cDNA library Establishment

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cDNA library Establishment

-- It’s a good idea to establish this library than DNA library. Why?

– Establishment need mRNA. Why?

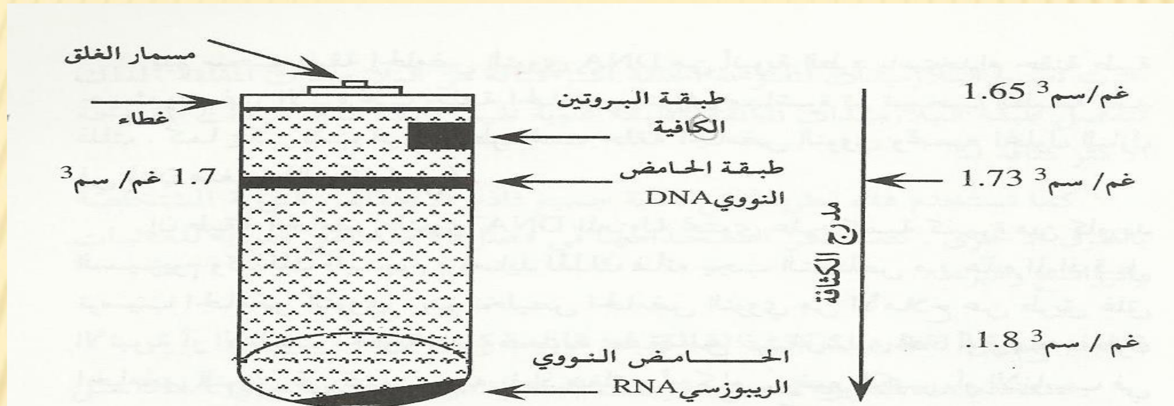
– Library size needed for cDNA is small. Why?

– Reverse transcriptase enzyme is needed for this assay. Why?

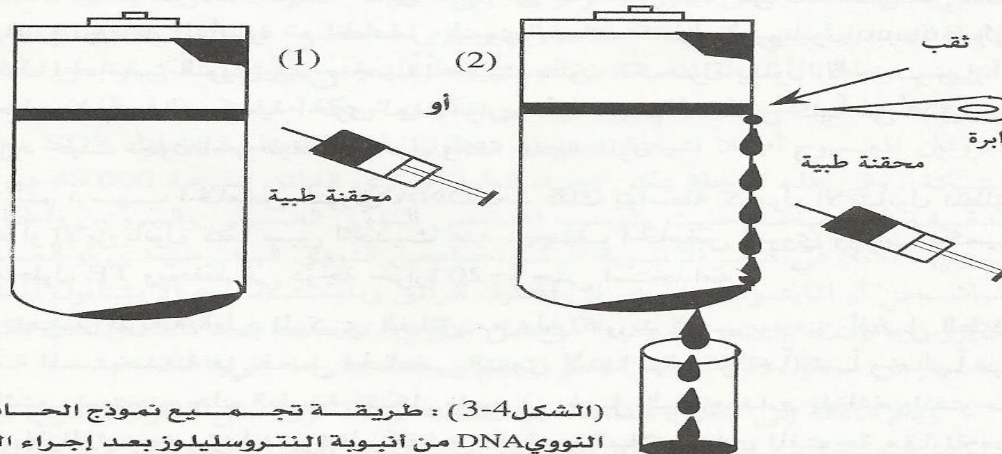
– Minigene will be produced. What this mean?

How can we establish cDNA library?

1. Extraction of total RNA from cells using Cesium Chloride-CsCl 5.6 M.



(الشكل 4-2) : انبوبة النتروسليلوز بعد إجراء الطرد المركزي الفائق مع ملح كلوريد السيزيوم 5.6 مولاري ويلاحظ بأن حلقة الحامض النووي DNA تقع في نفس موقع كثافة كلوريد السيزيوم المستخدم



(الشكل 4-3) : طريقة تجميع نموذج الحامض النووي DNA من انبوبة النتروسليلوز بعد إجراء الطرد المركزي الفائق بوجود كلوريد السيزيوم

2. Isolation of mRNA from the total RNA using Oligo T cellulose column- Chromatography.

– Wash column with 0.1 M NaoH solution

– Wash column with Low concentration salt solution.

– Wash column with High concentration salt solution.

– Load total RNA sample into the column.

– Allow the passed solution to be out.

– Wash column with Low concentration salt solution – collect the fraction-mRNA fraction.

