firefly® technical note

Kinnex full-length RNA Kit Library Prep

This technical note provides supporting information for automating PacBio's Kinnex full-length RNA Kit (103-238-700 REV06) Library Prep on SPT Labtech firefly liquid handler. These protocols are available to download from the firefly community. Here, we outline protocol run times, parts required and provide details on the steps performed in each protocol.

firefly protocols

Protocol number	Protocol name	Estimated run time (minutes)
Protocol 1 of 10	2.0A cDNA Synthesis	10
Protocol 2 of 10	2.0B 1.3X SMRTbell bead cleanup	35
Protocol 3 of 10	3.0A cDNA amplification	5
Protocol 4 of 10	3.0B 0.9X cDNA SMRTbell bead cleanup	35
Protocol 5 of 10	4.0A Kinnex PCR	15
Protocol 6 of 10	4.0B Pooling & 1.05X SMRTbell bead cleanup	40
Protocol 7 of 10	5.0A Kinnex array formation	10
Protocol 8 of 10	5.0B 1X SMRTbell bead cleanup	35
Protocol 9 of 10	6.0A Nuclease treatment	5
Protocol 10 of 10	6.0B Final SMRTbell cleanup	35

Table 1. Protocols & estimated run times used in Kinnex full-length RNA kit Library Prep on firefly.

sptlabtech

Input variables

Protocol number	Protocol name	Variable ID	Default Value
Protocol 1 of 10	2.0A cDNA Synthesis	Number of Samples	24
Protocol 1 of 10	2.0A cDNA Synthesis	LDV Reservoir Dead Volume (µL)	55
Protocol 2 of 10	2.0B 1.3X SMRTbell bead cleanup	Sample Start Column	1
Protocol 2 of 10	2.0B 1.3X SMRTbell bead cleanup	Number of Samples	24
Protocol 2 of 10	2.0B 1.3X SMRTbell bead cleanup	Supernatant Removal Starting Column	4
Protocol 3 of 10	3.0A cDNA amplification	Number of Samples	24
Protocol 3 of 10	3.0A cDNA amplification	LDV Reservoir Deav Volume (µL)	55
Protocol 3 of 10	3.0A cDNA amplification	Primer Plate Starting Column	1
Protocol 4 of 10	3.0B 0.9X cDNA SMRTbell bead cleanup	Sample Starting Column	1
Protocol 4 of 10	3.0B 0.9X cDNA SMRTbell bead cleanup	Number of Samples	24
Protocol 4 of 10	3.0B 0.9X cDNA SMRTbell bead cleanup	Elution Volume (µL)	24
Protocol 4 of 10	3.0B 0.9X cDNA SMRTbell bead cleanup	cDNA Resuspension Volume (µL)	55
Protocol 5 of 10	4.0A Kinnex PCR	LDV Reservoir dv (µL)	55
Protocol 5 of 10	4.0A Kinnex PCR	Number of PCR's / Kinnex Master Mix	8
Protocol 5 of 10	4.0A Kinnex PCR	Kinnex Master Mix Volume (µL)	22.5
Protocol 5 of 10	4.0A Kinnex PCR	Kinnex Primer Starting Column	1
Protocol 5 of 10	4.0A Kinnex PCR	Kinnex Master Mix's	6
Protocol 5 of 10	4.0A Kinnex PCR	Total Number of Kinnex PCRs	48
Protocol 6 of 10	4.0B Pooling & 1.05X SMRTbell bead cleanup	Sample Starting Column	1
Protocol 6 of 10	4.0B Pooling & 1.05X SMRTbell bead cleanup	Number of Samples	16
Protocol 6 of10	4.0B Pooling & 1.05X SMRTbell bead cleanup	Supernatant Removal Starting Column	3
Protocol 6 of 10	4.0B Pooling & 1.05X SMRTbell bead cleanup	QC Aliquot Starting Column	3
Protocol 7 of 10	5.0A Kinnex array formation	Number of Samples	8
Protocol 7 of 10	5.0A Kinnex array formation	LDV Reservoir dv (µL)	55
Protocol 8 of 10	5.0B 1X SMRTbell bead cleanup	Starting column	1
Protocol 8 of 10	5.0B 1X SMRTbell bead cleanup	Number of samples	8
Protocol 8 of 10	5.0B 1X SMRTbell bead cleanup	Supernatant Removal Starting Column	2
Protocol 8 of 10	5.0B 1X SMRTbell bead cleanup	Elution Volume (µL)	41
Protocol 8 of 10	5.0B 1X SMRTbell bead cleanup	QC Aliquot Starting Column	2
Protocol 9 of 10	6.0A Nuclease treatment	Number of Samples	8
Protocol 9 of 10	6.0A Nuclease treatment	LDV Reservoir dv (µL)	55
Protocol 10 of 10	6.0B Final SMRTbell cleanup	Starting Column	1
Protocol 10 of 10	6.0B Final SMRTbell cleanup	Number of Samples	8
Protocol 10 of 10	6.0B Final SMRTbell cleanup	Supernatant Removal Starting Column	2
Protocol 10 of 10	6.0B Final SMRTbell cleanup	Elution Volume (µL)	26
Protocol 10 of 10	6.0B Final SMRTbell cleanup	QC Aliquot Starting Column	2

