5.15.75

B5.3 Extraction of Cytoplasmic RNA

Dates:

| | | | | |
|---|---|---|---|---|
| 5.15 | * 200 plates | ~ most ~~½~~ heavy ¼ light | | ~~$5 \times 10^8$~~ ~~$1 \times 10^9$~~ cells (coulter) |
| 5.20 | 100 plates | ~ ⅓ light | | $3 \times 10^8$ cells (hemacytom.) |
| | 100 plates | | | $2.8 \times 10^8$ cells " " |
| 6.9 | 100 plates | heavy | | $5.1 \times 10^8$ cells |
| 6-13 | ~100 plates | some areas of plates light | | $2.5 \times 10^8$ |
| | | | total = | $1.84 \times 10^9$ cells |

General Procedure

1. Cells grown @ 37-38°C
2. When confluent (4-5 days) medium sucked off - 3 ml warm, Puck's EDTA added - 37°C × 5-10'
3. Cells scraped off, spun ↓ 1.6 K × 10'
4. Rinsed × 2 c̄ tris glucose
   Counted and resuspended in 0.1X RSB Buffer @ $5 \times 10^6$ cells/ml
5. Quenched × 15'
6. Dounced
   6 X c̄ LF's Dounce
   7 X c̄ Chun's
7. Spun ↓ 25' @ 1600
8. Cytoplasm poured off
   pronase added → 500 γ/ml
   SDS → 0.5%
   EDTA → 0.01 M
   37°C × 1 hr
9. 3 Room T° phenol extractions
   * Note: ½ of 5.15.75 prep lost - cracked bottle/centrifuge
10. 1/10 th vol of 2M NaAc ; 2 Vol ETOH → -20°C

B5-3 Cytoplasmic RNA - Cont.

All cytoplasmic RNA ETOH ppt's spun ↓ 2K for 20'
ppt's ↑ 4 ml 0.02 M Tris pH 8.1 ; 2ml / tube

| | | | | | |
|---|---|---|---|---|---|
| 1:500 dil | A. | .443 / .243 | 1.82 | 8.8 mg/ml | ml / run |
| | B. | .363 / .198 | 1.83 | mg/ml 7.26 mg/ml | |

total RNA recovered 32.24 mgm. ~ 64 γ/plate

Cytoplasmic RNA run over a poly dT column 5mgm / run
5 runs total
"Front" or Ribosomal fx's pooled → ETOH ppt'd
Messenger RNA fx's pooled ; 2/10th vol 2M NaAc + 2 Vol ETOH.

Estimated yield f 1st poly dT run ~ 630 γ
Spun ↓ ETOH ppt's ; ↑ 2 ml 0.02 M Tris pH 8.1

| O.D. | 260 | 280 | Ratio | Conc. | Total Recovered |
|---|---|---|---|---|---|
| 1:25 dil | 0.167 | / 0.082 | 2.04 | 168 γ/ml | 336 γ |

48% Rec.
1% of Cyto RNA is Messa

ETOH ppt ≈ 1/10th vol 2M NaAc to ↑ in smaller volume.

① —

5λ → 400λ water

20λ → 400 λ

a = .5 ml 3M KCl
.9 ml RNA
1.6 ml Tris

B. .5 ml 3M KCl
1.1 ml RNA
1.4 ml Tris

② Ribosomal Fx's

| | | |
|---|---|---|
| | 1. | 0.011 |
| pool ↓ | 2. | .067 |
| | 3. | .574 |
| | 4. | .602 |
| | 5. | .412 |
| | 6. | .110 |
| | 7. | .058 |
| | 8. | .034 |
| | 9. | .020 |

Messenger Fx's

| | | |
|---|---|---|
| | 1. | 0.006 |
| pool ↓ | 2. | .058 |
| | 3. | .057 |
| | 4. | .030 |
| | 5. | .007 |

[illegible] γ 2.7%

③

| | | |
|---|---|---|
| | 1. | .007 |
| pool ↓ | 2. | .185 |
| | 3. | .638 |
| | 4. | .675 |
| | 5. | .580 |
| | 6. | .065 |
| | 7. | .035 |
| | 8. | .040 |
| | 9. | .025 |
| | 10. | .015 |

| | | |
|---|---|---|
| pool ↓ | 1. | .025 |
| | 2. | .094 |
| | 3. | .065 |
| | 4. | .017 |
| | 5. | .011 |

