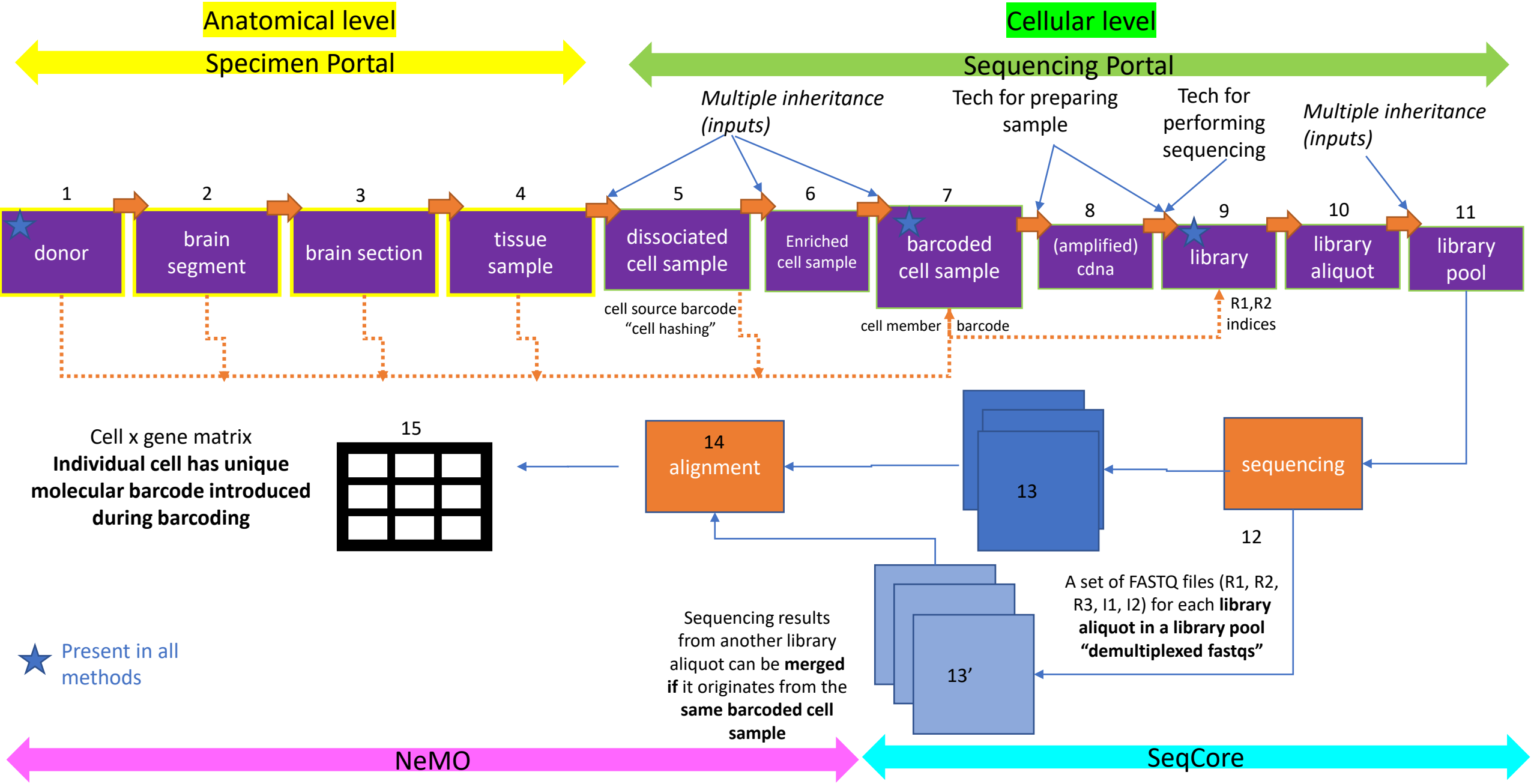
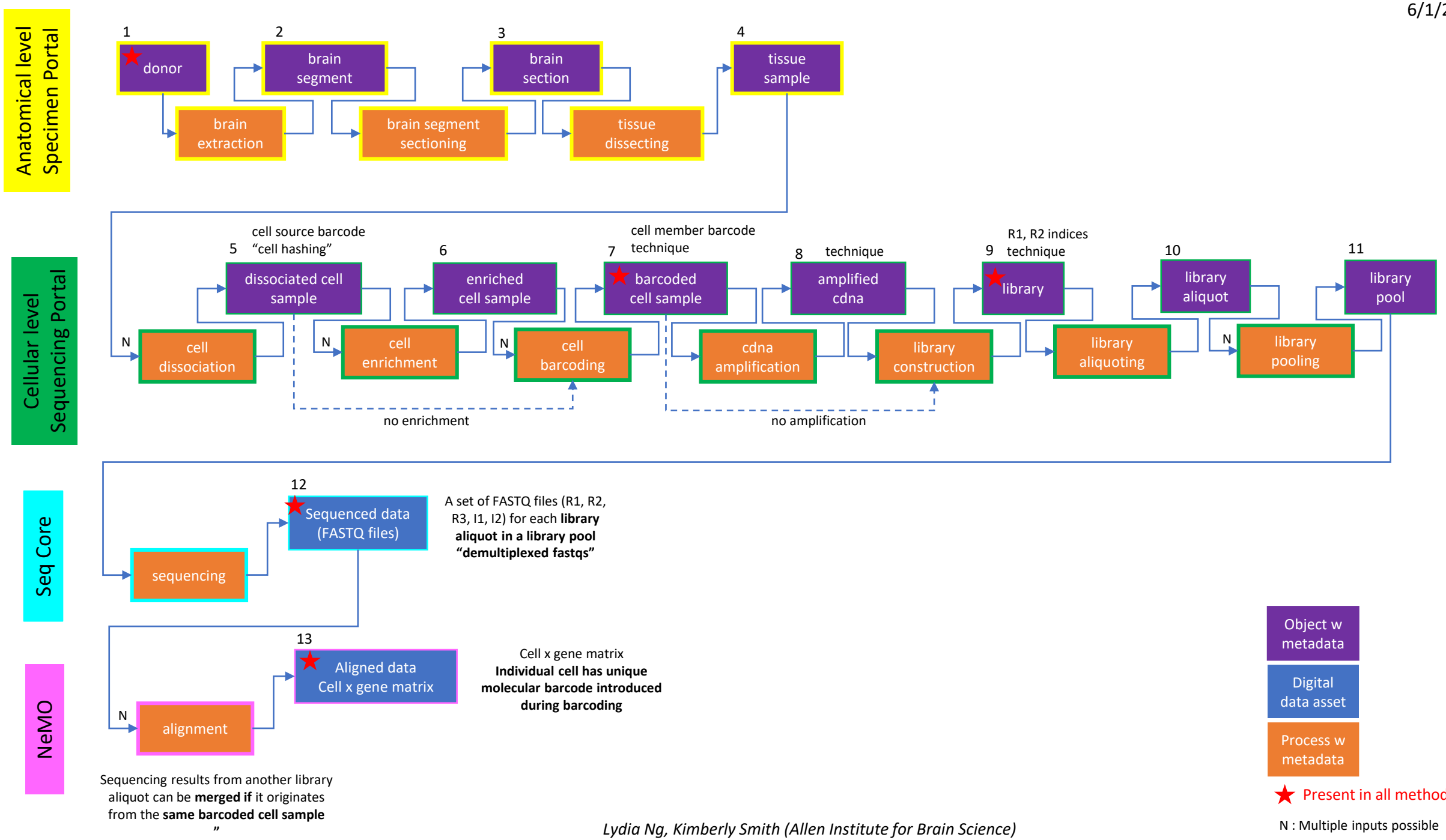
Critical Sequence Portal Use Cases

- Facilitates communication and instructions between services
  - Sample generation lab -> Sequencing Core (sequencing instructions)
  - Sample generation lab -> NeMO (alignment instructions)
- Dashboard and tracking
  - How many libraries? Which projects?
  - What is the status of my library through this workflow?
- Scientific analysis
  - Many different use cases
  - First critical one – assessment of batch effects
    - Need to understand where the batch (1:N, N:1) relationships
      - Sufficient metadata
      - example whole mouse 10x dataset provided


Anatomical level
Specimen Portal
Cellular level
Sequencing Portal
Multiple inheritance (inputs)
Tech for preparing sample
Tech for performing sequencing
Multiple inheritance (inputs)
1
2
3
4
5
6
7
8
9
10
11
donor
brain segment
brain section
tissue sample
dissociated cell sample
Enriched cell sample
barcoded cell sample
(amplified) cdna
library
library aliquot
library pool
cell source barcode "cell hashing"
cell member barcode
R1,R2 indices
Cell x gene matrix
Individual cell has unique molecular barcode introduced during barcoding
15
14 alignment
13
sequencing
12
13'
Sequencing results from another library aliquot can be merged if it originates from the same barcoded cell sample
A set of FASTQ files (R1, R2, R3, I1, I2) for each library aliquot in a library pool "demultiplexed fastqs"
NeMO
SeqCore