Table 2. Variables used in Kinnex full-length RNA kit Library Prep on firefly. Static variables, including those defined as algebraic expressions, are not shown.

Reagent volumes

The reagent volumes required to run Kinnex full-length RNA kit Library Prep on SPT Labtech firefly depend on the number of samples being processed. Default required minimum volumes for these reagents, based on the number of samples shown in the **Input variables** table, are shown below and in the EXECUTE section of the firefly software.

Protocol 1 of 10
2.0A cDNA Synthesis

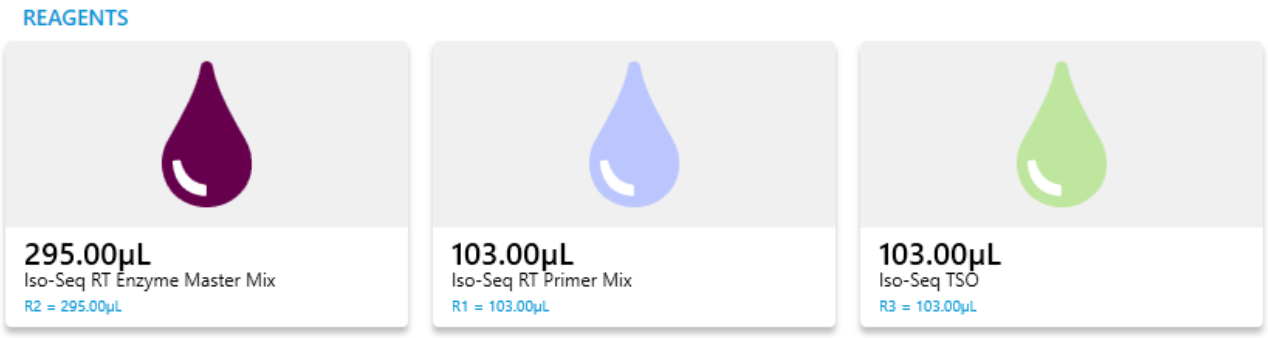

Figure 1. 2.0A cDNA Synthesis minimum required reagent volumes.

