Here is the complete protocol as originally received from the Schacherer Lab:

Here is the pSTHyg plasmid map:

Here is what the library prep should result in for sequencing (with primers, etc annotated):

Below I have reformatted it for readability, made notes, and changed some portions.
A - Preparation and Transformation

1. Streak out your strains from frozen stocks.
2. Verify that your strains are not hygromycin resistant by concurrently streaking to YPD + hygro, or by replica plating to hygro.
3. Verify that your strains are haploid by checking the ploidy by PI stain and flow cytometry.
   a. Strains must be haploid for this procedure (unless specifically designed otherwise), or else haplosufficiency will make your results useless.
4. Do a **High Efficiency Transformation Protocol** using 1\(\mu\)L of pSTHyg at 100ng/\(\mu\)L. Plate onto YPD + hygro to recover transformants.
   a. Note: As of August 2019, I have not tested the efficiency of the transformation with our transformation protocol. Transformation efficiency using EZ-Yeast transformation kit varies by strain.

B - Transposon induction

Note: for this part, I will be saying YPD, YPD + hygro, YPGal, and YPGal + hygro. You can use other media, however, it is important to note which steps there is Hygromycin B in the media to maintain or lose the plasmid, and to select for cells with the transposon integrated into the genome, and which steps use media with galactose, to drive the transposase.

1. Select a clone from your transformation, and inoculate it into 30 mL YPD + hygro. Incubate 24 h at 30°C under agitation.
   a. If you want, you can use part of this culture to check aspects of your transformants, for example, if they have maintained levels of fluorescence, or ploidy.
2. After the culture has reached saturation OD = 5, ~24 hours, back dilute at an OD of 0.05 (500 \(\mu\)L in 50mL of YPGal + Hygromycin B. Incubate 24h at 30°C under agitation. Dilute 4 times more in YPGalactose + Hygromycin B (one dilution every 24h).
   a. You could use potentially use the coulter counter to do your dilutions instead of OD.
   b. If some of your cultures have not reached saturation after 24 hours, transfer the same amount of cells by spinning down cells in whatever volume needed, and resuspending in 500 \(\mu\)L, then transferring.
   c. You could estimate the fraction of cells with a transposon integrated into the genome at each dilution by a outgrowth in YPD to lose the plasmid, then plating to YPD + hygro.
3. After the last 24h of galactose induction, dilute at an OD of 0.5 in 100mL of YPD to lose the plasmid. Centrifuge 5 minutes at 4000rpm the needed volume and remove the supernatant. Incubate 24h at 30°C under agitation. Dilute 1 time more in YPD after 24h (no need to remove the supernatant).
4. Dilute at an OD of 0.5 in 100mL of YPD+hygromycin B to only keep cells with the transposon in the genome. Incubate 24h at 30°C under agitation.
5. The next morning, dilute 100\(\mu\)L of each culture in 900\(\mu\)L of YPD+hygromycin B in 2mL tubes and incubate for 2h at 30°C under agitation and then prepare cells for ploidy control.
6. Centrifuge 100mL of each culture (2*50 mL Falcon) 5 minutes at 4000rpm, remove the supernatant and wash the cells with 1mL of sterile water. Cell pellets are split in aliquots of 500\(\mu\)L in 2mL tubes, centrifuge 2 minutes at 8000rpm and freeze the cells at -20°C.

D - DNA extraction

1. Rinse one frozen pellet for each isolate with 1mL of water, centrifuge (2 min / 10 000 rpm) and remove supernatant.
2. Extract genomic DNA from a cell pellet using Hoffman Winston DNA Prep (or other prep).
3. Check DNA extractions by gel electrophoresis, nanodrop, qubit

C - Library preparation

1. Tagmentation using Nextera XT
   a. 5 ng in 10 \(\mu\)L total
   b. 20 \(\mu\)L TD Buffer
   c. Add 10 \(\mu\)L ATM Buffer and mix
   d. Tagment on thermocycler
      i. 55C 10 min
      ii. 10C hold
   e. Add 10 \(\mu\)L NT Buffer and mix
   f. Incubate at RT 5 min
2. PCR1 Hermes enrichment
   a. Add 30 \(\mu\)L NPM Buffer
   b. Add 5 \(\mu\)L 5 um DG2152 Nextera_hermes_enrichment
   c. Add 5 \(\mu\)L 5 um DG2153 Nextera_i7_enrichment
   d. Add 10 \(\mu\)L H2O
   e. Program (100uL)
      i. 72C 3 min
      ii. 95C 30 sec
      iii. 3 cycles
      1. 95C 10sec; 55C 30sec; 72C 30sec
      iv. 72C 5min
     v. 10C hold
3. Ampure cleanup (remove primers - everything <200bp)
   a. Add 80 uL (0.8X) SPRIselect
   b. Incubate for 5 min
   c. Transfer to magnet, wait until clear, remove supernatant
   d. Wash 2X with 150 uL 80% EtOH
   e. Elute with 20.5 uL H2O and transfer 20 uL to new PCR tubes
4. PCR2 Indexing and library amplification (60 uL)
   a. Add to each tube (making sure to keep track of which tube has which i7 index)
      i. 5 uL index 1 (i7) adaptor
      ii. 5 uL 5 uM DG1954 (TruSeq, no index)
      iii. 30 uL 2x KAPA master mix (KK2611/KK2612)
   b. Program (60uL)
      i. 72C 3 min
      ii. 95C 30 sec
      iii. 9 cycles
      iv. 95C 10 sec; 55C 30 sec; 72C 30 sec
      v. 72C 5 min
   v. 10C hold
5. Double sided size selection (keep things between 200 and ~450 bp)
   a. Add 33 uL SPRIselect
   b. Incubate 5 minutes
   c. Transfer to magnet, wait until clear, transfer 93 uL supernatant to a new tube
   d. Add 15 uL (0.8X) SPRIselect to transferred supernatant
   e. Incubate for 5 min
   f. Transfer to magnet, wait until clear, remove supernatant
   g. Wash 2X with 150 uL 80% EtOH
   h. Elute with 25.5 uL 10mM Tris-HCl (pH 8) and transfer 25 uL to clean tubes
6. Library Quant
   a. Run D1000 tapestation and KAPA illumina library quant qPCR
   b. Pool