Pipetting worms in 96well plates for WmicroTracker reading

What you need:

- 2 NGM plates (10cm) with synchronized adult worms (5000 worms/plate aprox).
- 15 mL tube sterile.
- 96-well flat microplate sterile.
- WmicroTracker device.
- Pipette p20.
- Multichannel pipette P200.
- Multichannel pipette reservoir.
- Tips.

Protocol:

This protocol is recommended to be performed in an air conditioned room.
All buffers need to be at room temperature.

1. Collect young adult worms from 2 plates using M9 buffer and transfer them in a sterile 15 ml tube.
2. Let the worms settle for about 5 minutes (adult worm will fall by gravity to the bottom of tube).
3. Discard the supernatant using P1000 pipette taking care not to disturb the pellet.
4. Perform a wash with 5 ml of M9 buffer. Briefly shake or invert the tube.
5. Throw out the supernatant with the pipette and add 3 ml of M9 buffer.
6. Homogenize the suspension by shaking the tube by hand 3 times. Suck 10 µl of worm solution and count number of worms. Repeat three times, and calculate the average number of worms/10 µl.
7. Prepare a suspension to get [5 worms/10 µl]. Adjust volume in M9 buffer supplemented with bacteria OP50 (final OD600: 0.1).
8. Transfer the worm suspension to the multichannel pipette reservoir.
9. Transfer 90 µl of worm solution to 96-well microplates using multichannel pipette using recommended suggestions:

Suggestion for pippeting worms!

To Do:	**Reason:**
Add 25% volume excess into pipetting reservoir.	Pipetting conditions stable from beggining to end.
At first time of pipetting, wash the tip by pipetting the contents up and down in the *C. elegans* culture at least four times. (1)	Electrostatic removal.
Shake the pipette reservoir rotating gently before and while pipetting, using your free hand.	Worm suspension homogenization.

Maintain the level of pipetting about few milimeters from the bottom of the reservoir every time you pipette.	Pipetting conditions stable from beginning to end.
Check by eye no bubbles or unfilled tips are present every time.	Correct liquid dispensing.

(1). Tips could be reused for all the plate.

10. Let worms rest for 1 hour inside the incubator.

11. Before reading the plate, homogenize worm population by gently shake by hand (rotating the microplate on the bench) for 5 seconds. Register worm activity using WMicrotracker.