Protocol 2 of 10
2.0B 1.3X SMRTbell bead cleanup

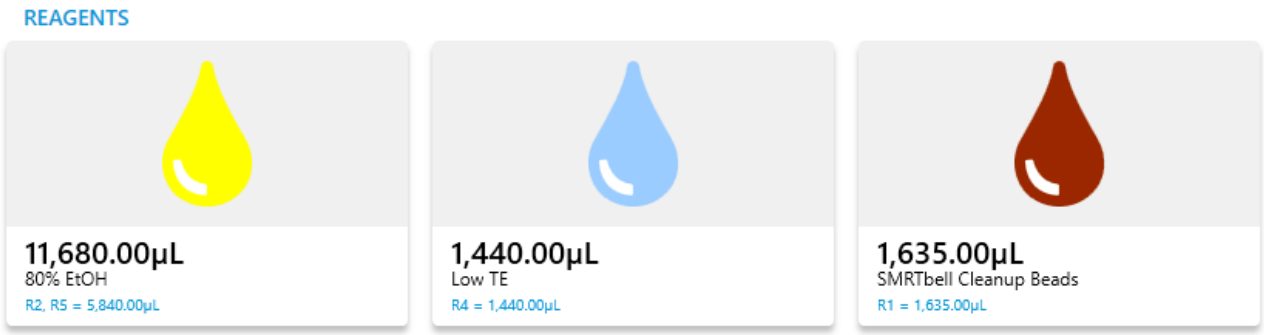

Figure 2. 2.0B 1.3X SMRTbell bead cleanup minimum required reagent.

Protocol 3 of 10
3.0A cDNA amplification

Figure 3. 3.0A cDNA amplification minimum required reagent volume.

Protocol 4 of 10
3.0B 0.9X cDNA SMRTbell bead cleanup

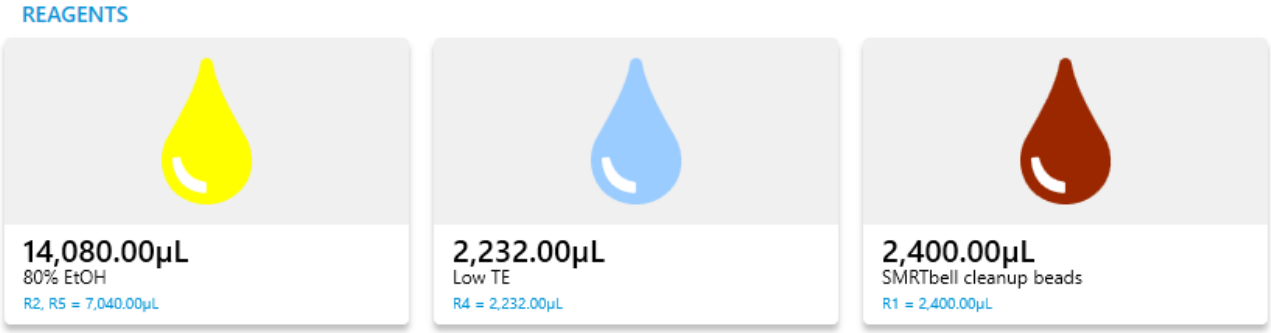

Figure 4. 3.0B 0.9X cDNA SMRTbell bead cleanup minimum required reagent volumes.

Protocol 5 of 10
4.0A Kinnex PCR

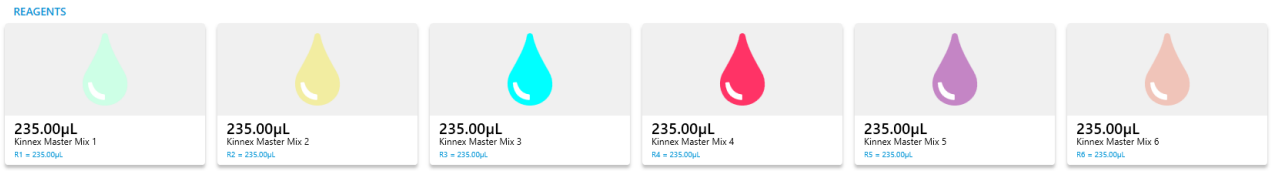

Figure 5. 4.0A Kinnex PCR minimum required reagent volumes.

