Protocol: Spatial Gene Expression Library Construction

Preparations:

- Bring out **Beckman Coulter SPRIselect reagents/AMPure XP beads** and bring to room temperature. Vortex immediately before use.
- Bring out the following reagents from the freezer and equilibrate to room temperature:
    - **Fragmentation Buffer** - Vortex, verify no precipitate, centrifuge briefly
    - **Adapter Oligos** - Vortex, centrifuge briefly
    - **Ligation Buffer** - Vortex, verify no precipitate, centrifuge briefly
    - **Dual Index Plate TT**
- Bring out the following reagents from the freezer and place on ice:
    - **Fragmentation enzyme** - Pipette mix, centrifuge briefly
    - **DNA Ligase** - Pipette mix, centrifuge briefly
    - **Amp Mix** - Vortex, centrifuge briefly
- Prepare fresh **80% ethanol**.
- Turn on the ThermoCube chiller and set to 0°C.

Prepare the room temperature (RT) reagent plate:

- In a square deepwell plate, add the following reagents:

Reagent	**Volume per well**	**Add to column:**	
		8 samples	*16 samples*
AMPure Beads	230 uL	Col 1	Col 1 & 2
80% ethanol	1500 uL	Col 2	Col 3 & 4
EB (Qiagen)	600 uL	Col 3	Col 5 & 6
Vapor-Lock	30 uL	Col 4	Col 7 & 8

Prepare the cold reagent plate:

- Prepare the Fragmentation Mix:

Fragmentation Mix	**1X (ul)**	**8X + 10% (ul)**	**16X + 10% (ul)**
Fragmentation Buffer	5	44	88
Fragmentation	10	88	176

Enzyme			
Total	15	132	264

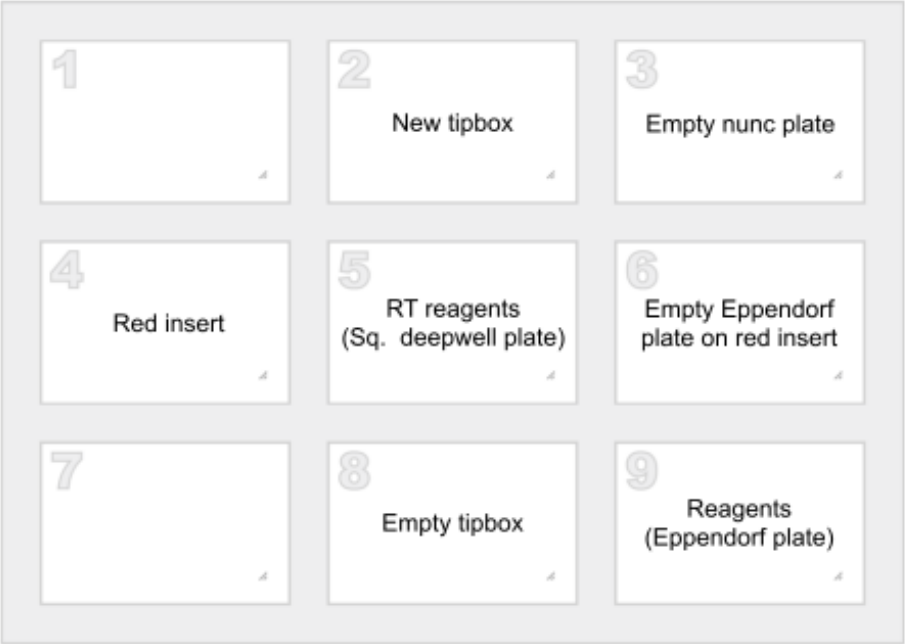
- Prepare the Ligation Mix:

Ligation Mix	**1X (ul)**	**8X + 10% (ul)**	**16X + 10% (ul)**
Ligation Buffer	20	176	352
DNA Ligase	10	88	176
Adapter Oligos	20	176	352
Total	50	440	880