3. Precipitate the mRNA using 2 volumes cold absolute ethanol + 0.3 M NaCl of ice solution.

4. Read the concentration at 260A using spectrophotometer.

5. Go for cDNA library assay.

cDNA assay

1.The primer which must be used is a T-oligo.? Why???

2.+ dNTP, G, C, T, A + Reverse transcriptase enzyme + Enzyme buffer + mRNA template.

3.Incubation at 37.5C? For several hours.

4. mRNA-DNA hybrid will be produced.

5.Remove mRNA strand and primer by adding NaOH solution.

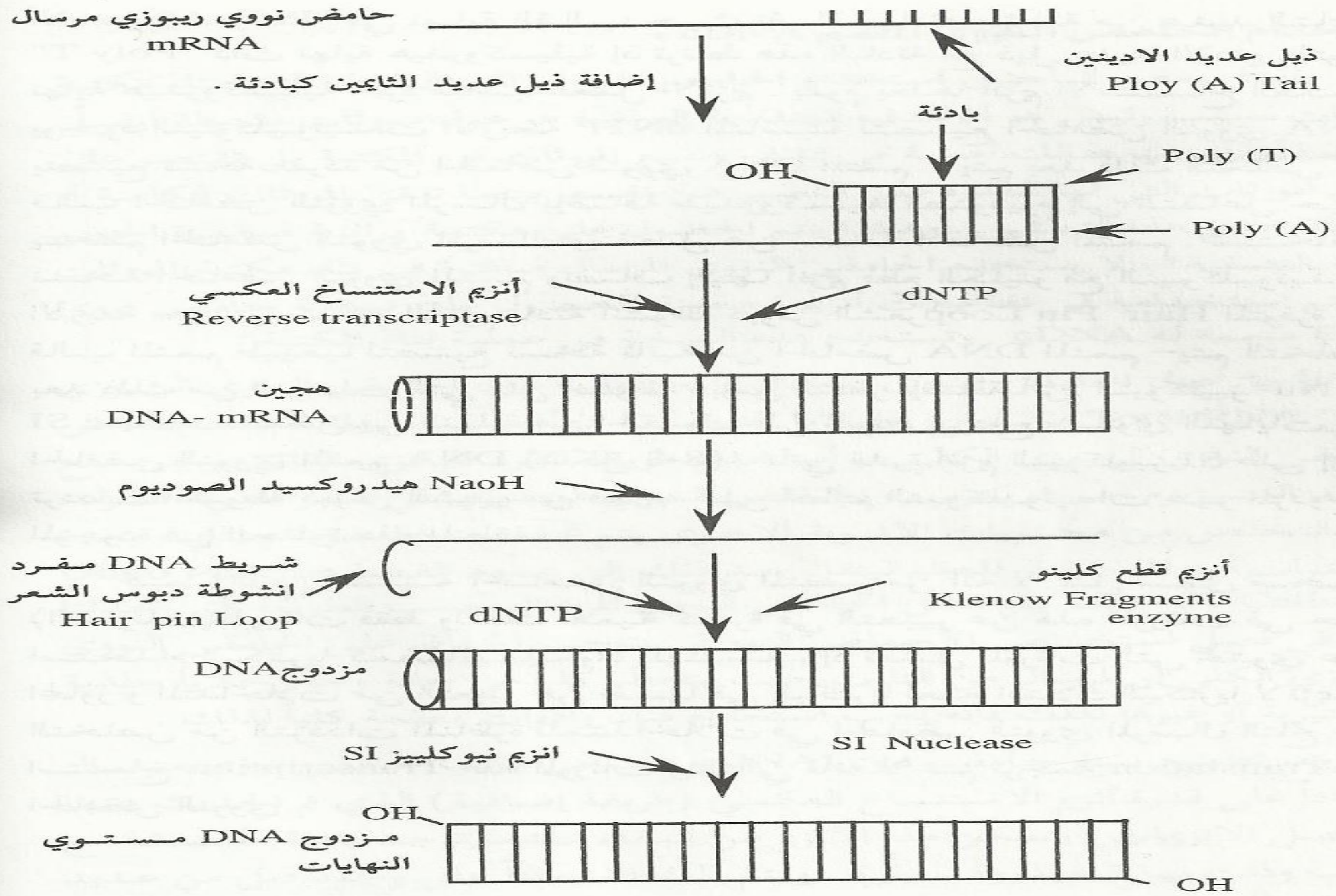
6. The remain DNA strand will have a hair pin loop. This loop ended with nucleotide with OH end.