KS updated 5/30/23





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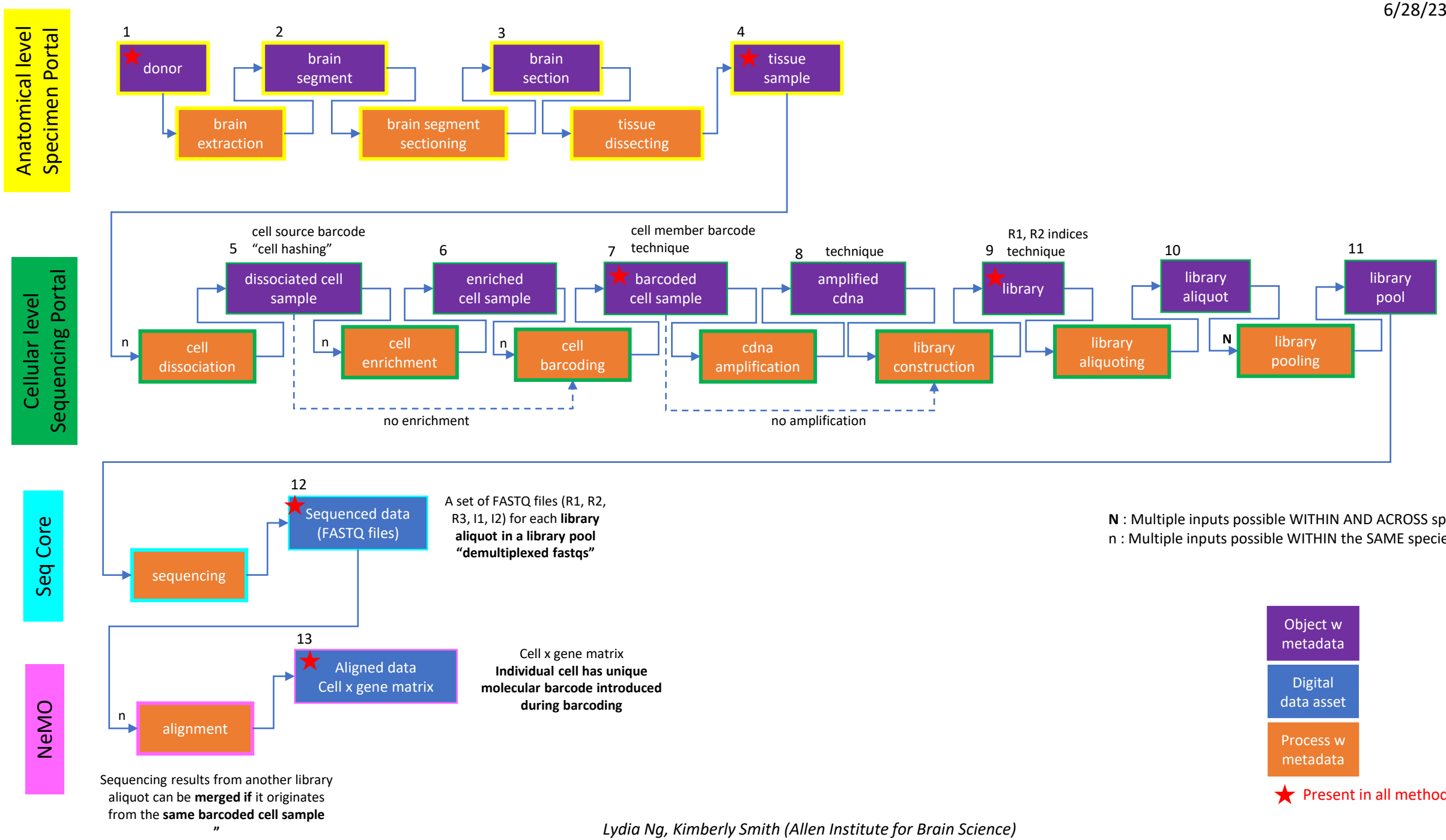
6/28/23



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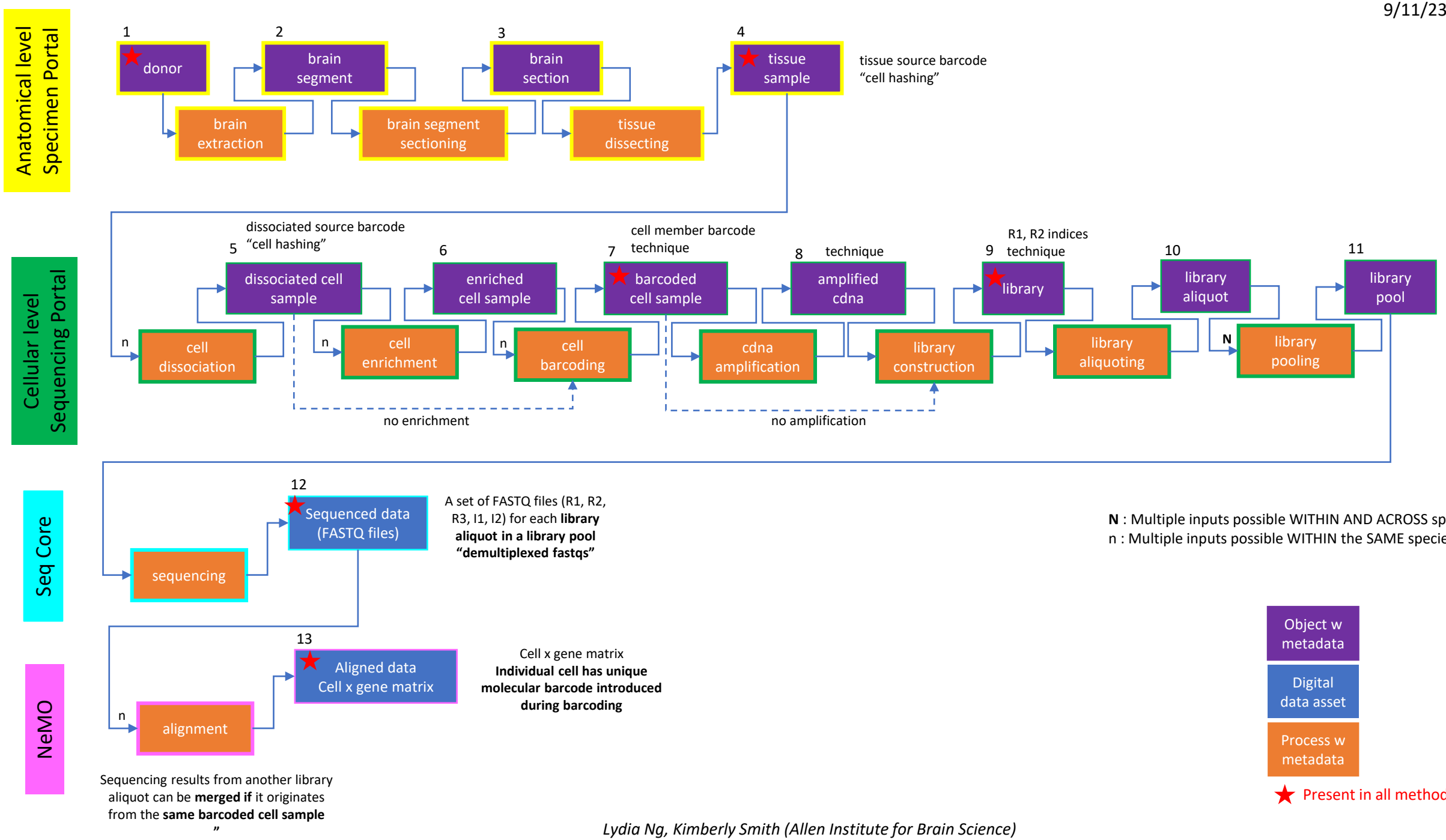

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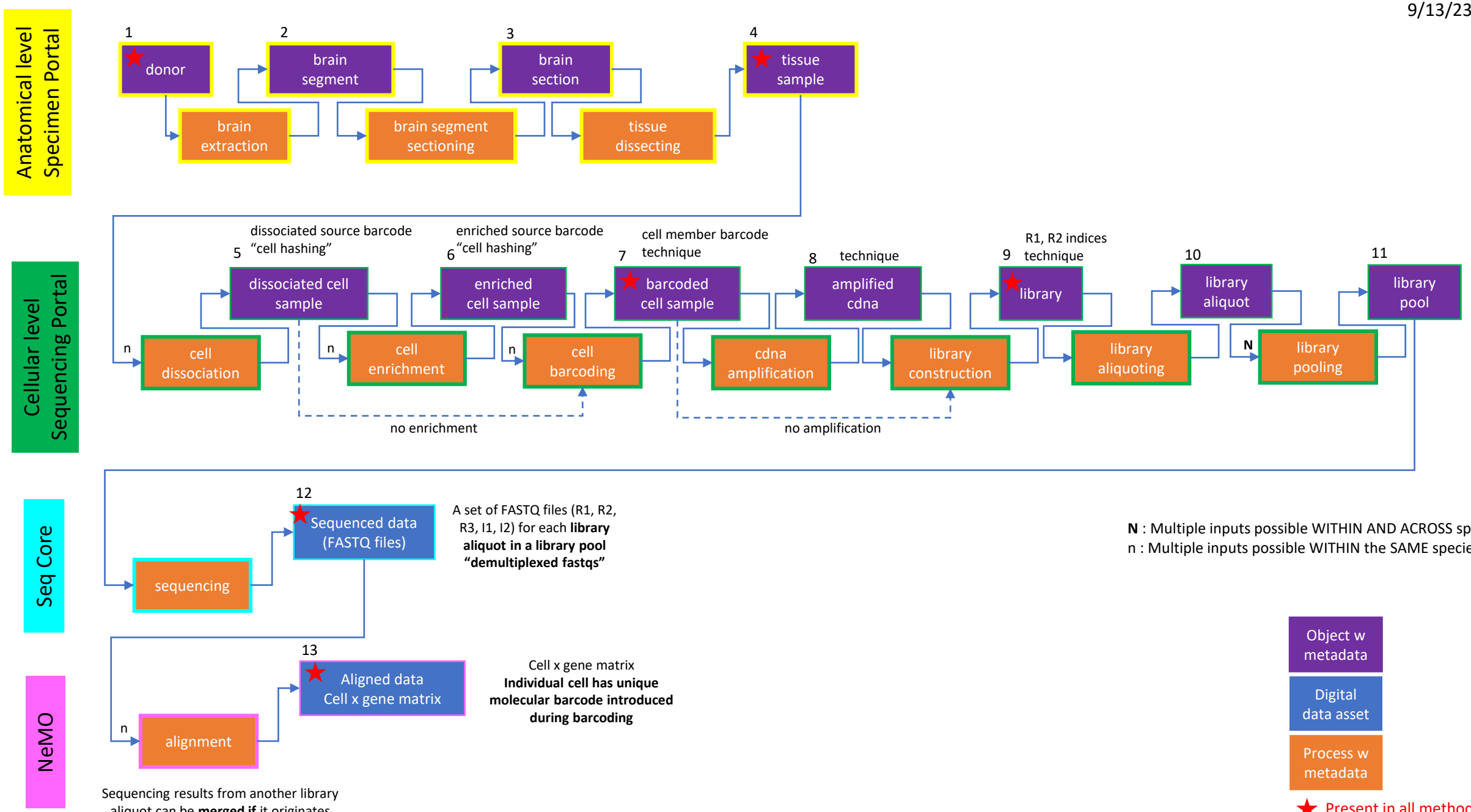
9/13/23



