**Genomic DNA isolation from fixed cells**

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**Reagents:**

Proteinase K (Zymo #D3001-2-20) (20mg/ml stock in storage buffer)

RNase A (Sigma #70856) (10mg/ml stock)

100% Molecular biology grade Ethanol

**Silica spin columns (Econospin)**

**Qiagen buffer AL**

**Qiagen buffer AW1**

**Qiagen buffer AW2**

**Nuclease free water**

* Resuspend 1-3 X106 cells in 250µl of PBS and transfer to a 2ml tube (this would represent one 3.5cm dish of 3T3 cells)
* Add 200µg Proteinase K (from a 20mg/ml stock) and 200µg RNase A (from a 20mg/ml stock)
* Incubate cells at 37°C for 30 min in a water bath
* Add 250µl Qiagen AL lysis buffer per 250µl of the protease and RNAase-containing cell suspension and mix thoroughly
* Place tubes in an incubator at 56°C with shaking at 800rpm overnight, capped
  + Each tube is Parafilm sealed to ensure safety
* Add 250µl, 100% molecular biology grade ethanol; mix slowly using a slow vortex for 5 sec
* With a razor blade, trim the tip of a 1ml pipet tip to enlarge the opening. Use this tip to pipet out DNA from the ethanol solution and apply it onto a silica spin DNA binding column (e.g. EconoSpin 1920-250)
* Spin at 6,000g for 1’ in a fixed angle tabletop microfuge; aspirate and discard flow-through
* Add 500 µl Qiagen **buffer AW1** to the column, spin again at 6,000g for 1’, aspirate and discard flow-through
* Add 500 µl Qiagen **buffer AW2**, spin at 8,000g for 1’, aspirate and discard flow-through
* Spin once more using microfuge to remove excess ethanol at 13,000 g for 1’
* Transfer column into a new, 1.5ml collection tube
* Elute with pre-warmed, 100µl nuclease-free **water or TE**
  + Volume depends on starting number of cells: use 100µl per 1 million cells
* Incubate for 1’, then spin at 13,000g for 1’
* Add another 50µl **nuclease free water** to accomplish a second elution
* Incubate at room temperature for 1’, then spin as before at 13,000g for 1’.
* **The two flow-through fractions contain the genomic DNA**
* Perform Nanodrop and Qubit HS DNA estimation to calculate yield
  + Theoretically, 1X106 cells should yield 6µg DNA

Buffer AL (storage: room temperature (RT))

50 mM Tris-HCl 7.4, 5.5 M guanidine HCl, 20 mM EDTA, 1.3% Triton X-100

Buffer AW1 (storage: RT)

1 M guanidine HCl, 57% EtOH, pH 5.5

Buffer AW2 / PE (storage: RT)

10 mM Tris-HCl pH 7.5, 80% ethanol

Buffer TE (storage: RT)

10 mM TrisHCl pH 9, 0.5 mM EDTA