

Cultivo Celular - Prática

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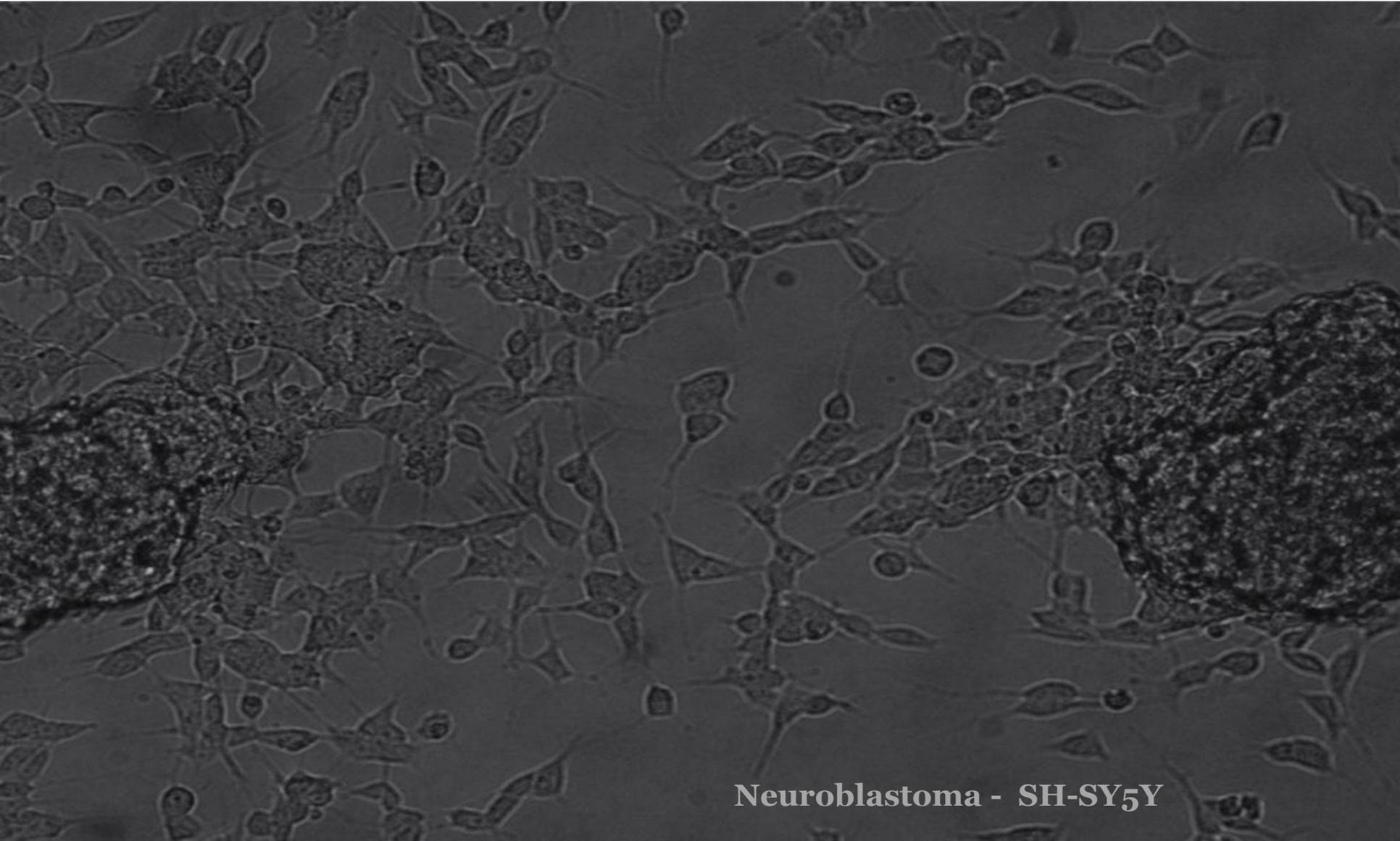
CDTec

centro de desenvolvimento tecnológico

Cronograma

- Descongelamento
- Manutenção da cultura
 - Troca de meio
 - Repique
- Congelamento

2. Descongelamento



Neuroblastoma - SH-SY5Y



Colocando diretamente na garrafa de cultura.



Utilizando a centrífuga.