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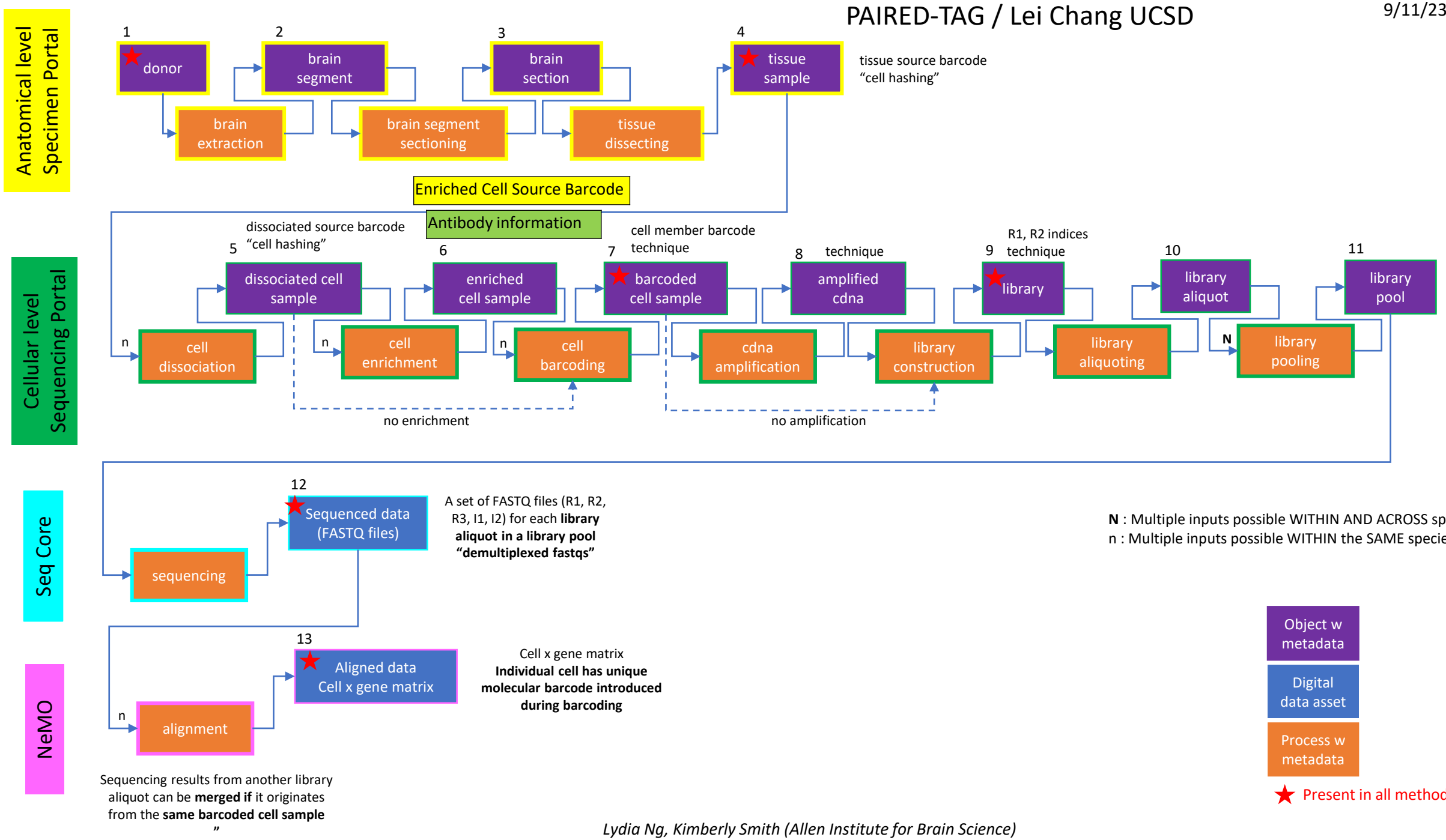
PAIRED-TAG / Lei Chang UCSD

9/11/23



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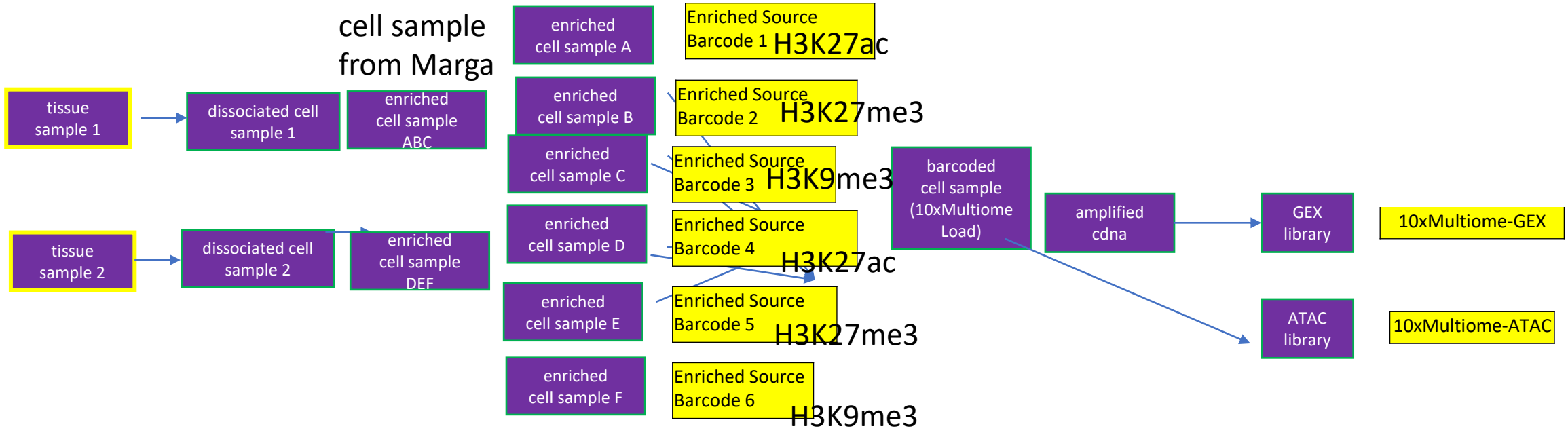
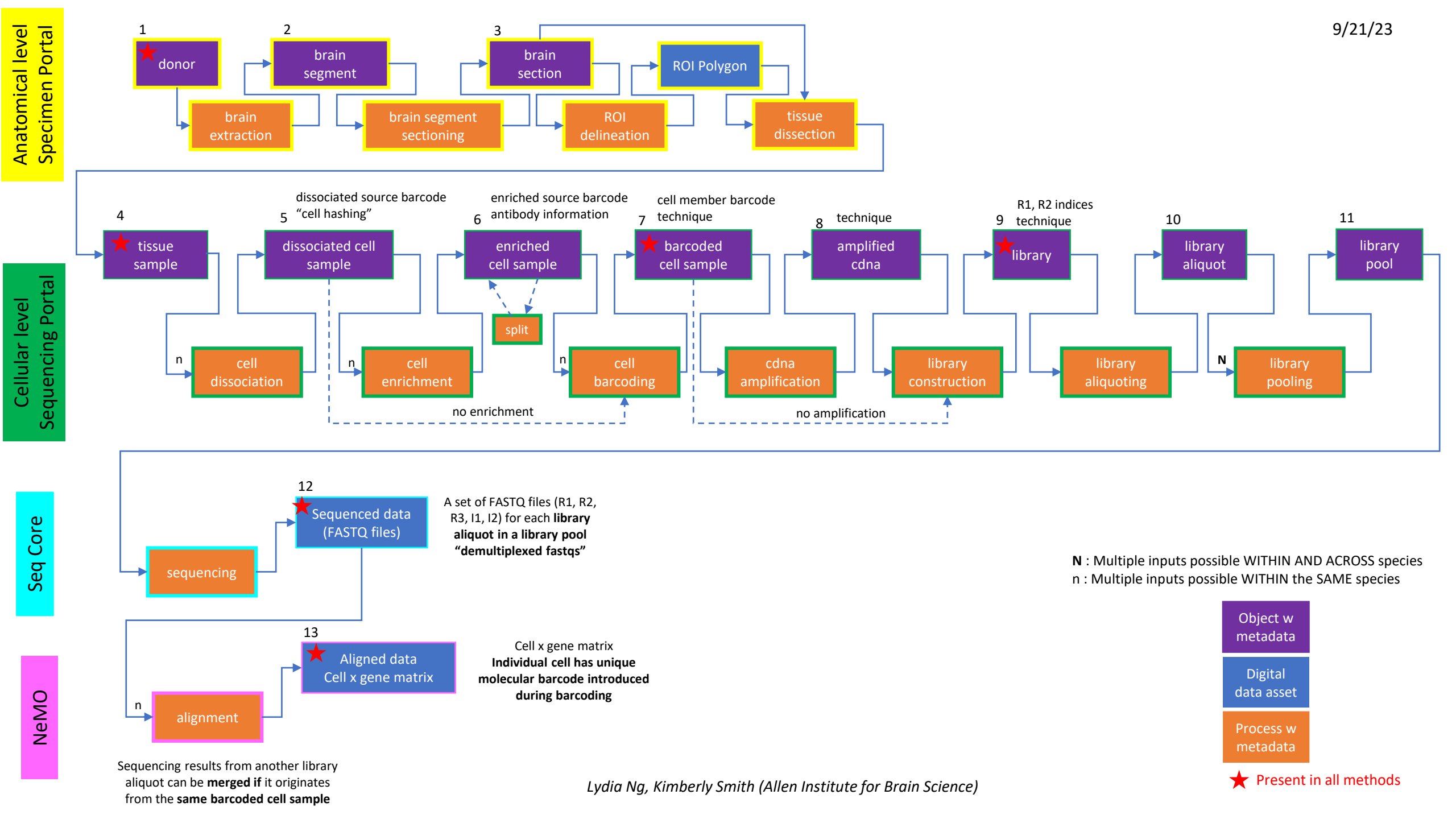
PAIRED-TAG / Lei Chang UCSD

