

# Computing as Biologists

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CEPLAS RNA-Seq Workshop 2022

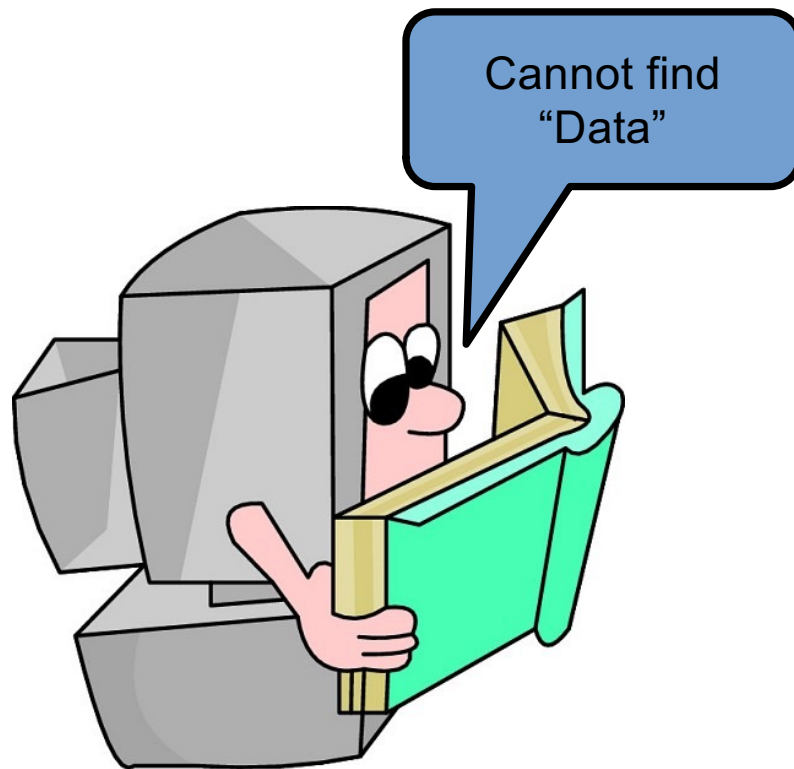
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```

- Computers will do exactly what you (the programmer) tells them to!

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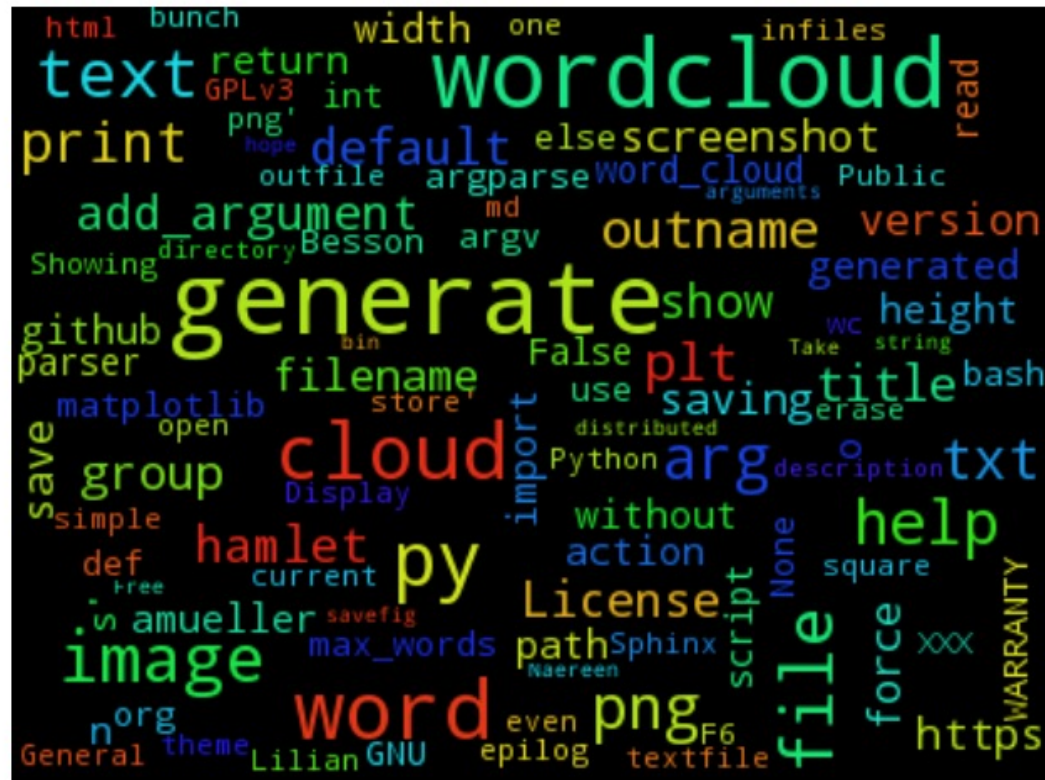
- Will: go through 100 Million data points without complaint
- Won't: figure out what you want and do that instead.



- Computers will do exactly what you (**the programmer**) tells them to!

