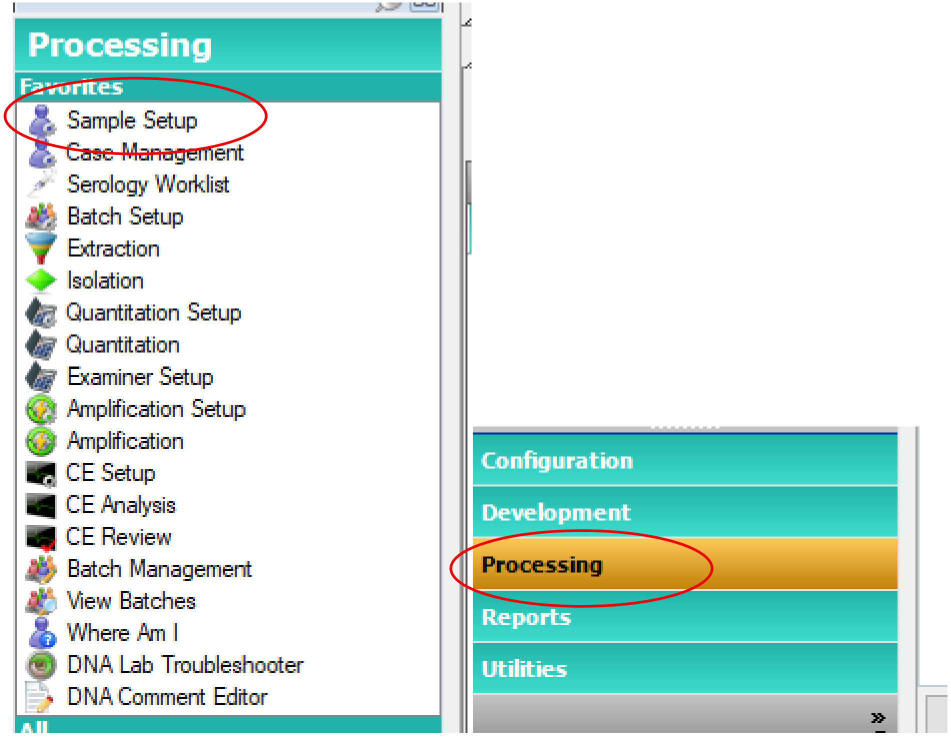
DNA Evidence Processing

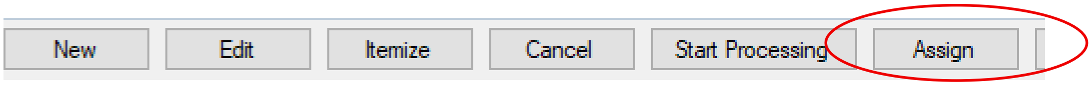
I. Batch Setup - Differentials

The Batch Setup module will be used to create an extraction batch and allocate samples/controls to the worksheet.

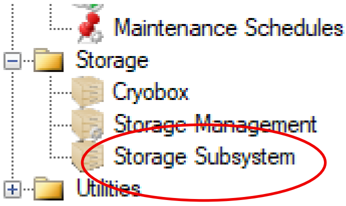
If the batch contains non-differential + differential samples, follow the *STACS Casework Non-Differential* instructions for the applicable samples. The non-differential samples may be added to the A-fraction isolation batch.

1. Upon batch assignment, assign the Justice-Trax STACS request to the processing examiner.
2. In STACS, assign the appropriate samples to the processing DNA examiner. This can be done in **Sample Setup**, under the **Sample Status** dropdown → **Processing.** Select the appropriate samples and choose **Assign.** Until this is done the sample will be assigned to the FB analyst who imported the sample. *The Reporting Analyst may be selected at this if known.* See below.

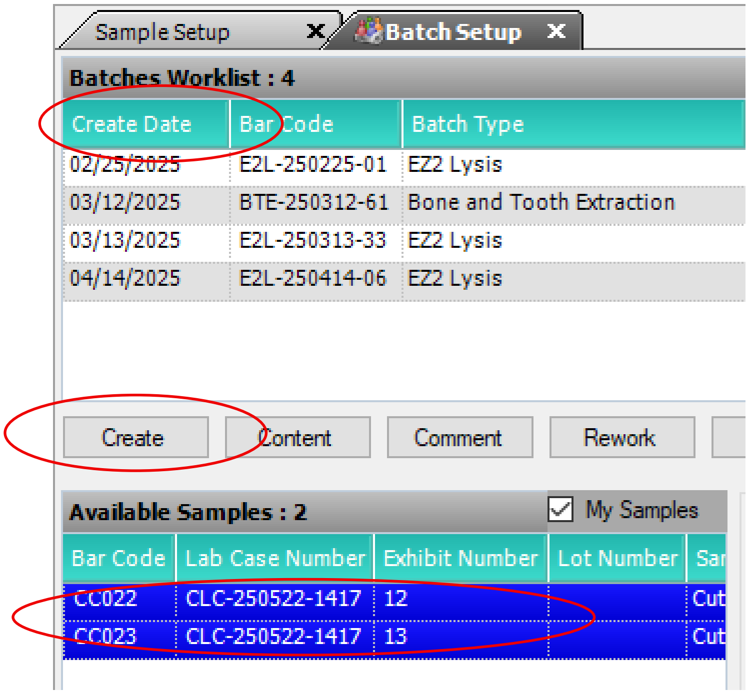




3. Take custody of the sample tube(s) to be processed. In **Storage Subsystem (Utilities → Storage → Storage Subsystem)**, select the **Retrieve** tab, scan the item bar code(s) to be put into your custody. Select **Save.**