Processo Rápido



Após ± 4 horas

Troca do Meio de Cultura



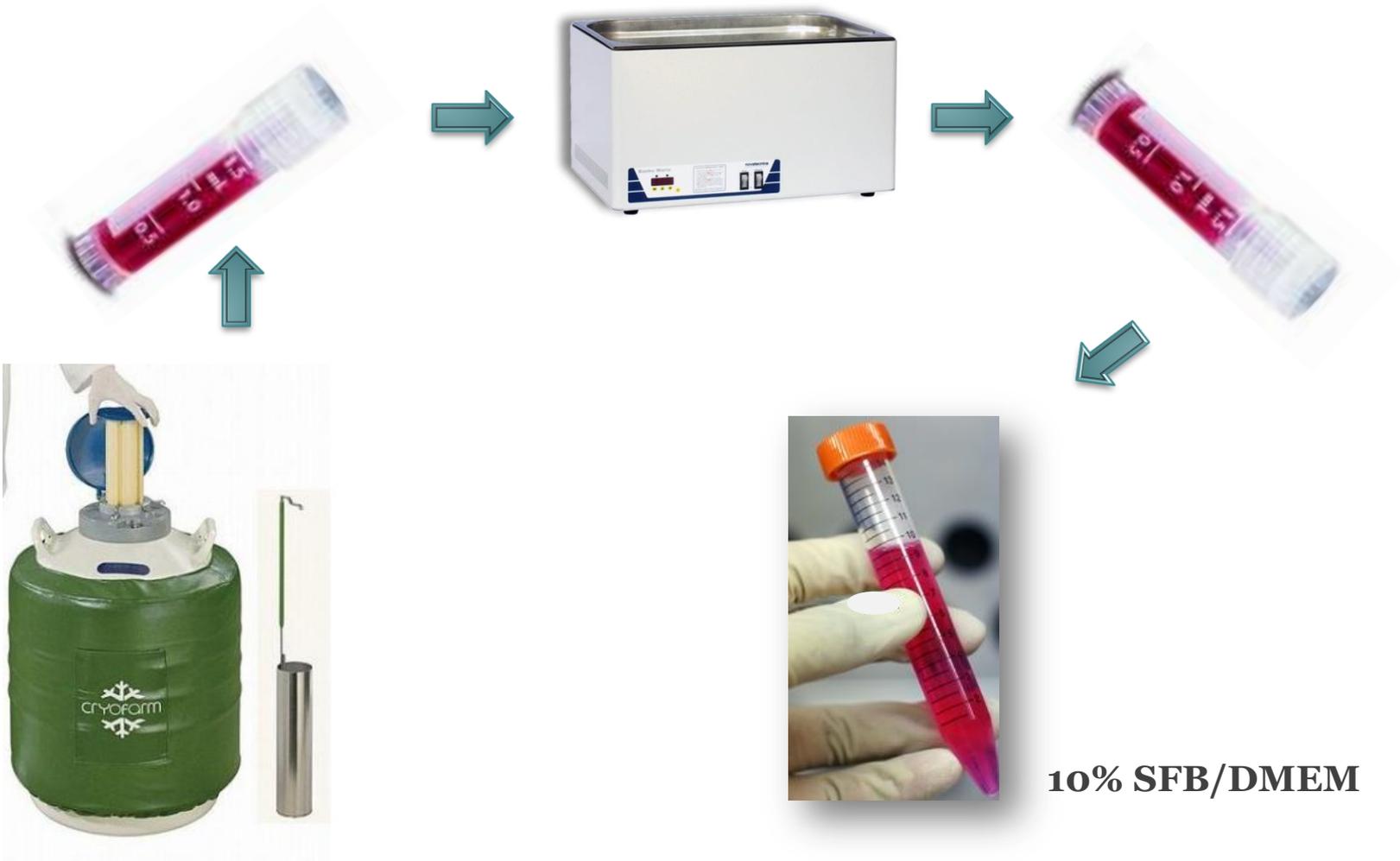
10% SFB



DMEM



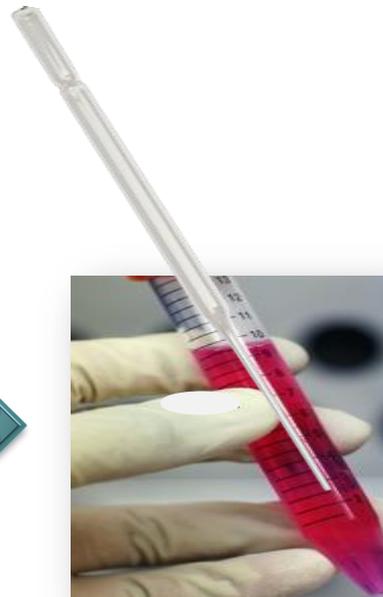
Utilizando a centrífuga.



2. Descongelamento – Centrífuga



1200 rpm
5 min
Banco de Células do Rio de
Janeiro (BCRJ/UFRJ)