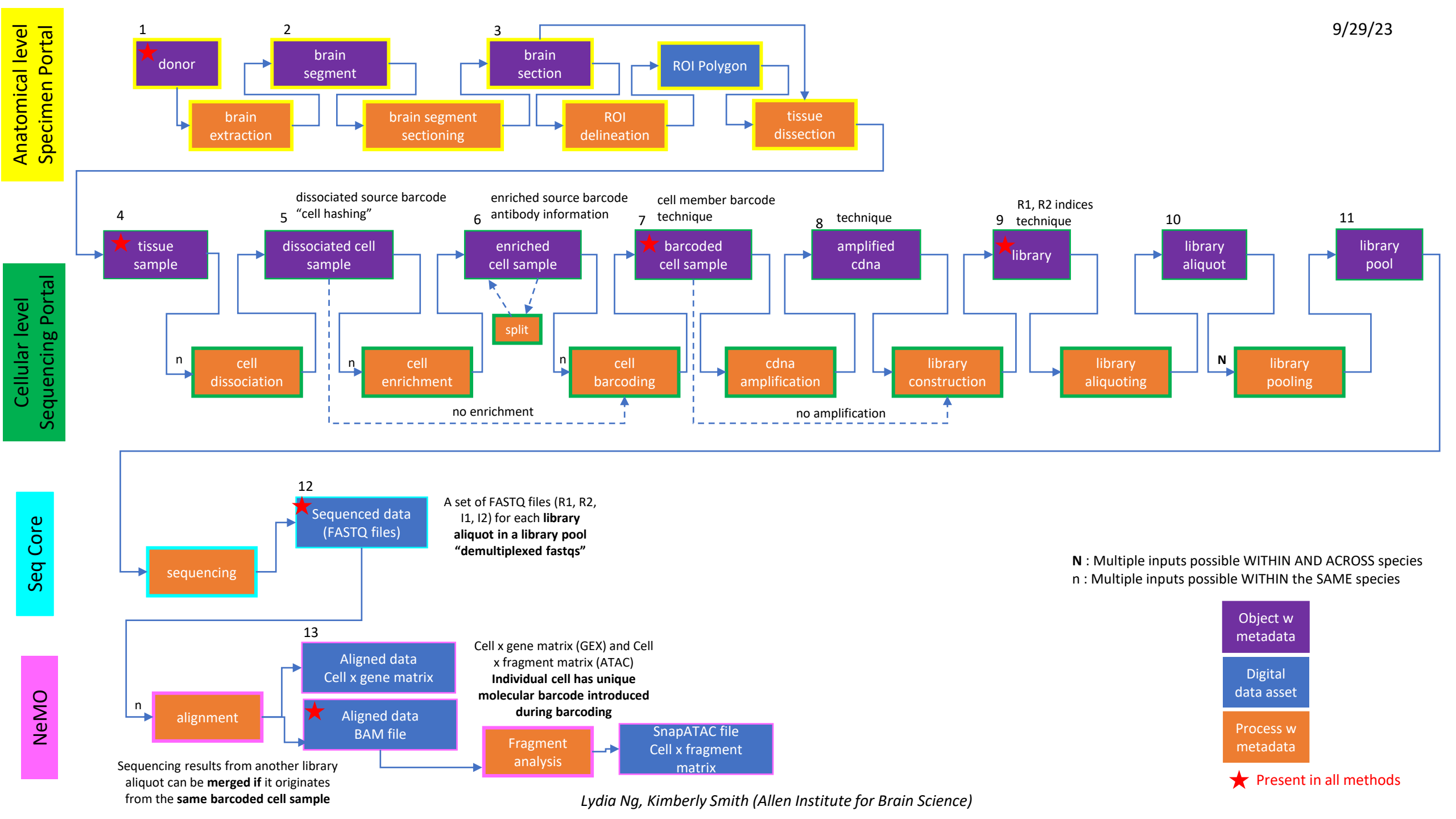


10xV3.1_CellPlex	GEXOnly	10xV3.1 (RNASeq) GEX transcript library, using multiple tagged tissue inputs from 10xCellPlex
10xV3.1_CellPlexTag	GEXOnly	10xV3.1 (RNASeq) Tissue Tag library, from using multiple tagged tissue inputs from 10xCellPlex
Paired-tag	REMOVE	No longer used – replaced by Multiome libraries

10xV3.1	GEXOnly	10xV3.1 (RNASeq)
10xV3.1_HT	GEXOnly	10xV3.1 (RNASeq) using High Throughput (HT) 10x chips
10xMultiome-GEX	GEXMultiome	10x RNASeq library from 10x Multiome parent Barcoded Cell Sample
10xMultiome-ATAC	ATACMultiome	10x ATACSeq library from 10x Multiome parent Barcoded Cell Sample

Timeframe: human pilot miniatlas, expect to start late September


9/21/23
Anatomical level Specimen Portal
Cellular level Sequencing Portal
Seq Core
NeMO
1
donor
2
brain segment
3
brain section
ROI Polygon
brain extraction
brain segment sectioning
ROI delineation
tissue dissection
4
tissue sample
5
dissociated source barcode
"cell hashing"
dissociated cell sample
6
enriched source barcode
antibody information
enriched cell sample
split
7
cell member barcode
technique
barcoded cell sample
8
technique
amplified cdna
9
R1, R2 indices
technique
library
10
library aliquot
11
library pool
n
cell dissociation
n
cell enrichment
n
cell barcoding
cdna amplification
library construction
library aliquoting
N
library pooling
no enrichment
no amplification
12
Sequenced data (FASTQ files)
A set of FASTQ files (R1, R2, R3, I1, I2) for each **library aliquot in a library pool "demultiplexed fastqs"**
sequencing
13
Aligned data Cell x gene matrix
Cell x gene matrix **Individual cell has unique molecular barcode introduced during barcoding**
n
alignment
Sequencing results from another library aliquot can be **merged if** it originates from the **same barcoded cell sample**
N : Multiple inputs possible WITHIN AND ACROSS species
n : Multiple inputs possible WITHIN the SAME species
Object w metadata
Digital data asset
Process w metadata
Present in all methods
Lydia Ng, Kimberly Smith (Allen Institute for Brain Science)


9/29/23
Anatomical level Specimen Portal
Cellular level Sequencing Portal
Seq Core
NeMO
1
donor
2
brain segment
3
brain section
ROI Polygon
brain extraction
brain segment sectioning
ROI delineation
tissue dissection
4
tissue sample
5
dissociated source barcode "cell hashing"
dissociated cell sample
6
enriched source barcode antibody information
enriched cell sample
split
7
cell member barcode technique
barcoded cell sample
8
technique
amplified cdna
9
R1, R2 indices technique
library
10
library aliquot
11
library pool
n
cell dissociation
n
cell enrichment
n
cell barcoding
cdna amplification
library construction
library aliquoting
N
library pooling
no enrichment
no amplification
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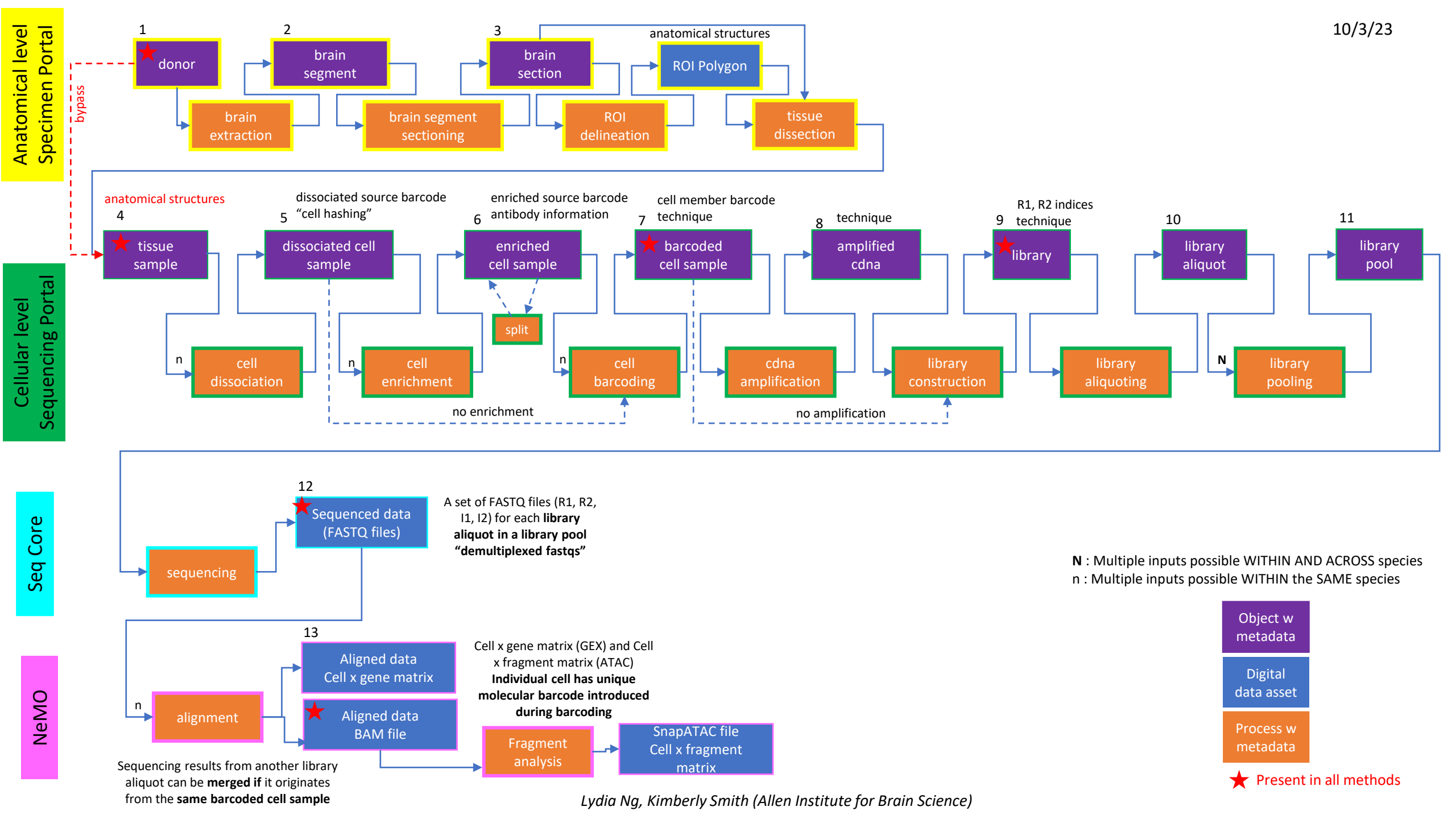
Sequenced data (FASTQ files)
A set of FASTQ files (R1, R2, I1, I2) for each **library aliquot in a library pool "demultiplexed fastqs"**
sequencing
13
Aligned data Cell x gene matrix
Aligned data BAM file
n
alignment
Cell x gene matrix (GEX) and Cell x fragment matrix (ATAC) **Individual cell has unique molecular barcode introduced during barcoding**
Fragment analysis
SnapATAC file Cell x fragment matrix
Sequencing results from another library aliquot can be **merged if** it originates from the **same barcoded cell sample**
N : Multiple inputs possible WITHIN AND ACROSS species
n : Multiple inputs possible WITHIN the SAME species
Object w metadata
Digital data asset
Process w metadata
Present in all methods
Lydia Ng, Kimberly Smith (Allen Institute for Brain Science)