4. Create a lysis worksheet: Open **Processing → DNA Processing → Batch Setup.** Click **Create.**



Batches Worklist : 4

Create Date	Bar Code	Batch Type
02/25/2025	E2L-250225-01	EZ2 Lysis
03/12/2025	BTE-250312-61	Bone and Tooth Extraction
03/13/2025	E2L-250313-33	EZ2 Lysis
04/14/2025	E2L-250414-06	EZ2 Lysis

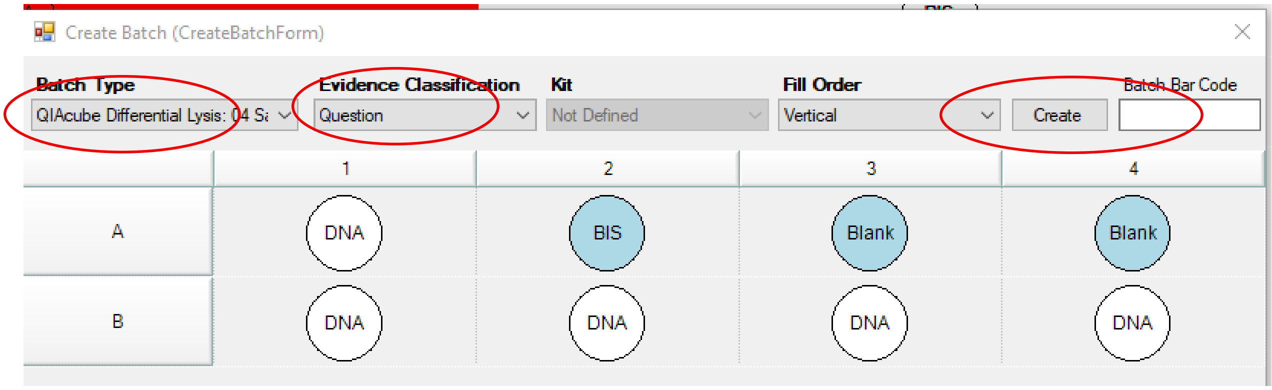
Available Samples : 2

Bar Code	Lab Case Number	Exhibit Number	Lot Number	Sar
CC022	CLC-250522-1417	12		Cut
CC023	CLC-250522-1417	13		Cut

5. Your samples should be in the **Available Samples** area above. Make sure to check the “My Samples” box to only show samples assigned to you. Click **Create.**

6. A **Create Batch** screen will appear as shown below.

7. Select the appropriate extraction under **Batch Type** (QIAcube Differential Lysis: **XX** samples), and the corresponding **Evidence Classification** (Question).
    a) Select the **total** number of questioned samples, EP2 and reagent blanks as the total sample number scenario.
        For example: 1 Q + EP2 + 2 RBs = 04 Samples
        (QIAcube Differential Lysis: 04 Samples)



Create Batch (CreateBatchForm)

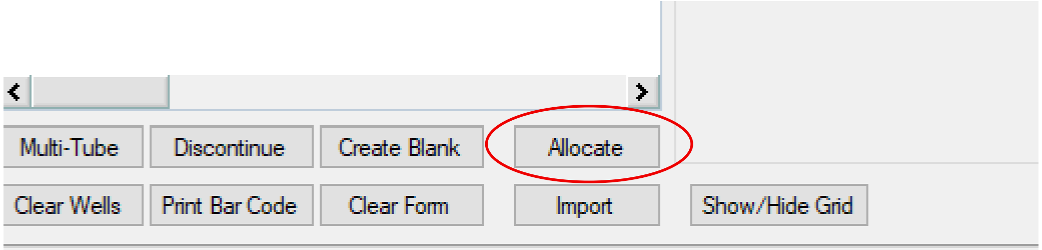
Batch Type: QIAcube Differential Lysis: 04 Sa
Evidence Classification: Question
Kit: Not Defined
Fill Order: Vertical
Batch Bar Code:

	1	2	3	4
A	DNA	BIS	Blank	Blank
B	DNA	DNA	DNA	DNA

8. Click **Create** (see above). A unique barcode associated with the extraction will be generated and printed. Moving forward, this barcode may be used to transfer the samples as a whole set. **Close** the Create Batch window at the bottom right corner.