- In an Eppendorf twin.tec PCR plate, add the following reagents:

Reagent	**Volume per well**	**Add to column:**	
		8 samples	*16 samples*
Sample	10 uL	Col 1	Col 1 & 2
Fragmentation Mix	15.5 uL	Col 2	Col 3 & 4
Ligation Mix	51 uL	Col 3	Col 5 & 6
Amp Mix	51 uL	Col 4	Col 7 & 8
Index	21 uL	Col 5	Col 9 & 10

Setup the Bravo as described:



Note: This protocol uses the red inserts. Make sure that the red inserts are in place on position 4 and 6 before attempting to start the protocol.

Starting and running the protocol:

1. Power on all devices and start VWorks via the shortcut on the desktop.
2. Make sure simulation mode is set to off in the VWorks toolbar.
3. Open the following form file:
   C:\VWorks Workspace\Protocol Files\development\FT\Visium\Visium.VWForm

   IMPORTANT: Make sure the full path matches the above, not just the file name.

4. Fill in the following:

   Columns: Select appropriate number of columns in the drop down-list: 1-2
   Protocol: Select “Library Prep”

   Note: It is possible to run the individual protocols as well, however they require different setups and different number of tips to be removed from the tip box. Due to this it is not recommended to run the individual protocols as this might cause the robot to crash.

5. Press the start button.
6. The protocol will pause once the Sample Index PCR reaction has been prepared. Take the plate from position 6, seal, place in a thermal cycler and run the following protocol:

Step	Temperature	Time
1	98°C	00:00:45
2	98°C	00:00:20
3	67°C	00:00:30
4	72°C	00:00:20
5	Go to step 2 for the desired number of cycles	
6	72°C	00:01:00
7	4°C	Hold

7. Once the incubation is finished, spin down the plate, remove the seal and place it back on position 6 and press continue.
8. The robot will proceed with the Post Sample Index PCR Double Sided Size Selection. The protocol will pause just before the elution. Replace the Eppendorf twin.tec PCR plate on position 4 with a clean Eppendorf twin.tec PCR plate and press continue.
9. The robot will proceed with the elution of the libraries. Once the protocol has finished the libraries will be in column 1/1-2 in the plate on position 4.

Explanation of the individual protocols of the library prep:
Fragmentation, End Repair and A-tailing (Visium_fragmentation.pro): *The robot will aspirate 25 uL of EB, immediately proceed to aspirate 15 ul of Fragmentation mix and dispense this in the sample column(s). It will then mix the reagents and transfer them to the plate on position 6 for incubation. After this the robot will add 15 ul Vapor-Lock on top of the reagents to avoid evaporation during the incubation. Incubation is performed on deck using position 4 and 6. The samples and reagents are in column 1 (1-2) in this step.*
Post Fragmentation Size selection (Visium_illumina_double-spri.pro): *The robot will perform a double sided size selection. The protocol uses column 1-2 (1-4) of the Nunc plate, the size selected samples are in column 2 (3-4) before elution. Elution is done in 50 ul to column 2 (3-4) of the reaction plate.*
Adaptor Ligation (Visium_ligation.pro): *The robot will aspirate 50 ul Ligation Mix and add to the samples in column 2 (3-4) in the reaction plate. The sample and reagents will be mixed and the incubation will be performed on deck.*
Post Ligation cleanup (Visium_illumina_spri.pro): *The robot will perform a single sided cleanup. The protocol uses column 3 (5-6) of the Nunc plate for the cleanup. Elution is done in 30 ul to column 3 (5-6) of the reaction plate.*
Sample Index PCR (Visium_amplification.pro): *The robot will aspirate 50 uL of Amp Mix, immediately proceed to aspirate 20 ul of Index and dispense this in the samples in column 3 (5-6) of the reaction plate. The protocol will then pause with a message instructing the user to*

place the plate in a thermal cycler, run the amplification program and place the plate back once the incubation is finished.