10/3/23
Anatomical level Specimen Portal
Cellular level Sequencing Portal
Seq Core
NeMO
bypass
1
donor
2
brain segment
3
brain section
anatomical structures
ROI Polygon
brain extraction
brain segment sectioning
ROI delineation
tissue dissection
anatomical structures
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tissue sample
dissociated source barcode
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dissociated cell sample
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technique
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library aliquot
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N
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n
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Aligned data BAM file
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SnapATAC file Cell x fragment matrix
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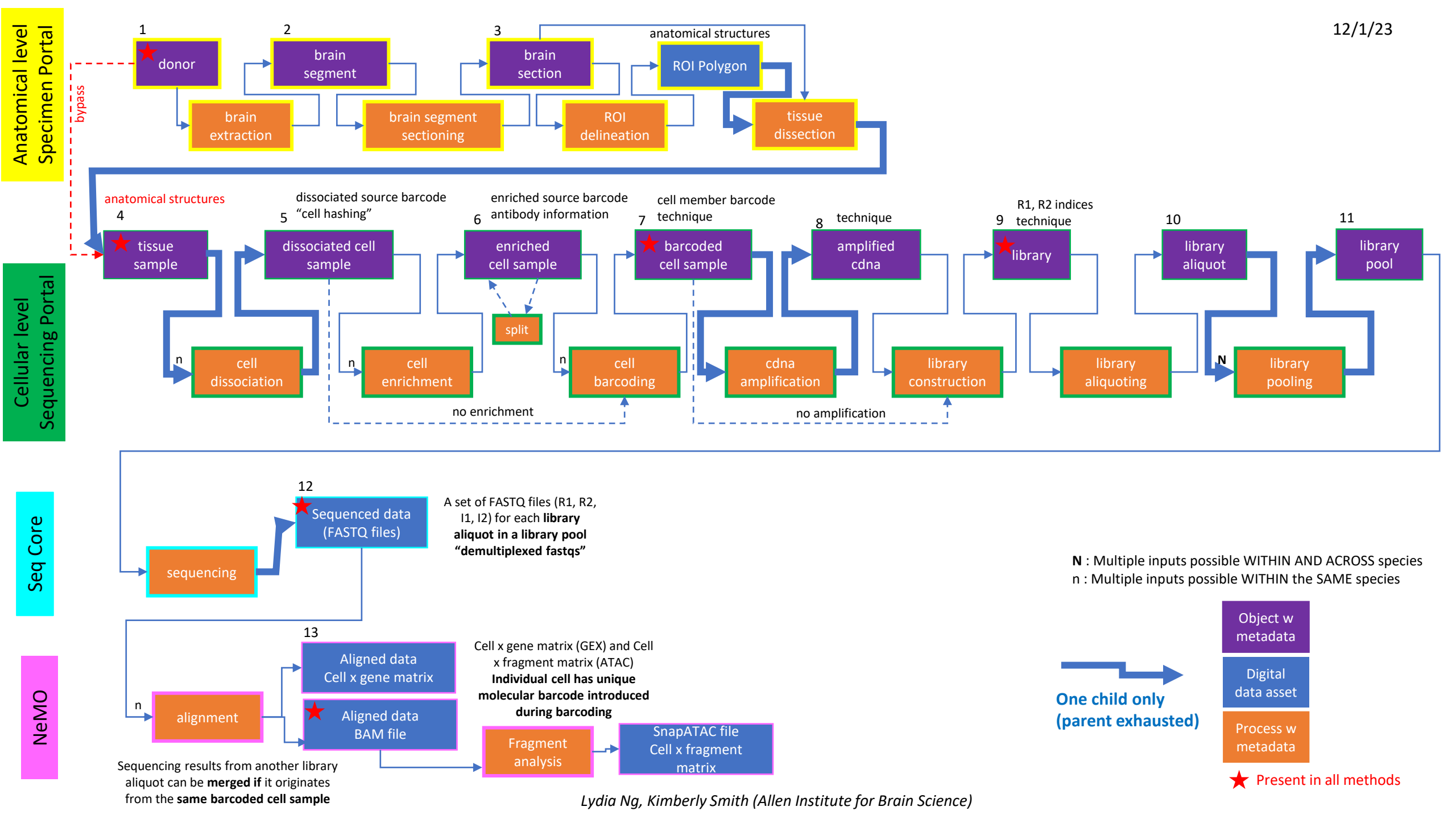
n : Multiple inputs possible WITHIN the SAME species
Object w metadata
Digital data asset
Process w metadata
Present in all methods
Lydia Ng, Kimberly Smith (Allen Institute for Brain Science)


12/1/23
Anatomical level Specimen Portal
Cellular level Sequencing Portal
Seq Core
NeMO
bypass
1
donor
2
brain segment
3
brain section
anatomical structures
ROI Polygon
brain extraction
brain segment sectioning
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barcoded cell sample
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amplified cdna
R1, R2 indices
technique
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library
10
library aliquot
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library pool
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cell dissociation
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cell enrichment
split
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cell barcoding
cdna amplification
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library aliquoting
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N : Multiple inputs possible WITHIN AND ACROSS species
n : Multiple inputs possible WITHIN the SAME species
One child only (parent exhausted)
Object w metadata
Digital data asset
Process w metadata
Present in all methods
Lydia Ng, Kimberly Smith (Allen Institute for Brain Science)

Resequencing Single Cell

10/24/23

Cellular level Sequencing Portal

7 cell member barcode technique

8 technique

9 R1, R2 indices technique

barcoded cell sample

Library GEX

Library ATAC

10

library aliquot

library aliquot

TBD

Pass

Fail

11

library pool

library pool

Sequenced data (FASTQ files)

Sequenced data (FASTQ files)

alignment

Assemble results

Aligned data Cell x gene matrix

Aligned data BAM file

Fragment analysis

SnapATAC file Cell x fragment matrix

Sequencing Result QC state set

Assembly step = Merge step
Alignment results are per cell-barcode. Multiple alignments mean multiple outputs for the same cell-barcode. So merged assembly using all available outputs for the cell-barcode.

10

library aliquot

library aliquot

Null

Pass

Fail

11

library pool

library pool

Sequenced data (FASTQ files)

Sequenced data (FASTQ files)

alignment

Aligned data Cell x gene matrix

Aligned data BAM file

Fragment analysis

SnapATAC file Cell x fragment matrix

End result: Merged

Assemble results

Merge

Aligned data Cell x gene matrix

Aligned data BAM file

SnapATAC file Cell x fragment matrix

Merge triggered by Pass

Sequencing results from another library aliquot can be **merged if** it originates from the **same barcoded cell sample**

Lydia Ng, Kimberly Smith (Allen Institute for Brain Science)

Resequencing Single Cell

12/1/23

Cellular level Sequencing Portal

7 cell member barcode technique

8 technique

9 R1, R2 indices technique

barcoded cell sample

Library GEX

Library ATAC

10

library aliquot

A, B

Null

Pass

Fail

library aliquot

Null

Pass

Fail

A', B'

11

library pool

library pool

Sequencing Result QC state set

A

Sequenced data (FASTQ files)

B

Sequenced data (FASTQ files)

alignment

Assemble results

Aligned data Cell x gene matrix

A+B

Aligned data BAM file

Fragment analysis

SnapATAC file Cell x fragment matrix

A'

Sequenced data (FASTQ files)

B'

Sequenced data (FASTQ files)

alignment

Aligned data Cell x gene matrix

A'+B'

Aligned data BAM file

Fragment analysis

SnapATAC file Cell x fragment matrix

A'', B''

10

library aliquot

Null

Pass

Fail

library aliquot

11

library pool

library pool

A''

Sequenced data (FASTQ files)

Sequenced data (FASTQ files)

B''

alignment

Aligned data Cell x gene matrix

A''+B''

Aligned data BAM file

Fragment analysis

SnapATAC file Cell x fragment matrix

Assembly step = Merge step
Alignment results are per cell-barcode. Multiple alignments mean multiple outputs for the same cell-barcode. So merged assembly using all available outputs for the cell-barcode.

End result: Merged

Assemble results

Merge

Aligned data Cell x gene matrix

Aligned data BAM file

SnapATAC file Cell x fragment matrix

Merge triggered by Pass

Sequencing results from another library aliquot can be **merged if** it originates from the **same barcoded cell sample**

A+B = first sequencing Pool-1
A'+B' = second sequencing Pool-1 (same library aliquot names as A+B)
A''+B'' = first sequencing Pool-2 (new library aliquot names)

Lydia Ng, Kimberly Smith (Allen Institute for Brain Science)

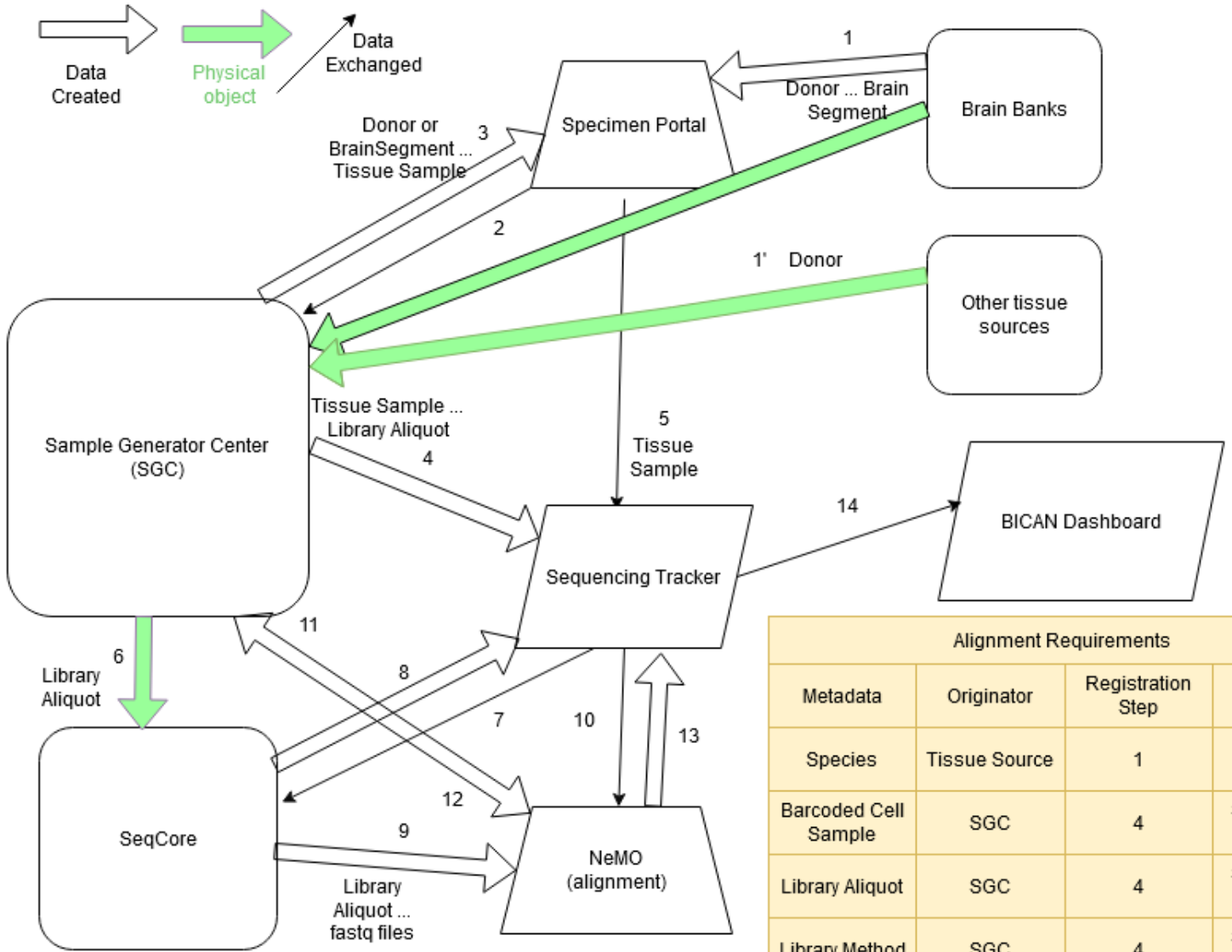
Other types discussion (10/18/23)

- 1) 'pilot' pre-SeqCore 10x datasets : it seems clear to me that these should be in the SeqPortal to ensure we have same databased elements for the datasets. Example: DevMouse P0-Male and early HMBA-Macaque specimen 01

- 2) PatchSeq : sequencing not done at SeqCores and fastqs deposited at NeMO with limited metadata (we do this),

- 3) SmartSeq3 : sequencing not done at SeqCores and fastqs should be going to NeMO (this is with Nowakowski team for DevMouse - we send them Enriched Cells).