9. To begin sample allocation, the batch should automatically populate into the "Sample Allocation" window. If it does not, scan the extraction barcode or double-click the batch in the upper window.

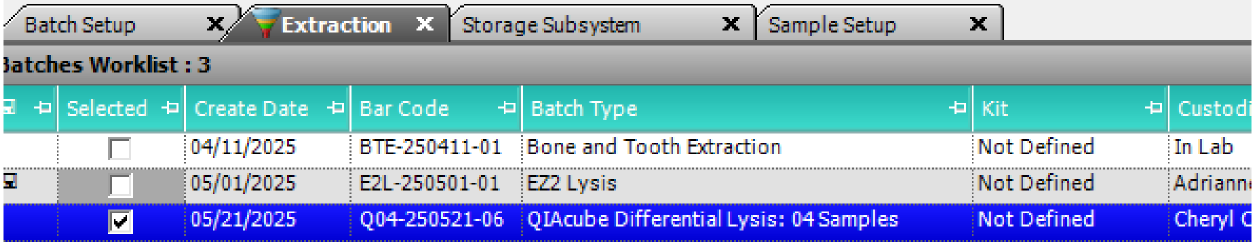
10. Select samples to be extracted from the available samples window on the left. Click **Allocate** to add selected samples to the extraction worksheet. You can also allocate samples by scanning the barcode on the sample tube.



11. To add the BIS to the worksheet, select the designated BIS well and scan the barcode label on the EP2 tube.
12. If more samples need to be added, click Save.
13. If no changes/additions are needed, click **Complete** in the **Batch Setup** window.
14. By completing the batch, a **Blank** sample will automatically be generated. If multiple RBs are used for the differential batch, each RB will have a unique barcode.
15. In the Lot field, you may distinguish RB1, RB2 with your initials/date – but it is not necessary as the barcodes are affixed to the tubes.
16. In the pop-up window, click Save.

II. Extraction (Lysis & QIAcube Processing)

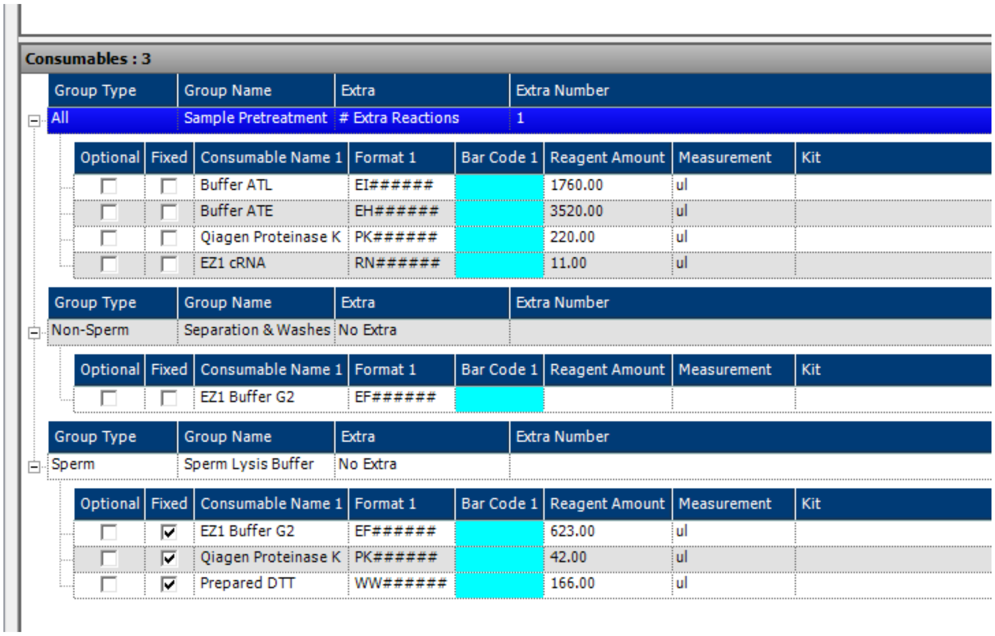
1. Open **Processing → DNA Processing → Extraction.**
2. Select/scan the batch to be processed from the worklist and click **Select Scenario (**on the right side**).**



Batches Worklist : 3

Selected	Create Date	Bar Code	Batch Type	Kit	Custodi
☐	04/11/2025	BTE-250411-01	Bone and Tooth Extraction	Not Defined	In Lab
☐	05/01/2025	E2L-250501-01	EZ2 Lysis	Not Defined	Adriann
☑	05/21/2025	Q04-250521-06	QIAcube Differential Lysis: 04 Samples	Not Defined	Cheryl C

3. Differential consumables are split into 3 groups.



Consumables : 3

Group Type	Group Name	Extra	Extra Number
All	Sample Pretreatment	# Extra Reactions	1