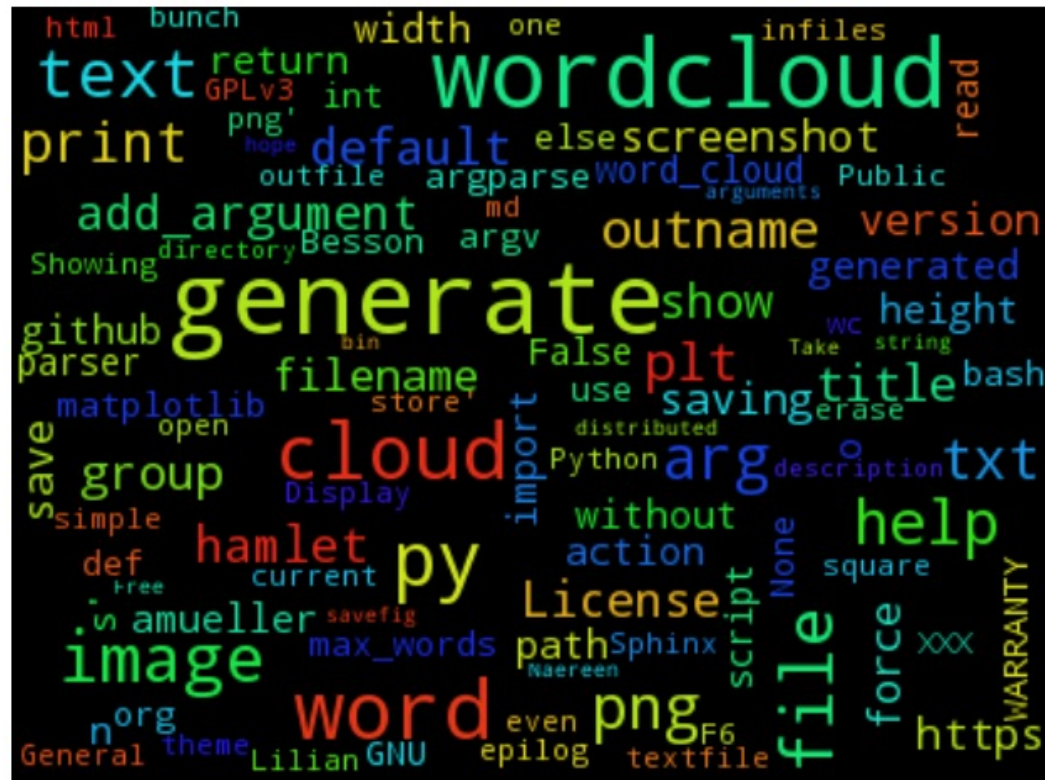


- Computers do however, speak their own languages



Img source: [nareen.github.io](https://nareen.github.io)

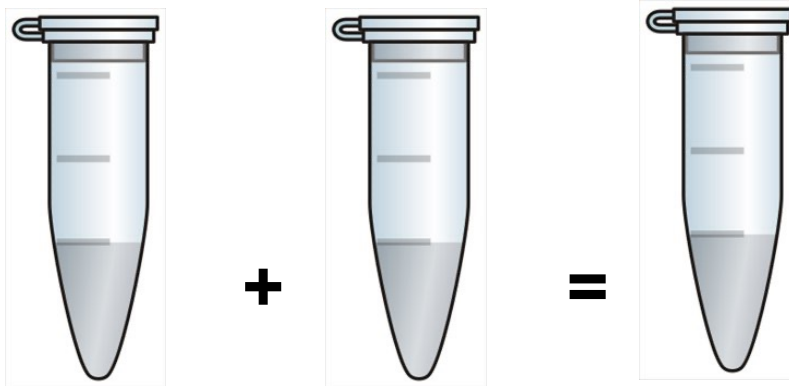
- Computers do however, speak their own languages
  - Precise
  - Simple
  - Foreign
  - Learn-able



Img source: [nareen.github.io](https://nareen.github.io)

- Please don't be scared of them!

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#### Embedding nuclei in agarose plugs ● **TIMING** 1 h + overnight

12| Prepare a 1% LMP agarose solution in 1× HBS and incubate at 45 °C in a waterbath.

13| Pre-warm the nuclei to 45 °C in a waterbath for 5–10 min. Mix the nuclei with an approximately equal volume (1 ml) of the 1% LMP agarose solution using a wide-bore pipette tip (prepared by slicing off the fine tip to produce a bore of 1.5–2 mm).

14| Aliquot 95 µl of the mixture into ice-cold CHEF disposable plug moulds on a level pre-chilled glass tray using a wide-bore pipette tip. A total of 20 g of leaf tissue should generate approximately 10–15 plugs.

■ **PAUSE POINT** Allow plugs to set fully overnight at 4 °C.

#### Lysis ● **TIMING** 24–48 h

15| Transfer the plugs (take care as they may be fragile) into lysis buffer in a 50-ml tube and incubate at 50 °C for 24–48 h with gentle shaking.

16| Wash the plugs in 0.5 M EDTA (pH 9.0–9.3) for 1 h at 50 °C with shaking.

17| Wash the plugs in 0.05 M EDTA (pH 8.0) for 1 h on ice with shaking.

▲ **CRITICAL STEP** Plugs should be washed a minimum of two times for 1 h in TE (20:50) with shaking to remove all traces of ethanol.

■ **PAUSE POINT** Store in 0.05 M EDTA (pH 8.0) at 4 °C. The plugs can be transported or shipped in 70% ethanol.

Programming draws on many of the same skills as lab work, for instance:

- Planning out a sequence of “reactions” to get a desired result
- Trouble shooting by looking at intermediate stages
- Patience

Img source: clker.com, nature.com/nprot