- 4) BulkRNA : sequencing not done at SeqCores, not at single cell level. Fastqs direct to NeMO (we do this, not frequently).



Alignment Requirements			
Metadata	Originator	Registration Step	Database
Species	Tissue Source	1	Specimen Portal
Barcoded Cell Sample	SGC	4	Sequencing Tracker
Library Aliquot	SGC	4	Sequencing Tracker
Library Method	SGC	4	Sequencing Tracker
r1 & r2 index sequences	SGC	4	Sequencing Tracker
demultiplexed fastq files	SeqCore	9	NeMO

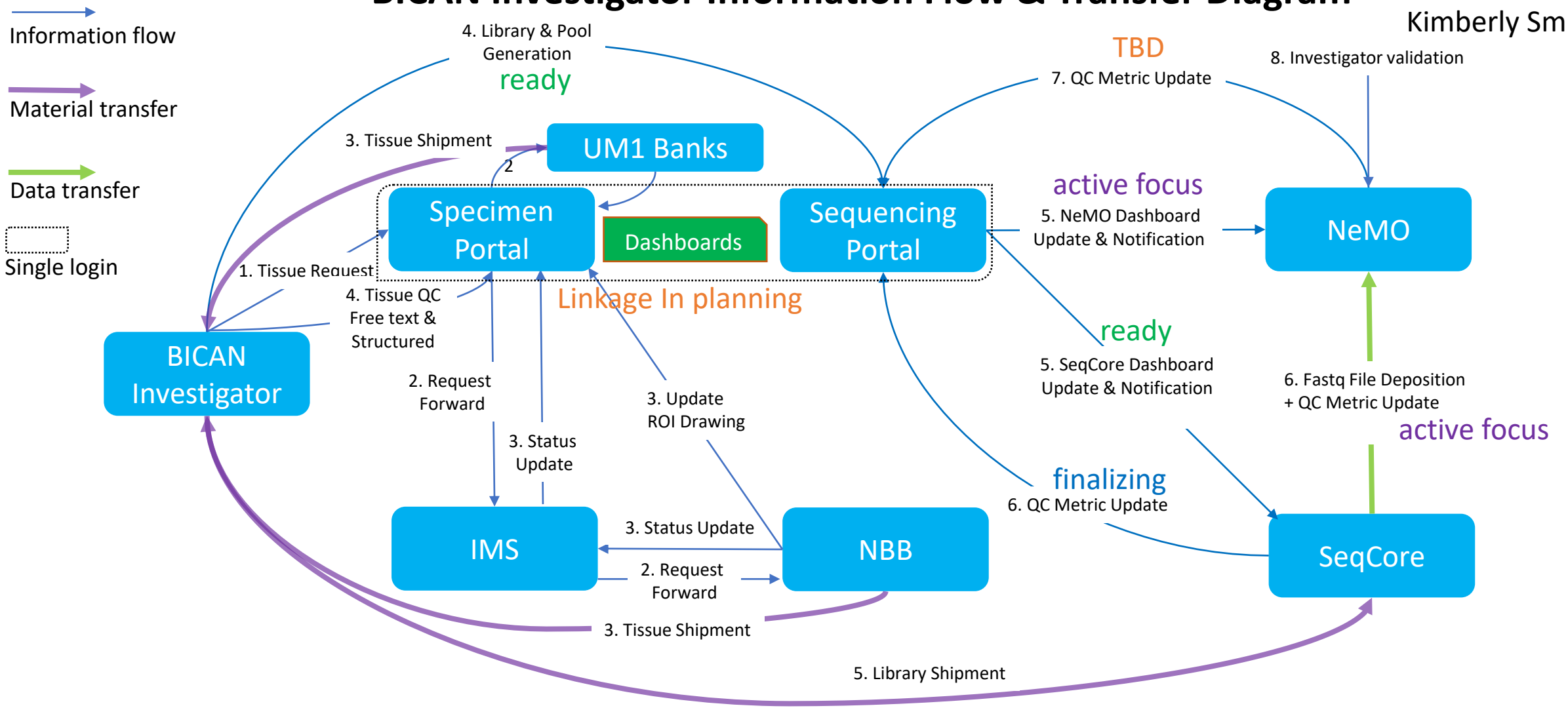
SeqCore Instructions : Required Elements

5. SeqCore Dashboard Update & Notification:: to streamline sequencing instructions, the SeqCore should retrieve all details from Sequencing Portal that are required for sequencing a library pool. This will include some elements that are required for dataset analysis, but also many elements that are required only for sequencing instructions.

SeqCore Instructions						
Metadata	Example	Originator	Database	Analysis	Instruction	Description
SeqCore_tube_name	SQ_MX2042	SeqCore/SGC	SeqTracker	yes	yes	This is the container of the library pool. It is a barcoded tube supplied to SGC by SeqCore ahead of pooling. Library aliquots assigned to batch_tube_name and registered into Sequencing Tracker.
Internal_Batch_name	MTX-2042	SGC	SeqTracker	yes	yes	SGC internal batch name. There may be more than one batch_tube_name associated to one Library_Lab_Batch_name
Library_pool_name	SQ_MX2042	SGC	SeqTracker	yes	yes	library pool tube name, internal. This may be the same as SeqCore_tube_name or may be different, depending on the lab.
tube_contents_nm	10	SGC	SeqTracker	yes	yes	molar concentration in nM of library pool provided in batch_tube_name
tube_avg_size_bp	474	SGC	SeqTracker	yes	yes	average size of library pool in base pairs provided in batch_tube_name
tube_volume_ul	200	SGC	SeqTracker	no	yes	volume of library pool in SeqCore_tube_name
library_aliquot	SQ_MX2042-5	SGC	SeqTracker	yes	yes	The identifer of the specific library aliquot. There will be multiple library_aliquots within one library pool.
Library_Method	10xMulti-RSeq	SGC	SeqTracker	yes	yes	Chemistry used to generate the library
r1_index_sequence	GTCCCATCAA	SGC	SeqTracker	yes	yes	Sequence of i7 index required by sequencing instrument for demultiplexing (could be sense or antisense). This is derived from a table of index name and sequences.
r2_index_sequence	GTCACGTTCG	SGC	SeqTracker	yes	yes	Sequence of i5 index required by sequencing instrument for demultiplexing (could be sense or antisense). This is derived from a table of index name and sequences.
r1_index	SI-TT-F9_i7	SGC	SeqTracker	yes	yes	Name of the library index used for Read-1 sequence. Indexes allow libraries to be pooled together for sequencing. Sequencing output (fastq) are demultiplexed by using the indexes for each library. The name will be associated with a oligo (string of bases). The required direction of the sequence (sense or antisense) of the index can differ depending on sequencing instruments.
r2_index	SI-TT-F9_ai5-as	SGC	SeqTracker	yes	yes	Name of the library index used for Read-2 sequence. Indexes allow libraries to be pooled together for sequencing. Sequencing output (fastq) are demultiplexed by using the indexes for each library. The name will be associated with a oligo (string of bases). The required direction of the sequence (sense or antisense) of the index can differ depending on sequencing instruments.
PhiX_Spike_In_percent	5	SGC	SeqTracker	no	yes	Percent of PhiX spike-in included in library pool
Sequencing_Platform	NovaSeq S4	SGC	SeqTracker	no	yes	Sequencing instrument to be used for sequencing library pool
Sequencing_Read_Length	R1: 28, R2: 90	SGC	SeqTracker	no	yes	Read 1 and Read 2 read lengths, required for each library pool and specific to Library_Method
Index_Length	i7:10 i5:10	SGC	SeqTracker	no	yes	I7 and I5 read lengths, required for each library pool and specific to indexes used in the Library_Method.
Primer_Sequence_Source	Illumina	SGC	SeqTracker	no	yes	Adapter Chemistry Type (TruSeq, Nextera, Custom)
Sample_Type	cDNA	SGC	SeqTracker	no	yes	Type of molecules in library pool to be sequenced
Sample_Source	Tissue	SGC	SeqTracker	no	yes	The source of the molecules in the library pool to be sequenced
Species	mouse	Tissue Source	Specimen Portal	yes	yes	Species of the molecules in the library pool to be sequenced
Custom primers	yes	SGC	SeqTracker	no	yes	yes/no if there are custom primers