Optional	Fixed	Consumable Name 1	Format 1	Bar Code 1	Reagent Amount	Measurement	Kit
☐	☐	Buffer ATL	EI######		1760.00	ul	
☐	☐	Buffer ATE	EH######		3520.00	ul	
☐	☐	Qiagen Proteinase K	PK######		220.00	ul	
☐	☐	EZ1 cRNA	RN######		11.00	ul	

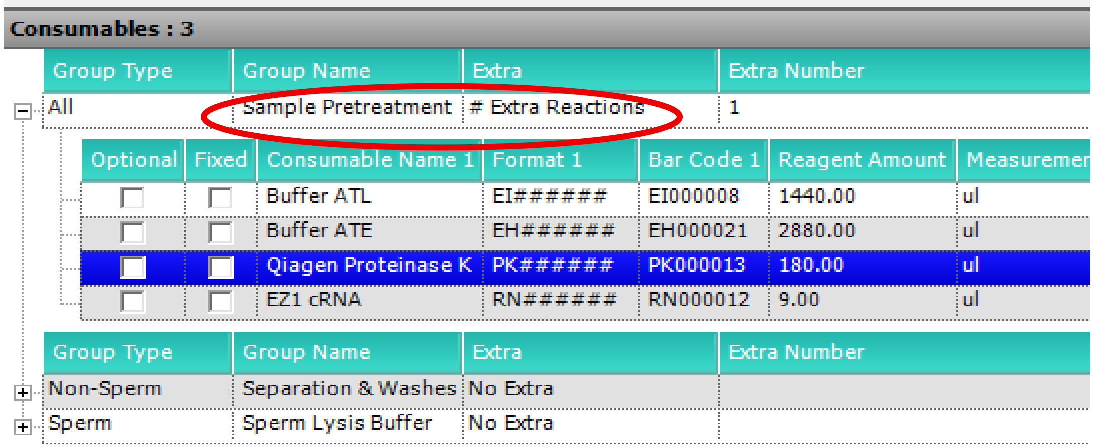
Group Type	Group Name	Extra	Extra Number
Non-Sperm	Separation & Washes	No Extra	

Optional	Fixed	Consumable Name 1	Format 1	Bar Code 1	Reagent Amount	Measurement	Kit
☐	☐	EZ1 Buffer G2	EF######				

Group Type	Group Name	Extra	Extra Number
Sperm	Sperm Lysis Buffer	No Extra	

Optional	Fixed	Consumable Name 1	Format 1	Bar Code 1	Reagent Amount	Measurement	Kit
☐	☑	EZ1 Buffer G2	EF######		623.00	ul	
☐	☑	Qiagen Proteinase K	PK######		42.00	ul	
☐	☑	Prepared DTT	WW######		166.00	ul	

4. Highlight the **Samples Pretreatment** header and scan the consumable barcodes in the **Sample Pretreatment** portion of the consumable window. This is for the incubation of your samples with the master mix.



Consumables : 3

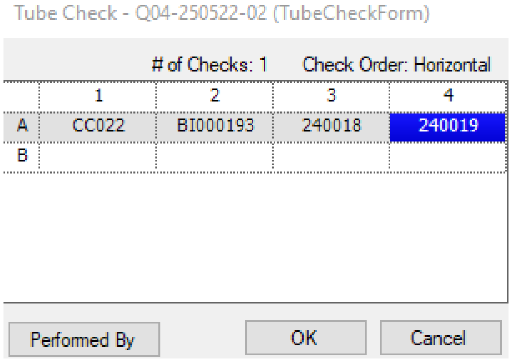
Group Type	Group Name	Extra	Extra Number
All	Sample Pretreatment	# Extra Reactions	1

Optional	Fixed	Consumable Name 1	Format 1	Bar Code 1	Reagent Amount	Measuremen
☐	☐	Buffer ATL	EI######	EI000008	1440.00	ul
☐	☐	Buffer ATE	EH######	EH000021	2880.00	ul
☐	☐	Qiagen Proteinase K	PK######	PK000013	180.00	ul
☐	☐	EZ1 cRNA	RN######	RN000012	9.00	ul

Group Type	Group Name	Extra	Extra Number
Non-Sperm	Separation & Washes	No Extra	
Sperm	Sperm Lysis Buffer	No Extra	

5. Scan the batch and click **Start Process** on the bottom right-hand corner.

6. Complete the tube check by scanning the tube labels and proceed with lysis. Click **OK**

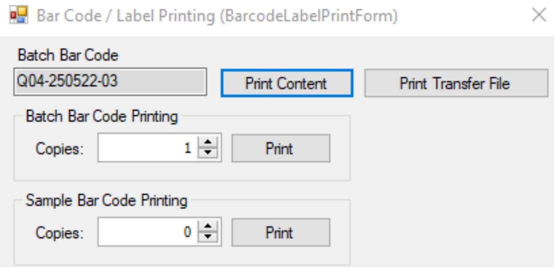


Tube Check - Q04-250522-02 (TubeCheckForm)

# of Checks: 1 Check Order: Horizontal

	1	2	3	4
A	CC022	BI000193	240018	240019
B				

7. A window with a .jpg picture will populate depicting the QIAcube Loading Scheme based on the sample quantity chosen.
8. Labels are needed for subsequent QIAcube loading and can be printed from the **Print Labels** button on top right. Print 2 copies. The A frac labels will go on the tubes in the shaker and your EZ2 elution tubes. An additional copy of the B frac labels can be printed from the “content” button. Highlight just the samples you want to print and press “print selected bar code” at the bottom. The B frac labels will go on the rotor adaptor, the tube inside the rotor adaptor and your elution tubes for your EZ2.