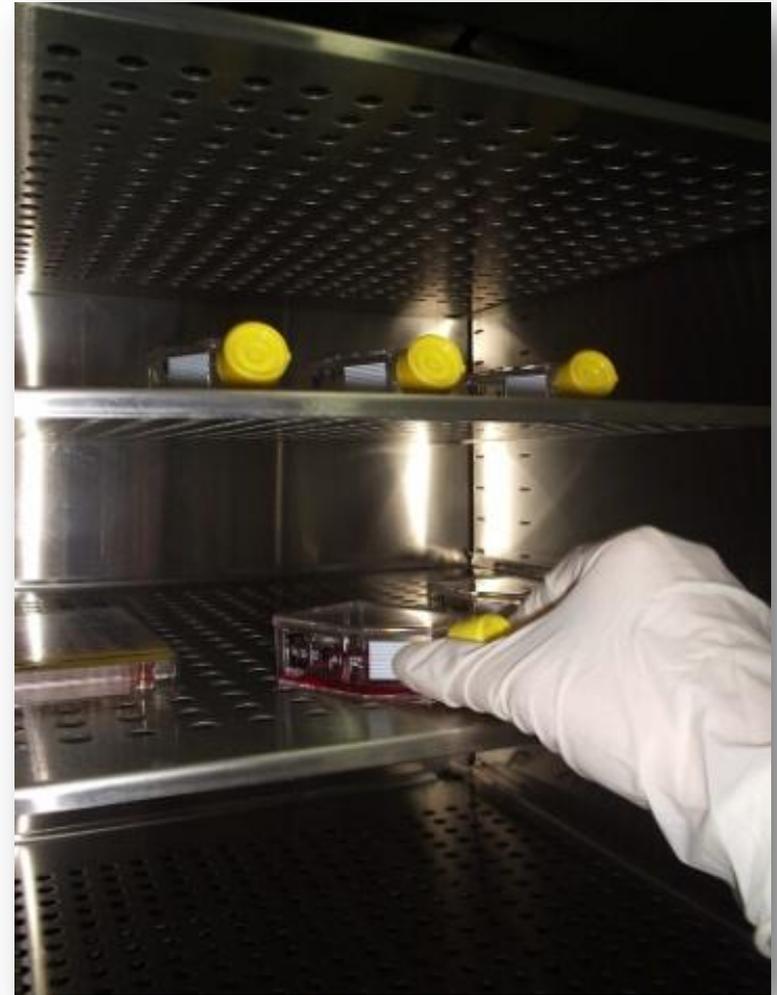
2. Descongelamento – Centrifuga



← **10% SFB/DMEM**



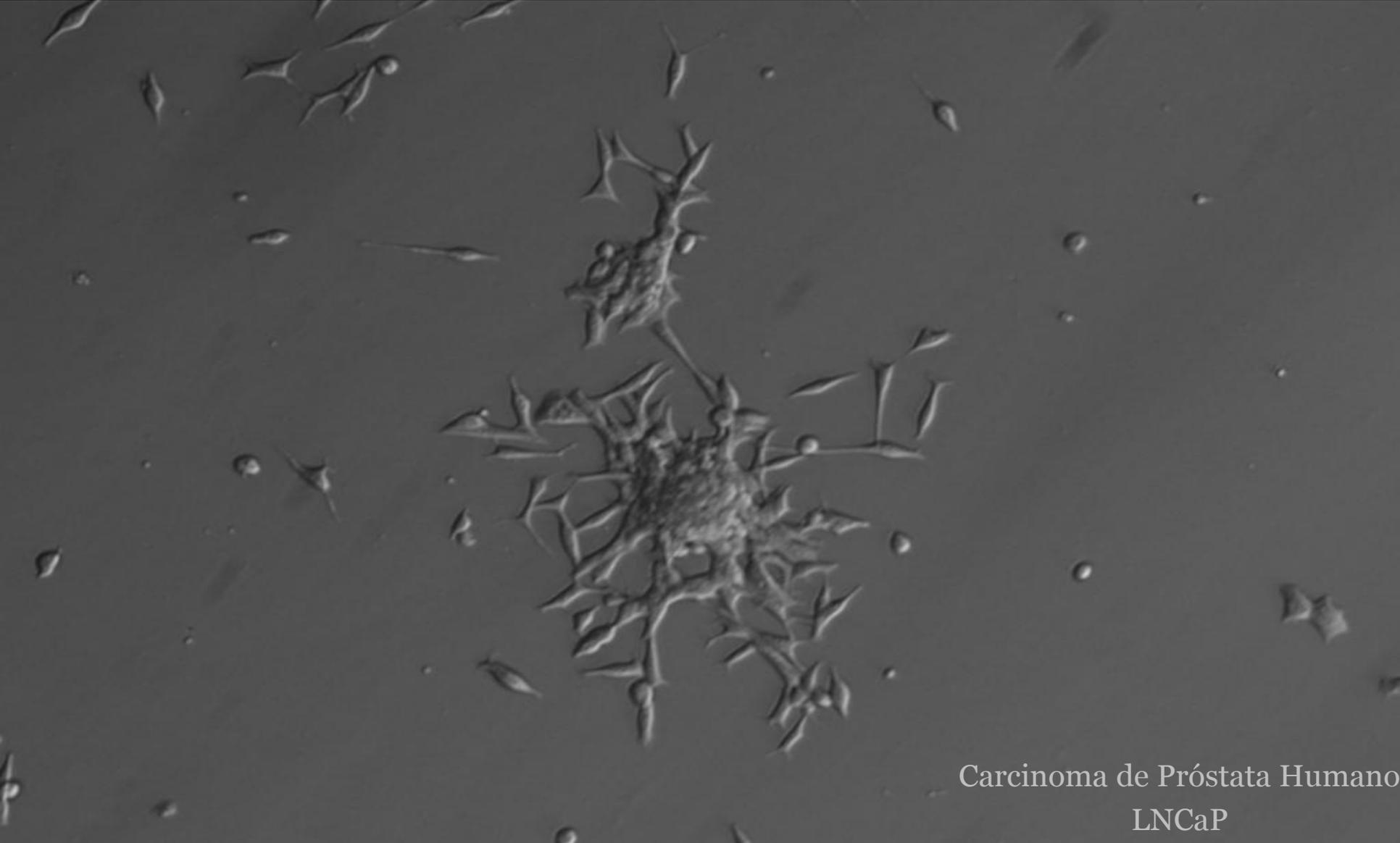
Estufa de Co₂
37 °C
5% de Co₂
95% de umidade



