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LVL0 cloning using annealed Oligos

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Protocol status: Working
We use this protocol and it's working

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Annealing of Oligos

1 Set up Annealing reaction in 1,5 mL microcentrifuge tube

	fwd Oligo	1,5 µL (10 µM)
	rev Oligo	1,5 µL (10 µM)
	T4 ligase buffer	5 µL (10x)
	ddH_20	42 µL

Incubate in heatblock for 10 min at 85°C
Turn off heatblock and allow samples to remain in the heatblock for slow cooling to room temperature.
Proceed with next step or freeze annealed oligos for long term storage.

Golden Gate Reaction

2 Set up Golden Gate Reaction

	Entry Vector	50 - 70 ng
	T7-Ligase (NEB)	1 µL
	BsmBI (NEB)	1 µL
	T4-Ligas Buffer	1 µL
	ddH_20	Ad 10 µL

Start Golden Gate Reaction in Thermocycler

	Digest	42°C	2 min
	Ligation	16°C	5 min
	Final Digest	60°C	30 min
	Inactivation	80°C	19 min

Transformation

3 Transform complete reaction mix into competent cells using a chosen protocol