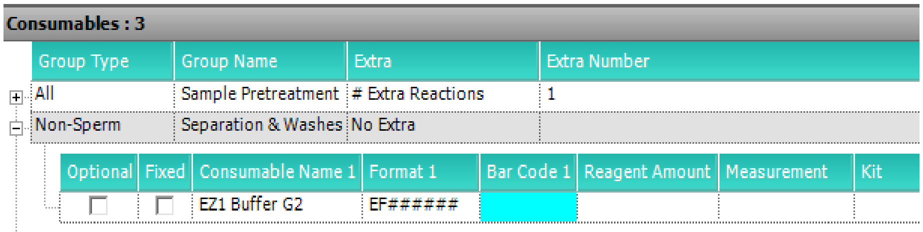
Bar Code / Label Printing (BarcodeLabelPrintForm)

Batch Bar Code: Q04-250522-03

Batch Bar Code Printing
Copies: 1

Sample Bar Code Printing
Copies: 0

9. Once lysis is completed, highlight the batch and click **Load Scenario** on the right-hand side.



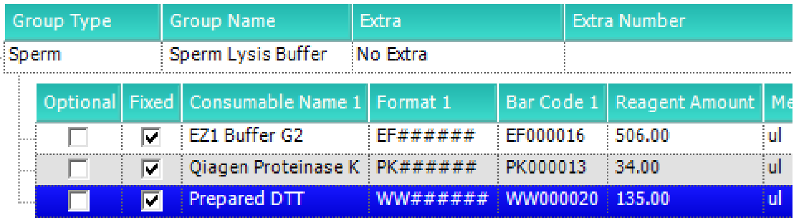
Consumables : 3

Group Type	Group Name	Extra	Extra Number
All	Sample Pretreatment	# Extra Reactions	1
Non-Sperm	Separation & Washes	No Extra	

Optional	Fixed	Consumable Name 1	Format 1	Bar Code 1	Reagent Amount	Measurement	Kit
☐	☐	EZ1 Buffer G2	EF######				

10. Highlight the **Separation and Washes** header and scan the Buffer G2 lot. Scan the QIAcube instrument barcode. Click **Continue** on right hand side.

11. Complete the tube check by scanning the tube labels and proceed with instrument setup.
12. Highlight the batch and click **Load Scenario**.
13. Highlight the **Sperm Lysis Buffer** header. Scan the appropriate consumables to prepare the Sperm Lysis Buffer.



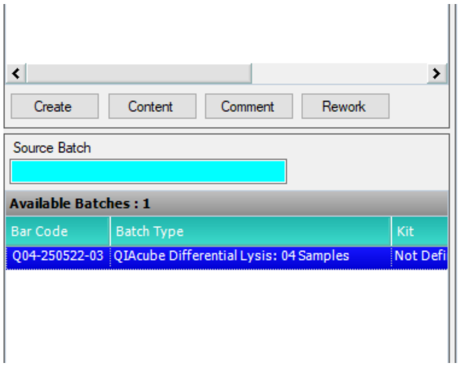
Group Type	Group Name	Extra	Extra Number
Sperm	Sperm Lysis Buffer	No Extra	

Optional	Fixed	Consumable Name 1	Format 1	Bar Code 1	Reagent Amount	Me
☐	☑	EZ1 Buffer G2	EF######	EF000016	506.00	ul
☐	☑	Qiagen Proteinase K	PK######	PK000013	34.00	ul
☐	☑	Prepared DTT	WW######	WW000020	135.00	ul

14. Click **Complete Process** (bottom right) and record the results using the Complete Batch Activity screen.
    - **Process Successful**: the batch advances to the next processing step.
    - **Process Aborted:** the batch remains on the Extraction Batches worklist. A **Batch Comment** is required with this option.
    - **Process Failed:** the batch is abandoned and all samples return to <u>**Batch Setup**</u>. A **Batch Comment** is required with this option. This option should only be used if the instrument crashes or something occurs that affects all samples.
15. Click **Save**.