Cronograma

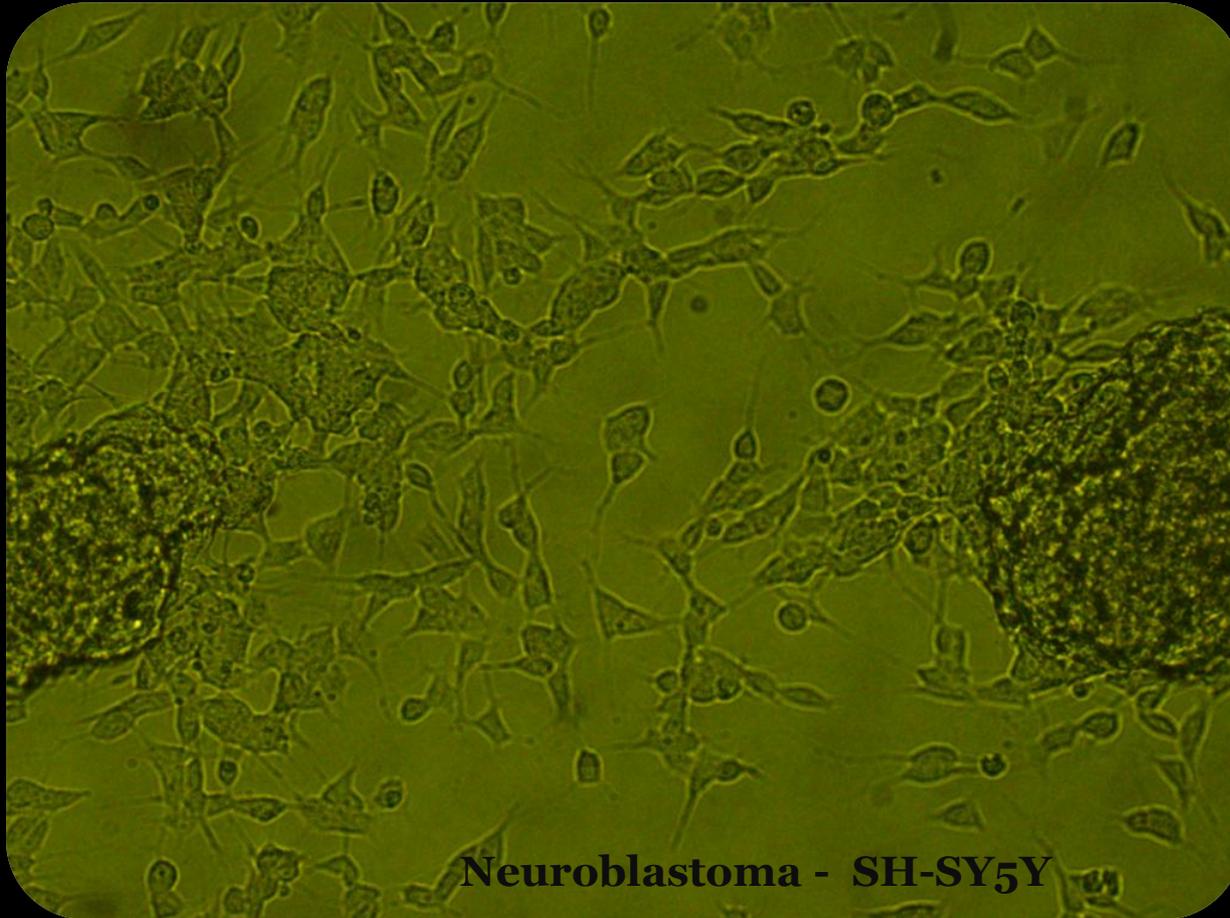
- ✓ Descongelamento
- Manutenção da cultura
 - Troca de meio
 - Repique
- Congelamento

3. Manutenção da Cultura

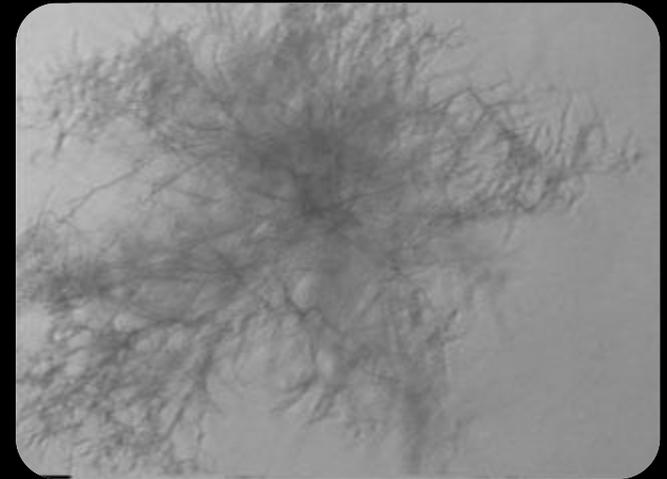
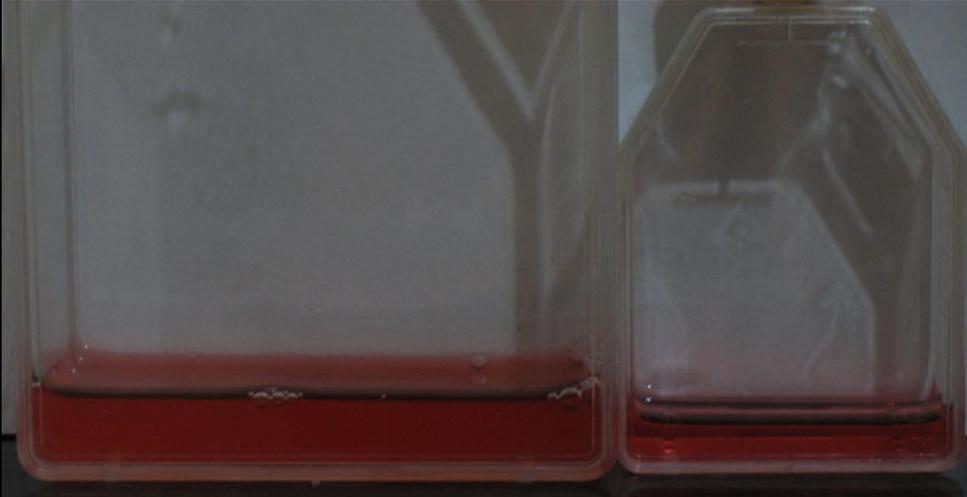