Protocol 6 of 10
4.0B Pooling & 1.05X SMRTbell bead cleanup

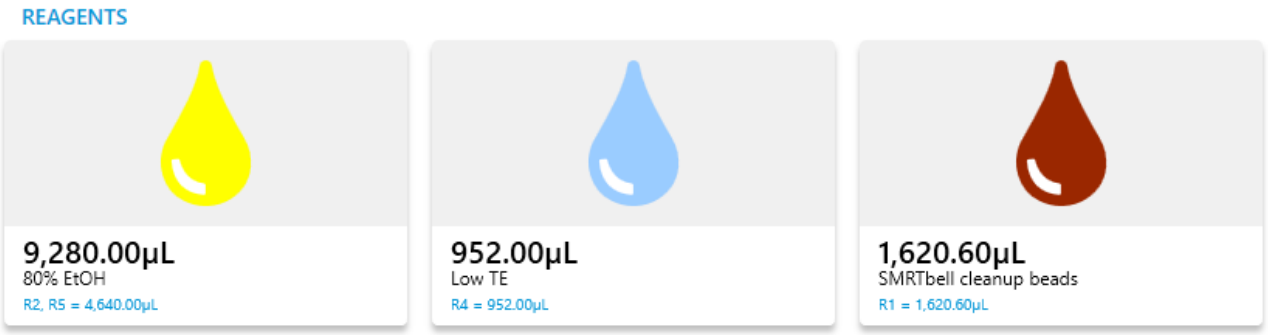

Figure 6. 4.0B Pooling & 1.05X SMRTbell bead cleanup minimum required reagent volumes.

Protocol 7 of 10
5.0A Kinnex array formation

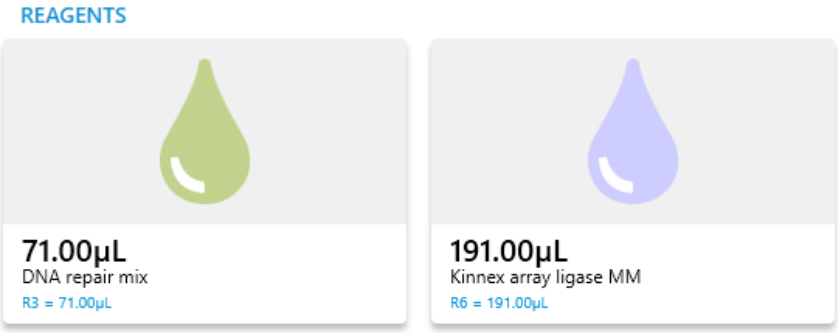

Figure 7. 5.0A Kinnex array formation minimum required reagent volumes.

Protocol 8 of 10
5.0B 1X SMRTbell bead cleanup

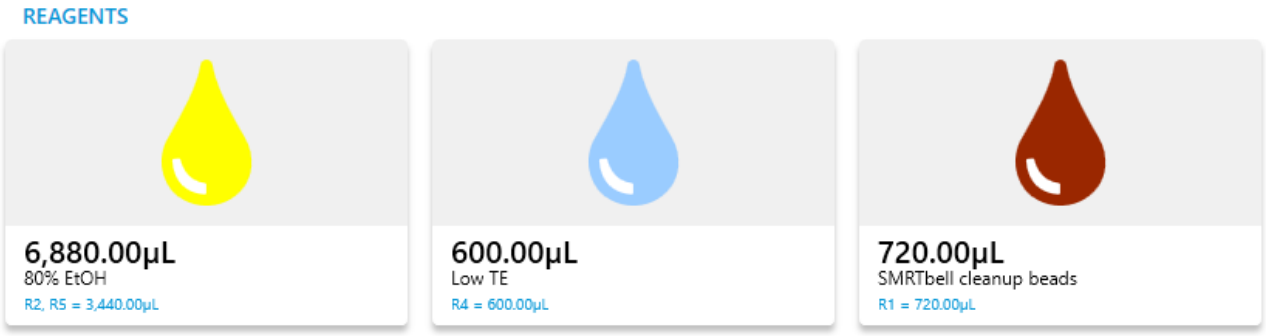

Figure 8. 5.0B 1X SMRTbell bead cleanup minimum required reagent volumes.

Protocol 9 of 10
6.0A Nuclease treatment

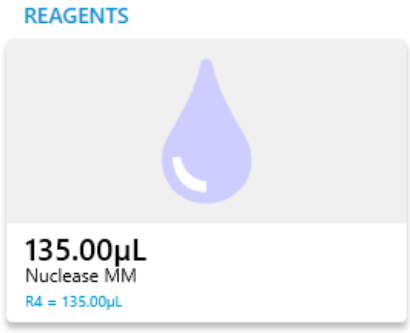

Figure 9. 6.0A Nuclease treatment minimum required reagent volumes.