BICAN Investigator Information Flow & Transfer Diagram

Status 8/21/23
Kimberly Smith

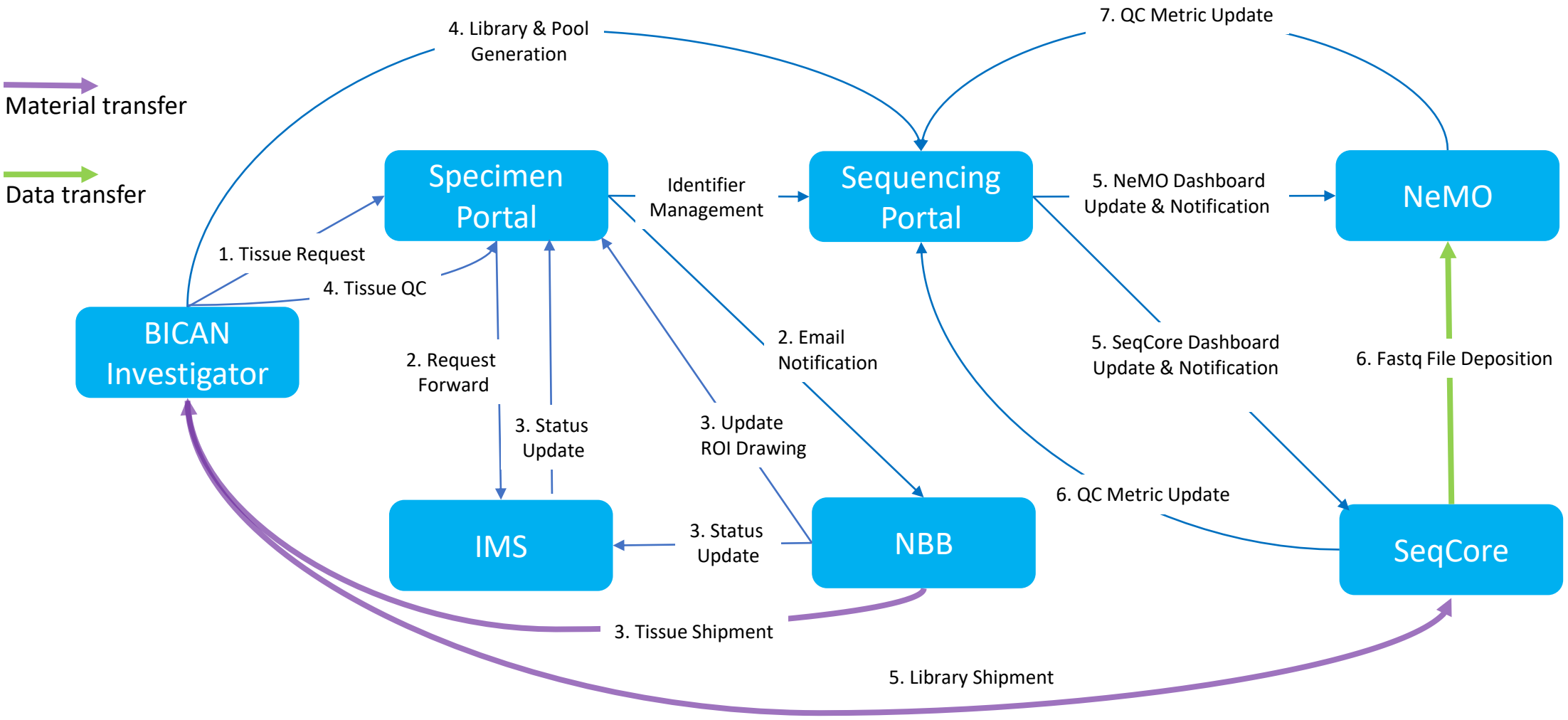


1. Request tissue; 2. Forwarding request to banks; 3. Fulfilling request by banks; 4. Library preparation; 5. Library shipment & notification to SeqCore; 6. SeqCore tasks; 7. Fastq file ingestion and QC metric report; 8: Investigator validation of Fastq files

Dashboards in Specimen or Sequencing Portal: A. Investigator dashboard; B. IMS dashboard; C. NBB dashboard; D. SeqCore dashboard; E. NeMO dashboard; F. UM1 banks dashboard; G. NIH dashboard

Note: all dashboards will offer downloading in xls or other formats + API

BICAN Investigator Information Flow Diagram



1. Request tissue; 2. Forwarding request to banks; 3. Fulfilling request by banks; 4. Library preparation; 5. Library shipment & notification to SeqCore; 6. SeqCore tasks; 7. Fastq file ingestion and QC metric report.

Dashboards: 1. Investigator dashboard; 2. IMS dashboard; 3. NBB dashboard; 4. SeqCore dashboard; 5. NeMO dashboard; 6. NIH dashboard

Flow step	Description	Information	Physical	Originator	Destination
1	Brain Banks register donor through brain segment at Specimen Portal. Not all tissue will be coming from Brain Banks.	Donor to Brain Segment	Tissue at brain segment level (eg brain slabs) (goes directly to Sample Generator Center)	Brain Banks	Specimen Portal
1'	Other tissue sources besides human brain banks will be used. These are not expected to interface with the Specimen Portal directly. Donor and tissue information will be provided to Sample Generator Centers.	Donor to Brain Segment	Tissue either at donor level (eg mouse) or brain segment level (eg brain slabs)	other tissue sources	SGC
2	If tissue is registered at Specimen Portal by Brain Bank, then Donor to Brain Segment information will be retrieved by SGC from Specimen Portal before further tissue processing	Donor to Brain Segment	none	Specimen Portal	SGC
3	SGC register at Specimen Portal the tissue partitioning, ending with Tissue Sample. This is the last physical piece of tissue before it becomes a collection of cells or nuclei.	Donor (or Brain Segment) to Tissue Sample	none	SGC	Specimen Portal
4	SGC registers at Sequencing Tracker the steps from Tissue to Library Aliquot. These include several steps with Multiple Inheritance.	Tissue Sample to Library Aliquot	none	SGC	Sequencing Tracker
5	Sequencing Tracker connects to Specimen Portal using the Tissue Sample associated with the Library Aliquot, provided by the SGC	Donor to Tissue Sample	none	Specimen Portal	Sequencing Tracker
6	SGC provides instructions for sequencing Library Aliquot, along with physical Library Pool containing multiple Library Aliquots. Note that this will include sequencing instructions that are not necessary for sample or dataset analysis.	Library Aliquot to Library Pool (includes specific sequencing instructions)	Library Pool containing multiple Library Aliquots	SGC	SeqCore
7	SeqCore confirms that Library Aliquot has been registered at Sequencing Tracker *and* has not yet been sequenced	Library Aliquot	none	Sequencing Tracker	SeqCore
8	SeqCore adds sequencing metadata/metrics to Library Aliquot at Sequencing Tracker	Metadata at Library Aliquot and Library Pool levels	none	SeqCore	Sequencing Tracker
9	SeqCore deposits demultiplexed fastq files at NeMO for each Library Aliquot	Demultiplexed fastq file set for each Library Aliquot	none	SeqCore	NeMO
10	NeMO retrieves metadata required for alignment from Sequencing Tracker	Species, Barcoded Cell Sample, Library Method, Library Aliquot, r1_index_sequence, r2_index_sequence	none	Sequencing Tracker	NeMO
11	SGC can retrieve fastqs and alignment outputs from NeMO	Output files by Library Aliquot	none	NeMO	SGC
12	SGC registers at NeMO final results of sequencing for each Library Aliquot (pass, fail, remove)	Library Aliquot sequence results	none	SGC	NeMO
13	Sequencing Tracker retrieves alignment status and library aliquot sequence result from NeMO	Sequence status and results for Library Aliquot	none	NeMO	Sequencing Tracker

Notes from meeting with Chongyuan, Aparna (5/12)

- Sex/race is unknown at time of submission
- Some lab have a highly 1:1 pipeline and will be using the same identifiers for each component – this should be allowed and still retain that there was a tissue sample, dissociated cell sample etc with the same label. Sequencing portal needs to be able to issue unique identifier
- We want to able to skip a box in some workflow
  - For example, for ATAC library does not go through amplified cdna

Notes from meeting with Tomas Nowakowski (5/18)

- Registering donor at Specimen Portal should not require use of drawing tools
- Registering donor at Specimen Portal needs to be minimalistic (“take a ticket”) and allow for filling in information at a later point in time without holding up downstream sequencing

Notes from meeting with Lei Chang and Specimen Portal team (5/19)

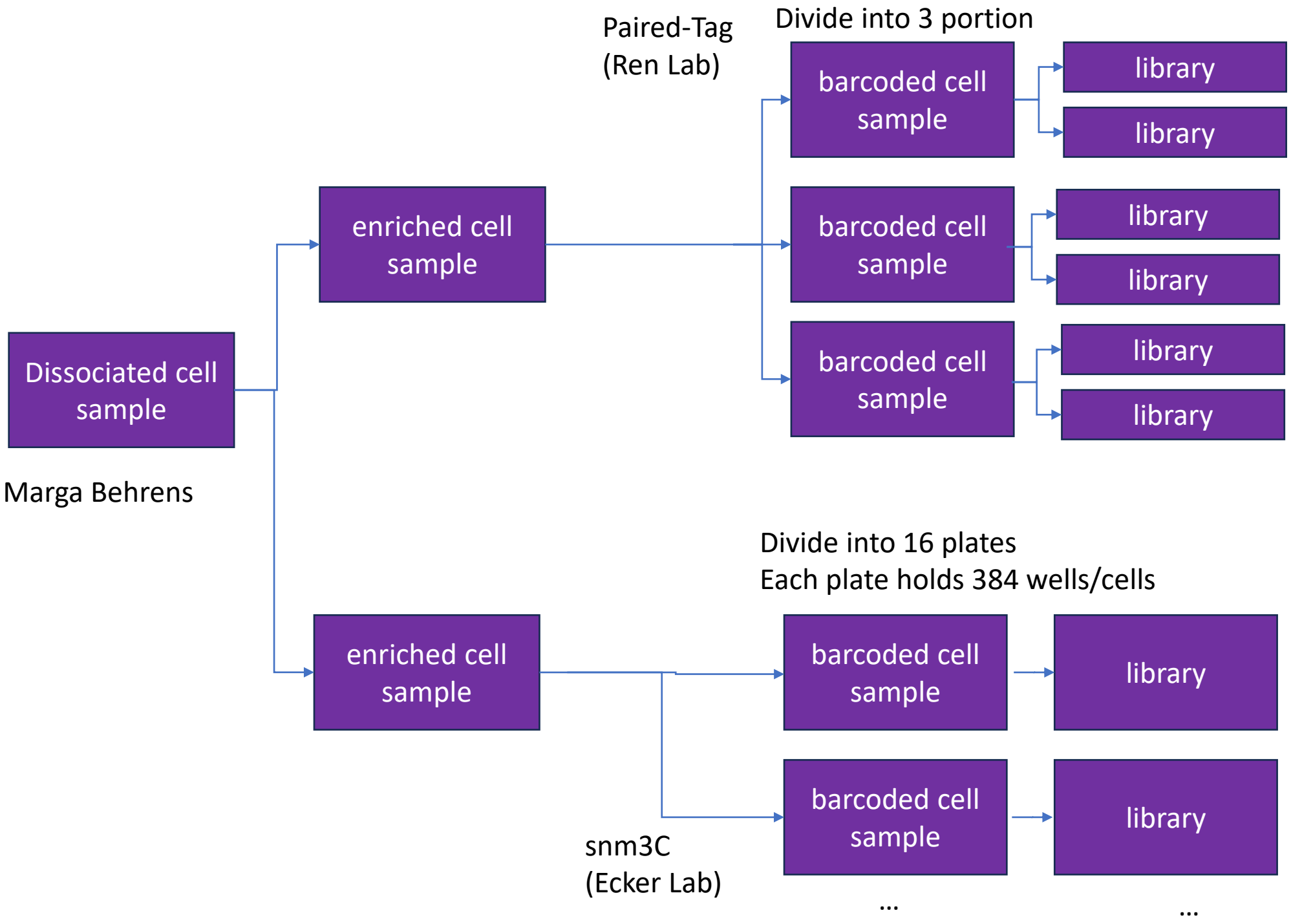
- Lei confirmed that the “purple box” diagram fits their library generation paradigm