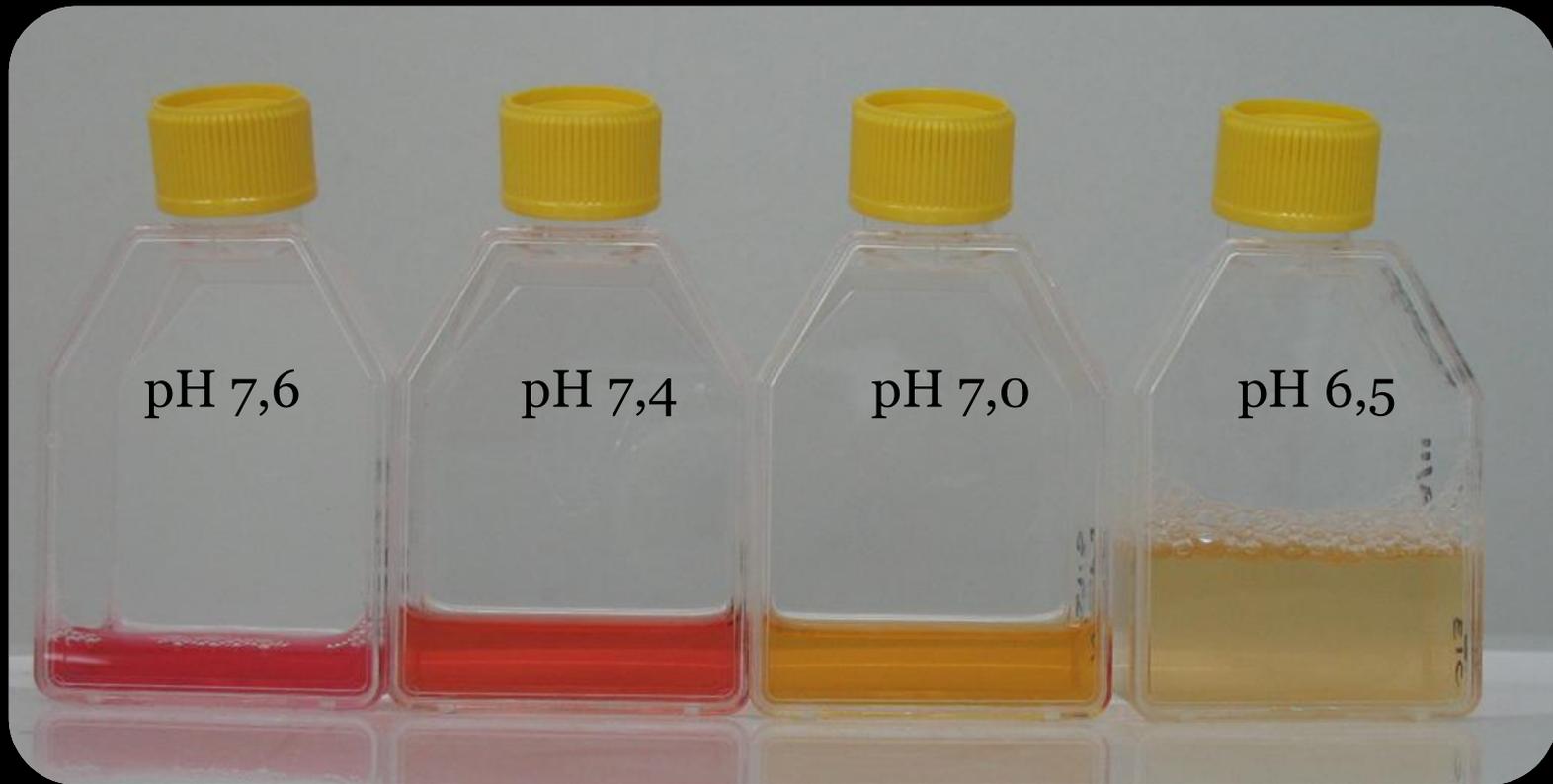
Carcinoma de Próstata Humano
LNCaP

Manutenção da Cultura



I. Visualização das Garrafas de Cultivo

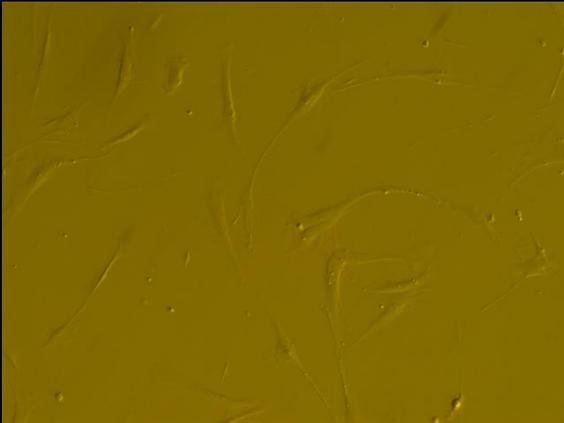




II. Visualização da morfologia e viabilidade celular



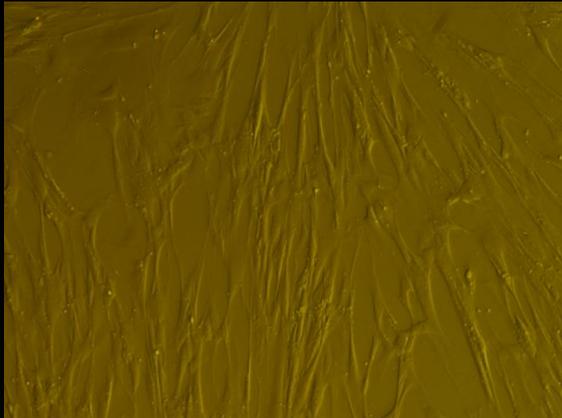
Fibroblasto de Pulmão Humano – IMR-90



Células não confluentes



Troca do meio



Células em confluência



Repique

III. Trocas do Meio



1. Remoção do Meio

