

FAQ for Asymmetric Template Prep Release

Asymmetric Template Prep Kit (TPK) Release FAQs

Q. What is in the SMRTbell™ Asymmetric TPK 1.0?

R. The SMRTbell™ Asymmetric TPK 1.0 is a bundle of both the SMRTbell Template Prep kit 1.0 and the SMRTbell Asymmetric Auxiliary TPK. This kit contains template preparation reagents required to perform asymmetric SMRTbell library preparations. Reagent quantities support 10 library preparations.

DESCRIPTION	PART NUMBER	PART TYPE
SMRTbell™ Asymmetric TPK 1.0	101-113-000	Bundle
➤ <i>SMRTbell™ Template Prep Kit 1.0</i>	<i>100-259-100</i>	<i>Kit</i>
➤ <i>SMRTbell™ Asymmetric Auxiliary TPK</i>	<i>101-080-400</i>	<i>Kit</i>

Q. What is in the SMRTbell™ Asymmetric Auxiliary TPK?

R. The SMRTbell™ Asymmetric Auxiliary TPK is a template preparation kit containing asymmetric SMRTbell adapters and the corresponding sequencing and capture primers. This kit contains auxiliary reagent supplies to perform asymmetric SMRTbell library preparations for amplicons on the Sequel™ System. It is intended to be used in conjunction with the SMRTbell Template Prep Kit 1.0. Reagent quantities support 10 library preparations.

DESCRIPTION	PART NUMBER	PART TYPE
SMRTbell Asymmetric Auxiliary TPK	101-080-400	Kit
➤ <i>Asymmetric Adapter B</i>		<i>Tube</i>
➤ <i>Asymmetric Adapter A</i>		<i>Tube</i>
➤ <i>Asymmetric Seq Primer A</i>		<i>Tube</i>
➤ <i>Asymmetric Capture Primer B</i>		<i>Tube</i>

Q. What reagents do I need outside of the SMRTbell™ Asymmetric TPK 1.0 to execute the workflow?

R. Amplification:

- KAPA HiFi HotStart Uracil+ PCR Kit from Kapa Biosystems (Kit Codes KK2801, KK2802)
- Primers for 1st and 2nd PCR:
 1. Target Specific Primers tailed with universal sequence
 2. Forward and Reverse Barcoded Universal Primers tailed with 4 bp containing dUTP

Library Construction:

- NEB USER™ Enzyme (Catalog #M5505)
- PacBio AMPure® PB beads

Q. What improvements can I expect?

R. At higher loading concentrations, customers can expect an increase in CCS reads compared to the symmetric template prep workflow. The latest enhancements in chemistry improve reliability and repeatability through enabling loading over a wider range of concentrations. Specifically, the new chemistry is more tolerant to overloading.

Q. When can I order and when will the shipments start?

New Products available for order on May 2, 2017:

DESCRIPTION	PART NUMBER	PART TYPE
SMRTbell™ Asymmetric TPK 1.0	101-113-000	Bundle
SMRTbell Asymmetric Auxiliary TPK	101-080-400	Kit

Q. What types of samples are appropriate for this workflow?

R. This workflow is best suited for sequencing amplicons in the 2 kb - 5 kb size range.

Q. Is this workflow compatible with both the PacBio RS II instrument and the Sequel instrument?

R. Asymmetric Template Prep workflow has only been validated for the Sequel instrument.

Q. Does the software need updating to call these new adapters?

R. No, a software upgrade is not required to run the asymmetric template prep workflow.

Q. How can I get started?

R. Review the [Overview Webinar](#) and determine if this workflow meets your sequencing needs. If so, please contact your Field Application Scientist Representative for additional details.

Q. How do I know if Asymmetric Template Prep is right for me?

R. The primary benefit is the chemistry is more tolerant to overloading. This may help in high throughput product labs or when achieving equimolar pooling is a challenge.

Q. What is the recommendation between for use of asymmetric template prep at each of the amplicon size bins?
R.

		Amplicons			
SIZE RANGE		2-5 kb*		>5 kb**	
TEMPLATE PREP KIT		Asymmetric	Symmetric	Asymmetric	Symmetric
LOADING CONDITIONS	Pre-Extension	✓		✓	
	Diffusion	✓		✓	
	MagBead				
BENEFIT OVER SYMMETRIC	Tolerance to Overloading	✓		✓	
	Increased CCS Performance (Readlength)	✓		✓	

*	Recommended
**	Guidance
	Do not see benefit with this workflow

Q. What are the input recommendations based on size range?

R.

Insert size range	1 – 2 kb	2 - 3 kb	3 - 4 kb	4 - 5 kb	>5 kb
Input DNA amount	1-2 µg	1-2 µg	2-3 µg	2-4 µg	3-5 µg