III. <u>Isolation</u>

1. Create an Isolation (purification) worksheet: Open **Processing → DNA Processing → Isolation.** Click **Create Batch.**

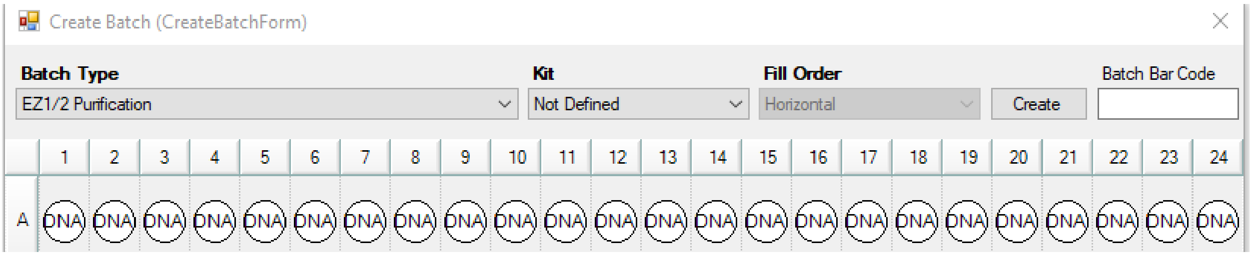


Available Batches : 1

Bar Code	Batch Type	Kit
Q04-250522-03	QIAcube Differential Lysis: 04 Samples	Not Defi

2. Click the **Create** button under the New Batches worklist.

3. Select a **Batch Type** and click **Create** to create the unique barcode associated with isolation.
    a. **EZ1/EZ2 Purification** = "A" fractions + any non-differentials lysed separately.

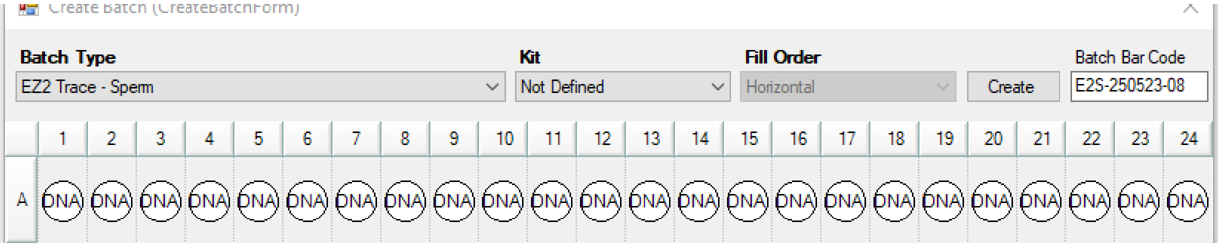


Create Batch (CreateBatchForm)

Batch Type: EZ1/2 Purification
Kit: Not Defined
Fill Order: Horizontal
Batch Bar Code:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA

    b. **EZ2 Trace – Sperm** = "B" fractions

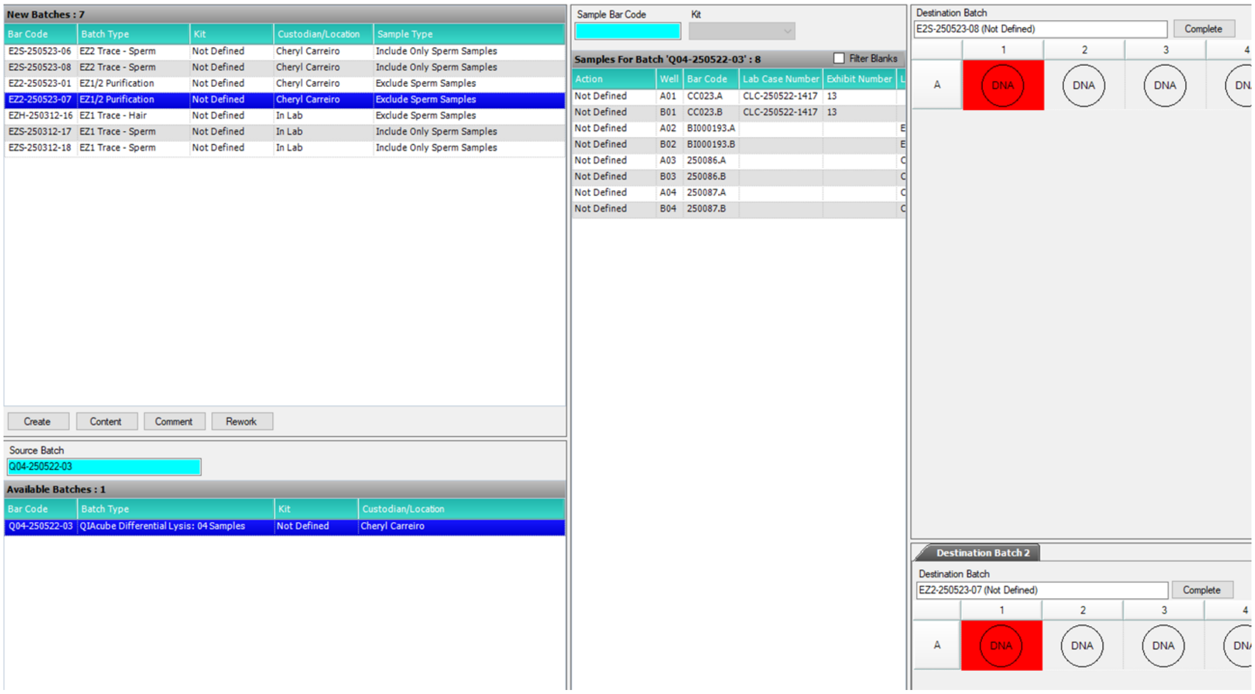


Create Batch (CreateBatchForm)