Post Amplification Size selection (Visium_illumina_double-spri.pro): *The robot will perform a double sided size selection. The protocol uses column 4-5 (7-10) of the Nunc plate, the size selected samples are in column 5 (9-10) before elution. Before elution the protocol will pause, asking the user to replace the Eppendorf twin.tec plate on position with a new Eppendorf twin.tec plate. Elution is done in 35 ul to column 1 (1-2) of the new plate.*

Protocol: cDNA Cleanup

Note: *This protocol needs to be tested before use!*

Preparations:

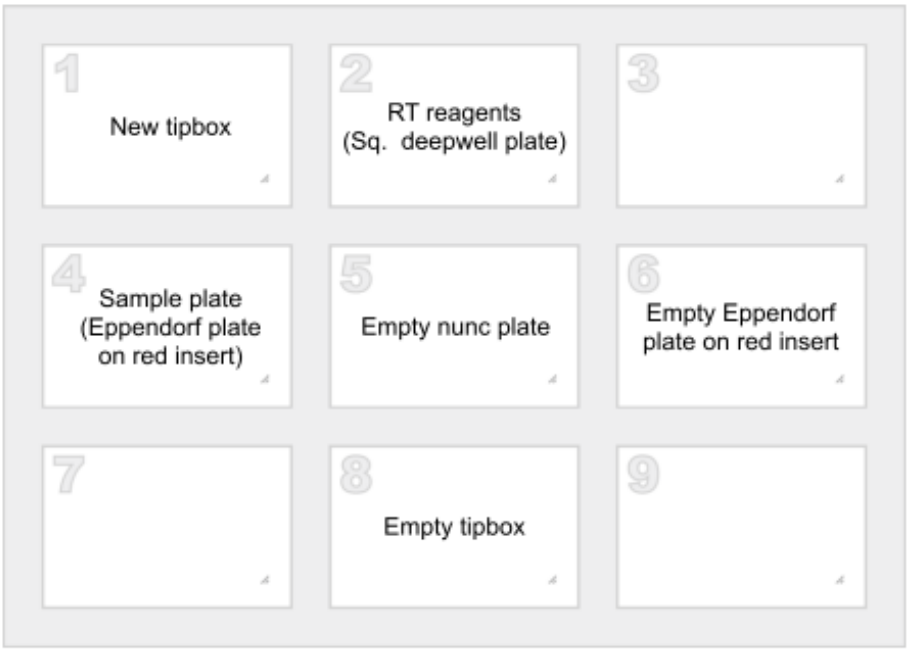
- Bring out **Beckman Coulter SPRIselect reagents/AMPure XP beads** and bring to room temperature. Vortex immediately before use.
- Prepare fresh **80% ethanol**.

Prepare the room temperature (RT) reagent plate:

- In a square deepwell plate, add the following reagents:

Reagent	**Volume per well**	**Add to column:**	
		8 samples	*16 samples*
AMPure Beads	80 uL	Col 1	Col 1 & 2
80% ethanol	600 uL	Col 2	Col 3 & 4
EB (Qiagen)	400 uL	Col 3	Col 5 & 6

Setup the Bravo as described:



Note: This protocol uses the red inserts. Make sure that the red inserts are in place on position 4 and 6 before attempting to start the protocol.

Starting and running the protocol:

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2. Make sure simulation mode is set to off in the VWorks toolbar.
3. Open the following form file:
   C:\VWorks Workspace\Protocol Files\development\FT\Visium\Visium.VWForm

   IMPORTANT: Make sure the full path matches the above, not just the file name.

4. Fill in the following:

   Columns: Select appropriate number of columns in the drop down-list: 1-2
   Protocol: Select “cDNA Cleanup”

   Note: It is possible to run the individual protocols as well, however they require different setups and different number of tips to be removed from the tip box. Due to this it is not recommended to run the individual protocols as this might cause the robot to crash.

5. Press the start button.
6. The protocol will proceed to perform the cDNA cleanup. The samples will be on position 4 once the protocol is finished.