Protocol 10 of 10
6.0B Final SMRTbell cleanup

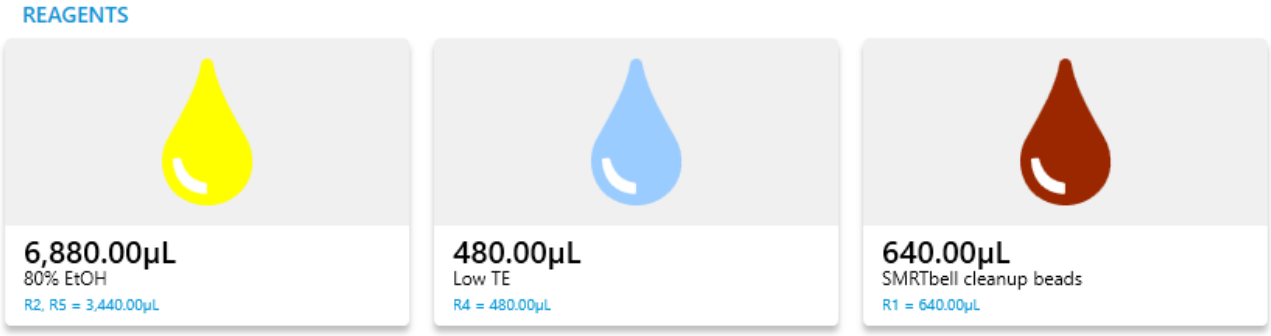

Figure 10. 6.0B Final SMRTbell cleanup minimum required reagent volumes.

Consumables

Supplier	Part Name	Part Number	Number Required	Note
SPT Labtech	40mm Upper Deck Riser	3276-01838	6	
SPT Labtech	dragonfly® discovery Sterile LDV Reservoirs	4150-07203	15	Reservoir types needed are dependent on the number of columns processed
SPT Labtech	dragonfly® discovery Sterile Reservoirs	4150-07204	23	
SPT Labtech	dragonfly® discovery Sterile Syringes	4150-07201	20	
SPT Labtech	dragonfly® discovery Sterile, Ultra Low Retention Syringes	4150-07209	18	Number required depends on the number of columns processed
SPT Labtech	firefly® Strip Tips, 100µl, with Filters, Sterile	125-008-FF-FS	25	
SPT Labtech	firefly® Strip Tips, 35µl, with Filters, Sterile	050-008-FF-FS	8	Waste plate
Alpaqua Engineering	Alpaqua Magnum FLX	A000400	1	
Thermo Fisher Scientific	Fisherbrand 1ml Deep Well	236600	4	
Bio-Rad	Hard Shell Plate (HSP)	HSP-9601	1	
Eppendorf	twin.tec PCR	30128648	4	

Table 3. Consumables & labware required for Kinnex full-length RNA kit Library Prep on firefly.

Protocol overview

This method was developed with an EZ-load 6 head genomics (v1.9.1 software) firefly using 24 samples, skirted twin.tec plates (30128648) and the Alpaqua Magnum FLX magnet. Use of alternative firefly configurations or labware may require further optimization.

This suite of protocols has been published with sample numbers assuming a 24 RNA sample input, with 4-sample multiplexing (3.3 Pooling Barcoded cDNA), resulting in 6 Kinnex PCR Pools & 48 Kinnex PCR samples (4.0 Kinnex PCR). Sample inputs may be adjusted using “Number of Samples” variables embedded in each protocol.

LDV reservoir dead volumes have been updated to 55 µL to account for limiting reagents where possible.

Protocol 1 of 10
2.0A cDNA Synthesis

This protocol performs sections 2.1 – 2.3 of PacBio’s Kinnex Library Prep Kit (103-238-700 REV06).

Prior to executing this protocol:

- **1.1** Measure RIN with Agilent 2100 Bioanalyzer Instrument using the RNA 6000 Nano kit
- **2.1.1** Thaw cDNA synthesis components as directed in protocol document

This protocol is compatible with 8 - 48 samples as written and has been published in the v1.8.6 firefly software.