2. Lavagem com PBS
3. Remoção do PBS



4. Colocação de Meio Novo



5. Estufa
37 °C
5% de CO₂
95% de umidade



IV. Repique



1. Remoção do Meio



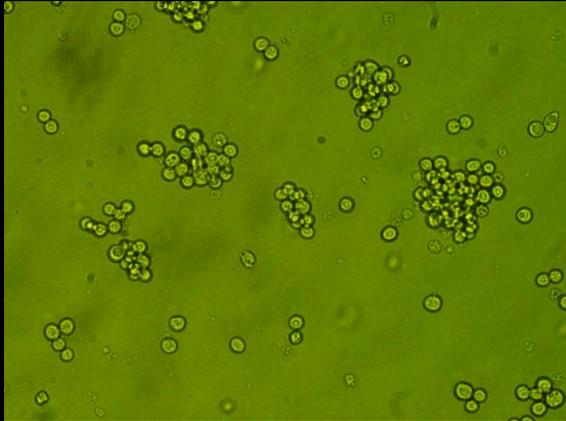
2. Lavagem com PBS

3. Remoção do PBS

4. 0,25% Tripsina/EDTA



3. Manutenção da Cultura



Trypsina



Sem Trypsina



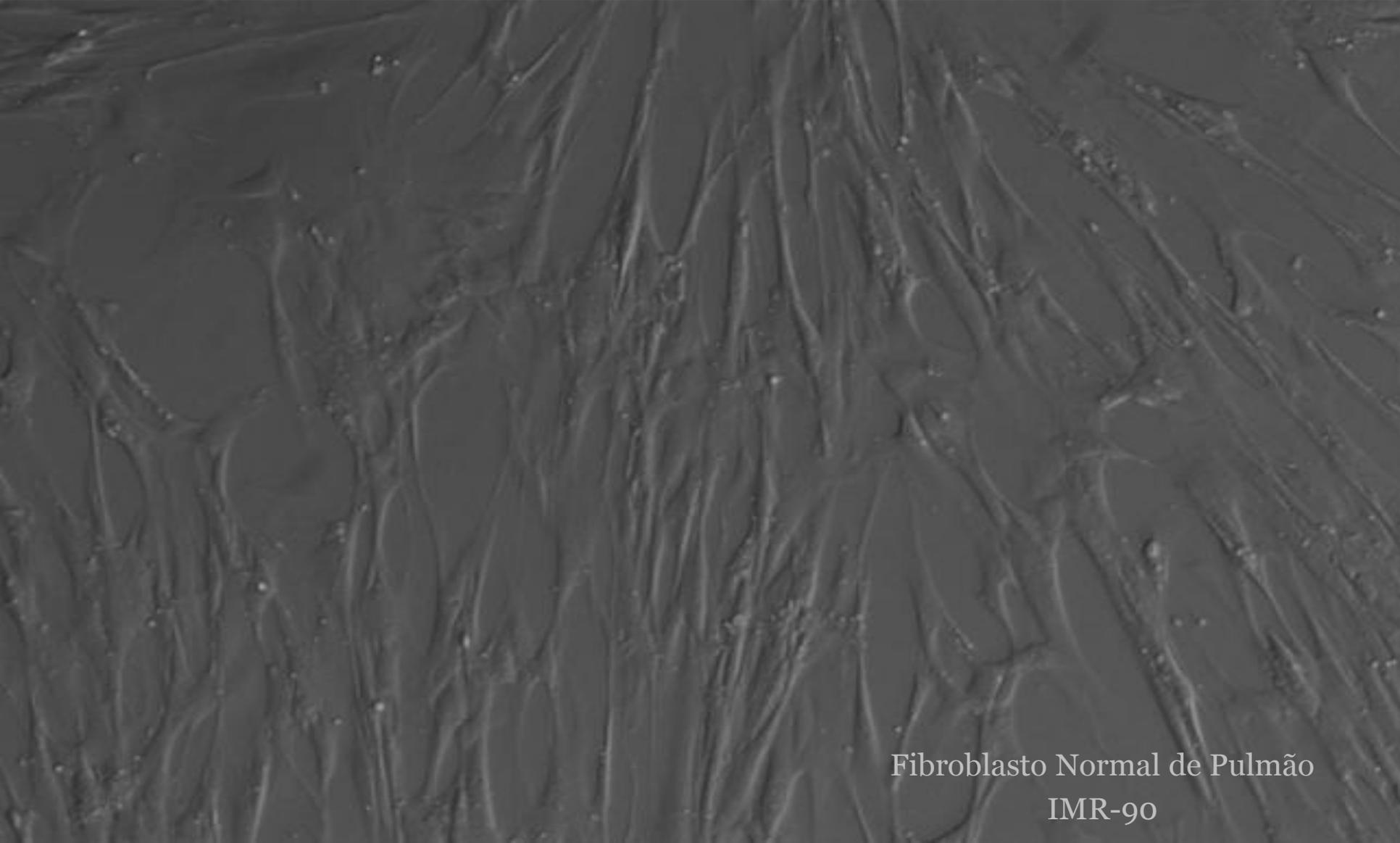
5. Colocação de Meio Novo
6. Pipetagem
7. Transferência para duas garrafas de cultivo



