**The list of protocol files in the runset file (ssDNAprep\_96samples\_lib\_prep\_and\_dilutions.rst)**

1. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_A1\_transfer\_master\_mixes.pro**
   1. Protocol starts with sorting the tips, following with aliquoting the master mix from 5ml tubes (24-96 ThermoScientific 5ml tube) to 96 format nunc plate.
   2. After aliquoting finish, left-over clean tips will be transfer to the tip trash box, waiting time is kind of long.
2. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_A2\_transfer\_extracts.pro**
   1. Protocol starts with bringing spike in reservoir and extract fluidX tube rack.
   2. First aspirates 1μl of spike in then goes to extract tubes, aspirates 30μl of extracts **with dynamic tip retraction of 0.06.**
   3. Spike-in with extract are transferred into fresh sample plate.
3. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_B\_dephosphorylation.pro**
   1. Protocol starts with aliquoting dephosphorate mix to temporary plate 1, transfer 17μl dephosphorate mix into each well of the temp plate 1.
   2. 96 Head pipette will aspirate 15.6μl from temporary plate 1 added into 31ul into sample plate with contains 31ul extracts including spikein.
   3. Sample plate will be incubated at 37°C in the position 4 for 5minutes.
   4. Then, sample plate will be transferred to position 7 and incubates at room temperature for 20 minutes (incubation will be carry out in the following protocol.
4. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_C\_ligation\_preparation.pro**
   1. Protocol starts with aliquoting 37μl ligation mix I to temporary plate 2.
   2. Position 4 will be cooling down to 4°C and position 6 will be heating up to 95°C.
   3. Once position 6 reaches to 95°C (actual temperature is 91°C, when the plate moved to pos.6 after 2 min the temp is 90.2°C) sample plate will be incubated at 95°C for 2 min, then, immediately moved to position 4 at 4°C for snap cooling .
   4. Sample plate will be incubates at 4°C for 2 min, then moves to pos.5
5. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_D\_ligation.pro**
   1. Protocol starts with adding 5μl of adapters/splinters column-wise one by one to sample plate.
   2. 34.4 μl of ligation mix I from temporary plate 2 gently added to samples with adapter mix. And mixed gently.
   3. The sample plate is incubated at 37°C degree for 50 minutes
   4. Then, incubate at room temperature for 15 minutes
   5. Pos.4 will be cool down to 4°C degree
6. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_E\_bead-binding.pro**
   1. Sample plate moved to incubate at 95°C for 2min (actual temperature is 88°C, after 2min is 90.2°C)
   2. Then moved to 4°C degree at position4
   3. Transfer 67μl 0.1xBWT+SDS to temporary plate 3
   4. Add 5μl of washed MyOne beads to the temporary plate 3
   5. Move sample plate to pos 1
   6. Prepare pos 4 to 37°C degree and pos 6 to 45°C degree
   7. Diluted 70μl beads added to sample, and incubate it with mixing gently for 30 min
   8. Incubate for 10 min on the magnetic rack.
7. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_F\_post\_binding\_washes.pro**
   1. Protocol starts with removing supernatants from sample plate after bead binding
   2. Wash the beads with 100μl Stringency buffer, and beads will be resuspended by mixing gently.
   3. Incubate the sample plate at 45°C degree for 5 min
   4. Move the plate to magnetic rack to collect beads for 2min
   5. Remove the supernatant and add 150μl of 0.1x BWT
8. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_G\_fill-in\_preparation.pro**
   1. Protocol starts with dispense 42μl fill-in mix into temp plate4
   2. Remove the supernatants (0.1x BWT) from sample plate.
   3. Add 40μl fill-in mix to sample plate from temp plate 4
   4. Mixing it by pipetting up and down.
   5. Incubate sample plate at 65°C degree for 1min, then move the plate to position 4 at 4°C degree.
9. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_H\_fill-in.pro**
   1. Protocol starts with dispensing 11μl of klenow mix into temporary plate 5
   2. Add 10μl of klenow mix from temporary plate 5 to sample plate mixing by pipetting and incubate it for 5 min at room temperatur.
   3. Move the samples plate to pos6 incubate it for 25 min at 35°C degree mixing gently every 5 min.
   4. Move the plate to magnetic rack, wait 2 min to collect the beads.
10. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_I\_post-fill-in-washes.pro**
    1. Protocol starts with removing supernatants (klenow mix) from the sample plate.
    2. Add 100μl of 0.1xBWT+SDS, resuspense the beads by pipetting up and down.
    3. Move plate to magnetic rack to collect the beads, wait 2 min before removing supernatant.
    4. Remove the supernatants and add 100ul stringency buffer. Resuspense the beads by gently pipetting up and dow.
    5. Move the plate to position 4 and incubate it at 45°C degree for 5 min before move the plate to magnetic rack.
    6. Move the plate to magnetic rack, wait 2 min to collect the beads.
    7. Remove the supernatant, add 150μl 0.1xBWT.
11. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_J\_2nd-ligation.pro**
    1. Protocol starts with dispensing 42μl ligation mix II to temporary plate 6
    2. After supernatants removed (0.1xBWT), sample plate moved to position 5 and 40μl ligation mix II added to sample plate from temporary plate 6.
    3. Mixed gently by pipetting up and down
    4. Incubate for one hour and mixing by pipetting every 5 min.
12. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_K\_post-ligation-washes.pro**
    1. Protocol starts with removing supernatants (ligation mix II) from the sample plate.
    2. Add 100μl of 0.1xBWT+SDS, resuspense the beads by pipetting up and down.
    3. Move plate to magnetic rack to collect the beads, wait 2 min before removing supernatant.
    4. Remove the supernatants and add 100ul stringency buffer. Resuspense the beads by gently pipetting up and down.
    5. Move the plate to position 4 and incubate it at 45°C degree for 5 min before move the plate to magnetic rack.
    6. Move the plate to magnetic rack, wait 2 min to collect the beads.
    7. Remove the supernatant, add 150μl 0.1xBWT.
13. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_L\_elution.pro**
    1. Protocol starts with removing the supernatants (0.1x BWT) from sample plate. Then sample plate moved to position 5.
    2. 50ul EBT added to sample plate and mixed by pipetting up and down.
    3. Sample plate move to position 6 (at 95°C) and incubated for 2 min. then immediately move to magnetic rack to transfer the libraries into a fresh plate.
14. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_M\_dispenseEBT.pro**
    1. Preparation of qPCR dilution plate.
    2. Transfer 49μl EBT to a 1:50 dilution plate.
15. **S:\Bravo\_protocols\MPI-EVAN-homebrew\protocols\ssDNAprep96wells\ssDNAprep96wells\ssDNAprep\_96samples\_N\_qPCR-dilutions.pro**
    1. 1μl Libraries added into 49μl EBT for qPCR dilution plate.