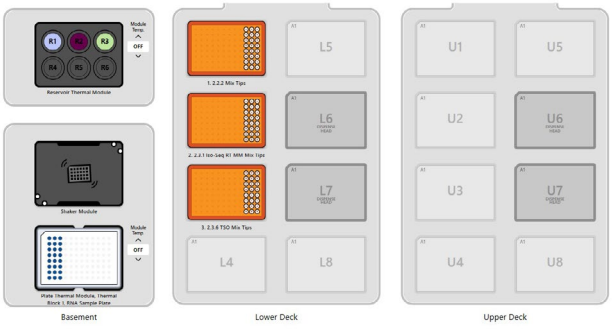

Figure 11. 2.0A cDNA Synthesis deck layout.

Protocol 2 of 10
2.0B 1.3X SMRTbell bead cleanup

This protocol performs section 2.4 of PacBio's Kinnex full-length RNA Kit (103-238-700 REV06).

Prior to executing this protocol:

- Equilibrate SMRTbell cleanups beads at room temperature for 30 minutes prior to use

This protocol is compatible with 8 - 48 samples as written and has been published in the v1.8.6 firefly software. To process > 48 samples, update 80% EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

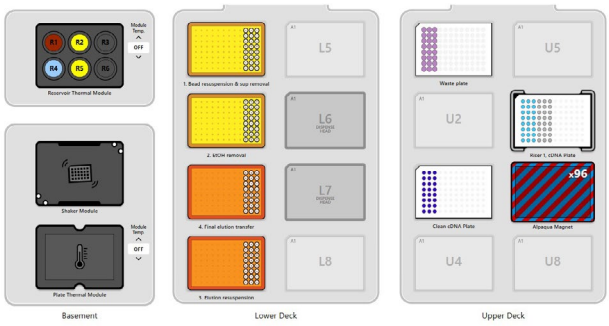

Figure 12. 2.0B 1.3X SMRTbell bead cleanup deck layout.

Protocol 3 of 10
3.0A cDNA amplification

This protocol performs section 3.1 of PacBio's Kinnex full-length RNA Kit (103-238-700 REV06).

Prior to executing this protocol:

- Aliquot Iso-Seq Primers to PCR Plate
- **3.1.1** Prepare reaction mix 3 (Iso-Seq cDNA PCR Master Mix)

This protocol is compatible with 8 - 96 samples as written and has been published in the v1.8.6 firefly software. To process > 48 samples, update 80% EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

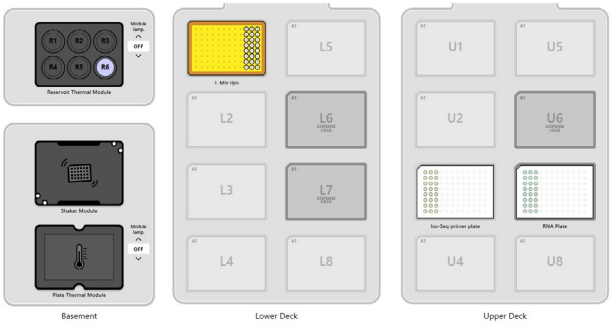

Figure 13. 3.0A cDNA amplification deck layout.

Protocol 4 of 10
3.0B 0.9X cDNA SMRTbell bead cleanup

This protocol performs section 3.2 of PacBio's Kinnex full-length RNA Kit (103-238-700 REV06).

Prior to executing this protocol:

- Equilibrate SMRTbell cleanups beads at room temperature for 30 minutes prior to use

This protocol is compatible with 8 - 96 samples as written and has been published in the v1.8.6 firefly software. To process > 48 samples, update 80% EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

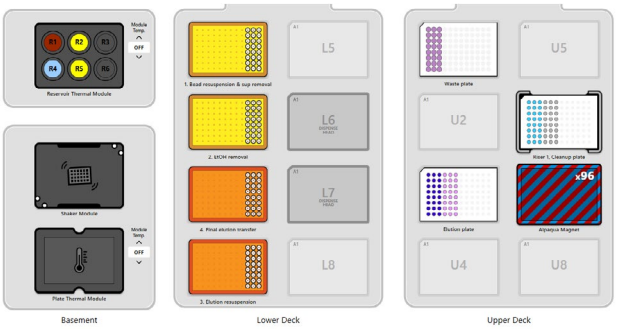

Figure 14. 3.0B 0.9X cDNA SMRTbell bead cleanup deck layout.

