Quick-RNATM MiniPrep

- <u>Sample Lysis</u>
 - Remove media and lyse cells in 300μ L (up to $5x10^6$ cells) or 600μ L
 - (>5x10⁶ cells) RNA Lysis Buffer by scraping
 - Put in 1.5mL tub
- <u>Sample Clearing and gDNA Removal</u>
 - Centrifuge lysate at 13,000g for 1min
 - Transfer the supernatant into a **Spin-Away Filter** in a Collection Tube and centrifuge at 13,000g for 1min
 - Save the flow-through
- <u>RNA Purification</u>
 - Add 1 volume ethanol (95-100%) to the sample in **RNA Lysis Buffer** (1:1). Mix well.
 - Transfer the mixture to a **Zymo-Spin IIICG Column** in a Collection Tube and centrifuge for 30 seconds. Discard the flow-through
 - In-column DNase I Treatment (optional)
 - Add 400µL **RNA Wash Buffer** to the column and centrifuge for 30sec. Discard the flow-through
 - Add 80µL **DNase I reaction mix** directly to the column matrix. Incubate at room temp for 15min, then centrifuge for 30 sec For each sample:

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DNase I		5μL
10X DNase I Reaction Bffr		8μL
DNase/RNase-Free Water		ĴμL
RNA Wash Bffr	64µL	-

- Add 400μ L **RNA Prep Buffer** to the column and centrifuge for 30 sec, discard the flow-through
- Add 700µL **RNA Wash Buffer** to the column and centrifuge for 30 sec, discard the flow-through
- Add 400µL **RNA Wash Buffer** and centrifuge the column for 2min
- Place the column in RNase-free tube. Add 30µL **RNase-Free Water** directly to the column, centrifuge at 16000g for 30sec
- Nanodrop RNA