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IN VITRO TRANSCRIPTION

Protocol for the *in vitro* transcription for RNAi libraries. The protocol is optimized for high-throughput *in vitro* transcription.

IVT reactions are performed in 96-well plates from Thermo-Scientific (cat# AB-0796) using a waterbath.

The IVT reaction are performed according to the manufacturer's description depending on the Supplier. Reactions should be performed in a volume of 50µl.

We use 15µl of the T7 containing 2ndPCR product as template. Incubate for several hours to overnight.

- DNAse treatment

	Per 50µl reaction
RNase free water (Acros Organics)	19.4µl
DNAseI (Fermentas)	0.6µl

- Incubate at room temperature for 30min
- Ethanol precipitation

	Per 50µl reaction
5M NH4OAc (5M, Ambion)	8µl
Ethanol absolut (Riedel-DeHaen)	200µl

- Incubate at 2-24h at -20°C
- Spin plates for 30min at 4000rpm
- Decant supernatant
- Dry pellet 1,5-2h
- Resuspend pellet in 100µl 10mM Tris pH7.0 (Ambion)
- Quality check on a gel (dilute IVT 1:50 and load 5µl dsRNA + 15µl loading dye)

Distribution: internal OK