Batch Type: EZ2 Trace - Sperm
Kit: Not Defined
Fill Order: Horizontal
Batch Bar Code: E2S-250523-08

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA	DNA

4. Scan the newly created barcode(s) into each **Destination Batch** section (top and bottom on right hand side).
5. Scan the extraction barcode into the **Source Batch** section. If necessary, non-differential samples can be merged with the A fractions here.



New Batches : 7

Bar Code	Batch Type	Kit	Custodian/Location	Sample Type
E2S-250523-06	EZ2 Trace - Sperm	Not Defined	Cheryl Carreiro	Include Only Sperm Samples
E2S-250523-08	EZ2 Trace - Sperm	Not Defined	Cheryl Carreiro	Include Only Sperm Samples
EZ2-250523-01	EZ1/2 Purification	Not Defined	Cheryl Carreiro	Exclude Sperm Samples
EZ2-250523-07	EZ1/2 Purification	Not Defined	Cheryl Carreiro	Exclude Sperm Samples
EZH-250312-16	EZ1 Trace - Hair	Not Defined	In Lab	Exclude Sperm Samples
EZS-250312-17	EZ1 Trace - Sperm	Not Defined	In Lab	Include Only Sperm Samples
EZS-250312-18	EZ1 Trace - Sperm	Not Defined	In Lab	Include Only Sperm Samples

Source Batch: Q04-250522-03

Available Batches : 1

Bar Code	Batch Type	Kit	Custodian/Location
Q04-250522-03	QIAcube Differential Lysis: 04 Samples	Not Defined	Cheryl Carreiro

Samples For Batch 'Q04-250522-03' : 8 ☐ Filter Blanks

Action	Well	Bar Code	Lab Case Number	Exhibit Number	L
Not Defined	A01	CC023.A	CLC-250522-1417	13	
Not Defined	B01	CC023.B	CLC-250522-1417	13	
Not Defined	A02	BI000193.A			E
Not Defined	B02	BI000193.B			E
Not Defined	A03	250086.A			C
Not Defined	B03	250086.B			C
Not Defined	A04	250087.A			C
Not Defined	B04	250087.B			C

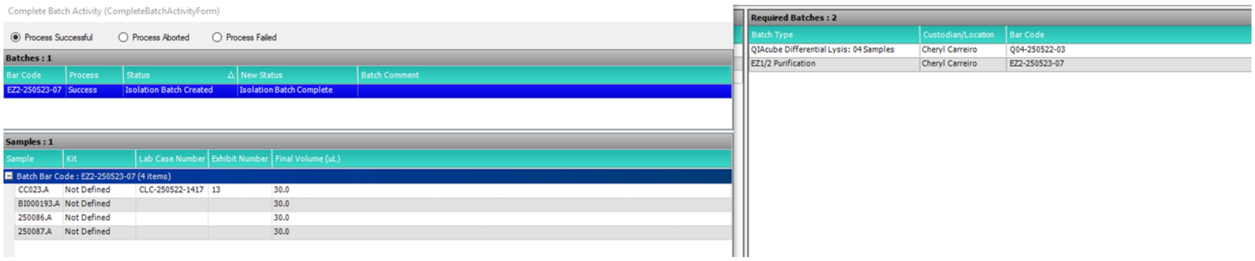
Destination Batch: E2S-250523-08 (Not Defined)

Destination Batch 2

Destination Batch: EZ2-250523-07 (Not Defined)

16. Click **Allocate** to add the samples to the isolation worksheet. “A” and “B” fractions will automatically separate on to the appropriate isolation batch. If sample(s) need to be moved, this can be done after the allocation is saved, followed by dragging the sample(s) as needed.
6. If more samples need to be added, click **Save**.
7. If finished, click **Complete** (next to Destination Batch Barcode) and then close.
8. In the Batches Worklist window, click **Select Scenario** for each Isolation worksheet.
    a. EZ1/EZ2 Purification = EZ1/2 Large Volume Protocol
    b. EZ2 Trace -Sperm
9. Scan the batch barcode and all necessary consumables and instrumentation.
10. Click **Start Process**.
11. Complete the tube check by scanning the lysis tube barcode labels.
12. Once the isolation is complete, click **Complete Process** and select the appropriate option. Adjust the elution volumes if needed.
    a. **Process Successful:** the batch advances to the next processing step.
    b. **Process Aborted**: the batch remains on the Isolation worklist. A **Batch Comment** is required with this option.

   c. **Process Failed:** the batch is assigned a **Status** of '**Abandoned**' and its samples are returned to <u>Isolation Batch Create</u> to be allocated to a new batch. A **Batch Comment** is required with this option.