Cronograma

- ✓ Descongelamento
- ✓ Manutenção da cultura
 - Troca de meio
 - Repique
- Congelamento

4. Congelamento



Fibroblasto Normal de Pulmão
IMR-90

Meio de Congelamento



95% SFB
5% DMSO

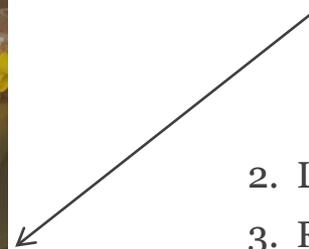
“Repique”



1. Remoção do Meio

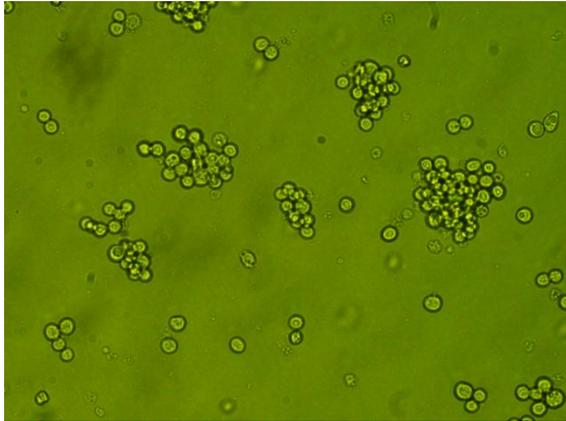


4. 0,25% Tripsina/EDTA

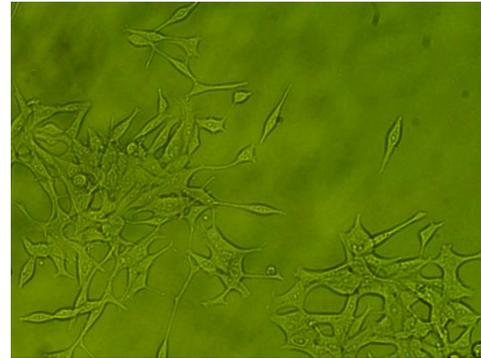


2. Lavagem com PBS

3. Remoção do PBS



Trypsina



Sem Trypsina



5. Colocação de Meio Novo
6. Pipetagem
7. Tra



4. Congelamento



← **10% SFB/DMEM**



1 mL

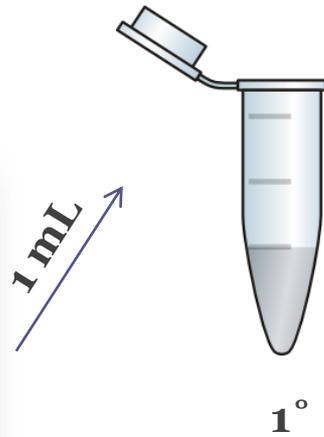


1. Contagem Celular

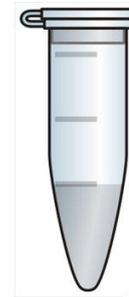


2. Congelamento

1. Contagem Celular

