Bead Assembly Protocol

General Tips:

Avoid letting the beads dry out. Prepare solutions ahead of time, so that they can be rapidly added after washes are removed.

When mixing and resuspending, make sure that there are no clumps of beads, and that the mixture looks uniformly coloured (no dark or light spots).

When pulling beads down, wait for the solution to turn totally clear before removing liquid – there should be no cloudiness left.

If droplets are stuck high in the tube, the tube can be pulsed, but this will also force the beads to the bottom of the tube.
Pulse for less than 1 second – if you then see the beads clumping, resuspend them before continuing the protocol.

You can prepare mid-protocol samples by stealing portions of the washes, just be sure you know how much you are taking out at each step.
Elute saved samples using the elution protocol, just as normal.

Prepare the Beads:

Retrieve storage tube, warm to room temperature and agitate to resuspend beads.

Remove 30uL of beads from the storage tube.

Pull down beads, remove storage solution.

Add 100uL of wash buffer and resuspend beads.

Pull down beads, remove wash buffer.

Add 100uL of wash buffer and resuspend beads.

Pull down beads, remove wash buffer.

Add 100uL of wash buffer and resuspend beads.

Pull down beads, remove wash buffer.

Add anchor DNA:

Add 387ng of A-Ori (1.17uL of the 331/uL stock).

Dilute to at least 15 uL total with wash buffer (add 13.83uL in this case).
Mix by pipetting.
Allow 15 minutes at room temperature to anneal.

Ligate the first gene:
Add 100uL of wash buffer, mix by pipetting.

Pull down beads, remove wash buffer.

Add 100uL of wash buffer, resuspend beads.

Pull down beads, remove wash buffer.

Add 2uL of ligase buffer.
Add 15.99uL of water.
Add a 2:1 molar ratio of the Kan gene. (1.01uL using the 365ng/uL stock).
Add 1uL of ligase enzyme.
Mix by pipetting.
Incubate at 4°C for 30 minutes.

Ligate the linker:
Add 100uL of wash buffer, mix by pipetting.

Pull down beads, remove wash buffer.

Add 100uL of wash buffer, resuspend beads.

Pull down beads, remove wash buffer.

Add 2uL ligase buffer.
Add 15.49uL of water.
Add 100:1 molar ratio of linker. (1.51uL using the 1/10 dilute linker stock).
Add 1uL of ligase enzyme.
Mix by pipetting.
Incubate at 4°C for 30 minutes.

Ligate next gene:
Add 100uL of wash buffer, mix by pipetting.

Pull down beads, remove wash buffer.

Add 100 uL of wash buffer, resuspend beads.

Pull down beads, remove wash buffer.

Add 100 uL of wash buffer, resuspend beads.

Pull down beads, remove wash buffer.

Add 2uL ligase buffer.
Add 9.55uL of water.
Add 4:1 molar ratio of Chlor gene. (7.45uL of the 1/10 chlor stock).
Add 1uL of ligase enzyme.
Mix by pipetting.
Incubate at 4°C for 30 minutes.

Elution:

Add 100uL of wash buffer, mix by pipetting.

Pull down beads, remove wash buffer.

Add 100uL of wash buffer, resuspend beads.

Prepare a sample tube containing 2uL of 50X TE buffer.

Pull down beads, remove wash buffer.

Add 20uL of 10mM NaOH, start a stopwatch, and begin resuspending the beads.

After 50 seconds have passed, pull the beads down.

Collect (but do not discard) the NaOH solution, and place it into the prepared sample tube.
Mix by pipetting.

Discard used beads.

Analysis:

Take 11uL from the sample tube, add 1.2uL of loading dye, and run on a standard agarose gel.
Save the remainder in case the gel is ruined, or in case other tests need to be performed on the same material.