Complete Batch Activity (CompleteBatchActivityForm)

● Process Successful ○ Process Aborted ○ Process Failed

Batches : 1

Bar Code	Process	Status	New Status	Batch Comment
EZ2-250523-07	Success	Isolation Batch Created	Isolation Batch Complete	

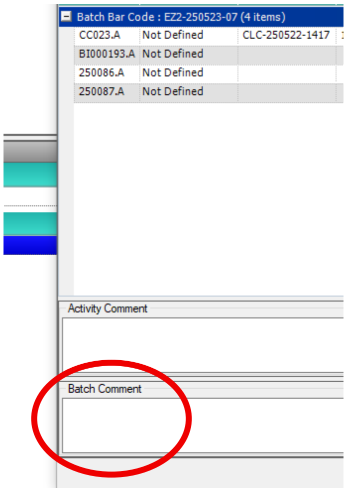
Samples : 1

Sample	Kit	Lab Case Number	Exhibit Number	Final Volume (uL)
Batch Bar Code : EZ2-250523-07 (4 items)				
CC023.A	Not Defined	CLC-250522-1417	13	30.0
BI000193.A	Not Defined			30.0
250086.A	Not Defined			30.0
250087.A	Not Defined			30.0

Required Batches : 2

Batch Type	Custodian/Location	Bar Code
QIAcube Differential Lysis: 04 Samples	Cheryl Carreiro	Q04-250522-03
EZ1/2 Purification	Cheryl Carreiro	EZ2-250523-07

13. If an elution volume discrepancy results in a manual volume check, this will be captured in the **Complete Process** window. Enter each sample's elution volume as measured off the instrument and select **Process Successful**. A note will be made (see red circle below) if water is added to an elution volume to bring up to RB volume, if necessary (the same water falcon tube is used throughout the entire process).

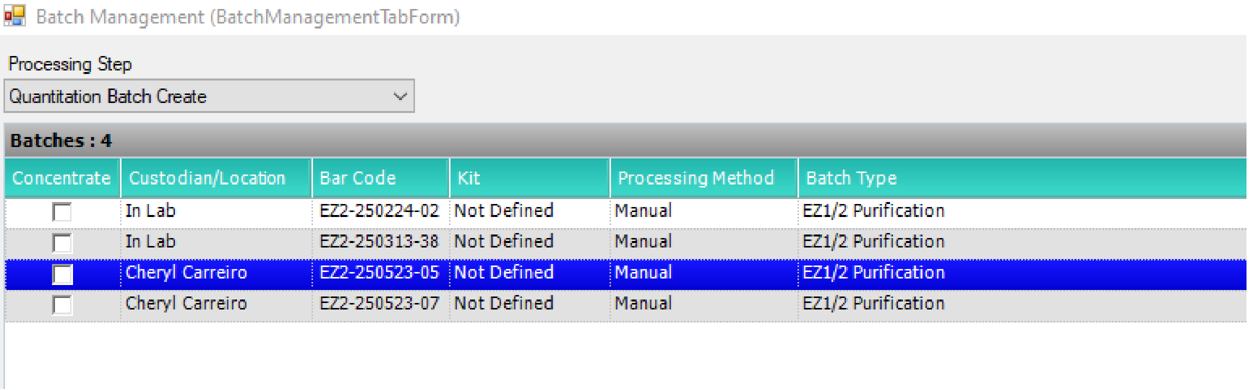


Batch Bar Code : EZ2-250523-07 (4 items)		
CC023.A	Not Defined	CLC-250522-1417
BI000193.A	Not Defined	
250086.A	Not Defined	
250087.A	Not Defined	

14. Proceed to the **Batch Management** module to document subsequent adjustments. Scan the extraction batch or locate using the **Quantitation Batch Create** option in the **Processing Step** dropdown
    a. If an elution volume is significantly increased (>40µL), select the sample(s) and control(s) and proceed to the **Concentrate** tab.
    b. If an elution volume is significantly reduced (0-20µL), select the sample(s) and control(s) and click on the **Rework** tab. Select **Isolation Batch Create** in the

Rework Entry Point dropdown and document the **Rework Reason**. Click **Save**. The sample(s)/control(s) can now be added to a new isolation batch.

c. If an eluate needs a volume adjustment, this can be done through the **Dilute** tab.


Batch Management (BatchManagementTabForm)

Processing Step: Quantitation Batch Create

Batches : 4

Concentrate	Custodian/Location	Bar Code	Kit	Processing Method	Batch Type
☐	In Lab	EZ2-250224-02	Not Defined	Manual	EZ1/2 Purification
☐	In Lab	EZ2-250313-38	Not Defined	Manual	EZ1/2 Purification
☐	Cheryl Carreiro	EZ2-250523-05	Not Defined	Manual	EZ1/2 Purification
☐	Cheryl Carreiro	EZ2-250523-07	Not Defined	Manual	EZ1/2 Purification