Protocol 5 of 10
4.0A Kinnex PCR

This protocol performs section 4.1 of PacBio's Kinnex full-length RNA Kit (103-238-700 REV06).

Prior to executing this protocol:

- **3.1.1** Aliquot Iso-Seq Primers to PCR Plate. Iso-Seq primer bc01–12 will be added to each sample individually and should not be added to the master mix
- **3.1.1** Prepare reaction mix 3 (Iso-Seq cDNA PCR Master Mix)

This protocol is compatible with 8 - 96 samples as written and has been published in the v1.8.6 firefly software. To process > 48 samples, update 80% EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

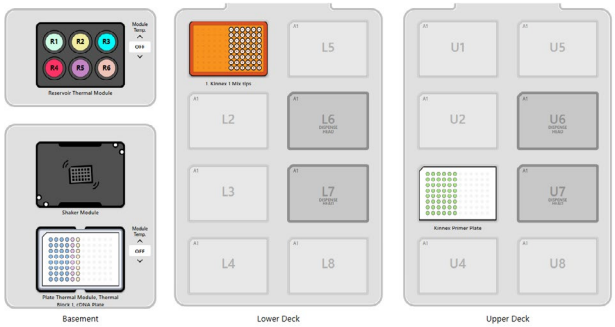

Figure 15. 4.0A Kinnex PCR deck layout.

Protocol 6 of 10
4.0B Pooling & 1.05X SMRTbell bead cleanup

This protocol performs section 4.2 of PacBio's Kinnex full length RNA Kit (103-238-700 REV06).

Prior to executing this protocol:

- **4.2.1** Add exactly 23 µL from each of the 8 PCR reactions into a 1.5 mL tube for a total volume of 184 µL. An equal volume of each PCR product is necessary for efficient array assembly. Equilibrate SMRTbell cleanups beads at room temperature for 30 minutes prior to use
- Protocol deviation: Split each pool into 2 pools equaling 92 µL for cleanup

This protocol is compatible with 8 - 96 samples as written and has been published in the v1.8.6 firefly software. To process > 48 samples, update 80% EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

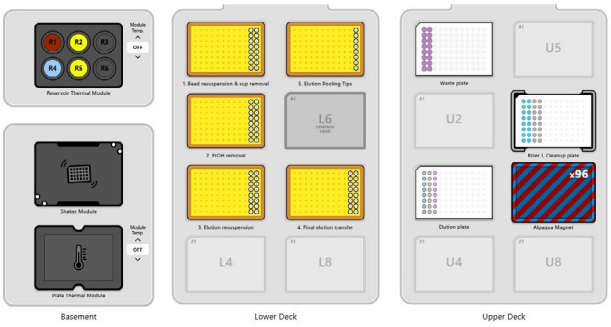

Figure 16. 4.0B Pooling & 1.05X SMRTbell bead cleanup deck layout.

Protocol 7 of 10
5.0A Kinnex array formation

This protocol performs section 5.1 of PacBio's Kinnex Library Prep Kit (103-238-700 REV06).

Prior to executing this protocol:

- **5.1.0** Place the DNA damage repair mix on ice at all times, and immediately return the DNA damage repair mix back to the freezer (-20°C) after use. Improper storage and handling of the DNA damage repair mix may result in poor library performance and should not be used for subsequent reactions
- **5.2.1** Prepare RM Master Mix(s)

This protocol is compatible with 8 - 96 samples as written and has been published in the v1.8.6 firefly software. To process > 48 samples, update 80% EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

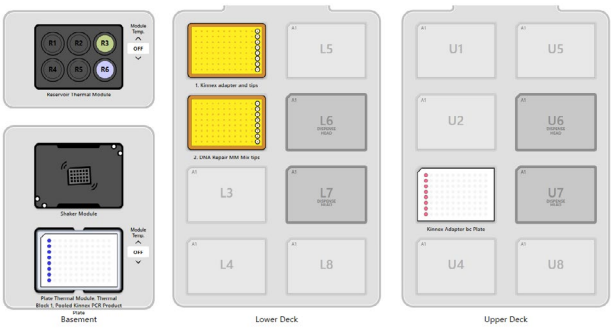

Figure 17. 5.0A Kinnex array formation deck layout.