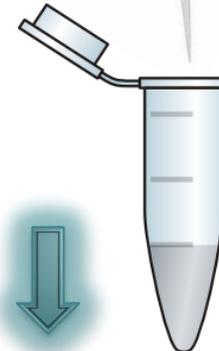


20 μ L

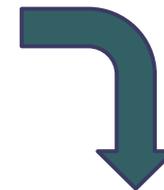
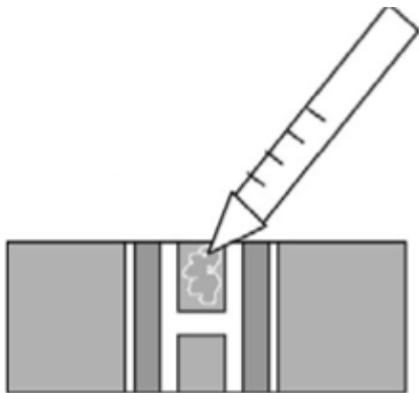
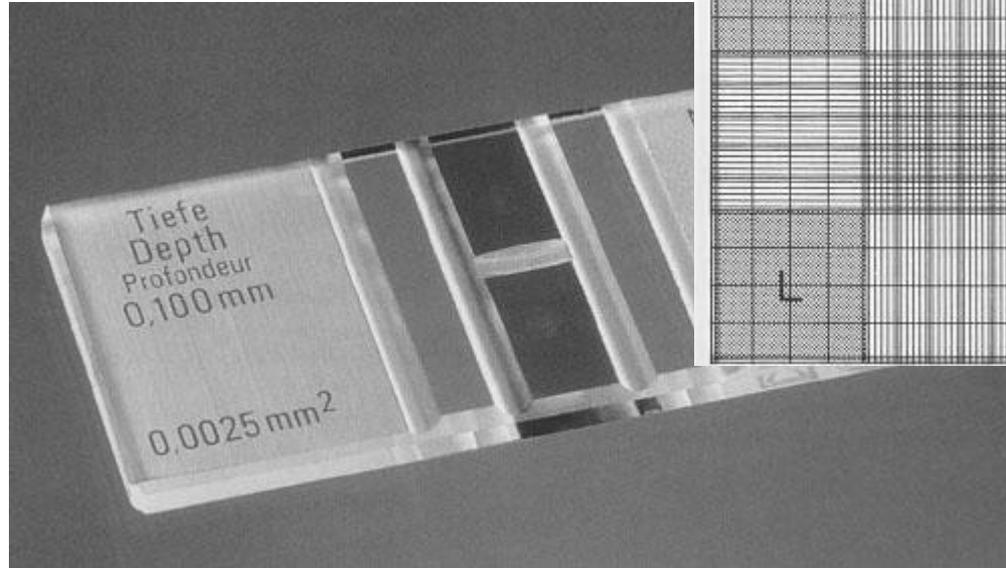
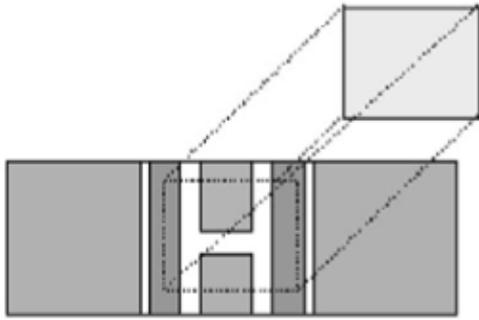


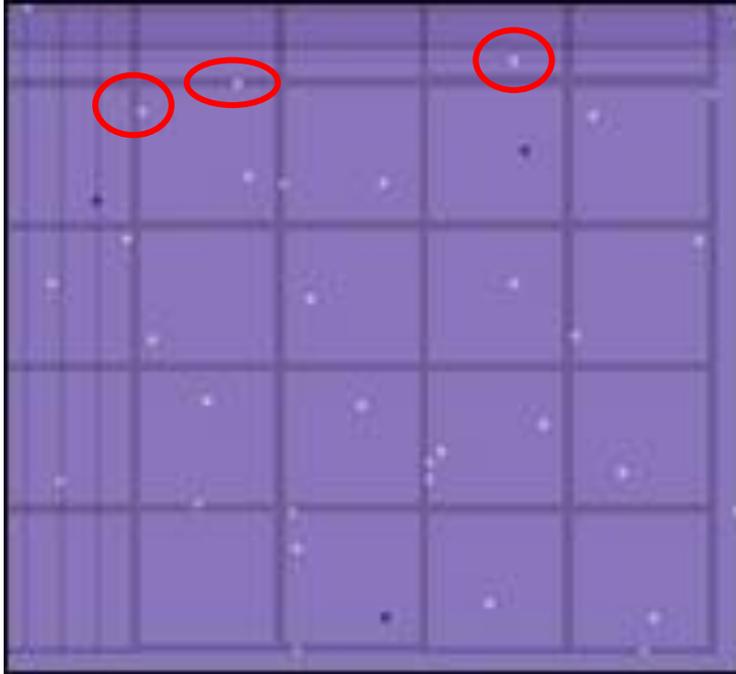
2°

20 μ L



4. Congelamento





$$\frac{Q_1+Q_2+Q_3+Q_4}{4} \times 10^4$$

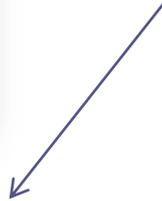
2. Congelamento



1 200 rpm

10 min

Banco de Células do Rio de Janeiro (BCRJ/UFRJ)

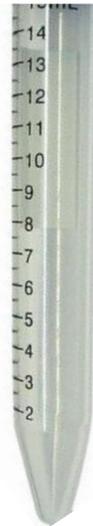




Meio de Congelamento
95% SFB
5% DMSO

Correta Identificação

- **Tipo de Cél.**
- **Data de Conge.**
- **Responsável**





Cooler "Mr. Frosty"
1 °C/min



Freezer -70



24 horas



Processo LENTO