170 γ or 2.4%

④

| | | |
|---|---|---|
| | 1. | .012 |
| | 2. | .080 |
| | 3. | .552 |
| | 4. | .620 |
| | 5. | .590 |
| | 6. | .127 |
| ↓ | 7. | .010 |
| | 8. | .085 |
| | 9. | .044 |
| | 10. | .035 |
| | 11. | .030 |

| | | |
|---|---|---|
| | 1. | .012 |
| | 2. | .032 |
| | 3. | .105 |
| | 4. | .012 |
| | 5. | .010 |
| | 6. | .006 |

[illegible] 2.7%

⑤

| | | |
|---|---|---|
| | 1. | .017 |
| pool | 2. | .157 |
| | 3. | .401 |
| | 4. | .394 |
| | 5. | .324 |
| | 6. | .080 |
| | 7. | .042 |
| | 8. | .035 |
| | 9. | .018 |
| | 10. | .010 |

| | | |
|---|---|---|
| pool | 1. | .025 |
| | 2. | .035 |
| | 3. | .087 |
| | 4. | .017 |
| | 5. | .014 |

[illegible] γ 2.8%

~[illegible] γ = 200 γ expected yield after 2nd run over poly dT

% of total

BS-3 Extraction of Nuclear RNA

6-9-75 100 plates used for extraction ~ $5.1 \times 10^8$ cells

1. Med. off ; 3 ml of Pucks EDTA ; 37°C X 5-10'
2. Cells scraped off ↓ 1.6 K X10'
3. Rinsed X2 c̄ tris-glucose
   Counted and resuspended in 0.1 X RSB Buffer @ $5 \times 10^6$ cells/ml ~ 100 ml
4. Quenched X 15', checked under 'scope for swelling
   Cells look good
5. Dounced c̄ LF's douce X 5 — good separation
6. ↓ 1.6K X 20'
7. Cytoplasm poured off ; Nuclei up in 50 ml of STE (sterile)
8. 20% SDS → .5% 2.5 ml
   pronase → 500 γ/ml 5 ml
   Dounced X 10
   37°C X 1 hr
9. 3-5' phenol ; 2M NaAc → 0.2 M ; 2 vol ETOH

B5-3 Extraction of Total RNA

Dates: 5-16-75 – 50 plates
6-1-75 – 50 plates

1. Media sucked off plates
2. STE Buffer, 0.5% SDS, 500 γ/ml pronase added
   3 ml/plate
3. 37°C × 1 hr 15′
4. Cells scraped → -20°C

4-11-75 Extraction of DNA from B5-1 cells

3-29-75 1 vial thawed → 2 plates ; cells grew slowly

4-5-75 Split 2 → 8 when ~~heavy~~ ~~not~~ growth ↑ but not yet confluent

4-7-75 Split 8 → 48

4-8-75 cells contaminated w/ Bact. ; cells left to grow

4-9- Sm... added

4-11-75 DNA extracted

Medium sucked off, rinsed w/ tris/glucose

Scraped into 4 ml DNA Buffer/plate, Spun 2K x 10'

↑ 50 ml DNA Buffer, Counted w/ Coulter : $1.1 \times 10^8$ cells total

pronase → 500 U/plate 2.5 ml

SDS → 0.5% 1.25 ml

37° C x 1hr

2 phenols, 2 vol EtOH

Extraction of DNA from B5-3 cells

| | |
|---|---|
| 3-29-75 | 1 vial → 2 |
| 4-[illegible] | plates contaminated ; Bacteria |
| | 1 vial → 2 |
| 4-4 | 2 plates → 12 |
| 4-7 | 12 plates → 64 |
| | Cells contaminated ; |
| 4-11 | Extracted ; B5-1 , same procedure |
| | Cell Count $2.9 \times 10^8$ cells total from 60 plates |
| | ↑ 60 ml ; pronased 3.0 ml , SDS 1.5 ml , phenols 2 vol ETOH → -20° |