Notes from meeting with Fenna Krienen (5/23)

- Fenna confirmed that the “purple box” diagram fits their library generation paradigm

Notes from meeting with Ren/Ecker Lab (6/28)

- Data includes whole human adult, childhood and adolescent
- Marga: produces enriched cell sample then send it to either:
  - PairTag
  - SNM3C
- Need to be able to add indexing after enriched cell sample “hashing/multiplexing”
- 2 different tubes one for each different destination lab
- Yang: PairTag using the same barcode as 10x
  - Make 3 separate batches to into separate barcoded_cell_sample
- Rosa/Anna: They do the **3C** process – sort into 384 plates individual cell
  - 16x plates of 384 wells
  - Separate single cell
  - Each well in plate get a barcode
  - Then each plate then get a barcode (= Illumina library indices)
  - No amplification
  - They get 16 different libraries


Paired-Tag
(Ren Lab)
Divide into 3 portion
Dissociated cell sample
Marga Behrens
enriched cell sample
barcoded cell sample
library
library
barcoded cell sample
library
library
barcoded cell sample
library
library
Divide into 16 plates
Each plate holds 384 wells/cells
enriched cell sample
snm3C
(Ecker Lab)
barcoded cell sample
library
barcoded cell sample
library
...
...

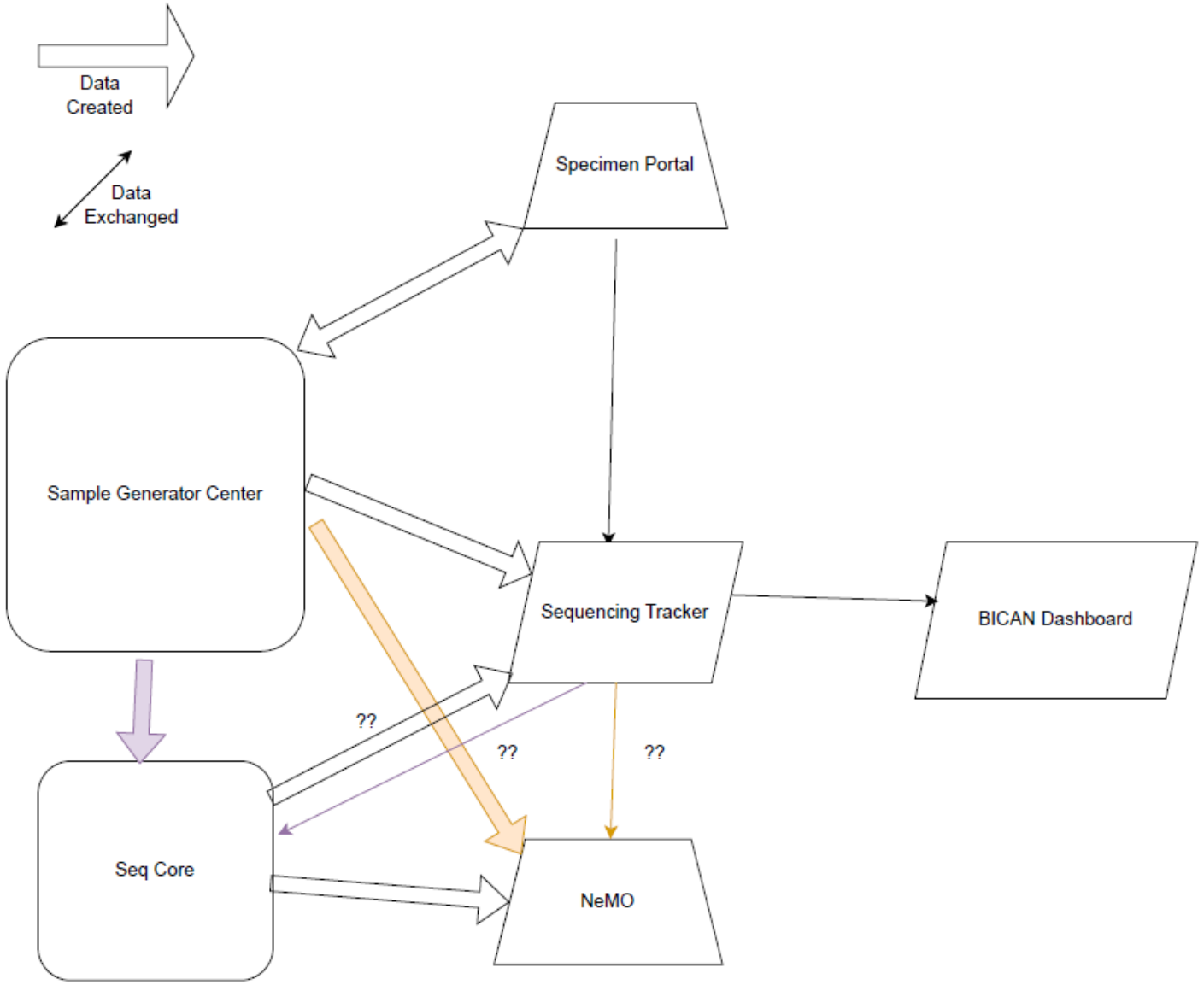
Notes on 7/11 Arlotta lab (Ashwin)

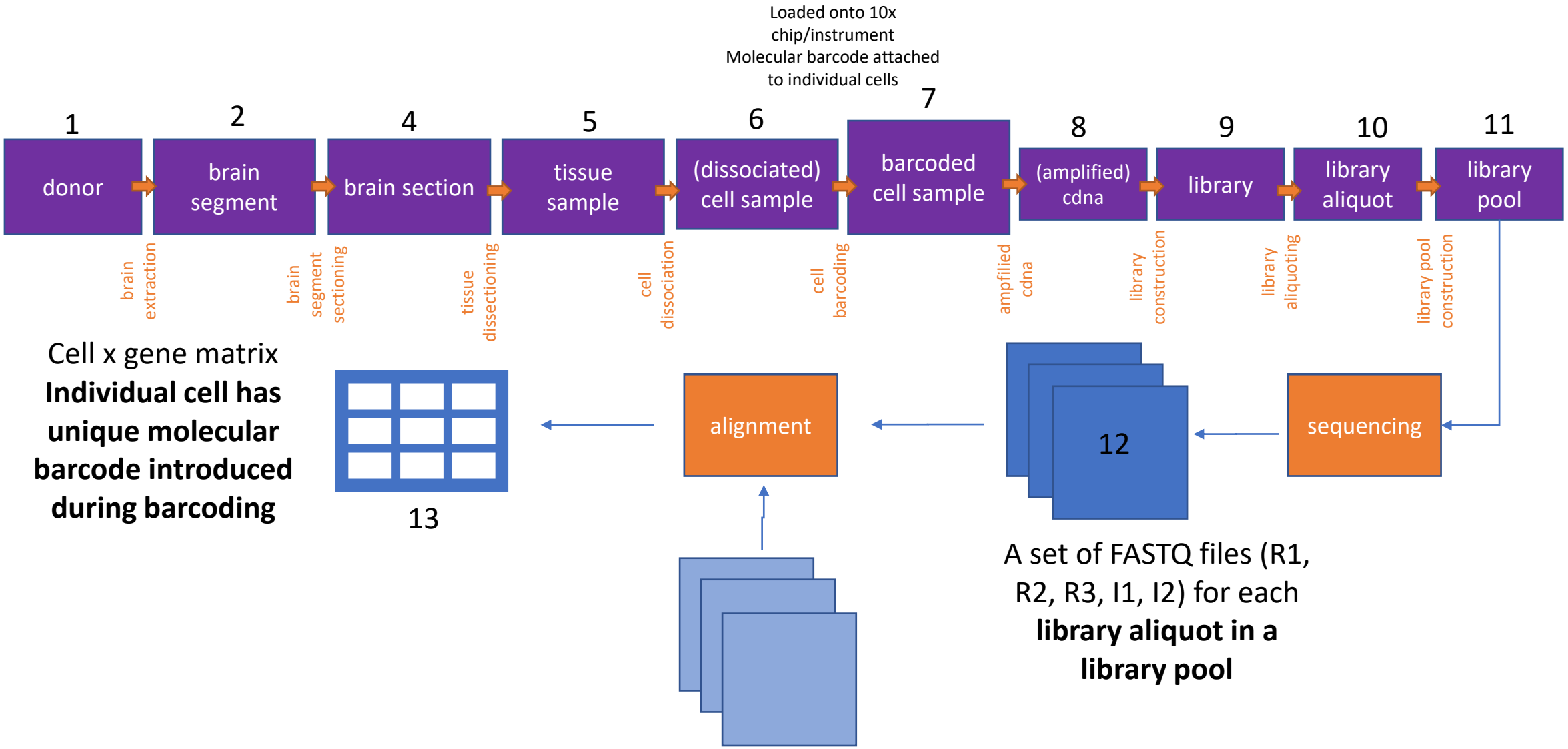
- C56 wildtype, Embryonic age
- Methods:
  - 10x Multiome
  - 10x RNA-seq
  - SMART-seq V3

Meeting with Anup/Michelle NEMO 7/19/23

Meeting with Lisa and Nick (Broad Seq Core?)

Old slides


Data
Created
Data
Exchanged
Specimen Portal
Sample Generator Center
Sequencing Tracker
BICAN Dashboard
??
??
??
Seq Core
NeMO


Loaded onto 10x chip/instrument
Molecular barcode attached to individual cells
1
donor
2
brain segment
4
brain section
5
tissue sample
6
(dissociated) cell sample
7
barcoded cell sample
8
(amplified) cdna
9
library
10
library aliquot
11
library pool
brain extraction
brain segment sectioning
tissue dissectioning
cell dissociation
cell barcoding
ampfilied cdna
library construction
library aliquoting
library pool construction
Cell x gene matrix
Individual cell has unique molecular barcode introduced during barcoding
13
alignment
12
sequencing
A set of FASTQ files (R1, R2, R3, I1, I2) for each library aliquot in a library pool
Sequencing results from another library aliquot can be merged if it originates from the same barcoded cell sample