Protocol 8 of 10
5.0B 1X SMRTbell bead cleanup

This protocol performs section 5.2 of PacBio's Kinnex full-length RNA Kit (103-238-700 REV06).

Prior to executing this protocol:

- Equilibrate SMRTbell cleanups beads at room temperature for 30 minutes prior to use

This protocol is compatible with 8 - 96 samples as written and has been published in the v1.8.6 firefly software. To process > 48 samples, update 80% EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

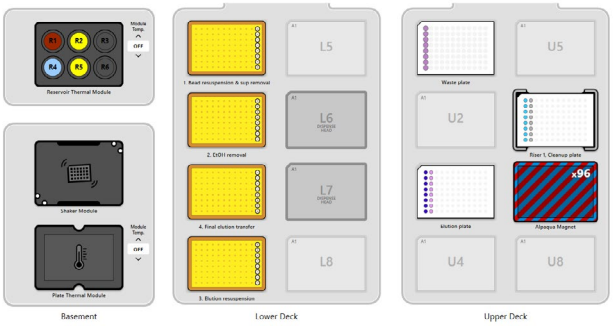

Figure 18. 5.0B 1X SMRTbell bead cleanup deck layout.

Protocol 9 of 10
6.0A Nuclease treatment

This protocol performs section 6.2 of PacBio's Kinnex full-length RNA Kit (103-238-700 REV06).

Prior to executing this protocol:

- **6.1.1** Prepare Nuclease Master Mix

This protocol is compatible with 8 - 96 samples as written and has been published in the v1.8.6 firefly software. To process > 48 samples, update 80% EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

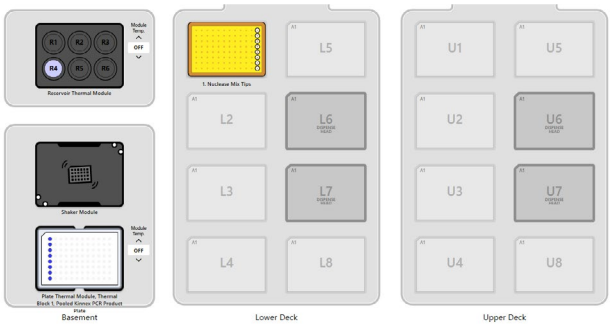

Figure 19. 6.0A Nuclease treatment deck layout.

Protocol 10 of 10
6.0B Final SMRTbell cleanup

This protocol performs section 6.2 of PacBio's Kinnex full-length RNA Kit (103-238-700 REV06).

Prior to executing this protocol:

- **6.1.1** Prepare Nuclease Master Mix

This protocol is compatible with 8 - 96 samples as written and has been published in the v1.8.6 firefly software. To process > 48 samples, update 80% EtOH reservoir asset definition and EtOH aspiration steps to use four syringes.

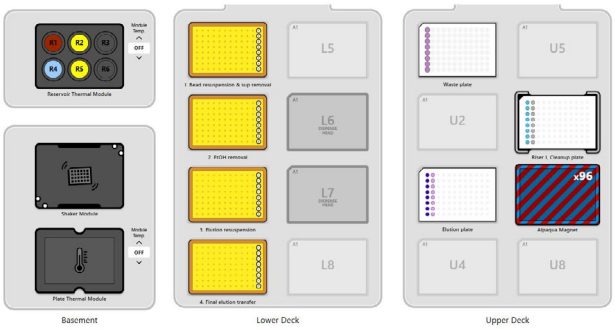

Figure 20. 6.0B Final SMRTbell cleanup deck layout.