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| Ad5CMVCre stock Ad3786 = 1.3 x 10^11 pfu/ml |
| Make 1:500 dilution (2 ul virus plus 998 ul conditioned media) = 2 x 10^8 pfu/ml, or 5 x 10^7 pfu in 250 ul (*Dilution factor may need to be adjusted/optimized for different viruses or organoid lines)* |
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| Added Y27632 ROCK inhibitor to tubes containing virus solution (1:1000 dilution of 10 mM stock to yield 10 uM final conc) |
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| Warm centrifuge to 32 C |
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| Harvested desired # of organoid wells with regular trypsin, low to moderate dissociation (pipet 5-10x after adding trypsin, pipet again after stopping trypsin) (too much dissociation does not work well) |
| Split into necessary # of groups (NEG no virus, and then multiple different virus titers or virus replicates) |
| Spin down, resuspend in 500 ul of conditioned media (NEG) or virus |
| Transfer to 24 well ultra low attachment plate |
| Seal with parafilm |
| Centrifuge 600g x 1 hr at 32 C |
|  |
| Remove parafilm |
| Incubate 37 C for 5-6 hrs |
|  |
| Add 800-1000 ul of wash media to each well |
| Transfer to 15 ml falcon |
| Add 2 ml wash media per tube |
| Spin down, resuspend in about 200 ul Matrigel (depending on final # of wells you want to plate), plate normally in nunclon delta plates with conditioned media plus 10 uM ROCK inhibitor |