7. Add dNTP + Klinow fragment Enzyme+enzyme buffer to the DNA solution. Incubate at 37.5C.

8.Double stranded DNA with hair pin end will be produced.

9.Treat the double strand DNA with SI nuclease to cut and remove the hair pin end.

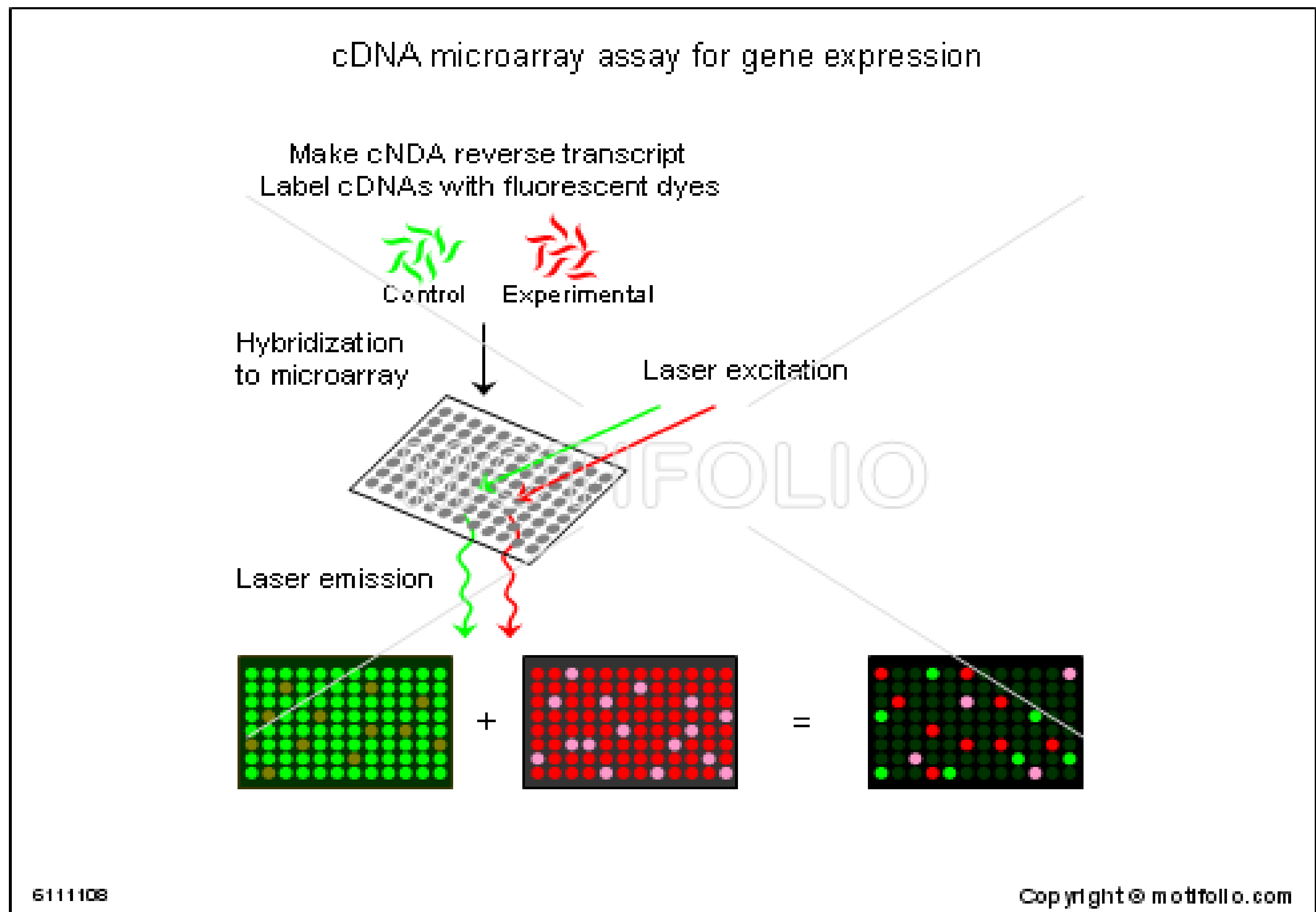
10. Minigene is ready now for cloning.



(الشكل 8-8): طريقة تصنيع مزدوج حامض نووي DNA مكمل من شريط حامض نووي مرسال باستخدام انزيم الاستنساخ العكسي وقطع كلينو

cDNA can be used to estimate the level of gene expression

+RT PCR



Thank you so much